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Review article

Biomarkers for successful IVF and ICSI: a systematic review

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ABSTRACT

Assisted reproductive technologies (ART), including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), have revolutionized infertility treatment. However, their success remains variable and depends on multiple biological and clinical factors. Biomarkers have emerged as promising tools for predicting, monitoring, and potentially improving ART outcomes. Despite extensive research, no biomarker has been adopted for routine clinical use. This systematic review aims to examine emerging biomarkers identified in serum, follicular fluid, follicular cells, culture medium, endometrial tissue, and cumulus granulosa cells, to provide a comprehensive understanding of their potential impact on ART outcomes. The publications were searched in PubMed and ScienceDirect from inception to May 25, 2024. Inclusion criteria were: (i) patients undergoing ICSI or IVF, (ii) biomarkers related to implantation and/or pregnancy outcomes, (iii) studies primarily focused on biomarkers, and (iv) full-text original articles. Exclusion criteria were: (i) non-English publications, (ii) review articles, (iii) unrelated outcomes, and (iv) studies involving reproductive system diseases or diagnosed reproductive disorders. A total of 47 studies met the inclusion criteria and were analyzed to synthesize current evidence on biomarkers and evaluate their potential relevance to ART outcomes. Numerous biomarkers were identified across various biological samples. Hormonal biomarkers are the most consistently reliable, with follicle-stimulating hormone to luteinizing hormone (FSH/LH) ratios and anti-Müllerian hormone (AMH) levels demonstrating utility in assessing ovarian reserve and predicting reproductive potential. In addition to hormonal indicators, molecular signatures (gene and protein expression profiles, cytokines, and other non-traditional biomarkers) have been widely investigated. However, no single biomarker or panel has yet shown sufficient predictive power, sensitivity, or specificity for routine clinical use. Nonetheless, certain biomarkers may offer therapeutic insights and inform novel intervention strategies. These findings underscore the complexity of human reproduction and highlight the need for integrated, large-scale research involving diverse populations to identify reliable biomarkers that can support evidence-based decision-making in reproductive medicine.

Keywords: biomarkers, assisted reproductive technologies, implantation, pregnancy outcome

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Introduction

Assisted reproductive technologies (ART) are medical procedures used to help individuals or couples conceive a child when they are unable to do so naturally due to infertility or other reproductive challenges. ART involves the manipulation of eggs, sperm, or embryos to achieve pregnancy. In 1978, the first successful ART was announced from England by using in vitro fertilization (IVF).[1] Later several ART have been developed to achieve pregnancy. Some common ART procedures include IVF and intracytoplasmic sperm injection (ICSI). While ART have helped countless individuals and couples achieve their dream of having children, the success of ART is not guaranteed and can be influenced by a range of factors. The problem with the success of ART lies in various challenges that affect the outcomes of treatment.

Biomarkers are biological indicators that can help predict, monitor, or improve the outcomes of fertility treatments such as IVF and other assisted reproductive procedures. The discovery of reliable biomarkers has been crucial for improving clinical outcomes. A wide range of biological samples, such as serum, follicular fluids (FFs), follicular cells, culture medium, endometrial tissue, and cumulus granulosa cells, have been investigated for their capacity to predict and enhance fertilization, implantation, and live birth rates.

Biomarkers can be used to assess the health and quality of eggs, sperm, embryos, and endometrium (the uterine lining) to enhance the chances of a successful pregnancy. Well-known biomarkers such as follicle-stimulating hormone (FSH),[2] serum anti-Müllerian hormone (AMH),[3–7] cell-free DNA (cfDNA),[8,9] human chorionic gonadotropin (hCG),[10] activated NK cells ($CD56^{dim}$, $CD56^{bright}$, $CD16^{+}$, $CD69^{+}$, and $CD94^{+}$) ,[11] CA-125,[12] TNF polymorphisms (TNF-308, rs1800629 and TNF-238, rs361525),[13] growth hormone (GH),[14] apolipoprotein B (APOB),[15] several miRNAs,[16,17] proinflammatory cytokines,[18,19] the fragile X mental retardation 1 gene (FMR1),[20] mitochondrial DNA (mtDNA),[21] immunosuppressive activity,[22] granulocyte colony-stimulating factor (G-CSF),[23] and macrophage colony-stimulating factor (M-CSF).[24]

Several biomarkers have been reported in the context of ART; however, none have yet been adopted for routine clinical use in IVF or ICSI procedures. Therefore, this systematic review analyzes emerging biomarkers identified in serum, follicular fluid, follicular cells, culture medium, endometrial tissue, and cumulus granulosa cells to provide a comprehensive understanding of their potential impact on ART outcomes.

Methods

Publication searching was performed on PubMed and ScienceDirect from inception to May 25, 2024. The search terms used were "biomarker," "ICSI," "IVF," and "implantation and/or pregnancy outcome." No other search conditions were applied. All articles obtained from the two databases were checked for duplication. The remaining articles were initially screened as per the inclusion criteria based on the content of the abstract section. The criteria for inclusion of the articles in this systematic review were as follows: (i) patients undergoing ICSI or IVF treatment, (ii) biomarkers associated with implantation and/or pregnancy outcomes, (iii) studies on biomarkers, (iv) full published research articles. The exclusion criteria included at least one of the following criteria: (i) articles published in a language other than English, (ii) review articles, (iii) not related to implantation and/or pregnancy outcome, and (iv) diseases related to reproductive system or diagnosis of reproductive disorders.

Data extraction from all articles was performed by two independent researchers. When conflicting opinions arose, the decision was sought from higher professional level personnel and the decision was considered final. The information extracted included: type of study, objective, participants,

type of sample, biomarkers, method, and main finding.

A formal quality or risk-of-bias appraisal of the included studies was not conducted in this review. Given the considerable heterogeneity in study designs, populations, and reported outcomes, a meta-analysis was not feasible. Instead, we conducted a qualitative descriptive synthesis. Findings were narratively summarized and categorized by biomarker type, including hormonal, genetic, protein-based, cytokines, and others. This approach enabled the identification of consistent trends, evaluation of predictive potential, and critical appraisal of study limitations, thereby supporting the objective of assessing biomarkers associated with ART outcomes. The absence of a standardized quality assessment is acknowledged and should be considered when interpreting the findings.

Results

Enrollment of study articles

A total 2,010 articles from PubMed and ScienceDirect databases were retrieved. Two hundred and ninety-seven articles were excluded as duplicates, 12 articles were review articles, 23 articles were excluded as non-full-text. 1,788 articles were excluded from the analysis as they did not fulfill the inclusion criteria. Out of 222 remaining articles, only 47 articles were finally included in the analysis (Figure 1).

Characterization of study articles

The articles included in the systematic review are summarized in **Tables 1** and **2**. Of these, 46 articles were about patients undergoing IVF treatment and only one article was about patients undergoing ICSI treatment. Of the 47 articles, 20[2–13,25–32], 12[14–19,33–38], 3[39–41], 5[22,42–45], 2[46,47], 2[20,48], 1[21], and 2[23,24] articles, respectively, were related to the investigation of biomarkers in the serum, FFs, follicular cells, culture medium, endometrial, oocyte, cumulus granulosa cells, and serum and FFs samples.

The enzyme-linked immunosorbent assay (ELISA) is widely utilized in research due to its exceptional sensitivity and specificity in identifying biomarkers essential for diagnosing and comprehending various reproductive disorders. This method depends on accurate antigen-antibody interactions to measure even trace amounts of specific molecules in biological samples, facilitating early detection and monitoring of hormonal imbalances, infections, and other reproductive abnormalities.[49] The cost-effectiveness, reliability, and versatility of ELISA have established it as a standard method in research focused on clarifying the complicated mechanisms of reproductive health and disease, thereby significantly advancing the development of more effective diagnostic and therapeutic strategies in the field.[50]

The investigated biomarkers are varied. Biomarkers are categorized into four main groups to facilitate comprehensive research and interpretation. These categories include hormones, genes/proteins, cytokines, and other groups include biomarkers that are unrelated to the previous classifications.

Biomarkers that can be identified in serum or/and blood

The potential serum biomarkers (**Table 1**) can be categorized into four distinct groups. The hormone category encompasses reproductive and growth-related hormones including the FSH:LH ratio,[2,25] FSH, progesterone (P), and estrogen (E_2),[2,7] AMH,[3–7] insulin-like growth factor-1 (IGF-1) and IGF-2,[31] hCG,[10] β-hCG,[30] and its hyperglycosylated form (hhCG).[29] The gene/protein group includes markers indicative of gene/protein mutation and expression, such as TNF polymorphisms (TNF-308, rs1800629, and TNF-238, rs361525),[13] insulin-like growth factor binding protein-1 (IGFBP-1) and soluble FMS-like tyrosine kinase-1 (sFLT-1).[31] The biomarkers in the cytokine category are activated NK cells ($CD56^{dim}$, $CD56^{bright}$, $CD16^{+}$, $CD69^{+}$, and $CD94^{+}$),[11] CA-125,[12] soluble major histocompatibility complex Class I chain-related molecule (sMIC).[26]

Other biomarkers include antral follicle count (AFC),[3] free fatty acids (FFA),[2,8] cfDNA,[8,9] and inflammatory hematological parameters.[32]

