RBMO

REVIEW

A systematic review identifying seminal plasma biomarkers and their predictive ability on IVF and ICSI outcomes

BIOGRAPHY

Gaby Steba is a biomolecular scientist and has held a postdoctoral position in reproductive medicine at the University Medical Centre Utrecht since 2019. Her translational research focuses on the role of the endometrium in unexplained infertility, the endometrium, its microbiome and seminal plasma.

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KEY MESSAGE

Seminal plasma biomarkers show potential for predicting assisted reproductive technology outcomes. This comprehensive review identifies 32 molecules, including IL-18 and TGF-β1/IL-18 ratio, as promising biomarkers. Further research is needed to explore their predictive ability and to develop non-invasive diagnostic tools for infertility investigation and assisted reproduction.

ABSTRACT

The diverse nature and high molecule concentration of seminal plasma (SP) makes this fluid a good potential source for a potential biomarker that could predict assisted reproductive technology (ART) outcomes. Currently, semen quality parameters cannot accurately predict ART outcomes. A systematic literature search was conducted to identify human SP biomarkers with potential predictive ability for the outcomes of IVF and intracytoplasmic sperm injection. Observational cohort and case-control studies describing the association between biomarkers in human SP and the outcome of infertile men attending for ART were included. Forty-three studies were selected, reporting on 89 potential SP biomarkers (grouped as oxidative stress, proteins glycoproteins, metabolites, immune system components, metals and trace elements and nucleic acids). The present review supports 32 molecules in SP as potentially relevant biomarkers for predicting ART outcomes; 23 molecules were reported once and nine molecules were reported in more than one study; IL-18 and TGF-β1—IL-18 ratio were confirmed in distinct studies. This review presents the most comprehensive overview of relevant SP biomarkers to predict ART outcomes to date, which is of clinical interest for infertility investigations and assisted reproduction; nevertheless, its potential is under-exploited. This review could serve as starting point for designing an all-encompassing study for biomarkers in SP and their predictive ability for ART outcomes, and for developing a non-invasive diagnostic tool.

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KEYWORDS

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INTRODUCTION

All 8 billion people on earth have one thing in common: the start of life; the fusion of one sperm cell with one oocyte. Approximately 250 babies are born each minute; however, not all babies are conceived via natural conception. Global infertility prevalence is increasing and currently affects around 15–20% of all couples (*Chandra et al., 2013*; *Rezaeiyeh et al., 2022*). Infertility is defined by the World Health Organization as the 'failure to achieve pregnancy after 12 months of regular unprotected sexual intercourse'. Assisted reproductive technology (ART) programmes have helped millions of couples to become pregnant; however, the results of these techniques are still unsatisfactory. Approximately one-third of the IVF and intracytoplasmic sperm injection (ICSI) transfers result in live birth (*DeGeyter et al., 2020*; *Sunderam et al., 2022*). Unfortunately, recurrent ART failure is often caused by unexplained infertility. The prevalence of male unexplained infertility among men with infertility is about 15% and is accompanied by normal semen analysis without any physical and endocrine abnormalities (*Hamada et al., 2012*). To date, no biomarkers can accurately predict ART success (*Wang and Swerdloff, 2014*).

An important male factor for successful pregnancy, which is often neglected, is the whole semen omitted from spermatozoa, defined as seminal plasma (SP). Seminal plasma is a combination of secretions from the testis, epididymis and accessory sex glands, i.e. seminal vesicles, prostate and bulbourethral glands, and comprises approximately 90–95% of semen volume (*Samanta et al., 2018*; *Anamthathmakula et al., 2020*). Seminal plasma has a complex and diverse nature and contains high concentrations of molecules, including proteins (antioxidative) enzymes, nucleic acids, metabolites, inorganic ions, microbes and hormones (*Juyena and Stelletta, 2012*; *Altmäe et al., 2019*). Seminal plasma was always considered to be a passive transport vector for spermatozoa. During ejaculation, however, spermatozoa come into contact with SP components, which triggers a maturation process that confers sperm motility and fertilization capacity (*Rodríguez-Martínez et al., 2011*; *Milardi et al., 2012*). Therefore, it has been suggested that SP factors have the ability to either stimulate or inhibit sperm viability, motility and fertilization capacity before insemination (*Rodríguez-Martínez et al., 2011*; *Mei et al., 2019*). To emphasize the importance of SP on pregnancy, clinical studies have shown that intravaginal SP insemination during ART yields higher implantation rates and clinical pregnancy compared with women who do not receive intravaginal SP insemination (*Chicea et al., 2013*; *Friedler et al., 2013*; *Nikolaeva et al., 2016*). The presence of SP, however, is considered non-essential for fertilization, as proven by successful fertilization using washed ejaculated spermatozoa in ART procedures (*Rodríguez-Martínez et al., 2011*; *Kanannejad and Gharesi-Fard, 2019*).

Before removing SP during ART, SP factors can already execute their function on spermatozoa by altering functionality and quality, indirectly affecting IVF and ICSI outcomes. Because of these important functions, the easy accessibility of SP during ART treatment and the high concentration of diverse molecules makes SP an interesting source for biomarkers predicting ART outcomes. Therefore, the aim of this systematic review was to describe potential biomarkers in SP and their ability to predict ART outcomes. To the best of our knowledge, this systematic review is the first to report solely the relationship between biomarkers in SP and the potential predictive ability of ART outcomes. Identifying novel molecular biomarkers in SP may give insight into unexplained male infertility and recurrent ART failure. It could also serve as a non-invasive diagnostic tool during fertility treatment.

MATERIALS AND METHODS

The search strategy was conducted in accordance with the Preferred Reporting Items For Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (*Page et al., 2021*). The review protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO; CRD42023404113).

Data sources and search strategy

A systematic search was conducted using two online databases: *MEDLINE-PubMed* and *EMBASE*. The search strategy combined 'seminal plasma', 'ART' and 'biomarker' related words, using keywords and medical subject heading (MeSH) terms. No language, geographic, demographic, time-specific or other inclusion filters were applied. The search in both databases was conducted on 22 February 2023. Output of the databases included studies up to February 2023. The exact *MEDLINE-PubMed* and *EMBASE* search query is presented in Supplementary Table 1. Additional sources were identified from citations of retrieved studies.

Study selection

Studies were selected independently by two investigators (JSB and NMM) and discrepancies were discussed and solved by involving a third independent researcher (GS). Articles were initially screened by title and abstract, and irrelevant articles subsequently removed. Full-text screening of the remaining articles was then conducted. The primary outcome of this review was to identify biomarkers in SP with the potential ability to predict IVF and ICSI outcomes. Observational cohort and case-control studies describing the association between SP biomarkers in infertile men, and the outcomes of ART, were evaluated. Exclusion criteria were identified as follows: review articles, editorials and opinions, case reports, abstracts and posters, animal studies, studies evaluating biomarker in something other than SP, e.g. whole semen, spermatozoa, blood, blood plasma, or studies evaluating SP from men not attending an ART programme.

Following systematic search and article selection, additional records were retrieved using the snowballing technique and evaluated in the same way as articles retrieved from the systematic search. This method helps to ensure that all relevant studies have been identified. The reference lists of review articles and relevant studies were hand-searched to identify other potentially eligible studies.

Outcome measures

At least one of the following ART outcomes must have been reported in association with a SP biomarker: biochemical pregnancy (defined as positive β-HCG measurement 2 weeks after embryo transfer), clinical pregnancy (defined as visible pregnancy using an ultrasound after 12 weeks of gestation), undefined pregnancy (defined as biochemical or clinical pregnancy), fertilization rate (defined as the percentage of fertilized oocytes), fertilization failure (defined as failure of fertilization in all oocytes), time to pregnancy (defined as the number of IVF or ICSI cycles until successful pregnancy)

or live birth (defined as the birth of a live child after 24 weeks' gestation).

Data extraction and synthesis

Data from articles were manually extrapolated by one investigator (JSB). For every eligible study, the following data were extracted: reference information, study design, study population (number of participants, subgroups, age, body mass index [BMI], country), SP biomarker, biomarker measurement technique, ART outcome and all relevant results (*P*-values, conclusion). Subsequently, SP biomarkers were categorized, and all extracted data were tabulated based on biomarker category (TABLE 1).

Quality assessment

To evaluate the quality and possible bias of the selected articles, two investigators (JSB and NMM) independently scored each included work using the Quality Assessment Tool from the National Heart, Lung, and Blood Institute-National Institute of Health (*NHLBI-NIH, 2021*). Possible inconsistencies were resolved through common agreement. Observational cohort studies were scored on 14 criteria using the 'Quality Assessment Tool for observational cohort and Cross-Sectional studies', and quality was categorized as poor quality (<5 points), fair quality (5–8 points), or good quality (>8 points). Case-control studies were scored on 12 criteria using the 'Quality Assessment of Case-Control studies', and the quality was categorized as follows: poor (<4 points), fair (4–7 points), or good (>7 points). All studies were included in this review regardless of the quality score.

RESULTS

Identification and selection of articles

The PRISMA flowchart of the search strategy, identification and selection process is presented in FIGURE 1. Initial *MEDLINE-PubMed* (n = 1020) and *EMBASE* (n = 271) searches identified a total of 1291 articles, including 94 duplicates, which were removed using Mendeley Desktop version 1.19.8 and Rayyan (*Ouzzani et al., 2016*). The remaining 1197 articles were screened for title and abstract; 1161 records were instantly excluded, and 36 articles were selected for full-text evaluation. Five records identified as abstracts or posters could not be retrieved and were subsequently excluded. Six articles were excluded based on inclusion and exclusion criteria: incorrect outcome parameters (n = 5) and incorrect semen fraction (n = 1). Twenty-five articles met the inclusion criteria and were selected via the systematic search. Additional records were included using snowballing method (n = 18). Eventually, 43 studies were included and classified into seven categories: oxidative stress (n = 8), proteins (n = 7), glycoproteins (n = 6), metabolites (n = 2), immune system components (n = 13), metals and trace elements (n = 5) and nucleic acids (n = 3) (FIGURE 2). One study reported biomarkers belonging to oxidative stress and the immune system component categories. In total, 43 studies reported on 89 potential SP biomarkers.