Among these biomarkers, the FSH:LH ratio, AMH, hCG, and cfDNA are prominent, with two or more studies investigating them. The FSH:LH ratio was addressed in two articles.[2,25] This ratio could represent a disruption in hormonal control, essential for follicular growth and oocyte quality.[51] An increased FSH:LH ratio has been recognized as an early indicator of poor ovarian response, resulting in affected patients producing significantly fewer mature oocytes during retrieval procedures which lead to low pregnancy rates. Serum AMH was presented in five articles.[3–7] Low AMH levels are associated with elevated rates of cycle cancellations.[4,5] An article indicates that women aged 40 and above have a correlation with negative outcomes in IVF/ICSI treatment.[4] However, another article indicates that high AMH levels are associated with an increased risk of early miscarriage, although this effect is limited to women under 35, whereas women over 35 showed consistency across low, medium, and high AMH groups.[7] The cut-off value of AMH varied depending on the study. However, the combination of AMH with AFC [3] showed to be a promising predictor for oocyte yield during IVF treatment, although one article [6] showed no significant relationship of AHM and oocytes fertilized and pregnancy outcomes. hCG serves as the biomarker for predicting reproductive outcomes in IVF cycles, as its early identification and increasing levels (>5 IU/l) are essential for higher ongoing pregnancy rates, higher multiple pregnancy rates, lower ectopic pregnancy rates, lower biochemical pregnancy loss rates, and lower spontaneous abortion rates.[10] β-hCG, another marker as part of the whole hCG profile, has a defined clinical cut-off to indicate the development of pregnancy; nevertheless, it may not accurately predict outcomes beyond the early stages of pregnancy.[30] Within this group, hhCG is a more precise subgroup that enhances specificity in predicting ongoing pregnancies, providing greater precision in differentiating viable from nonviable pregnancies relative to conventional hCG measurements.[29] However, there are not enough articles relating hCG, β-hCG, and hhCG, and therefore no particularly outstanding information appears. cfDNA was presented in two articles.[8,9] Increased cfDNA levels [9] on the day of the β-hCG test could represent heightened apoptotic activity. Apoptosis, or programmed cell death, can impact reproductive outcomes by affecting the quality of oocytes and embryos. Elevated apoptotic rates in granulosa and cumulus cells have been associated with diminished oocyte quality and unsuccessful IVF outcomes.[52,53] However, another article indicates no significant difference in cfDNA levels between pregnant and non-pregnant individuals following ART treatment.[8] Evidence on cfDNA is lacking; further research is required for additional insights.

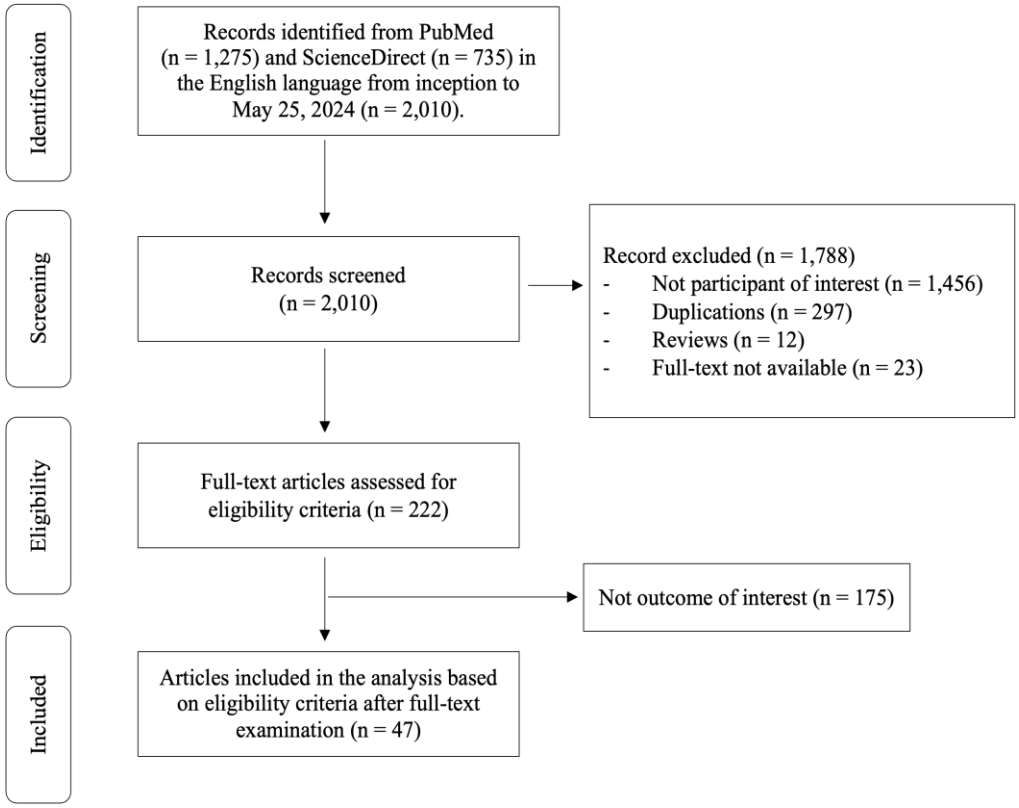

Fig. 1. Flowchart summarizing inclusion and exclusion of the articles for this study.

Table 1. Biomarkers in serum or blood (n = 20), in follicular fluids (n = 12), and in both serum and follicular fluids (n = 2).

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
Serum or blood							
25	Retrospective	To examine the impact of low basal cycle day 3 serum LH levels or a high FSH:LH ratio on IVF results.	N = 186 • *Based on serum day 3 LH levels alone:* ▪ Study group: LH values < 3 mIU/ml (n = 38), age 33.2 ± 3.4 years ▪ Control group: LH values ≥ 3 mIU/ml (n = 148), age 33.6 ± 3.5 years • *Based on FSH:LH ratio:* ▪ Study group: FSH:LH ratio > 3 (n = 28), age 33.3 ± 3.3 years • *Control group:* ▪ FSH:LH ratio ≤ 3 (n = 158), age 33.5 ± 3.5 years	Serum	FSH:LH ratio	Microparticle enzyme Immunoassay (MEIA-IMX)	• Basal LH levels showed no significant difference in response to ovarian stimulation between study *vs*. control group. • High FSH:LH ratio had significantly fewer mature oocytes, lower implantation, and clinical pregnancy rates. • High FSH:LH ratio on the 3 days of the menstrual cycle can be used as an early biomarker of poor ovarian response in IVF treatment, with a positive predictive value of 80% and a specificity of 96%.
2	Prospective cohort	To evaluate the value of elevated day 3 FSH/LH ratio in predicting IVF results in young and older women.	N = 174 *FSH/LH ratio:* • Group 1 (FSH/LH ≥ 3, n = 43); age 33.93 ± 5.24 years. • Group 2 (FSH/LH < 3, n = 131); age 30.98 ± 5.43 years. *Age + FSH/LH ratio:*	Serum	FSH/LH ratio	Electrochemiluminescense immunoassay	• Higher FSH/LH ratio had significantly lower pregnancy rates and fewer oocytes retrieved. • Older women with a higher FSH/LH ratio had significantly fewer good grade embryos transferred and lower pregnancy rates.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
			• Older women (≥ 35 years) (n = 61) ▪ FSH/LH ratio ≥ 3 (n = 23); age 38.00 ± 2.44 years. ▪ FSH/LH < 3 (n = 38); age 37.76 ± 2.17 years. • Younger women (<35 years) (n = 113) ▪ FSH/LH ratio ≥ 3 (n = 20); age 29.25 ± 3.29 years. ▪ FSH/LH < 3 (n = 93); age 28.21 ± 3.61 years.				• In younger women, the FSH/LH ratio did not significantly affect pregnancy rates. • The FSH/LH ratio should be considered alongside a patient's age when predicting IVF success; a higher day 3 FSH/LH ratio is a useful predictor of IVF outcome in older women but not in younger women.
11	Prospective	To evaluate the effect of the absolute count of the activation marker (CD69), IgG Fc receptor (CD16) and inhibitor marker (CD94) expression on peripheral blood natural killer (NK) cells on implantation and miscarriage rates after IVF treatment.	N = 138 • Pregnant (n = 51), age 34.16 ± 4.0 years • Non-pregnant (n = 75), age 35.45 ± 3.8 years	Peripheral blood (Heparinized tubes)	Activated NK cells: $CD56^{dim}$, $CD56^{bright}$, $CD16^{+}$, $CD69^{+}$, and $CD94^{+}$	Flow cytometry	• A higher absolute count of $CD56^{dim}$, $CD16^{+}$, $CD69^{+}$ NK cells were associated with a higher miscarriage rate. • $CD56^{dim}$, $CD16^{+}$, $CD69^{+}$ NK cells cut off value > 1.0 x 10^{6}/l, women with this value above the threshold had significantly lower implantation and pregnancy rates, and a higher miscarriage rate. • No significant difference in IVF outcomes was found with $CD56^{dim}$ *vs.* $CD56^{bright}$ NK cells. • The elevated peripheral activated NK cells may negatively affect IVF

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
							treatment outcomes; women with a high count of activated NK cells had a lower chance of embryo implantation after IVF treatment.
8	Cross-sectional	To determine and compare the relative concentration of cell-free DNA (cfDNA) in the sera of nonpregnant versus pregnant patients after ART treatment.	N = 30, age 30.7 ± 0.6 years • Pregnant (n = 16) • Non-pregnant (n = 14) **Controls male sera (n = 8) **Negative control: electrophoresis buffer	Serum	cfDNA	Modified isocratic capillary electrophoresis and ultra-violet epifluorescent microscope	• The study found no significant difference in cfDNA levels between pregnant and nonpregnant patients after ART treatment. • Both high (12 kb) and low (1 kb) molecular weight cfDNA concentrations were similar in nonpregnant and pregnant patients. • Male control sera showed higher cfDNA concentrations compared to female sera. • No significant daily variation in cfDNA concentration was observed during the menstrual cycle of a nonpregnant patient. • The study concluded that luteal phase cfDNA is not a reliable marker for predicting failed pregnancies after ART.
9	Prospective	To examine the cfDNA concentrations during ovarian stimulation and the relationship	N = 37 • Pregnant (n = 16); age 29.8 ± 4.3 years	Plasma	cfDNA	Fluorescence assay	• Plasma cfDNA concentrations may be linked to lower chances of pregnancy after IVF treatment; cfDNA was higher in nonpregnant

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		between cfDNA concentration and pregnancy rates in women undergoing IVF–embryo transfer.	• Non-pregnant (n = 21); age 27.9 ± 4.1 years .				women on the day of the β-hCG test. • No significant difference in cfDNA concentrations at different stages of the IVF cycle (T1 = 2 weeks after GnRH agonist administration; T2 = during oocyte retrieval prior to an aesthesia; T3 = during the routine blood test for β-hCG, 2 weeks after embryo transfer). • No relationship was found between smoking status, implantation rate, and mean plasma cfDNA concentration. • No significant age difference was found between women who became pregnant and those who did not. • Using a cfDNA concentration cutoff of 750 ng/ml, the sensitivity of cfDNA as a predictor of IVF failure was 39% with a specificity of 86%.
12	Retrospective	To correlate CA-125 concentrations in serum samples collected for routine hCG measurements 11 ± 2 days after embryo transfer in	N = 182 • Pregnant (n = 182), age 28 - 35 years. • Non-pregnant (n = 41) from the same patients, age 30 - 36 years.	Serum	CA-125	Microparticle enzyme immunoassay (MEIA)	• CA-125 levels were positively correlated with hCG and inhibin A levels in pregnant cycles. • CA-125 levels were lower in preclinical abortions than in clinical pregnancies. • No significant difference in CA-125 levels between

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
		relation to pregnancy and its outcome.					early pregnancy losses and ongoing pregnancies or between singleton and multiple pregnancies. • The predictive accuracy of CA-125 for clinical pregnancy was lower than that of hCG or inhibin A. • CA-125 levels may indicate endometrial receptivity but do not predict the number or viability of embryos.
3	Retrospective	To measure baseline concentrations of serum anti-Müllerian hormone (AMH) and FSH, and antral follicle count (AFC), then relate to IVF outcomes.	N = 126 • Low AMH (n = 54), age 36.6 ± 0.6 years • High AMH (n = 72), age 34.3 ± 0.8 years	Serum	• Serum AMH • AFC	• Serum AMH: Immunoenzymetric assay • AFC: Transvaginal ultrasound and GE scanner	• Low AMH had significantly inferior fertilization rates, high miscarried after pregnancy with fresh embryo transfer. • High AMH had more oocytes collected after ovarian stimulation, more embryos generated, high incidence of clinical pregnancy rate, greater number of embryos available for cryopreservation per cycle. • Serum AMH and AFC are good predictors of oocyte yield during IVF treatment, with positive associated with oocyte yield, fertilization rates, and embryos generated. • AFC was positively correlated with the number of oocytes retrieved during ovarian stimulation.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
4	Retrospective	To investigate the practicability of combining serum anti-Müllerian hormone (AMH) level with biological age as a simple screening method for counseling IVF candidates of advanced reproductive age with potential poor outcomes prior to treatment initiation.	N = 116 • Low AMH ≤ 0.48 ng/ml (n = 21); age 42.8 ± 2.3 years • Middle AMH = 0.49 - 1.22 ng/ml (n = 38); age 41.1 ± 1.3 years • High AMH ≥ 1.23 ng/ml (n = 57); 41.3 ± 1.4 years	Serum	Serum AMH	Enzyme-linked immunosorbent assay (ELISA)	• Serum AMH levels decline with age, and lower levels are associated with negative IVF/ICSI outcomes for women aged 40 and above. • Women aged 40 with low AMH levels had a higher cycle cancellation rate and no clinical pregnancies. • The lowest AMH level that resulted in a live birth for women aged 40 was 0.56 ng/ml. • AMH levels can predict IVF cycle cancellation and nonpregnancy with high accuracy. • The optimal AMH cut-off levels for predicting IVF cycle cancellation and nonpregnancy were 0.68 ng/ml and 1.05 ng/mL, respectively. • The study suggests using age and serum AMH levels as a screening tool for counseling advanced-aged IVF candidates.
5	Retrospective	To determine the predictive attributes of anti-Müllerian hormone (AMH) in terms of oocyte yield, cycle cancellation, and pregnancy outcomes.	N = 2,760 • Five age groups according to Society for Assisted Reproductive Technology categories: < 35, 35-37, 38-40, 41-42, and > 42 years	Serum	Serum AMH	Enzyme-linked immunosorbent assay (ELISA)	• AMH is a good predictor of predicting the number of eggs and cycle cancellation. ▪ Higher AMH levels: lower cancellation rates with an area under the curve (AUC) of 0.74. ▪ Undetectable AMH: 13.3-fold increased risk of cancellation as

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							compared with patients with an AMH >2.0 ng/mL. ▪ AMH had AUC of 0.83 for prediction of three or fewer oocytes retrieved. ▪ Undetectable AMH exhibited sensitivity and specificity of 21.1% and 98.2%, respectively, for three or fewer oocytes retrieved. ▪ AMH is a poor predictor for pregnancy after IVF; with AUCs 0.55 to 0.65; undetectable AMH, 23.5% of patients <40 years old achieved live birth after transfer.
6	Prospective	To compare the relationship between anti-Müllerian hormone (AMH) levels and pregnancy outcomes in patients undergoing ICSI.	N = 42, age 20 - 45 years • High AMH ≥ 3.0 ng/ml (n = 22) • Normal AMH ≥ 1.0 ng/ml (n = 17) • Low Normal Range ≤ 0.3-0.9 ng/ml (n = 3)	Serum	• Serum AMH • E_2	Enzyme-linked immunosorbent assay (ELISA)	• No significant relationship between AMH levels *vs.* number of oocytes fertilized and AMH levels *vs.* pregnancy outcomes. • A significant relationship between AMH levels and the day of embryo transfer. • AMH is a valuable marker for ovarian reserve but not a reliable predictor of the number of oocytes, mature oocytes, and fertilized oocytes. • E_2 was a better predictor than AMH for clinical pregnancy rates.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
31	Prospective	To identify biomarkers that prospectively predict IVF cycle cancellation.	N = 205 • Cancelled cycle due to poor ovarian response (n = 27), age 29 - 44 years • Ongoing cycle (n = 178), age 22 - 45 years	Serum	• Insulin-like growth factor-1 (IGF-1) • Insulin-like growth factor-2 (IGF-2) • Insulin-like growth factor binding protein-1 (IGFBP-1) • Soluble FMS-like tyrosine kinase-1 (sFLT-1)	Enzyme-linked immunosorbent assay (ELISA)	• IGF-2 and IGFBP-1: no significant differences between women with cancelled and ongoing cycles. • Elevated baseline of serum IGF-1 and sFLT-1 were found in women whose IVF cycles were cancelled. • IGF-1: negative correlate with the number of immature oocytes; it may affect folliculogenesis and oocyte maturation. • sFLT-1: higher in women with cancelled cycles even after controlling for AMH levels. • The study suggests that measuring IGF-1 and sFLT-1 levels before starting an IVF cycle could help identify women at risk of cycle cancellation.
26	Prospective	To investigate serum levels of the stress-inducible soluble major histocompatibility complex class I chain-related molecule, MICA, (sMIC), a regulator of cellular immunity, can be predictive of implantation or	N = 170 • Non-pregnant implantation failure (n = 132), age 32.07 ± 3.742 years. • Spontaneous abortion after implantation (n = 8), age 32.00 ± 3.721 years. • Evolutive pregnancy live born baby (n =	Serum	sMIC	Enzyme-linked immunosorbent assay (ELISA)	• Higher serum sMIC are linked to implantation failure after IVF treatment. • Serum sMIC < 2.45 ng/ml had a higher chance of giving birth to a viable baby. • Serum sMIC > 3.2 ng/ml were predictive of spontaneous abortion after successful implantation. • sMIC is suggested as a new blood biomarker for predicting IVF outcomes before treatment begins.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
		pregnancy failure after IVF.	30), age 32.51 ± 3.373 years.				
27	Retrospective	To analyze the role of luteal phase estradiol (E_2), progesterone (P), and follicle-stimulating hormone (FSH) levels in the prediction of clinical pregnancy in stimulated cycles.	N = 268 • Pregnant (n = 113), age 32.16 ± 3.83 years • Non-pregnant (n = 155), age 32.22 ± 4.74 years	Serum	• E_2 • P • FSH	Two-site chemiluminescent sandwich immunoassay	• E_2 was the best marker for predicting pregnancy with a classification accuracy of 82.1%; E_2 levels on days 7 and 14 after embryo transfer were significantly higher in pregnant women compared to nonpregnant women. • P showed a lower classification accuracy of 60.8% for predicting pregnancy, P levels were similar in both groups on days 0 and 14, but on day 7, nonpregnant women had significantly lower P levels. • FSH had a classification accuracy of 67.5% for predicting pregnancy, but was lower accuracy; FSH levels were significantly lower in the pregnant group on days 0, 7, and 14. • Among three markers, E_2 levels may be a good indicator of successful pregnancy in IVF/ICSI treatments.
28	Prospective cohort	To analyze relationships between serum free fatty acid (FFA) concentrations	N = 91 • Pregnant (n = 57), age 32.9 ± 3. 3 years	Serum	• Total free fatty acid • Myristic acid • Palmitic acid • Stearic acid • Oleic acid	CH_3I/SPE method (methylation with iodomethane and purification by solid phase extraction chromatography)	• High levels of serum ALA had a lower chance of pregnancy after IVF; after adjusting for other factors (age, BMI, and history of