The quality assessment of observational cohort- and case-control studies is presented in FIGURE 3. The complete quality assessment is available in Supplementary Table 2. Overall, the quality of the studies identified in this systematic literature search was fair. One of the 43 studies was categorized as poor quality, 34 studies were categorized as fair quality and eight studies were good quality. Information, such as inclusion criteria, sample size justification, and whether the outcome assessment was carried out blind, was unaccounted in 36, 40 and 37 studies, respectively.

Primary outcome: potential biomarkers

Oxidative stress

Oxidative stress (OS) is defined as the imbalance between the formation of oxidants (reactive oxygen species [ROS]) and (non)enzymatic antioxidants. Currently, OS is suggested to be one of the major causes of male infertility by altering sperm function and quality. Indeed, around 40% of men with infertility have shown elevated ROS levels in their SP (*Lanzafame et al., 2009*; *Mahfouz et al., 2009*). A total of eight studies reporting 10 potential OS biomarkers in SP were identified (TABLE 1). Noteworthy, two publications reported identical data and study population, but nothing about potential duplication was disclosed (*Al-Saleh et al., 2019*; *2021*). Two studies evaluated the relationship between SP ROS levels and IVF/ICSI outcomes (*Zorn et al., 2003*; *Hammadeh et al., 2008*). *Hammadeh et al. (2008)* reported a non-significant correlation between SP ROS levels and IVF and ICSI fertilization rates (n = 36, P = 0.187; and n = 22, P = 0.280, respectively) and pregnancy rate (n = 36; P = 0.976 and n = 22; P = 0.683, respectively). Surprisingly, a significant negative correlation between SP ROS levels and fertilization rate was established when IVF and ICSI groups were combined (n = 58; P = 0.045), but this was not observed for pregnancy rate (n = 58, P = 0.730) (*Hammadeh et al., 2008*).

Contrary to the continuous analysis by *Hammadeh et al. (2008)*, *Zorn et al. (2003)* analysed the fertilization rate as a categorical variable. Participating men were categorized according to fertilization rate (<25% or >25%). A significant association between high SP ROS concentration and low fertilization rates in IVF was reported (n = 41; P = 0.031), but not in ICSI (n = 106; P-value not reported). Additionally, ROS levels were significantly lower in SP of men whose partner achieved successful clinical pregnancy compared with unsuccessful pregnancy after IVF (n = 41, P = 0.041), but no discrepancies were observed after ICSI (n = 106, P = 0.718). Noteworthy, the IVF and ICSI groups did not contain identical populations, as the IVF group included men diagnosed with normospermia, whereas the ICSI group comprised men diagnosed with oligoasthenospermia (*Zorn et al., 2003*).

Among the generated ROS is H_2O_2 analysed by two studies by *Al-Saleh et al. (2019*; *2021*) who reported identical data concerning the association between SP H_2O_2 levels and IVF outcomes (n = 599). No statistically significant associations were identified between H_2O_2 and fertilization (P = 0.802), biochemical pregnancy (P = 0.757), clinical pregnancy (P = 0.545) and live birth rates (P = 0.494).

Oxidative DNA damage is related to the pathogenesis of numerous diseases and is particularly destructive for the sperm genome and consequently an important cause of male infertility (*Tunc and Tremellen, 2009*). DNA damage caused by OS results in single and double-strand DNA breaks as well as oxidative nucleotides (*Hegde et al., 2012*). One of these oxidative nucleotides is 8-hydroxy-2'-deoxyguanosine (8-OHdG), which can subsequently be used as a biomarker for measuring oxidative DNA damage (*Cambi et al., 2013*). Three studies have evaluated the relationship between SP 8-OHdG levels and IVF/ICSI outcomes (*Ahelik et al., 2015*; *Al-Saleh et al., 2019*; *2021*). Both studies by *Al-Saleh et al. (2019*; *2021*) reported identical non-statistical associations between 8-OHdG and

TABLE 1 COMPREHENSIVE OVERVIEW OF DATA EXTRACTED FROM ALL SELECTED ARTICLES IN THIS SYSTEMATIC REVIEW

Reference	Population (*n*)	Country; age; BMI	Seminal plasma biomarker	Measurement technique	Outcome	Main conclusion
Oxidative stress						
Ahelik et al. (2015)	IVF (58) ICSI (21)	Estonia: 36.3 ± 6.3; 26.6 ± 2.9	8-OHdG	ELISA	BP, CP	No conclusion can be drawn owing to lack of well-defined results.
Al-Saleh et al. (2019)	IVF (599)	Saudi Arabia: 32.8; 29,8	8-OHdG, H_2O_2, CAT, TAC	ELISA	FR, BP, CP, LB	No significant correlations between 8-OHdG, H2O2, CAT, TAC and IVF outcomes.
Al-Saleh et al. (2021)	IVF (599)	Saudi Arabia: 37.9 ± 7.4; 29.8 ± 6.1	8-OHdG, H_2O_2, CAT, TAC, MDA	ELISA MDA: HPLC	FR, BP, CP, LB	No significant correlations between 8-OHdG, H2O2, CAT, TAC, MDA and IVF outcomes.
Crisol et al. (2012)	IVF (71) ICSI (181) IVF/ICSI (48)	Spain: IVF: 36.6 ± 3.7; NR ICSI: 37.3 ± 4.6; NR	GPX	Spectrophotometry	FR, PR	No significant correlations between GPX activity and fertilization and pregnancy rates.
Hammadeh et al. (2008)	IVF (36) ICSI (22)	Germany: NR; NR	ROS, TAC	Colorimetric assay	FR, PR	Significant correlation between ROS and fertilization rate of IVF and ICSI combined. No significant correlation with TAC.
Jędrzejczak et al. (2005)	IVF (79)	Poland: successful: 33.3 ± 3.9; NR Unsuccessful: 35 ± 4.9; NR	MDA	Colorimetric assay	FR	Significant negative correlation between MDA and fertilization rate.
Yeung et al. (1998)	IVF (89)	Germany: 35.2; NR	GPX, GRD, SOD, catalase like	Spectrophotometer	FR, PR	No significant correlations between GPX, GRD, SOD, and catalase like activity with fertilization and pregnancy rates
Zorn et al. (2003)	IVF (41) ICSI (106)	Slovenia: IVF: 35.2 ± 6.1; NR ICSI: 34.1 ± 6.0; NR	ROS	Colorimetric assay	FR, CP	Significantly higher ROS level in fertilization rates <25% versus >25% after IVF. Significant lower ROS level in successful pregnancy versus unsuccessful pregnancy after IVF.
Proteins						
Kanannejad and Gharesi-Fard, (2019)	IVF (13): Successful (5) Unsuccessful (8)	Iran: successful: 33.8 ± 3.7; NR Unsuccessful: 34 ± 3.9; NR	Clusterin, NPC2, PSA	2D-PAGE, mass spectrometry	BP	Clusterin and NPC2 significantly overexpressed while PSA was significantly downregulated in successful pregnancy versus unsuccessful pregnancy.
Koistinen et al. (2002)	IVF (96)	Finland: NR; NR	Semenogelin, PSA	Immunofluorometric assay	FR	No significant correlation between semenogelin and PSA with fertilization rate.
Martinez-Soto et al. (2018)	ICSI (22)	Spain: 36.41 ± 0.42; NR	UPA	ELISA	CP	Significantly higher total UPA level in successful pregnancy versus unsuccessful pregnancy.
Mei et al. (2019)	IVF (97)	China: NR; NR	Galectin-3	ELISA	FR	Significant positive correlation between fertilization rate and galectin-3 concentration in seminal plasma derived extracellular vesicles.
Rolf et al. (1998)	IVF (73)	Germany: NR; NR	Creatine kinase	Spectrophotometry	PR	No significant difference in creatine kinase between successful and unsuccessful pregnancy.

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TABLE 1 (*Continued*)

Reference	Population (*n*)	Country; age; BMI	Seminal plasma biomarker	Measurement technique	Outcome	Main conclusion
Spiessens et al. (1998)	IVF (125)	Belgium: NR; NR	α-glycosidase	Spectrophotometry	FR, PR	No significant difference in α-glycosidase level between successful and unsuccessful pregnancy.
Xu et al. (2019)	IVF (166)	China: median 32; 23.51	Prosaposin	ELISA	FR	Significant positive correlation between prosaposin and fertilization rate
Glycoproteins						
Chen et al. (2021)	IVF (63)	China: 32.9 ± 4; NR	Soluble CD147	ELISA	FR, PR	Significant positive correlation between soluble CD147 with fertilization rate. Significant higher CD147 level in pregnancy versus non-pregnancy group.
Geva et al. (1997)	ICSI (50): Infertile (25) fertile (25)	Israel: infertile: 27.8 ± 5.6; NR fertile: 28.2 ± 4.3; NR	CA-125	Enzyme immune assay	FR, PR	No significant correlations between CA-125 and fertilization and pregnancy rates.
Koistinen et al. (2000)	IVF (112)	Norway: NR; NR	Glycodelin-A	Immunofluorometric assay	FR	Significantly higher glycodelin-A in fertilization rates <25% versus >25%. No continuous correlation between glycodelin-A and fertilization rate
Matorras et al. (1995)	IVF (46)	Spain: 34.5 ± 5.4; NR	CA-19.9, CA-125, CA-195	Immunoradiometric assay	FR, PR	Significantly higher CA-19.9 and CA-195 levels in fertilization rates ≥66% versus <66%. No significant correlations with fertilization rate as continuous variable.
Meisser et al. (1996)	IVF (97)	Switzerland: 26–64; NR	CA-125	Immunoradiometric assay	FR, PR	No significant correlations between CA-125 and fertilization and pregnancy rates.
Ovayolu et al. (2016)	IVF (113): Successful (42) Unsuccessful (71)	Turkey: successful: 33.5 ± 4.8; NR unsuccessful: 34.3 ± 7.6; NR	Soluble CD14	Chemiluminescence immune assay	BP, LB	Significantly higher soluble CD14 level in successful pregnancy and live birth versus unsuccessful pregnancy.
Metabolites						
Allahkarami et al. (2017)	ICSI (50)	Iran: 25-40; NR	Ammonia, urea, uric acid, creatinine	Spectrophotometry, diacetyl monoxime, enzyme assay	FR	Significant negative correlations between uric acid and urea with fertilization rate.
Lay et al. (2001)	IVF (24)	USA: 33.9 ± 4.8; NR	Fructose, lactic acid, citric acid, carnitine	HPLC	FR	No significant correlations between fructose, lactic acid, citric acid, and carnitine with fertilization rate
Immune system components						
Dahl et al. (2014)	ART (54)	Denmark NR; NR	sHLA-G	ELISA	PR	No significant difference in sHLA-G levels between pregnancy and non-pregnancy group.
El-Halawaty et al. (2011)	ICSI (33)	Egypt:NR; NR	AMH	ELISA	FR	No significant correlations between AMH and fertilization rates.