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
		and pregnancy.	• Non-pregnant (n = 34), age 32.1 ± 3.7 years		• Linoleic acid • Alpha-linolenic acids (ALA)		endometriosis or previous live birth). • No significant differences in pregnancy chances were found with other free fatty acids.
10	Retrospective	To investigate the relationship between serum concentrations of human chorionic gonadotrophin (hCG) measured in the peri-implantation period and various outcome measures following blastocyst transfer in IVF cycles.	N = 767 • Day-5 HCG < 5 IU/l (n = 363); age 33.0 ± 4.8 years • Day-5 HCG ≥ 5 IU/l (n = 404); 32.1 ± 4.6 years	Serum	hCG	Enzyme-linked immunosorbent assay (ELISA)	• Serum hCG levels on 5 days after blastocyst transfer is a strong predictor of IVF cycle outcomes. • Higher day-5 hCG levels (above 5 IU/l) were associated with higher ongoing pregnancy rates, higher multiple pregnancy rates, lower ectopic pregnancy rates, lower biochemical pregnancy loss rates, and lower spontaneous abortion rates. • Early rising hCG levels are critical to pregnancy success in IVF, delayed rise in hCG suggests nonviable pregnancy.
29	Case-control, retrospective cohort	To determine if hyperglycosylated human chorionic gonadotropin (hhCG), produced by invasive trophoblasts, measured as early as 9 days after egg retrieval can predict ongoing	Case-control study (N = 52), age 35.7 ± 5.7 years: • Ongoing pregnancies (n = 26) • Spontaneous abortions (n = 20) • Negative hCG tests (n = 6)	Serum	• hhCG • hCG	• hhCG: Electrochemiluminescence (ECL) • hCG: Enzyme-linked immunosorbent assay (ELISA)	• hhCG (Day 9 and 16) had significantly higher in participants with ongoing pregnancies. • hhCG is more sensitive and has a larger area under the curve compared to hCG. • A day-9 hhCG level > 110 pg/ml was 96% specific for ongoing pregnancy.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
		pregnancies after in vitro fertilization and fresh embryo transfer.	Retrospective study (N = 112), age 36.4 ± 4.2 years.				• hhCG was a more reliable early predictor of pregnancy outcomes than traditional hCG, high hhCG on day 9 after egg retrieval are predictive of ongoing pregnancies after IVF.
13	Retrospective cohort	To evaluate the implantation rate in women with and without tumor necrosis factor (TNF) polymorphisms (-308 and -238) during the course IVF procedures.	N = 424 • Entire patient group (n = 424), age 31.9 ± 4.5 years • Selected group (n = 120): a normal karyotype, age under 38 years, serum follicle-stimulating hormone (Day-3 FSH) levels below 10 IU/l, a long agonist desensitization protocol associated with recombinant FSH treatment and a Caucasian background, age 30.7 ± 3.6 years	Blood samples (DNA)	• TNF-308, *rs1800629* • TNF-238, *rs361525*	Allele-Specific Polymerase Chain Reaction (ASPCR)	• TNF-238 polymorphism did not impact implantation or pregnancy rates. • TNF-308A allele: higher day 3-E_2 levels, increased embryo implantation, pregnancy rates, and take-home baby rate, with a decrease in miscarriage rate in women undergoing IVF without known infertility factors. • The TNF-308A allele might be a potential biomarker for predicting implantation rates.
32	Retrospective cohort	To investigate the predictive role of inflammatory hematological markers on treatment success in IVF patients.	N = 115 • HCG was positive (n = 28), age 20 - 36 years • HCG was negative (n = 87), age 20 - 43 years	Complete blood count (CBC)	• White blood cell (WBC) counts • Neutrophil/lymphocyte ratio (NLR) • Monocyte/lymphocyte ratio (MLR) • Platelet/lymphocyte ratio (PLR)	CBC automate	• Younger age and a higher number of mature (MII) oocytes were significant in predicting IVF success. • Inflammatory hematological markers like WBC, NLR, MLR, PLR, MPV, and PDW

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
					• Mean platelet volume (MPV) • Platelet distribution width (PDW)		were not effective in predicting IVF success. • A lower PLR was observed in patients with a positive hCG test. • PLR might be a promising marker for predicting IVF success, but larger studies are needed to confirm its value.
7	Retrospective cohort	To explore whether serum anti-Müllerian hormone (AMH) levels are associated with early miscarriage rates after IVF/ICSI with fresh embryo transfer.	N = 2,246 *Age:* • Young-age group (≤ 35 years, n = 1,531) • Advanced-age group (> 35 years, n = 715) *AMH level:* • Low AMH (0.06 - 1.60 ng/ml) • Medium AMH (1.61 - 3.98 ng/ml) • High AMH (3.99 - 20.20 ng/ml)	Serum	Serum AMH	Enzyme-linked immunosorbent assay (ELISA)	• Serum AMH: a valuable marker to oocyte quality and the risk of early miscarriage in IVF/ICSI. ▪ Women under 35 with high AMH levels had a higher risk of early miscarriage. ▪ In women over 35, early miscarriage rates were not significantly different across low, medium, and high AMH groups. ▪ High AMH levels were not associated with pregnancy rates after adjusting for confounders in women over 35. • No significant differences in implantation rates, biochemical pregnancy rates, ectopic pregnancy rates, and live birth rates among different age and AMH groups.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
30	Retrospective cross-sectional	• To evaluate the value of serum beta human chorionic gonadotropin (β-hCG) levels in discriminating biochemical and clinical pregnancies 12 days after embryo transfer. • To determine the factors predicting ongoing pregnancy.	N = 445 • Biochemical pregnancy (n = 87), age 30.4 ± 5.4 years • Clinical pregnancy (n = 358), age 30.1 ± 4.9 years	Serum	β-hCG	Enzyme-linked immunosorbent assay (ELISA)	• β-hCG cut-off values ▪ Day 3 after ET: 57 mIU/mL ▪ Day 5 after ET: 87 mIU/mL ▪ Day 12 after ET: 86.8 IU/mL • Factors such as the duration of infertility and levels of E_2 on the day of hCG administration were significant in predicting ongoing pregnancy. • β-hCG on day 12 (86.8 IU/mL) after ET was the best threshold for predicting clinical pregnancy with a sensitivity of 65.1% and specificity of 74.7%. Other factors are also involved in predicting ongoing pregnancies.
Follicular fluids							
33	Prospective	To evaluate survivin gene expression in granulosa cells from infertile patients and examine the relationship between survivin gene expression and infertile clinical background.	N = 28 *Disease of endometriotic lesions:* • Endometriosis patients (n = 9), age 35.0 ± 0.77 years • Tubal infertility patients (n = 9), age 32.8 ± 0.87 years • Male factor infertility patients	Follicular fluids	Survivin	Quantitative Reverse Transcription-Polymerase Chain Reaction	• Survivin gene expression was found in all granulosa cells from the patients studied. • Endometriosis patients had significantly lower survivin gene expression compared to those with male factor infertility. • Higher survivin gene expression levels were observed in pregnant *vs.* nonpregnant patients.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
			(n = 10), age 32.0 ± 1.42 years *Pregnancy status:* • Pregnant (n = 10) • Non-pregnant (n = 18)				• No significant correlation was found between survivin gene expression level and serum estradiol (E_2) level. • Survivin could potentially be used to predict the chances of success with IVF-ET, and endometriosis might lead to more cell death in granulosa cells.
34	Retrospective cohort	To compare the effects of the telomerase activity (TA) and telomere length (TL) on IVF treatment outcomes in the same individuals.	N = 76 • Pregnant (n = 29), age 29.13 ± 1.32 years • Non-pregnant (n = 47), age 28.80 ± 1.74 years	Follicular fluids	• Telomerase activity (TA) • Telomere length (TL)	• TA: the telomeric repeat amplification protocol (TRAP) followed by non-denaturing PAGE silver staining • TL: quantitative real-time PCR (qPCR)	• TA in granulosa cells is a better predictor of pregnancy outcomes from IVF treatment than TL. • High TA: more retrieved oocytes and a higher rate of blastocyst transfer in pregnant group compared to the non-pregnant group. • No significant difference in TL between the pregnant *vs*. non-pregnant groups. • An increase in TA of 1 ODmm increased the chance of becoming pregnant by approximately 4.769-fold. • The cut-off points for predicting pregnancy outcomes of TA were 0.65 ODmm.
36	Cross-sectional	To investigate polymorphisms in the follicle-stimulating hormone receptor (FSHR) and	N = 606, age 22 - 39 years • First cohort (n = 373) • Validation cohort (n = 233)	Follicular fluids	• LHCGR variant N312S • FSHR variant N680S	• LHCGR: Sequencing • FSHR: Allele-specific PCR	• Genetic variants in the LHCGR and FSHR genes can predict pregnancy chances in IVF treatments. • Serine variant (S) in both LHCGR and FSHR genes