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TABLE 1 (*Continued*)

Reference	Population (*n*)	Country; age; BMI	Seminal plasma biomarker	Measurement technique	Outcome	Main conclusion
El-Sherbiny et al. (2021)	ICSI (184)	Egypt: NR; NR	ASA	ELISA	FR, PR	No significant differences in fertilization and pregnancy rates between couples with positive and negative ASA in seminal plasma.
Ford et al. (1996)	IVF (63)	UK: NR; NR	ASA	Immunobead assay	FR	Suggested a decrease in fertilization rate as ASA IgA or IgG concentration increased.
Jędrzejczak et al. (2005)	IVF (79)	Poland: successful: 33.3 ± 3.9; NR; unsuccessful: 35 ± 4.9; NR	IL-8	enzyme immunoassay	FR	No significant correlations between IL8 and fertilization rates.
Li et al. (2021)	IVF (102) R-ICSI (58)	China: IVF: 32.9 ± 4.8; 23.3 ± 1.7; R-ICSI: 33.1 ± 4.1; 23.2 ± 1.5	AMH, INHB	ELISA	FR	Significantly higher AMH and INHB levels in fertilization rates >30% versus <30%.
Nikolaeva et al. (2016)	IVF (40), ICSI (31): successful (32); unsuccessful (39)	Russia: successful: median 35.6; NR; unsuccessful: median 34.2; NR	TGF-β1, IL-18, TGF-β1/IL-18 ratio	Flow cytometry	CP	Significantly lower IL-18 level in successful pregnancy versus unsuccessful pregnancy. Significantly higher TGF-β1/IL-18 ratio in successful pregnancy versus unsuccessful pregnancy.
Nikolaeva et al. (2019)	IVF (20), ICSI (9): successful (14); unsuccessful (15)	Russia: successful: median 34.5; NR; unsuccessful: median 34; NR	TGF-β1, IL-18, TGF-β1/IL-18 ratio	Flow cytometry	CP	Significantly lower IL-18 level in successful pregnancy versus unsuccessful pregnancy. Significantly higher TGF-β1-IL/18 ratio in successful pregnancy versus unsuccessful pregnancy.
Nilsson et al. (2020)	IVF/ICSI (126)	Denmark: 33; NR	sHLA-G, TGF-β1,2,3	ELISA	TTP	No significant correlations between sHLA-G and TGF-β1,2,3 with TTP.
Schallmoser et al. (2019)	IVF/ICSI (106)	Germany: 36.9; NR	sHLA-G	ELISA	CP	No significant difference in sHLA-G between successful and unsuccessful pregnancy.
Seshadri et al. (2011)	IVF (36)	UK: median 34; NR	IL-6, IL-8, IL-10, IL-11, IL-12, IFN-γ, TNF-α	ELISA	FR	Significantly higher IL-11 level in fertilization rates >60% versus <35%. No significant correlation with fertilization rate as continuous variable
Seshadri et al. (2012)	IVF (36)	UK: median 34; NR	T cells, macrophages/monocytes, granulocytes, B cells, pan leucocytes, natural killer cells, activated T and B cells,	Immunohistochemical staining	FR	Significantly elevated macrophage and monocyte (CD14) concentration in fertilization rates >60% versus <35%. No significant correlation with fertilization rate as continuous variable.
Zorn et al. (2004)	IVF (104)	Slovenia: median 35; NR	elastase inhibitor complex	Immunoassay	FR, PR	No significant correlations between elastase inhibitor complex and fertilization and pregnancy rates.
Metals and trace elements						
Benoff et al. (2003)	IVF (78) ICSI (18)	USA: NR; NR	Pb	Atomic absorption spectrometer	FR	Significant negative correlation between Pb and fertilization rate.
Benoff et al. (2009)	IVF (96)	USA: NR; NR	Cd	Atomic absorption spectrometer	FR	No significant correlation between Cd and fertilization rate.

(*continued on next page*)

TABLE 1 (*Continued*)

Reference	Population (*n*)	Country; age; BMI	Seminal plasma biomarker	Measurement technique	Outcome	Main conclusion
Kim et al. (2014)	IVF (30)	USA: median 37; NR	Cd, Pb, Hg	Mass spectrometry	FR, CP, LB	No significant correlations between Cd, Hg, and Pb with either fertilization, pregnancy or live birth rates.
Rodríguez-Díaz et al. (2022)	IVF/ICSI (92)	Spain: pathological: 38.0 ± 5.4; 25.8 ± 3.5; normal: 38.0 ± 6.2; 27.3 ± 5.2	Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Si, Sn, Sr, V, Zn	Optical emission spectrophotometry	FR, CP, LB	Significantly lower V level in fertilization rates ≥75% versus <75%. Significantly higher Zn, Ca, K, and Mg levels in successful pregnancy versus unsuccessful pregnancy.
Zhou et al. (2021)	IVF (195)	China: 31.6 ± 3.6; 24.1 ± 3.8	As, Ba, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Tl, V, Zn	IPC-MS	FR, CP, LB	No significant correlations between metals and trace elements with IVF outcomes.
Nucleic acids						
Bounartzi et al. (2016)	IVF/ICSI (55)	Greece: 38.2 ± 0.6; 27.6 ± 0.5	f-spDNA	RT-PCR	FR, CP	No significant correlations between f-spDNA and fertilization and pregnancy rates.
Grosso et al. (2021)	ICSI (56)	Argentina: NR; NR	5′tRF-Glu-CTC, 5′tRF-Lys-CTT, 5′tRF-Gly-GCC	qRT-PCR	PR	Significant elevated 5′tRF-Glu-CTC and 5′tRF-Lys-CTT levels in unsuccessful pregnancy versus successful pregnancy.
Sukhikh et al. (2012)	IVF (79): successful (13); unsuccessful (66)	Russia: NR; NR	*PRM1*, *PRM2*, *ADAM-2* mRNA	Reverse qPCR	CP, BP, IVF failure	*ADAM-2* significantly overexpressed whereas *PRM1* and *PRM2* significantly downregulated in successful pregnancy versus unsuccessful pregnancy.

Al, aluminium; AMH, anti-Müllerian hormone; ART, assisted reproductive technology; As, arsenic; ASA, anti-sperm antibodies; B, boron; Ba, barium; BP, biochemical pregnancy; CAT, catalase; Cd, cadmium; Co, cobolt; f-spDNA, free-sperm plasma DNA; Ca, calcium; Cd, cadmium; CP, clinical pregnancy; Cr, chromium; Cu, copper; ELISA, enzyme-linked immunosorbent assay; Fe, iron; FR, fertilization rate; GPX, glutathione peroxidase; GRD, glutathione reductase; Hg, mercury; HPLC, high-performance liquid chromatography; ICSI, intracytoplasmic sperm injection; INHB, inhibin B; IPC-MS, inductively coupled plasma mass spectrometry; K, potassium; LB, live birth; Li, lithium; MDA, malondialdehyde; Mg, magnesium; Mn, manganese; Mo, Molybdenum; Na, sodium; Ni, nickel; NPC2, epididymal secretory protein E1; NR, not reported; Pb, lead; PR, undefined pregnancy rate, i.e. it can be biochemical or clinical; PSA, prostate specific antigen; qRT-PCR, quantitative reverse transcription polymerase chain reaction; ROS, reactive oxygen species; RT-PCR, reverse transcription polymerase chain reaction; sHLA-G, serum human leukocyte antigen G; Si, silicon; Sn, tin; SOD, superoxide dismutase; Sr, strontium; TAC, total antioxidant capacity; Tl, thallium; TTP, time to pregnancy; UPA, urokinase-type plasminogen activator, V, vanadium; Zn zinc; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

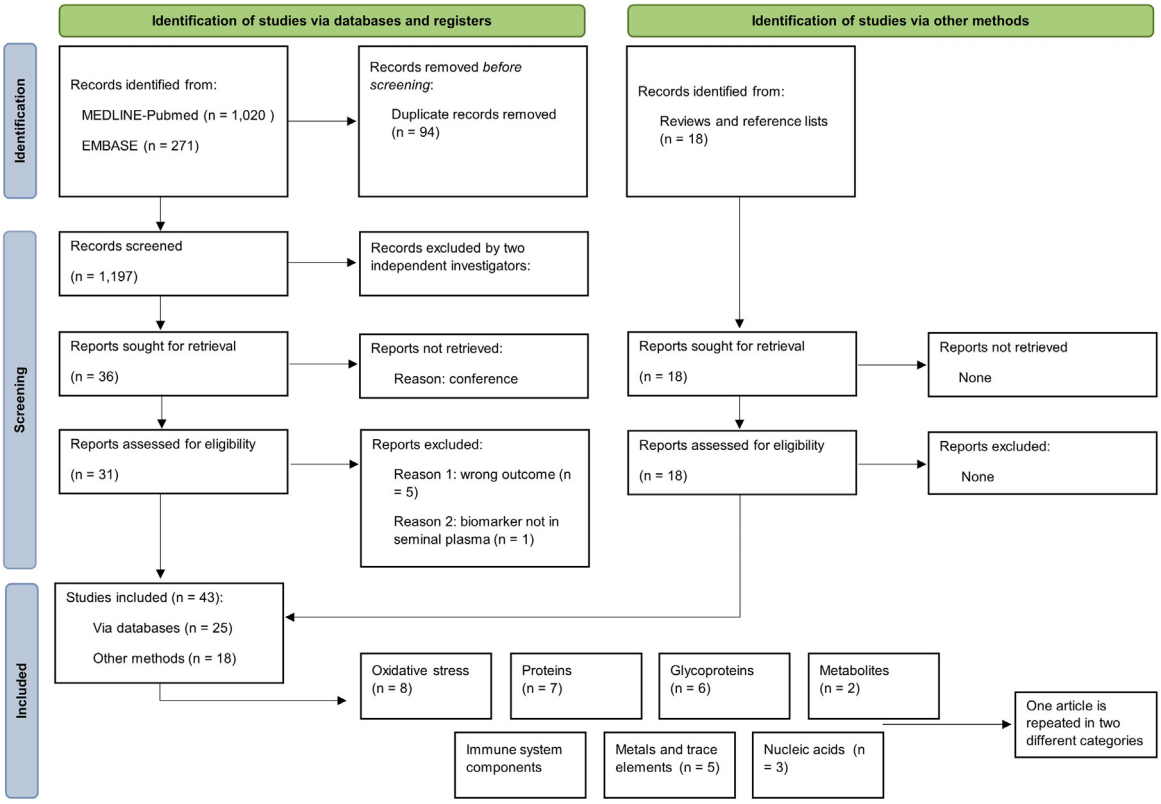

FIGURE 1 The Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of the systematic literature search. Study identification, screening, and eligibility. Included studies were classified into seven groups: oxidative stress (n = 8), proteins (n = 7), glycoproteins (n = 6), metabolites (n = 2), immune system (n = 13), metals and trace elements (n =5) and nucleic acids (n = 3). One article reported biomarkers belonging to both oxidative stress and immune system categories.

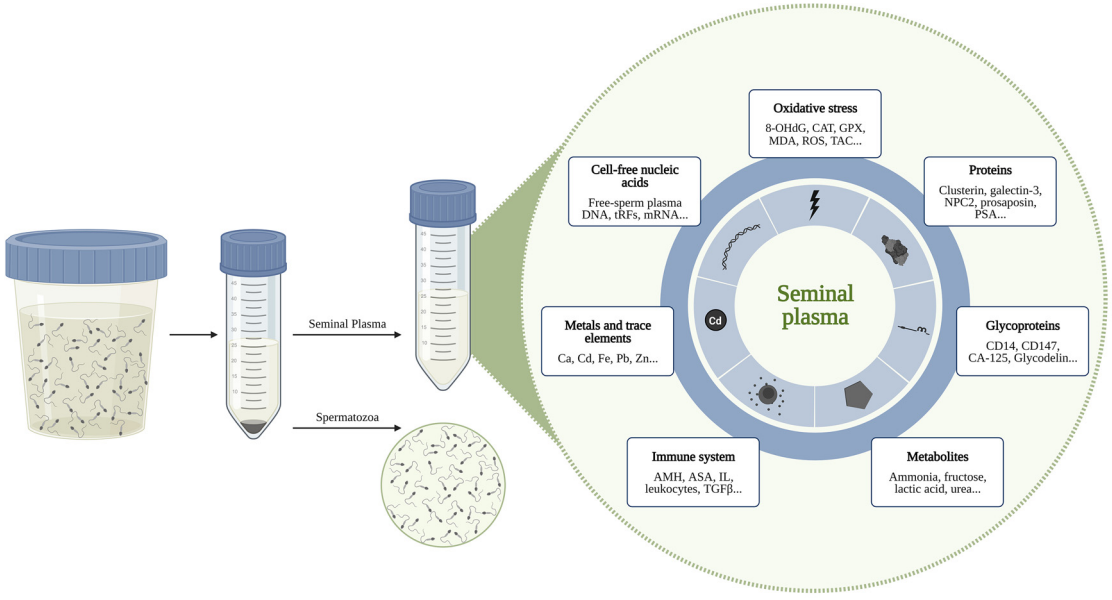

FIGURE 2 Visual representation of potential seminal plasma biomarkers and their categories. AMH, anti-Müllerian hormone; ASA, anti-sperm antibodies; Ca, calcium; CA, carcinoma antigen; CAT, catalase; Cd, cadmium; CD, cluster of differentiation; E1, epididymal secretory protein; Fe, iron; GPX, glutathione peroxidase; IL, interleukin; MDA, malondialdehyde; Pb, lead; PSA, prostate specific antigen; ROS, reactive oxygen species; TAC, total antioxidant capacity; TGFβ, transforming growth factor β; tRFs, tRNA-derived fragments; Zn, zinc; 8-hydroxy-2′-deoxyguanosine (8-OHdG). Figure created with BioRender.

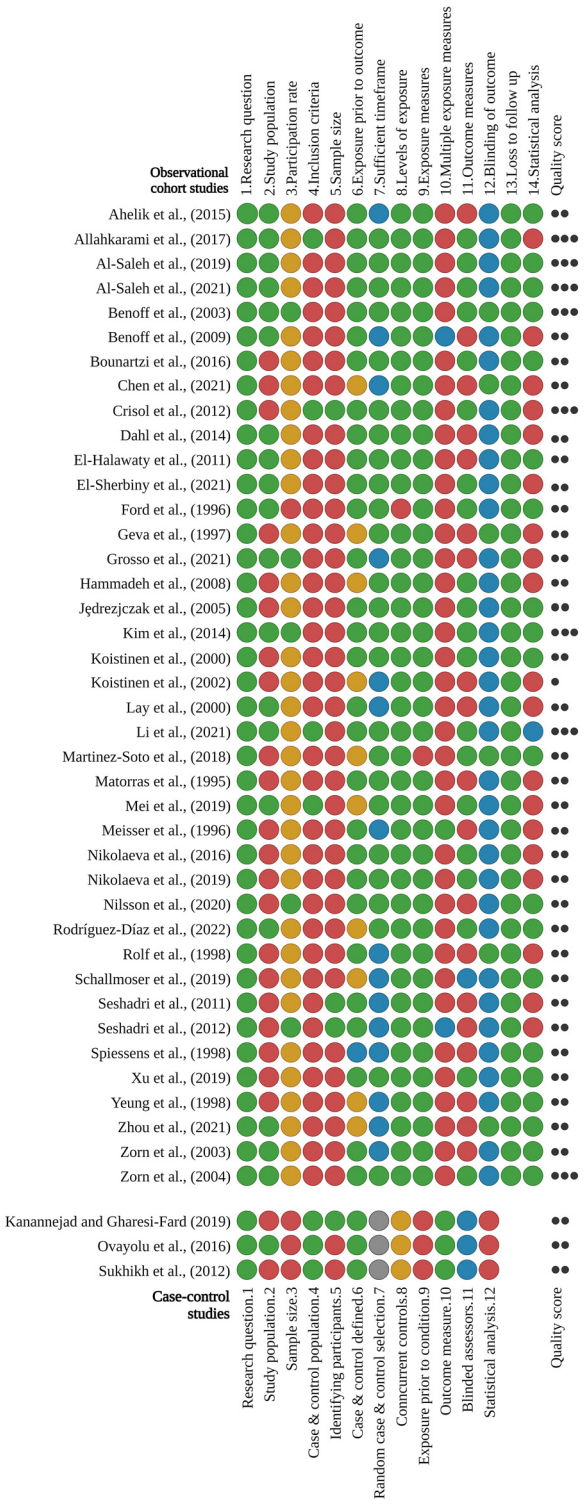

FIGURE 3 Quality assessment of observational cohort studies and case-control studies. Yes (green), no (red), cannot determine (blue), not applicable (grey), not reported (yellow). Quality score observational cohort studies: • Poor (<5), •• Fair (5-8), ••• Good (>8). Quality score case-control studies: • Poor (<4), •• Fair (4-7), ••• Good (>7). Figure created with BioRender.

fertilization ($P = 0.625$), biochemical pregnancy ($P = 0.054$), clinical pregnancy ($P = 0.055$) and live birth rates ($P = 0.105$) ($n = 599$). In the third study, 8-OHdG was tested in parallel with lipid peroxidation marker 8-iso prostaglandin $F_{2\alpha}$ (8-EPI), a urinary OS marker (*Ahelik et al., 2015*). *Ahelik et al. (2015)* reported a significant association between OS in the male partners and pregnancy rate ($P = 0.022$); however, the OS marker (8-OHdG or 8-EPI) is not specified as well as the source of the marker (seminal plasma or urine) and the ART programme (IVF or ICSI). In addition, a significant association between urinary 8-EPI and pregnancy was reported (n = not reported, $P = 0.049$) (*Ahelik et al., 2015*). Ultimately, because of the lack of well-defined results, no clear conclusion regarding the SP OS marker 8-OHdG can be drawn.

Besides oxidative DNA damage, OS can induce cell membrane damage in spermatozoa. Specifically, ROS induces lipid peroxidation in the sperm membrane, which can reduce membrane fluidity and hinder sperm–oocyte fusion (*Aitken et al., 1989*; *De Lamirande et al., 1993*). A quantitative end-product of lipid peroxidation is malondialdehyde (MDA), which can subsequently be used as an OS marker (*Requena et al., 1996*). Two studies evaluated MDA levels in SP and IVF outcomes (*Jędrzejczak et al., 2005*; *Al-Saleh et al., 2021*). *Al-Saleh et al. (2021)* observed no significant association between MDA levels and fertilization ($P = 0.288$), biochemical pregnancy ($P = 0.851$), clinical pregnancy ($P = 0.552$) and live birth rates ($P = 0.38$) ($n = 599$). When *Jędrzejczak et al. (2005)* compared SP MDA levels between successful and unsuccessful fertilization, no discrepancies were observed ($P > 0.05$).

Contrary to this, analysing fertilization rate as a continuous variable, a statistically negative correlation between seminal MDA levels and the percentage of fertilized oocytes was observed ($n = 79$; $r = -0.27$; $P < 0.05$). This negative correlation enhanced when the proportion of successfully fertilized oocytes increased (>10%; $r = -0.36$, >20%; $r = -0.44$, >30%; $r = -0.49$; $P < 0.05$ for all correlations) (*Jędrzejczak et al., 2005*).