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
		luteinizing hormone/human chorionic gonadotrophin receptor (LHCGR) genes, separately and in combination, impact IVF outcomes and clinical parameters in IVF trials.					had higher pregnancy rates compared to asparagine variant (N). • Women carrying the LHCGR S312 variant had higher pregnancy rates, regardless of their FSHR variant. • Women with the N variant in both genes may have a lower hormonal response in their granulosa cells.
16	Prospective	To evaluate the expression profiles of seven extracellular miRNAs (miR-7-5p, miR-202-5p, miR-378-3p, miR-224, miR-320a, miR-212-3p, and miR-21-5p) in human follicular fluid (FF) to explore the outcomes of IVF.	N = 255 • Polycystic ovary syndrome (PCOS) (n = 110) • Without PCOS having normal ovarian reserve (NOR) (n = 145)	Follicular fluids	miRNAs: • miR-7-5p • miR-202-5p • miR-378-3p • miR-224 • miR-320a • miR-212-3p • miR-21-5p	• qRT-PCR with TaqMan™ MicroRNA assay	• A combination of 6 microRNAs (miR-7-5p, miR-202-5p, miR-378-3p, miR-224, miR-21- 5p, miR-212-3p) could distinguish between women with normo-androgenic PCOS and those without PCOS with high sensitivity and specificity. • miR-320a: significantly different in top-quality embryos *vs.* non-top-quality embryos on day 3 in patients with normal ovarian reserve (NOR). • miR-212-3p expression level was related to high-quality blastocyst development. • miR-21-5p showed high sensitivity and specificity for predicting clinical pregnancy outcomes.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
							• miR-7-5p, miR-378-3p, miR-224, miR-212-3p were differentially expressed in PCOS patients. • miR-202-5p and miR-21-5p were significantly higher in NOR patients.
17	Observation	To investigate miRNAs represent potential biomarkers for predicting the outcomes of IVF–ET.	N = 106, median age of 37 years	Follicular fluids	miRNAs: • miR-103a-3p • miR-10a-5p	• miScript PCR System	• miR-103a-3p and miR-10a-5p in follicular fluid (FF) negatively correlate with brain-derived neurotrophic factor (BDNF) mRNA levels, suggesting they may inhibit BDNF expression. • High levels of miR-103a-3p and miR-10a-5p in FF could predict poorer outcomes for embryo development in IVF-ET treatment. • miR-103a-3p and miR-10a-5p affect oocyte maturation by regulating BDNF expression in human FF. • The expression of miR-10a-5p was found to increase with higher levels of certain hormones like FSH, LH, and testosterone.
35	Prospective	To investigate the peptide profiling of follicular fluids (FFs) and its potential use as a biomarker for oocyte quality.	N = 50, age 18 - 36 years <u>*Data set*</u>: • Biomarkers identification (n = 17) • Biomarkers validation (n = 32)	Follicular fluids	Peptide (Proteins)	• Nano-scale liquid chromatography coupled to tandem mass spectrometry (nano LC-MS/MS).	• 53 potential peptide biomarkers in human FFs that could predict the outcome of IVF. • These biomarkers were able to predict fertilization outcomes with a sensitivity of 81.3%, specificity of

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
							68.8%, and an area under the curve (AUC) of 0.86. • 7 peptides: insulin-like growth factor binding protein-5, alpha-2-antiplasmin, complement component 3, inter-alpha-trypsin inhibitor heavy chain H1, serum albumin, protein diaphanous homolog 1 and plastin-3 were among the identified biomarkers.
37	Observation	To investigate the protein profile of human follicular fluid from women undergoing successful IVF.	N = 10, age ≤ 38 years	Follicular fluids	Proteins	Reverse phase high performance liquid chromatography (RP-HPLC) coupled to matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry (LC-MALDI TOF/TOF MS)	• 219 unique proteins were identified in human follicular fluid (HFF). • These proteins are involved in immunity, blood coagulation, and angiogenesis, which might be biomarkers for female infertility and IVF outcomes. • Some proteins identified are not previously reported in human follicular fluid or plasma, suggesting new areas for research.
18	Retrospective	To explore oocyte competence for subsequent birth. The modified natural IVF/ICSI cycle was used as an experimental model by measuring levels	N = 83 • Pregnant (n = 25): deliveries (n = 19), and spontaneous abortions (n = 6), mean age 33.5 years	Follicular fluids	• Granulocyte colony-stimulating factor (G-CSF) • IL-15	Multiplex bead-based immunoassays	• Combination of G-CSF and IL-15 levels could predict successful birth in modified natural IVF/ICSI cycles with an accuracy of 85%. • FF G-CSF levels were consistent over two cycles which means it could be a

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
		of cytokines, chemokines, and growth factors in individual follicular fluids (FFs).	• Non-pregnant (n = 29), mean age 33.8 years • No embryo transfer (n = 19): an oocyte was collected but not successfully fertilized, ICSI (n = 12), and IVF (n = 7), mean age 34.3 years • No oocyte collected (n = 10): no oocyte was retrieved, mean age 33.7 years				useful predictor of IVF/ICSI outcome. • FF G-CSF (> 12 pg/mL) and IL-15 (< 7 pg/mL): a better chance of having a baby (birth rate per cycle = 48.9%). When only one or none of these good-prognosis criteria were present, the birth rates per cycle dropped to 8% and 0%, respectively.
19	Clinical study design	To analyze follicular cytokines (proinflammatory: IL-1β, IL-6, IL-18, IFN-γ, IFN-α, TNF-α, IL-12, and IL-23;, and anti-inflammatory: G-CSF), chemokines (MIP-1α, MIP-1β, MCP-1, RANTES, and IL-8), and other biomarkers (sAPO-1/Fas, CD44(v6)) in 153 women undergoing IVF.	N = 153 • Male factor infertility (n = 67); age 32.6 ± 4.3 years • Tubal factor infertility (TFI; n = 44), age 34.7 ± 5.0 years • Polycystic ovary syndrome (PCOS; n = 8), age 34.8 ± 3.2 years • Endometriosis (n = 23), age 31.8 ± 3.9 years • Unexplained infertility (n = 7), 32.7 ± 2.9 years	Follicular fluids	Proinflammatory cytokines: • IL-1β • IL-6 • IL-18 • IFN-γ • IFN- α • TNF-α • IL-12 • IL-23 Anti-inflammatory cytokine: • G-CSF Chemokines • MIP-1α • MIP-1β • MCP-1 • RANTES	Flow Cytometry	• Proinflammatory cytokines and chemokines in follicular fluid (FF) are associated with IVF success rates. • Cytokines (IL-12, IL-18, IL-8, MIP-1β) are correlated with successful pregnancy following IVF treatment. • Elevated levels of MIP-1α in PCOS patients indicate increased inflammation. • Higher MIP-1β levels correlate with follicular growth and achieving pregnancy. • TNF-α levels are elevated in endometriosis patients. • IL-18 levels were positively correlated with increased

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
			• Other reasons (n = 4), age 36.0 ± 5.9 years		• IL-8 Other biomarkers • sAPO-1/Fas CD44(v6)		chances of intrauterine pregnancy and the number of fetuses detected by ultrasonography. Low IL-18 levels in unexplained infertility patients. • Lower concentrations of IL-1β and IFN-α were significantly associated with tubal factor infertility (TFI). mRNA analysis showed that most of the studied transcripts were more abundantly expressed in mural granulosa cells (MGC) compared to cumulus granulosa cells (CGC).
15	Prospective	• To investigate relationships between intra-follicular apolipoprotein B (APOB) levels and IVF/ICSI outcomes. • To explore hormonal regulation by gonadotropins of APOB expression in cultured human granulosa cells (GC).	N = 61, age 336.6 ± 4.8 years: obtained follicular fluid (FF sample; n = 201) The concentration of APOB defined as: • Q1: APOB <112 ng/ml • Q2: APOB 112-229 ng/ml • Q3: APOB 230-329 ng/ml • Q4: APOB >330 ng/ml	Follicular fluids,	Apolipoprotein B	Enzyme-linked immunosorbent assay (ELISA)	• APOB concentrations in follicular fluid (FF): significantly higher in young, normal BMI, and in their first or second attempt IVF. • FF with oocyte and with normal fertilized oocytes had significantly higher APOB levels. • High APOB in FF were associated with the increase probabilities of obtaining an oocyte, a fertilized oocyte, an embryo, and a top-quality embryo on day 2. • In vitro experiments showed that APOB gene

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
							expression in GC was significantly under expressed when stimulated with gonadotropins.
38	Cross-sectional	To study the possibility of using adipokines levels in the follicular fluid to predict IVF efficiency.	N = 53 • Normal body weight (BW) with non-pregnant (nPN); (n = 16), age 31.8 ± 0.8 years • Normal BW with pregnant (PN); (n = 9), age 32.1 ± 1.6 years • Increase BW with non-pregnant (nPI); (n = 21), age 34.3 ± 1.1 years • Increase BW with pregnant (PI); (n = 7), age 31.6 ± 1.3 years	Follicular fluids	Adipokines • leptin • adiponectin • ghrelin	Enzyme-linked immunosorbent assay (ELISA)	• The leptin/ghrelin ratio in follicular fluid (FF) is a good indicator for predicting IVF success in women with normal body weight. • In normal body weight: higher levels of leptin in pregnant compared to non-pregnant. • The leptin levels and leptin/adiponectin ratio serve as dependable predictors of IVF success. • In increase body weight: no significant difference of leptin between pregnant vs. non-pregnant.
14	Cross-sectional	• To compare mean concentrations of growth factors (IGF1 and GH) and interleukins (IL-6 and IL-17) in follicular fluid (FF) between good or bad prognosis.	N = 140 • Group 1 (bad prognosis): women aged ≥ 35 years or attempt with embryo development failure or recurrent implantation failure (RIF) antecedent (n = 72). • Group 2 (good prognosis): women	Follicular fluids	• Growth hormone (GH) • Insulin growth factor I (IGF1) • Interleukins-6 (IL-6) • IL-17	Enzyme-linked immunosorbent assay (ELISA)	• In group 2: higher number of oocytes, better oocyte maturation, blastulation rates, ongoing pregnancy rate and cumulative pregnancy rate compared to group 1. • GH, IGF1, and IL-6 levels were significantly higher in the good prognosis group and found to be predictive of oocyte quality, with GH being the best biomarker for this quality.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
		• To investigate the relationships between these FF component levels and the main parameters of controlled ovarian stimulation, embryo laboratory outcomes, and pregnancy outcomes.	aged < 35 years, first or second attempt (n = 68).				• GH: negatively correlated with age but positively correlated with maturity rate and IGF1. • GH and IGF1: correlated with top embryo rate and cumulative pregnancy rate. • IL-6: correlated with IGF1 level, endometrium thickness, and implantation rate. • IL-17: no correlation with pregnancy outcome and oocyte quality, but correlate to IL-6 levels.
Serum and follicular fluids							
23	Observation	• To investigate granulocyte colony-stimulating factor (G-CSF) in human reproduction.	N = 93, age 20 - 42 years • Patients with the aetiology of tubal or male factor infertility (n = 82) • Patients with moderate response to ovarian stimulation (n = 23 of the 82 patients in group 1) • Patients with endometriosis were assessed for G-CSF and estradiol in serum and FF on the day of FP (n = 11)	Serum and follicular fluid	• Granulocyte colony-stimulating factor (G-CSF)	Enzyme-linked immunosorbent assay (ELISA)	• G-CSF levels in follicular fluid (FF) were higher than in serum. • Serum G-CSF levels increased from low to high response patients, correlating with pregnancy rates. • G-CSF levels increase throughout ovarian stimulation and peak at ovulation induction. • Pregnant patients showed a continuous increase in G-CSF from embryo transfer to implantation and gestation. • Patients with endometriosis had lower G-CSF levels in