Opposed to the above-described SP factors related to ROS, multiple studies have evaluated elements in SP that are known to provide protection against OS: enzymatic and non-enzymatic antioxidants. Interestingly, men with infertility have shown impaired non-enzymatic antioxidant capacity in their SP compared with fertile men (*Hammadeh et al., 2008*). The total antioxidant capacity (TAC) provides information about the status of all antioxidants in biological samples. Three studies have evaluated the relationship

between SP TAC levels and IVF/ICSI outcomes. Both studies of *Al-Saleh et al. (2019; 2021)* reported identical data: non-significant associations between TAC and IVF fertilization ($P = 0.823$), biochemical ($P = 0.815$), clinical pregnancy ($P = 0.834$) and live birth rates ($P = 0.743$) ($n = 599$). *Hammadeh et al. (2008)* observed no correlations between SP TAC levels and fertilization- or pregnancy rate after IVF ($n = 36$, $P = 0.881$ and $P = 0.98$, respectively) and ICSI ($n = 22$, $P = 0.412$ and $P = 0.859$, respectively).

During sperm maturation and transport, cytoplasmatic enzymes, such as protective antioxidant enzymes, are lost (*Hwangbo et al., 2016*). Accordingly, SP enzymatic oxidants need to create a protective environment for spermatozoa, e.g. by preventing the formation of reactive oxidants or by interfering with radical chain reactions and thereby scavenging free radicals) (*Huang et al., 2005*). Antioxidant enzymes were found to be excreted by epididymal principal cells and include catalase (CAT), catalase-like, superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione reductase (*Yeung et al., 1998*; *Mahfouz et al., 2009*). *Al-Saleh et al. (2019; 2021)* reported the lack of associations between SP CAT levels with fertilization ($P = 0.536$), biochemical pregnancy ($P = 0.218$), clinical pregnancy ($P = 0.218$) and live birth rates ($P = 0.534$) ($n = 599$). *Yeung et al. (1998)* reported no statistically significant correlations between SP catalase-like activity, SOD, GPX and glutathione reductase with IVF fertilization rate (*P*-values not reported). The relationship between SP GPX activity and IVF outcomes was also evaluated by *Crisol et al. (2012)*. Here, also no correlation between GPX activity in SP and fertilization failure after IVF and ICSI was observed ($n = 252$, $P = 0.55$), along with no GPX activity discrepancies between successful and unsuccessful pregnancy ($n = 252$, $P = 0.44$).

Enzymatic antioxidants, such as CAT and SOD, are dependent on co-factors: calcium, iron and zinc. Live birth was associated with significantly elevated levels of zinc and calcium in SP, whereas no correlation with iron was observed ($n = 92$, $P = 0.004$, $P = 0.013$ and *P*-value not reported, respectively) (*Rodríguez-Díaz et al., 2022*). Contrary to this, *Zhou et al. (2021)* did not detect any correlations between zinc and fertilization, clinical pregnancy or live birth rates (*P*-values not reported). A more comprehensive analysis of trace elements is described in the section 'Metals and trace elements'.

Proteins

Seminal plasma contains a high protein concentration with a comprehensive range of action. The proteins are involved in sperm maturation, capacitation, acrosome reaction, modulation of the immune response and providing a protective environment for spermatozoa (*Rodríguez-Martínez et al., 2011*). A total of seven studies reported on nine proteins as potential SP biomarkers. *Kanannejad and Gharesi-Fard (2019)* examined differential SP protein expression in association with a biochemical pregnancy test after IVF. Mass spectrometry was used to identify three differentially expressed proteins: clusterin, epididymal secretory protein E1 (NPC2) and prostate-specific antigen (PSA). Clusterin is one of the most abundant glycoproteins in semen and is associated with sperm quality, sperm maturation and protecting spermatozoa against oxidative damage (*Salehi et al., 2013*; *Janiszewska et al., 2022*). The pregnancy group presented significantly higher clusterin expression ($P = 0.04$) (*Kanannejad and Gharesi-Fard, 2019*). Similar to clusterin, NPC2, which is associated with cholesterol regulation in spermatozoa and sperm cell stabilization (*Sullivan et al., 2011*), was significantly overexpressed in the pregnancy group ($n = 13$, $P = 0.02$) (*Kanannejad and Gharesi-Fard, 2019*). The third identified protein, PSA, is explicitly produced by the prostate and plays a key role in semen liquefaction by degrading fibronectin and semenogelin (*Lilja et al., 1987*). High PSA levels have been an early prostate cancer diagnostic marker as well as an indicator for benign prostate hyperplasia (*Catalona et al., 1994*; *Nadler et al., 1995*). *Kanannejad and Gharesi-Fard (2019)* observed a significant overexpression of PSA in the non-pregnant group ($n = 13$, $P = 0.003$); however, another study found no significant correlation between PSA levels and IVF fertilization rates ($n = 13$, $P = 0.42$) (*Koistinen et al., 2002*). *Koistinen et al. (2002)* evaluated the correlation between PSA and semenogelin with IVF fertilization rates. Semenogelin is a gel matrix protein preventing sperm capacitation and motility. Semenogelin expression is most abundant in seminal vesicles (*Lilja et al., 1989*). An inverse correlation between the semenogelin and PSA concentration in SP was observed ($n = 96$, $P = 0.015$). Nonetheless, no correlation between SP semenogelin levels and IVF fertilization rates was observed ($n = 96$, $P = 0.25$).

Seminal plasma extracellular vesicles (EV) expressed galectin-3 mediates spermatozoa-zona pellucida binding during fertilization (*Deschildre et al., 2007*). *Mei et al. (2019)* assessed the relationship of galectin-3 in EV-free SP and galectin-3 bound to seminal plasma-derived EV with IVF fertilization rate. No significant correlations between the galectin-3 concentration in EV-free SP and fertilization rate was observed ($n = 97$, $P = 0.127$) Interestingly, a significant positive correlation between IVF fertilization rate and galectin-3 bound to SP-derived EV was detected ($n = 97$; $P = 0.0083$) (*Mei et al., 2019*).

Urokinase-type plasminogen activator (UPA) is crucial for activating the plasminogen system, which operates as an extracellular protease system, and has been implicated in the male reproductive function (*Gunnarsson et al., 1999*; *Castellino and Ploplis, 2005*). Animal studies have shown the necessity of UPA for normal male fertility: downregulation of UPA in mice resulted in decreased fertility and sperm motility (*Qin et al., 2015*; *Zhao et al., 2017*). An association of UPA with sperm capacitation and acrosome reaction has also been suggested (*Martinez-Soto et al., 2018*). A study evaluated total UPA and active UPA levels in SP and its association with clinical pregnancy outcome after ICSI, revealing that total UPA levels were significantly higher in the pregnancy group compared with the non-pregnancy group ($n = 22$, $P = 0.01$); however, no significant differences in active UPA were observed ($n = 22$, $P = 0.38$) (*Martinez-Soto et al., 2018*).

Ultimately, three SP proteins were evaluated in three distinct studies: prosaposin (*Xu et al., 2019*), creatine kinase (*Rolf et al., 1998*) and α-glucosidase (*Spiessens et al., 1998*). No statistically significant differences in SP creatine kinase and α-glucosidase levels between the men whose partners achieved pregnancy and those who did not achieve pregnancy after IVF were observed ($n = 73$ and $n = 125$, respectively; *P*-values not reported) (*Rolf et al., 1998*; *Spiessens et al., 1998*). Mice studies showed that prosaposin, a lysosomal protein identified in Sertoli cells and the epididymis, is involved in sperm–oocyte binding and fertilization (*Magargee et al., 2000*). *Xu et al. (2019)* showed a significantly positive correlation

between SP prosaposin levels and IVF fertilization rate (n = 166; P = 0.005).

Glycoproteins

Seminal plasma is rich in glycosylated proteins, which are indispensable during spermatogenesis, sperm maturation, capacitation and fertilization (*Seppälä et al., 2007*; *Cheon and Kim, 2015*; *Maric et al., 2021*). A total of six studies reporting on six glycoproteins as potential biomarkers for the prediction of ART outcomes were identified. *Koistinen et al. (2000)* evaluated the glycoprotein glycodelin-A in SP, which is produced in seminal vesicles (*Koistinen et al., 1997*). Glycodelin is suggested to reduce cholesterol influx and maintaining spermatozoa in incapacitated state (*Yeung et al., 2009*). No linear correlation between SP glycodelin-A levels and IVF fertilization rate was observed (n = 112; P = 0.14) (*Koistinen et al., 2000*). When categorizing men based on fertilization rate (<25% or >25%), however, significantly higher glycodelin-A levels were observed in men with lower fertilization rates (n = 112; P = 0.010). Additionally, total SP protein concentration was significantly higher in men with high fertilization rates (n = 112; P = 0.015) (*Koistinen et al., 2000*).

Carcinoma antigens (CA), commonly known as tumour markers, are not strictly associated with cancer. They can form structural units of glycoproteins and have been identified in SP (*Hanisch et al., 1985*). The relationship between SP CA-125 levels and IVF/ICSI outcomes was reported in three studies (*Matorras et al., 1995*; *Meisser et al., 1996*; *Geva et al., 1997*). *Geva et al. (1997)* compared men diagnosed with severe oligoasthenospermia and fertile men with normospermia. Although the SP CA-125 levels were significantly higher in patients with severe oligoasthenospermia (n = 50, P < 0.04), no direct correlations between CA-125 and fertilization and clinical pregnancy rates were reported. In agreement with *Geva et al. (1997)* both *Matorras et al. (1995)* and *Meisser et al. (1996)* reported no significant correlations between SP CA-125 and fertilization, and pregnancy outcomes (n = 46 and n = 97, respectively; P-values not reported). *Matorras et al. (1995)* also evaluated SP CA-19.9 and CA-195 levels; however, no correlations with fertilization and pregnancy rates were observed. Nonetheless, a statistically significant association was found when categorizing men based on fertilization rate (<66% or ≥66%); higher CA-19.9 and CA-195 levels were observed in men with fertilization rates 66% or above (n = 97, P = 0.02 and n = 97, P = 0.01, respectively) (*Matorras et al., 1995*).