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
							serum and FF compared to non-endometriosis patients. • G-CSF is involved in follicle development and may predict IVF outcomes. • Measuring G-CSF in FF is more reflective of follicular development, as levels are significantly higher in FF than in serum. However, serum G-CSF can provide insights into ovarian stimulation response and pregnancy progression.
24	Randomized controlled trial	To evaluate the level of macrophage colony-stimulating factor (M-CSF) in serum in response to ovarian stimulation in low-response, moderate-response and high-response patients and compare its changes throughout the menstrual cycle between pregnant and nonpregnant patients.	N = 95, age 20 - 42 years • Low-response (n = 26), • Moderate-response (n = 40), • High-response (n = 29)	Serum and follicular fluid	• Macrophage colony-stimulating factor (M-CSF)	Enzyme-linked immunosorbent assay (ELISA)	• M-CSF levels in serum could help predict the success of IVF treatment. • M-CSF levels in serum and follicular fluid (FF) are increase during ovarian stimulation and peak around the time of oocyte retrieval. • M-CSF levels higher in patients who become pregnant. • A significant correlation between M-CSF levels in serum and FF. • M-CSF in FF at higher concentrations than in serum.

Table 2. Biomarkers in other sample types [Follicular cells (n = 3); Culture medium (n = 5); Endometrial (n = 2); Oocyte (n = 2); Cumulus granulosa cells (n = 1)].

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
40	Retrospective	To investigate the value of candidate genes in follicular cells (FCs) with are expected to improve oocyte and embryo selection with higher implantation and pregnancy rates using single embryo transfer.	N = 34, average age of 36 years (***more than one sample from a single patient) • Pregnant: a transferred embryo with successful pregnancy (n = 14) • Non-pregnant: a transferred embryo with unsuccessful pregnancy (n = 15) • Embryo Failure: embryos arrested in their development before the 8 cell-stage (n = 9)	Follicular cells	• Phosphoglycerate kinase 1 (PGK1) • Regulator of G-protein signaling 2 (RGS2) • Regulator of G-protein signaling 3 (RGS3) • Cell division cycle 42 (CDC42)	Quantitative RT–PCR	• Certain genes in FCs can predict pregnancy success in IVF treatments. • PGK1 and RGS2: significant difference in expression between embryos that led to pregnancy, they are the best predictors for ongoing pregnancy. • Differences in FCs expression for PGK1 and CDC42 were found between follicles with positive and negative pregnancy results.
39	Case-control study	To determine biomarkers expression in the surrounding follicular cells (FCs) and the oocyte led to a successful or unsuccessful pregnancy outcome.	N = 18, average age of 36 years • Pregnant (n = 9) • Non-pregnant (n = 9)	Follicular cells	• Epiregulin (EREG) • Dihydropyrimidinase-like 3 (DPYSL3) • Progesterone receptor (PGR) • Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein (YWHAZ)	• Microarray hybridizations • Quantitative RT–PCR	• Identified follicular marker genes that can predict pregnancy outcomes in human IVF treatments. • UGP2 and PHLDA1 are associated with successful pregnancies. • GABPB1 may be more expressed in cases of non-pregnancy, suggesting a potential marker for follicular incompetence.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
					• Myristoylated alanine-rich protein kinase C sub-strate (MARCKS) • UDP-glucose pyrophosphorylase 2 (UGP2) • Sema-phorin 3A (SEMA3A) • Low-density lipoprotein receptor-related protein (LRP8) • Pirin (PIR) • Pleckstrin homology-like domain, family A, member 1 (PHLDA1) • Secreted frizzled-related protein 1 (SFRP1) • Homer homolog 1 (HOMER1) • GA-binding protein transcription factor b1 (GABPB1)		• No significant candidate genes were associated with non-pregnancy outcomes. • These markers could improve embryo selection for single embryo transfer.
41	Prospective cohort study design	• To introduce a new tool (the follicular sensitivity index; FSI) for objective assessment of follicular responsiveness to exogenous gonadotropins. • To evaluate its ability to predict the clinical pregnancy rate in women with unexplained	N = 1,000 • Low FSI with FSI values below the 33rd percentile (n = 329), age 32.9 ± 4.9 years • Moderate FSI with FSI values from zero to 43.01 (n = 340), age 32.8 ± 4.6 years • High FSI with FSI more than 43.01 (n = 331), age 32.2 ± 5 years	Follicular cells	Follicular Sensitivity Index (FSI)	The FSI = PFC * 10,000/(AFC * Total dose of FSH). PFC = preovulatory follicle count AFC = antral follicle count	• FSI is a better predictor of clinical pregnancy from IVF/ICSI treatments; increase in the clinical pregnancy rate with higher FSI values. • High FSI: high oocytes retrieved, high fertilization rates, and more embryos available for transfer. • FSI can predict follicular response to exogenous FSH. • FSI could help in managing future IVF cycles and in deciding when to cancel a

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
		infertility or tubal factor undergoing IVF/ICSI.					cycle due to poor ovarian response.
22	Prospective cohort	To evaluate potential correlations between establishment of pregnancy and immunosuppressive activity secreted by the preimplantation embryo.	N = 27, age < 40 years *Immunosuppression* (n = 11): • Pregnant (n = 5) • Non-pregnant (n = 6) *Alpha interferon* (n = 16): • Pregnant (n = 9) • Non-pregnant (n = 7)	Embryo culture medium	• Immunosuppressive activity • Alpha interferon concentrations	*Immunosuppression:* Lymphocyte proliferation assays *Alpha interferon*: Radioimmunoassay	• Immunosuppressive activity was higher in women who became pregnant vs. non-pregnant after IVF treatment. • Alpha interferon levels were not significantly different between pregnant and nonpregnant women. • Immunosuppressive activity might be related to successful pregnancy, but alpha interferon may not be.
42	Experiment	To determine whether human blastocysts secrete microRNA (miRNAs) into culture media and whether these reflect embryonic ploidy status and can predict IVF outcomes.	N = 13 couples donated 91 cryopreserved pronuclear-stage embryos	Blastocyst culture medium	miRNAs	qRT-PCR and TLDA array	• Human blastocysts secrete miRNAs into IVF culture media which could be potential biomarkers for implantation success. • 10 miRNAs: miR-106b, miR-191, miR-30c, miR-372, miR-376a, miR-548a-3p, miR-548c, miR-548d-3p, miR-576–3p, miR-603, and miR-645 were identified in the culture media. • miR-191 was found in higher concentrations in media from aneuploid embryos and in failed IVF/non-ICSI cycles. • miR-372 was significantly more concentrated in day-5 media than in day-4 media.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
							• Higher levels of miR-645 correlated with poor pregnancy outcomes in non-ICSI inseminated embryos. • miR-191 and miR-372 were more concentrated in media from embryos inseminated by ICSI compared to regular insemination in day-5 media.
43	Prospective cohort	To employ NMR based metabolic profiling analysis of spent embryo culture media to identify novel biomarkers of embryo viability and provide insight into the metabolism of a viable embryo.	N = 37, age 32 - 40 years: 58 media samples collected from embryos transferred to the uterus • Pregnant: 27 media samples • Non-pregnant: 31 media samples	Embryo culture medium	• 2-methylglutarate • 3-aminoiso- butyrate, 3-hydroxyisovalerate • Acetate • Acetoacetate • Alanine • Citrate • Formate • Glutamate • Glycine • Lactate • Tryptophan	Nuclear magnetic resonance (NMR) spectrometer	• NMR based metabolic profiling can identify biomarkers for embryo viability. • Metabolic profiling analysis using ^{1}H NMR spectra quantified 12 metabolites in the media samples. • Significant differences in metabolite ratios between positive and negative pregnancy groups. • Some of the most biologically relevant differences included a 17% increase in the formate to glycine ratio and a 22% decrease in the citrate to alanine ratio in the spent embryo media from the positive pregnancy group.
44	Cross-sectional	To investigate whether selected cytokines are detectable in the embryo culture medium (EM) of	N = 330, age 21 - 46 years • IL-8 positive (n = 107) • IL-8 negative (n = 223) groups	Embryo culture medium	13 cytokines: GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, interferon-γ, and TNF-α	Luminex's xMAP assay	• Interleukin-8 (IL-8) was the only cytokine significantly associated with clinical outcome, could be used to predict the potential of

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
		human preimplantation embryos and what the relationship is of the cytokines with clinical outcomes.					embryos to develop before they are transferred. • IL-8 in EM was associated with higher pregnancy rates, implantation rates, number of live births and lower abortion rate per IVF patient. • IL-8 may be an independent predictor for assessing embryo development potential in IVF patients.
45	Case-control	To characterize the profile of glycans secreted in spent culture medium of blastocyst and to investigate whether the profile of glycans could be used as predictive markers of embryo viability in ART.	N = 78 • Successful implantation (n = 39), age 29.31 ± 3.58 years • Implantation failure (n = 39), age 30.28 ± 3.87 years	Blastocyst culture medium	• Glycan profiles • Lectin (NPA, UEA-I, MAL-I, LCA, PHA-E +L, GNA, AAL, LTL, DBA, BPL)	Microarray	• Glycan profile in culture medium can be used as a biomarker for predicting embryo implantation potential. • Glycan profiles in spent blastocyst culture medium differ between successful and failed implantation cases. • Glycan binding to certain lectins was significantly increased in successful implantation. • No significant glycan profile differences were found in embryos of different morphological grades, except for UEA-I between poor and medium blastocysts.
46	Prospective cohort	To explore whether a cytokine profile predictive of implantation and	N = 210 • Pregnant (n = 68), age 34.2 ± 4.0 years	Endometrial secretions	17 soluble regulators: IL-1β, IL-5, IL-6, IL-10, IL-12, IL-15, IL-17, IL-18, tumor necrosis factor (TNF)-α,	Multiplex immunoassay	• Profiles of mediators in endometrial secretions are correlated to implantation and pregnancy.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
		clinical pregnancy can be identified in endometrial secretions aspirated immediately prior to embryo transfer following IVF.	• Non-pregnant (n = 142), age 35.3 ± 4.2		interferon (IFN)-γ, macrophage migration inhibitory factor, eotaxin,IFN-γ-inducible 10 kDa protein (IP- 10), monocyte chemo-attractant protein-1 (MCP-1), Dickkopf homolog 1, heparin-binding epidermal growth factor, and vascular endothelial growth factor (VEGF)		• Higher levels of MCP-1 were negatively associated with implantation, while higher levels of IP-10 were positively associated. • For clinical pregnancy: higher levels of TNF-α were positively associated, while higher levels of IL-1β were negatively associated. • The ratio of TNF-α and IL-1β may indicate endometrial receptivity. • IL-1β and TNF-α, pro-inflammatory cytokines, play a role in implantation. • Chemokines MCP-1 and IP-10 are associated with initial embryo implantation.
47	Observation	To evaluate whether the number of p16-positive cells in the functional layer of the endometrium could be a useful biomarker to identify women with recurrent implantation failure (RIF) undergoing IVF at risk of miscarriage.	N = 311, age 22 - 48 years • Unsuccessful embryo implantation (n = 151) • Miscarriage (n = 66) • Live birth (n = 94)	Endometrial biopsies	p16-positive cells	Immunohistochemistry	• Women with live births had a higher percentage of p16-positive cells in the glandular and luminal epithelial cells of the endometrium compared to women who had miscarriages. • p16-positive cells of live birth vs. miscarriage: o glandular cells; 9.3% vs. 2.9% o luminal cells; 35.2% vs. 11.7% • Stromal cells: no significant difference of p16-positive between women with live births and those who miscarried.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
							• Cut-off values: o less than 12.5% of p16-positive luminal cells o less than 3.2% of p16-positive glandular cells • A decreased number of senescent p16-positive cells in the endometrium may be associated with implantation failures and the risk of recurrent miscarriage.
48	Retrospective	To identify the cumulus cell gene expression associated with oocyte developmental competence, specifically live birth, after single ET (SET) assisted reproductive technology.	N = 38 • Pregnant (n = 12), age 32.9 ± 1.13years • Non-pregnancy (n = 26), age 33.6 ± 0.64 years	Human cumulus cells from oocytes	• Metabolic genes: ALDOA, LDHA, PFKP, PKM2 • Signaling genes: AHR, GREM1, PTGS2, STS • Extracellular matrix genes: HAS2, PTX3, TNFAIP6, VCAN	qRT-PCR	• Gene expression can be a biomarker for predicting successful pregnancy outcomes after SET. • VCAN and PTGS2, may help identify oocytes with a higher chance of leading to a live birth after SET--high expression of these genes associated with oocyte quality, maturation timing, and pregnancy. • PTX3 showed a trend of association with oocyte developmental competence. • VCAN, GREM1, and PFKP correlated with birth weight at 38 weeks of gestation. • No significant correlation was found between cumulus cell gene expression and embryo morphology.
20	Cross-sectional	• To confirm in human that FMR1 affects IVF outcomes.	N = 598, age 33.4 ± 3.4 years • The first section infertile women of	Oocyte	Fragile X mental retardation 1 (FMR1) gene	CGG_n of the FMR1 gene by Polymerase Chain Reaction (PCR), Southern Blot	• FMR1 gene mutations are associated with distinct ovarian aging patterns.

Reference	Type of study	Objective	Participants	Type of sample	Biomarkers	Method	Main finding
		• To determine whether this reflects differences in ovarian aging between FMR1 mutations, egg/embryo quality or an effect on implantation.	all ages undergoing IVF treatment (n = 125) • The second section presumed fertile women undergoing IVF for pre-implantation genetic diagnosis (n = 121) • The third section infertile patients using their own eggs in first IVF cycles and donor recipient cycles (n = 352)			Analysis, and Fragment Analysis by Capillary Electrophoresis.	• FMR1 gene mutations were not linked to embryo aneuploidy, but aneuploidy increased with female age. • Recipient pregnancy rates were not affected by donor age or donor FMR1 genotype. • Women without a low FMR1 allele had a higher chance of clinical pregnancy in IVF treatments. • Low FMR1 alleles are associated with reduced egg/embryo quality and pregnancy chances as women age.
21	Observation	To evaluate the link between the amount of mitochondrial DNA (mtDNA) in CGCs surrounding an oocyte and the chances for the corresponding embryo of implanting and leading to an ongoing pregnancy.	N = 71, age 31.3 ± 4.3 years; obtained 84 oocyte-cumulus-complexes (OCCs) • OCCs having led to an implanted embryo (n = 26) • OCCs having led to an implanted embryo (n = 58)	Cumulus granulosa cells (CGCs)	mtDNA	qRT-PCR	• mtDNA content in CGCs as a potential biomarker for predicting embryo implantation potential. • Higher mtDNA content is associated with embryos that successfully implanted.

Biomarkers that can be identified in follicular fluids

The biomarkers identified in FFs (**Table 1**) can be classified into four main categories. The hormone category includes GH and IGF-1,[14] the gene/protein expression category includes the luteinizing hormone/human chorionic gonadotrophin receptor (LHCGR) variant N312S and the follicle-stimulating hormone receptor (FSHR) variant N680S,[36] survivin,[33] telomerase activity (TA) and telomere length (TL),[34] APOB,[15] several miRNAs (miR-7-5p, miR-202-5p, miR-378-3p, miR-224, miR-320a, miR-212-3p, and miR-21-5p,[16] miR-103a-3p and miR-10a-5p[17]), peptides,[35] and proteins.[37] The cytokine category is extensive, including adipokines (leptin, adiponectin, ghrelin),[38] G-CSF,[18,19] pro-inflammatory cytokines (IL-15[18], IL-1β, IL-6, IL-18, IFN-γ, IFN-α, TNF-α, IL-12, and IL-23 [19]), chemokines (MIP-1α, MIP-1β, MCP-1, RANTES, IL-8, sAPO-1/Fas, and CD44(v6) [19]), along with interleukins IL-6 and IL-17.[14]

Among the 12 articles that investigated biomarkers in FFs, miRNA and protein markers were observed in two studies.[16,17] Significant miRNAs identified in these two studies were of different types, including miR-7-5p, miR-202-5p, miR-378-3p, miR-224, miR-320a, miR-212-3p, and miR-21-5p,[16] as well as miR-103a-3p and miR-10a-5p from another study.[17] Similar to the proteins/peptides, no identical protein markers were identified in these two studies.[35,37] In the other group, several biomarkers are present.

Biomarkers that can be identified in serum and follicular fluids

The cytokine group includes two significant biomarkers detectable in both serum and FFs (**Table 1**): G-CSF [23] and M-CSF.[24]

Biomarkers that can be identified in follicular cells, culture medium, endometrial tissue, oocytes, and cumulus granulosa cells

Biomarkers detectable in other sample types are shown in **Table 2**. The hormone-associated biomarker in this collection is the progesterone receptor (PGR),[39] which is crucial for supporting hormonal responses. In the category of gene/protein expression, including microRNAs and protein markers, there is a diverse set of molecules such as phosphoglycerate kinase 1 (PGK1), regulator of G-protein signaling 2 (RGS2), RGS3, and cell division cycle 42 (CDC42),[40] and other markers like epiregulin (EREG), dihydropyrimidinase-like 3 (DPYSL3), tyrosine 3-monooxygenase/tryptophan 5 monooxygenase activation protein (YWHAZ), myristoylated alanine-rich protein kinase C substrate (MARCKS), UDP-glucose pyrophosphorylase 2 (UGP2), semaphorin 3A (SEMA3A), low-density lipoprotein receptor-related protein (LRP8), pirin (PIR), pleckstrin homology-like domain, family A member 1 (PHLDA1), secreted frizzled-related protein 1 (SFRP1), homer homolog 1 (HOMER1), and GA-binding protein transcription factor b1 (GABPB1).[39] The group is additionally enhanced by indicators of cell cycle status (p16-positive cells),[47] various metabolic genes (ALDOA, LDHA, PFKP, PKM2), signaling genes (AHR, GREM1, PTGS2, STS), and extracellular matrix genes (HAS2, PTX3, TNFAIP6, VCAN),[48] FMR1,[20] mtDNA,[21] and microRNAs.[42] The cytokine classification comprises a wide range of immunomodulatory and inflammatory mediators, including traditional cytokines such as GM-CSF, IL-2, IL-4, IL-7, IL-8, and IL-13,[44] IL-1β, IL-5, IL-6, IL-10, IL-12, interferon-γ (IFN-γ), and TNF-α,[44,46] and IFN-α,[22] as well as a further group of cytokines—IL-15, IL-17, IL-18, macrophage migration inhibitory factor, eotaxin, IFN-γ-inducible 10 kDa protein (IP-10), monocyte chemoattractant protein-1 (MCP-1), dickkopf homolog 1, heparin-binding epidermal growth factor, and vascular endothelial growth factor (VEGF)[46]. Other biomarkers in this comprehensive list include functional indicators such as the follicular sensitivity index (FSI),[41] immuno-suppressive activity,[22] metabolites (including 2-methylglutarate, 3-aminoiso-butyrate, 3-hydroxyisovalerate, acetate, acetoacetate, alanine, citrate, formate, glutamate, glycine,

lactate, tryptophan),[43] as well as components of glycan profiles and lectins (NPA, UEA-I, MAL-I, LCA, PHA-E +L, GNA, AAL, LTL, DBA, BPL).[4 5] Among all samples, several cytokine biomarkers showed duplication; however, the main findings regarding these cytokines differ.