Cluster of differentiation (CD) glycoproteins belong to the immunoglobulin (Ig) superfamily and are generally found on the membrane of immune cells. Aside from membrane-bound expression, for some CDs, a soluble isoform has been identified (*Ovayolu et al., 2016*; *Chen et al., 2021*). Levels of a subtype of soluble CD14 (sCD14-ST), also known as presepsin, increase during inflammation (*Yaegashi et al., 2005*). *Ovayolu et al. (2016)* assessed, in a case-control study, the relationship between SP presepsin and clinical pregnancy after ICSI. Seminal plasma presepsin levels were significantly higher in the pregnancy group compared with the non-pregnancy group, in terms of successful pregnancy and live birth (n = 113, P = 0.004; and n = 113, P = 0.037, respectively). As for biochemical pregnancy, SP presepsin levels were non-significantly higher in the pregnancy group (n = 113; P = 0.060) (*Ovayolu et al., 2016*).

Another soluble CD is CD147. On the basis of reports on CD147 in the mice female reproductive tract, a role in fertilization of CD147 expressed in the cumulus cell has been suggested (*Kuno et al., 1998*). CD147 are present in mice spermatozoa and may be involved in sperm motility and acrosome reaction (*Chen et al., 2012*). The relationship between soluble CD147 levels in SP and IVF fertilization rate and pregnancy outcome was evaluated by *Chen et al. (2021)*. Seminal plasma soluble CD147 was significantly positively correlated with fertilization rate (n = 63; P = 0.0023). Additionally, soluble CD147 levels were significantly lower in the SP of men whose partners did not achieve pregnancy after IVF (n = 63; P = 0.045).

Metabolites

Metabolites in SP are associated with sperm energy metabolism, metabolic activity and motility (*Luiza et al., 2018*). Two studies evaluated eight potential metabolites as SP biomarker. *Lay et al. (2001)* evaluated the association between fructose, lactic acid, carnitine, citric acid and total protein concentration with IVF fertilization rates. Fructose is suggested to be the primary energy source for sperm motility, and related to anaerobic fructose catabolism is lactic acid (*Mann and Lutwak-Mann, 1948*; *Tsujii et al., 2006*). Citric acid has been associated with semen liquefaction (*Huggins and Neal, 1942*) and macronutrient metabolism (*Toragall et al., 2019*), whereas carnitine is involved in metabolic processes like fatty acid transportation into mitochondria and acetylated co-enzyme A storage (*Ruiz-Pesini et al., 2001*). No associations between these SP metabolites and fertilization rate were found, using continuous and categorical analysis (n = 24; fertilization rate <30%, 31–70%, and ≥71%) (*Lay et al., 2001*).

Non-protein nitrogenous compounds (NPN), such as uric acid, urea, ammonia and creatinine, are produced during protein and nucleic acid catabolism (*Dimski, 1994*). *Allahkarami et al. (2017)* evaluated the correlation between these NPN and ICSI fertilization rates. Significant negative correlations between SP uric acid and urea levels with fertilization rate were reported (n = 50, P = 0.043 and n = 50, P = 0.03, respectively). Contrary, no significant correlations between SP ammonia and creatinine with fertilization rate were established (n = 50; P-values not reported) (*Allahkarami et al., 2017*).

Immune system components

Seminal plasma contains immune system components, such as cytokines, immune cells and antigens (*Samanta et al., 2018*). During natural conception, these components may be responsible for conditioning the female immune system for allogenic spermatozoa and embryos (*Ahmadi et al., 2022*). A total of 13 studies reported on 24 potential SP biomarkers related to the immune system. An increase in the number of white blood cells in semen is referred to as leukocytospermia and can be a sign of infection or inflammation (*WHO, 2021*). Leukocytospermia has been associated with infertility, reduced sperm motility, fertilization and pregnancy rate in ART (*Yilmaz et al., 2005*). *Seshadri et al. (2012)* studied the effect of SP leukocytes and its subpopulations on IVF fertilization rates, whereas evaluating fertilization rate, participating men were categorized based on fertilization rate (<35% or >60%), excluding cases falling between these categories (*Seshadri et al., 2012*). Immunohistochemistry was used to detect leukocytes and subpopulations in SP, and the immune cells were identified by membrane-bound CD glycoproteins. SP CD14, as a marker of monocytes and macrophages, was significantly higher in

the high fertilization group compared with the low fertilization group ($n = 36$; $P < 0.05$) (*Seshadri et al., 2012*). Therefore, monocytes and macrophages were significantly elevated in the high fertilization group. A non-significant reduction of CD69 (activated T- and B cells) was observed in the higher fertilization rate group ($n = 36$; $P = 0.06$). Ultimately, no significant differences between T cells (CD3, CD4, CD8), granulocytes (CD16), B cells (CD20), pan leukocytes (CD45) and natural killer cells (CD56) were observed ($n = 36$; $P > 0.15$). Additionally, when analysing fertilization rate as a continuous variable, none of the immune cells presented significant correlations with fertilization rates (*P*-values not reported) (*Seshadri et al., 2012*).

Inverse relationships of SP cytokine levels with sperm motility and migration have been described, showing high cytokine levels in men with infertility and leukocytospermia (*Seshadri et al., 2011*). Pro- and anti-inflammatory cytokines are produced by various immune-competent cells in the male reproductive tract (*Hill et al., 1987*). Three studies reported on the anti-inflammatory cytokine transforming growth factor β (TGF-β) isoform 1 in SP (*Nikolaeva et al., 2016*; *2019*; *Nilsson et al., 2020*). Both studies by *Nikolaeva et al. (2016*; *2019*) reported no significant difference in SP TGF-β1 concentration between successful and unsuccessful pregnancy in women exposed to seminal plasma during IVF/ICSI treatment (2016: $n = 71$; *P*-value not reported; 2019: $n = 29$; $P = 0.077$). Additionally, *Nikolaeva et al. (2016)* reported no significant difference in TGF-β1 between successful and unsuccessful pregnancy groups in terms of live birth rate (*P*-value not reported). Noteworthy, the results of *Nikolaeva et al. (2016*; *2019*) are exceedingly similar: the 2016 study population consisted of 71 couples, the 2019 study included 29 couples. As the time frame of study population recruitment and demographic information are not reported, there could be an overlap in study population. *Nilsson et al. (2020)* evaluated the relationships between SP levels of three TGF-β isoforms (1, 2 and 3) with time to pregnancy. All three isoforms, TGF-β1, 2 and 3, had no significant effect on time to pregnancy after IVF/ICSI ($n = 126$, $P = 0.60$, $P = 0.18$ and $P = 0.85$, respectively). In addition to the three TGF-β isoforms, two other members of the TGF-β family were evaluated: anti-Müllerian hormone (AMH) and inhibin B (INHB) (*El-Halawaty et al., 2011*; *Li et al., 2021*). Anti-Müllerian hormone and INHB, besides cytokines, are also classified as protein hormones. Both AMH and INHB are produced by the Sertoli cells in the testis and have been associated with semen quality and spermatogenesis (*Fujisawa et al., 2002*; *Barbotin et al., 2015*). *Li et al. (2021)* categorized men based on fertilization rate; when the fertilization rate was less than 30%, couples would undergo rescue ICSI ($n = 58$), and when the fertilization rate was more than 30%, conventional IVF ($n = 102$) was carried out. The SP of men undergoing IVF, which had a good fertilizing capacity, contained significantly higher AMH and INHB levels compared with men undergoing rescue ICSI ($P = 0.000$ and $P = 0.000$, respectively) (*Li et al., 2021*). When analysing AMH with fertilization rate as continuous variable, *El-Halawaty et al. (2011)* observed no significant correlation between SP AMH levels and ICSI fertilization rate ($n = 33$; $P = 0.08$). Furthermore, no significant difference in AMH levels between males with infertility and fertile males attending for ICSI were reported ($n = 33$, $P = 0.21$).

Besides TGF-β1, both studies by *Nikolaeva et al. (2016*; *2019*) compared pro-inflammatory interleukin-18 (IL-18) levels between the SP of men whose partner achieved a pregnancy and those who did not achieve a pregnancy. Both studies reported significantly lower SP IL-18 levels in the pregnancy group (2016: $n = 71$, $P = 0.018$; 2019: $n = 29$, $P = 0.02$). Regardless of the non-significant difference in SP TGF-β1 levels between the successful and unsuccessful pregnancy groups, a significantly higher TGF-β1/IL-18 ratio was found in the successful pregnancy group (2016: $n = 71$, $P = 0.026$; 2019: $n = 29$, $P = 0.033$) (*Nikolaeva et al. 2016*; *2016*).

Seshadri et al. (2011), evaluated the relationship between SP pro- and anti-inflammatory cytokines and IVF fertilization rate. The following cytokines were analysed: pro-inflammatory (IL-8, IL-12, interferon gamma; INF-γ, and tumour necrosis factor alpha; TNF-α), anti-inflammatory (IL-10) and both pro- and anti-inflammatory (IL-6 and IL-11). None of these SP cytokines correlated with continuous IVF fertilization rates. Nonetheless, when categorizing the participating men based on fertilization rate (<35% or ≥60%), SP IL-11 levels seemed to be significantly higher in the high fertilization group ($n = 36$; $P \leq 0.05$) (*Seshadri et al., 2011*).

An antigen identified in SP is the soluble form of human leukocyte antigen-G (sHLA-G), which is expressed by the epididymis, testis and prostate gland (*Larsen et al., 2011*). The sHLA-G has mostly been investigated in the female reproductive system where it is associated with pregnancy complications and immunomodulation of the fetal—maternal niche (*Ishitani et al., 2003*). Moreover, the homozygous HLA-G genotype is associated with reduced fertility and unsuccessful ART outcomes (*Hviid et al., 2004*; *Dahl et al., 2014*). Three studies evaluated SP sHLA-G with ART outcome (*Dahl et al., 2014*; *Schallmoser et al., 2019*; *Nilsson et al., 2020*). *Dahl et al. (2014)* divided men based on normal or reduced semen quality parameters. For both categories, no significant difference in SP sHLA-G concentration between men whose partner achieved pregnancy and those who did not was observed ($n = 17$; $P = 0.740$ and $n = 21$; $P = 0.161$, respectively). Similarly, *Schallmoser et al. (2019)* reported no difference in SP sHLA-G concentration between the pregnancy and non-pregnancy groups ($n = 106$; $P = 0.484$), regardless of the males semen parameters. In addition, *Nilsson et al. (2020)* reported no association between SP sHLA-G concentration and time to pregnancy after IVF/ICSI ($n = 80$; $P = 0.484$).