Discussion

Sample collection

The identification of biomarkers from different types of samples revealed distinct types of biomarkers. The most commonly used sample type was serum/blood and FFs. Serum or blood sampling is the traditional method due to its convenience and standardized collection processes. The primary advantage of blood sampling is its minimal invasiveness, requiring only a simple venipuncture that may be performed in almost every clinical setting without the need for specialist equipment beyond regular phlebotomy equipment. Blood samples offer a comprehensive assessment of hormonal profiles and metabolic indicators, facilitating continuous monitoring during treatment cycles. Blood measures may not precisely represent the current microenvironment of developing oocytes, thereby missing critical localized biochemical alterations that directly affect oocyte quality and developmental potential.[54] Conversely, FF sampling provides direct access to the oocyte's current biochemical environment, yielding more relevant data about folliculogenesis and oocyte maturation processes. FF contains specific metabolites, growth factors, hormones, and cytokines that directly affect oocyte development and are inadequately reflected in peripheral circulation. This microenvironmental evaluation may provide enhanced prediction indicators for oocyte quality and embryo developmental potential.[55] The primary limitation of FF sampling is its invasiveness, as collection may only occur during oocyte retrieval procedures for assisted reproductive technology processes, requiring transvaginal ultrasound-guided aspiration under sedation or anesthesia. Furthermore, FF analysis presents technological difficulties such as the risk of blood contamination during collection and the necessity for immediate processing to avoid the degeneration of labile components.[56] The technical complexity of FF analysis further differentiates it from blood sampling. Although blood processing refers to established methods with standardized reference ranges, FF analysis requires specialized handling and lacks universal standardization. This presents difficulties in comparing outcomes across various laboratories and studies. Recent advances in proteomics, metabolomics, and lipidomics have improved the ability to study FF composition, indicating its potential as a non-invasive predictor of oocyte quality and IVF outcomes.[57] Despite these obstacles, FF analysis is increasingly significant in reproductive research, as it offers distinctive insights into the follicular environment that peripheral blood collection cannot provide.[58] This review includes two articles that compared two samples,[23,24] both demonstrating more significant outcomes in FF than in blood. However, the insufficient findings limit conclusive evidence that FF is superior to serum.

Hormones

Biomarkers were classified into four categories to enhance thorough study and analysis. These categories include hormones, gene and protein expression, cytokines, and others. Various biomarkers are derived from the hormonal group in blood samples, although none are distinctive or significant, with the exception of FSH:LH [2,25] and AMH [3–7]. Hormones regulate reproductive outcomes by organizing essential processes including gamete maturation, ovulation, and uterine receptivity; consequently, disruptions in hormones such as GnRH, FSH, LH, E_2, and P may result in problems like anovulation and implantation failure, whereas hormonal therapies in reproductive technologies assist in restoring balance and enhancing fertility outcomes.[59,60] FSH and LH are gonadotropins

predominantly identified in blood serum through immunoassay techniques, with LH being measurable in urine for ovulation prediction kits.[61] Both hormones, released by the anterior pituitary gland under hypothalamic regulation,[62,63] are essential for reproductive function. FSH stimulates follicular development[64] and E_2 production in females, while promoting spermatogenesis in males; LH triggers ovulation through its mid-cycle surge[64] and stimulates testosterone synthesis in males. In typical menstrual cycles, these hormones function synergistically[65] - FSH predominates during the follicular phase, facilitating follicle development, whereas the mid-cycle LH surge triggers ovulation, followed by corpus luteum production in the luteal phase.[64] In IVF procedures, FSH medications facilitate controlled ovarian stimulation to develop multiple follicles simultaneously,[66] while LH activity (often supplemented) promotes follicular development and oocyte quality[67,68]; hCG, displaying LH-like activity, functions as the "trigger shot" to initiate final oocyte maturation prior to retrieval. The FSH:LH ratio offers critical diagnostic information, as variations from the standard 1:1 ratio may suggest conditions such as polycystic ovary syndrome (increased LH:FSH) [69] or diminished ovarian reserve (increased FSH:LH),[68,70] thereby emphasizing the importance of these hormones in natural reproduction and assisted reproductive technologies. AMH is a dimeric glycoprotein classified within the transforming growth factor beta (TGF-β) superfamily.[71] It is expressed in the granulosa cells of ovarian follicles, including in pre-antral and early antral follicles,[72] and is detectable in blood serum. The levels serve as a crucial sign of ovarian reserve, assisting clinicians in evaluating a woman's remaining egg supply and overall reproductive capacity. [73–75] During typical reproductive processes, AMH remains largely constant throughout the menstrual cycle, indicating follicular recruitment without significant variations, consequently serving as an effective marker for ovarian function. In the field of IVF, AMH measurements are commonly employed to predict ovarian responsiveness to stimulation protocols, personalized treatment strategies, and the probability of retrieving enough oocytes while minimizing the risk of ovarian hyperstimulation syndrome.[76,77]

Gene and protein expression

Most of the gene and protein expression categories of biological markers can be identified in FFs samples. These biomarkers are various, and none are particularly significant. Consequently, it might be insufficient to predict the outcome independently, suggesting further investigation. Genes and proteins, including miRNAs, are crucial in reproductive outcomes by regulating essential pathways that regulate gamete maturation, endometrial receptivity, and embryo development. For instance, miRNA dysregulation can cause abnormal gene expression in the endometrium, leading to impaired implantation or heightened risk of early pregnancy loss.[78,79] Additionally, changes in protein expression profiles in oocytes and embryos are closely associated with gamete quality and developmental competence, eventually impacting the success rates of assisted reproductive technologies.[80]

Cytokines

Cytokines are essential for reproductive outcomes by regulating complicated immunological interactions during the reproductive process, including gametogenesis, implantation, and pregnancy maintenance.[81] These small signaling proteins regulate inflammation, immune cell recruitment, angiogenesis, and tissue remodeling at the maternal-fetal interface, with balanced cytokine profiles being crucial for successful reproduction. Pro-inflammatory cytokines (e.g., IL-1β, IL-6, TNF-α)[82] and anti-inflammatory cytokines (such as IL-10, TGF-β) must maintain homeostasis, as dysregulation is associated with several reproductive disorders, including recurrent

pregnancy loss, implantation failure, preeclampsia, and infertility.[83,84] Excessive expression of pro-inflammatory cytokines may inhibit embryo development and implantation, whereas insufficient levels of regulatory cytokines could compromise the immune tolerance essential for pregnancy maintenance, emphasizing the uncertain immunological balance necessary for reproductive success.[85] Nevertheless, cytokines require a short-term storage protocol due to their rapid degradation and low abundance, as their short half-life indicates that even small delays in processing can result in considerable loss of biological activity, thereby affecting assessment reliability. This intrinsic instability requires immediate sample processing and precise analytical methods to accurately measure the true in vivo concentrations of these temporary signaling molecules.[82,86] This may result in cytokine biomarkers being neither particularly distinguished nor remarkable, despite the presence of several cytokine biomarkers in the sample.

Others

There are additional biomarkers that, although identifiable, lack the significance or distinctiveness of the primary ones. These additional markers may provide insights into subtle physiological variations or enhance the understanding of reproductive function.

In a previous systematic review report from Berg and colleagues about seminal plasma biomarkers and their predictive ability on IVF and ICSI outcomes, the authors have studied 89 biomarkers including oxidative stress markers, proteins, glycoproteins, metabolites, immune system components, metals and trace elements and nucleic acids. The major difference to our study was the subjects' sex and biological fluid (seminal fluid). Biomarkers found in both reviews were immune system markers (IL-18 and TGF-β1–IL-18 ratio).[87]

Several limitations of the present review should be acknowledged. First, no formal risk-of-bias or quality assessment of the included studies was undertaken. As a result, the overall strength and reliability of the evidence may depend on the methodological rigor of the primary studies, which could introduce bias and affect the interpretation of our findings. Therefore, the conclusions drawn from this review should be interpreted with caution. Future systematic reviews on this topic would benefit from incorporating standardized critical appraisal tools to strengthen the validity of the evidence synthesis. Second, there was a lack of clarity in the included studies regarding whether the reported success rates of IVF and ICSI procedures were assessed in the context of fresh or frozen embryo transfer cycles. Most studies reported clinical outcomes—such as implantation, pregnancy, or live birth rates—without specifying the type of embryo transfer cycle. This lack of detail limits our ability to perform stratified analyses and reduces the depth and specificity of our findings.

Conclusion

The current status of biomarkers for ART demonstrates that AMH, FSH, and circulating nucleic acids are widely used to assess ovarian reserve and predict ART outcomes. However, as no single biomarker provides a comprehensive prediction, a combined approach using multiple biomarkers and clinical indicators is recommended for improving prognostic accuracy.[88] The variability in secreted biomarkers across different sample types suggests that reproductive outcomes may vary considerably. This heterogeneity poses a significant challenge in identifying a dominant biomarker capable of consistently predicting reproductive outcomes or serving as a reliable indicator across research studies. Additionally, current investigations remain largely preliminary, leaving a gap in our understanding of the broader implications and biological interactions of these biomarkers. Consequently, there is a clear

need for further validation studies to assess their reliability and predictive value, ensuring that future clinical applications are grounded in robust and comprehensive evidence. Moreover, many fertility-related biomarkers may represent promising therapeutic targets, offering potential avenues for intervention and treatment. These biomarkers can support the development of pharmacological strategies to improve ovulation and follicular development, enhance endometrial receptivity, and reduce inflammation or oxidative stress associated with infertility.

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Conflicts of Interest

The authors report no conflicts of interest in this work.

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