Spermatozoa are considered to be antigenic and present sperm-specific surface antigens on their membrane (*Bohring and Krause, 2003*). By unfortunate events, sperm surface antigens can be recognized as foreign by immune competent cells, resulting in the production of anti-sperm antibodies (ASA). The ASA have been identified in SP and may affect sperm quality, motility and capacitation (*Vickram et al., 2019*). *El-Sherbiny et al. (2021)* evaluated the differences in fertilization rate and successful clinical pregnancy after ICSI between men positive or negative for ASA in their SP. Nineteen per cent of included men proved positive for SP ASA. A non-significant trend of lower fertilization rate in ASA-positive men ($n = 35$) compared with ASA-negative men ($n = 149$) was observed ($P = 0.091$), whereas successful pregnancy rate was almost identical between both groups ($P = 0.98$) (*El-Sherbiny et al., 2021*). Moreover, *Ford et al. (1996)* evaluated the relationship between SP ASA isoform IgA and IgG levels with IVF fertilization rate. It was reported that the percentage of fertilized

oocytes tended to decrease as ASA IgA or IgG increased; however, no concrete data or *P*-values were reported (*Ford et al., 1996*).

Polymorphonuclear neutrophils in SP secrete elastase (*Korkmaz et al., 2010*). In SP, elastase is bound to an inhibitor, forming an elastase-inhibitor complex (s-EI) (*Remold-O'Donnell et al., 1989*). High levels of s-EI are a marker for genital tract inflammation and are more regularly associated with infertile men rather than fertile men (*Wolff and Anderson, 1988*). While increased s-EI levels were significantly associated with reduced blastocyst development and increased embryo arrest, no significant correlations between SP s-EI levels and fertilization and pregnancy rates were observed ($n = 104$; *P*-values not reported) (*Zorn et al., 2004*).

Metals and trace elements

As a result of increasing environmental pollution, exposure to toxic heavy metals, such as cadmium (Cd), mercury (Hg) and lead (Pb) via inhalation, digestion and dermal contact has increased (*Jaishankar et al., 2014*; *Witkowska et al., 2021*). These metals may interfere with endocrine function, spermatogenesis, sperm quality and fertilization capacity (*Ng et al., 1991*; *López-Botella et al., 2021*). Besides heavy metals, trace elements like magnesium (Mg), iron (Fe), and zinc (Zn) are reportedly associated with male reproduction (*Chyra-Jach et al., 2020*). A total of five studies reported 25 heavy metals or trace elements as potential biomarkers for ART outcomes.

Four studies reported on the relationship between SP Pb and IVF outcomes (*Benoff et al., 2003*; *Kim et al., 2014*; *Zhou et al., 2021*; *Rodríguez-Díaz et al., 2022*). *Benoff et al. (2009)* reported a significant negative correlation between SP Pb and IVF fertilization rate ($n = 78$ $P < 0.0001$). In contrast, *Kim et al. (2014)* ($n = 30$), *Rodríguez-Díaz et al. (2022)* ($n = 92$), and *Zhou et al. (2021)* ($n = 195$) evaluated SP Pb with fertilization, clinical pregnancy and live birth rates, and did not establish any significant relationship (*P*-values not reported). Noteworthy, although *Benoff et al. (2009)*, *Kim et al. (2014)* and *Zhou et al. (2021)* analysed fertilization rate as a continuous variable, *Rodríguez-Díaz et al. (2022)* categorized men based on fertilization rate (<75% or ≥75%). The relationships between SP Cd, another toxic metal, and IVF outcomes were also evaluated in four studies (*Benoff et al., 2009*; *Kim et al., 2014*; *Zhou et al., 2021*; *Rodríguez-Díaz et al., 2022*). *Benoff et al. (2009)* reported no significant correlation between Cd and fertilization rate ($n = 96$; $P = 0.455$). Moreover, *Rodríguez-Díaz et al. (2022)* and *Zhou et al. (2021)* reported no relationships with fertilization, clinical pregnancy or live birth rates (*P*-values not reported). Interestingly, *Kim et al. (2014)* suggested a negative association between Cd and clinical pregnancy; however, this association seemed to be non-significant (*P*-value not reported). A third toxic metal, Hg, was assessed by *Kim et al. (2014)* and *Zhou et al (2021)*; both studies reported no significant relationship between SP Hg levels and fertilization, clinical pregnancy or live birth rates (*P*-values not reported) (*Kim et al., 2014*; *Zhou et al., 2021*).

Rodríguez-Díaz et al. (2022) conducted a comprehensive analysis. Besides the previously described Pb and Cd, the following metals and trace elements in SP were evaluated: aluminium (Al), boron (B), barium (Ba), calcium (Ca), cobalt (Co), chrome (Cr), copper (Cu), Fe, potassium (K), lithium (Li), Mg, manganese (Mn), molybdenum (Mo), natrium (Na), nickel (Ni), silica (Si), tin (Sn), strontium (Sr), vanadium (V), Zn. When categorizing fertilization rate, a significantly lower V concentration in the group with high fertilization rate was observed ($P = 0.039$). Furthermore, live birth was associated with significantly elevated levels of Zn, Ca, K, and Mg in SP ($P = 0.004$, $P = 0.013$, $P = 0.002$, $P = 0.009$, respectively) (*Rodríguez-Díaz et al., 2022*). Interestingly, *Zhou et al. (2021)* reported no significant continuous correlation between SP V and Zn levels with fertilization, clinical pregnancy or live birth rates (*P*-values not reported) (*Zhou et al., 2021*).

In addition to the preceding mentioned metals and trace elements, *Zhou et al. (2021)* analysed arsenic (As), Ba, Cr, Cu, Hg, Mn, Mo, Ni, and thallium (Tl). No significant correlations for either of these metals and trace elements with fertilization, clinical pregnancy or live birth rates were established (*P*-values not reported). Statistically significant relationship of these elements was only observed regarding blastocyst formation and quality (*Zhou et al., 2021*).

Nucleic acids

A total of three studies reported on seven nucleic acids in SP as potential biomarkers for ART outcomes. *Bounartzi et al. (2016)* studied the relationship between SP-free sperm DNA (f-spDNA) and IVF/ICSI outcome. Semifinal plasm free sperm DNA may be associated with sperm apoptosis, lysis or active secretion. No significant correlations between SP f-spDNA and fertilization ($n = 55$; $P > 0.05$) or clinical pregnancy rates ($n = 55$; $P > 0.05$) have been established (*Bounartzi et al., 2016*).

It has been demonstrated that SP contains numerous mRNAs, including protamines 1 and 2 (*PRM1*, *PRM2*) mRNA and fertillin- β (*ADAM-2*) mRNA (*Sukhikh et al., 2012*). Although *PRM* transcripts quantity relates to sperm motility and fertilization capacity (*Depa-martynów et al., 2007*), no such relation for *ADAM-2* transcripts has been described. *Sukhikh et al. (2012)* evaluated discrepancies of SP *PRM1*, *PRM2*, and *ADAM-2* mRNA levels of men whose partner achieved clinical pregnancy ($n = 13$) with SP of men whose partner did not achieve pregnancy ($n = 66$). The latter was categorized into three groups: spontaneous miscarriage before week 11 ($n = 10$), only biochemical pregnancy ($n = 19$) and IVF failure ($n = 37$). *PRM1*, *PRM2* and *ADAM-2* levels were not significantly different between clinical pregnancy group and the miscarriage group ($P = 0.73$, $P = 0.92$ and $P = 0.077$, respectively). *ADAM-2* expression was significantly decreased in both the biochemical pregnancy and IVF failure group ($P = 0.002$ and $P = 0.012$, respectively). Additionally, *PRM1* and *PRM2* mRNA expression were significantly decreased in the biochemical pregnancy group ($P = 0.023$ and $P = 0.008$, respectively), but no statistically significant difference with the IVF failure group was established ($P = 0.13$ and $P = 0.15$, respectively) (*Sukhikh et al., 2012*).

Small non-coding RNAs (sRNA) are particularly involved in gene expression regulation, through, for example, RNA modification and interference (*Raina et al., 2018*; *Grosso et al., 2021*). Small non-coding RNAs include transfer RNAs (tRNAs), which are involved in protein transcription. By cleavage of the anticodon loop of a tRNA, tRNA-derived fragments (tRF) are produced (*Odonoghue et al., 2018*). Mice experiments showed the involvement of tRF in post-testicular spermatozoa regulation (*Sharma et al., 2016*). tRNA-derived fragments have been detected in SP, although their function remains unclear. *Grosso et al. (2021)* evaluated the difference of three SP tRFs (5'tRF Glu-CTC, 5'tRF Lys-CTT, and 5'tRF Gly-GCC) between men whose partner

achieved a pregnancy and those who did not. SP 5′tRF Glu-CTC and 5′tRF Lys-CTT levels were significantly higher when pregnancy was not achieved ($n = 56$; $P < 0.05$, $n = 56$; $P < 0.05$, respectively) (*Grosso et al., 2021*).

Significant seminal plasma biomarkers and their outcome

In total, 26 studies reported a significant relationship for 32 SP biomarkers with at least one ART associated outcome (TABLE 2). Twenty-three biomarkers were evaluated once, whereas nine biomarkers were assessed in more than one study. For two out of these nine biomarkers a significant relationship was reported in both studies (IL-18 and TGF-β1/IL-18 ratio), whereas the other seven were not supported by other studies. Both studies by *Nikolaeva et al. (2016; 2019)*, however, reported a significant relationship between IL-18 and TGF-β1/IL-18 ratio with pregnancy rate; the data of these studies are most likely partially overlapping.

DISCUSSION

This is the first comprehensive systematic review of observational cohort- and case-control studies describing potential biomarkers in seminal plasma (SP) of men attending assisted reproductive technology (ART) programmes, aligned with the predictive ability of these biomarkers on ART outcomes. A comprehensive analysis yielded a total of 43 studies reporting on 88 potential SP biomarkers. Here, we described evidence for 32 potential biomarkers in SP, whereas no statistically significant evidence was reported for 56 molecules. Of these 32 biomarkers, 23 were evaluated in a single study (5′tRF Glu-CTC, 5′tRF Lys-CTT, *ADAM-2* mRNA, Ca, CA-19.9, CA-195, Clusterin, Galectin-3, glycodelin-A, IL-11, INHB, K, macrophages/monocytes, Mg, NPC2, *PRM1* mRNA, *PRM2* mRNA, prosaposin, soluble CD14, soluble CD147, uPA, urea, and uric acid), whereas nine biomarkers were evaluated in more than one study (AMH, IL-18, MDA, Pb, PSA, ROS, TGF-

TABLE 2 STATISTICALLY SIGNIFICANT BIOMARKERS IDENTIFIED IN THE SYSTEMATIC REVIEW

Biomarker	Reference	IVF/ICSI	Outcome	*P*-value	Variable
Oxidative stress					
MDA	*Jędrzejczak et al. (2005)*	IVF	FR	<0.05[a]	Continuous and categorical: >10%; >20%; >30%
	Al-Saleh et al. (2021)	IVF	FR	0.288	Continuous
			BP, CP, LB	NS	Continuous
ROS	*Zorn et al. (2003)*	IVF	FR	0.031[a]	Categorical: <25% versus >25%
			CP	0.041[a]	Categorical: P versus NP
		ICSI	FR	NS	Categorical: <25% versus >25%
			CP	0.718	Categorical: P versus NP
	Hammadeh et al. (2008)	IVF	FR	0.187	Continuous
			PR	0.976	Continuous
		ICSI	FR	0.280	Continuous
			PR	0.683	Continuous
		IVF/ICSI	FR	0.045[a]	Continuous
			PR	0.730	Continuous
Proteins					
Clusterin	*Kanannejad and Gharesi-Fard (2019)*	IVF	BP	0.04[a]	Categorical: P versus NP
Galectin-3	*Mei et al. (2019)*	IVF	FR	0.0083[a]	Continuous
NPC2	*Kanannejad and Gharesi-Fard (2019)*	IVF	BP	0.02[a]	Categorical: P versus NP
Prosaposin	*Xu et al. (2019)*	IVF	FR	0.005[a]	Continuous
PSA	*Kanannejad and Gharesi-Fard (2019)*	IVF	BP	0.00 [a]	Categorical: P versus NP
	Koistinen et al. (2002)	IVF	FR	0.42	Continuous
UPA	*Martinez-Soto et al. (2018)*	ICSI	CP	0.01[a]	Categorical: P versus NP
Glycoproteins					
CA-19.9	*Matorras et al. (1995)*	IVF	FR	0.02[a]	Categorical: <66% versus ≥66%
			FR	NS	Continuous
CA-195	*Matorras et al. (1995)*	IVF	FR	0.01[a]	Categorical: <66% versus ≥66%
			FR	NS	Continuous
Glycodelin-A	*Koistinen et al. (2000)*	IVF	FR	0.010[a]	Categorical: <25% versus >25%
			FR	.0.14	Continuous

(*continued on next page*)

TABLE 2 (*Continued*)

Biomarker	Reference	IVF/ICSI	Outcome	*P*-value	Variable
Soluble CD14	*Ovayolu et al. (2016)*	ICSI	FR	0.004[a]	Categorical: P versus NP
			LB	0.037	Categorical: P versus NP
Soluble CD147	*Chen et al. (2021)*	IVF	FR	0.0023[a]	Continuous
			PR	0.045[a]	Categorical: P versus NP
Metabolites					
Urea	*Allahkarami et al. (2017)*	ICSI	FR	0.03[a]	Continuous
Uric acid	*Allahkarami et al. (2017)*	ICSI	FR	0.043[a]	Continuous
Immune system components					
AMH	*Li et al. (2021)*	IVF/R-ICSI	FR	0.000[a]	Categorical: <30% versus >30%
	El-Halawaty et al. (2011)	ICSI	FR	0.08	Continuous
IL-11	*Seshadri et al. (2011)*	IVF	FR	≤ 0.05[a]	Categorical: <35% versus >60%
			FR	NS	Continuous
IL-18[b]	*Nikolaeva et al. (2016*)	IVF/ICSI	CP	0.018[a]	Categorical: P versus NP
	Nikolaeva et al. (2019)	IVF/ICSI	CP	0.02[a]	Categorical: P versus NP
INHB	*Li et al. (2021)*	IVF/R-ICSI	FR	0.000[a]	Categorical: <30% versus >30%
Macrophage/monocyte	*Seshadri et al. (2012)*	IVF	FR	<0.05[a]	Categorical: <35% vs >60%
			FR	NS	Continuous
TGF-β1–IL-18 ratio[b]	*Nikolaeva et al. (2016*)	IVF/ICSI	CP	0.026[a]	Categorical: P versus NP
	Nikolaeva et al. (2019)	IVF/ICSI	CP	0.033[a]	Categorical: P versus NP
Metals and trace elements					
Ca	*Rodríguez-Díaz et al. (2022)*	IVF/ICSI	FR	0.013[a]	Categorical: <75% versus ≥75%
Pb	*Benoff et al. (2003)*	IVF	FR	<0.0001[a]	Continuous
	Kim et al. (2014)	IVF	FR, CP, LB	NS	Continuous
	Zhou et al. (2021)	IVF	FR, CP, LB	NS	Continuous
	Rodríguez-Díaz et al. (2022)	IVF/ICSI	FR, CP	NS	Categorical: <75% versus ≥75%
Mg	*Rodríguez-Díaz et al. (2022)*	IVF/ICSI	FR	0.009[a]	Categorical: <75% versus ≥75%
K	*Rodríguez-Díaz et al. (2022)*	IVF/ICSI	FR	0.002[a]	Categorical: <75% versus ≥75%
V	*Rodríguez-Díaz et al. (2022)*	IVF/ICSI	FR	0.039[a]	Categorical: <75% versus ≥75%
	Zhou et al. (2021)	IVF	FR, CP, LB	NS	Continuous
Zn	*Rodríguez-Díaz et al. (2022)*	IVF/ICSI	FR	0.004[a]	Categorical: <75% versus ≥75%
	Zhou et al. (2021)	IVF	FR, CP, LB	NS	Continuous
Nucleic acids					
5′tRF Glu-CTC	*Grosso et al. (2021)*	ICSI	PR	<0.05[a]	Categorical: P versus NP
5′tRF Lys-CTT	*Grosso et al. (2021)*	ICSI	PR	<0.05[a]	Categorical: P versus NP
ADAM-2 mRNA	*Sukhikh et al. (2012)*	IVF	BP	0.002[a]	Categorical: P versus NP
			IVF failure	0.012[a]	Categorical: P versus NP
PRM1 mRNA	*Sukhikh et al. (2012)*	IVF	BP	0.023[a]	Categorical: P versus NP
PRM2 mRNA	*Sukhikh et al. (2012)*	IVF	BP	0.008[a]	Categorical: P versus NP

[a] Statistically significant.

[b] Biomarkers analysed more than once with consistent results.

ADAM, fertillin-β; AMH, anti-Müllerian hormone; BP, biochemical pregnancy rate; Ca, calcium; CP, clinical pregnancy rate; FR, fertilization rate; ICSI, intracytoplasmic sperm injection; INHB, inhibin B; K, potassium; LB, live birth; MDA, malondialdehyde; Mg, magnesium; NP, not pregnant; NPC2, epididymal secretory protein E1; NP, not pregnant; NS, not significant; P, pregnant; PB, lead; PRM, protamine; PSA, prostate-specific antigen; ROS, reactive oxygen species; PR, undefined pregnancy rate, i.e. it can be biochemical or clinical; UPA, urokinase-type plasminogen activator; V, vanadium; Zn, zinc.

β/IL-18 ratio, V, and Zn). Except for IL-18 and TGF-β/IL-18 ratio, different studies presented conflicting results.

Two studies reported significantly higher SP TGF-β/IL-18 ratio in the group that achieved clinical pregnancy (*Nikolaeva et al., 2016*; *2019*). *Nikolaeva et al. (2019)* suggested the necessity of TGF-β1 levels exceeding IL-18 levels for IVF/ICSI success. It is necessary, however, to draw attention to the possible overlap in the study population for these two studies, resulting in a high probability of duplication of the results instead of confirmation (*Nikolaeva et al., 2016*; *2019*). IL-18 and TGF-β have also been associated with fertility in other studies. IL-18 in uterine luminal secretions has been described to be essential for endometrial angiogenesis and blastocyst implantation (*Lédée-Bataille et al., 2004*). Excess IL-18 levels in the uterine lumen, however, may be detrimental for implantation (*Lédée-Bataille et al., 2004*; *2005*). This could potentially relate to the significantly higher SP IL-18 levels in the non-pregnancy group (*Nikolaeva et al., 2016*; *2019*). Additionally, high IL-18 levels have been associated with urogenital infections and leukocytospermia (*Matalliotakis et al., 2006*); however, men with either diagnoses were excluded from both studies (*Nikolaeva et al., 2016*; *2019*). Furthermore, excess IL-18 levels may interfere with TGF-β1 signalling, which is indispensable for regulating the fetal–maternal immune tolerance (*Yang et al., 2021*). Taken together, the cytokines IL-18 and TGF- β1 may play a role in reproductive outcome and might serve as a potential biomarker for pregnancy success. It is not yet clear, however, whether the SP IL-18/ TGF- β1 levels affect the fertilization capacity or have a direct influence on the female endometrium, as patients were exposed to SP before treatment in the studies by *Nikolaeva et al. (2016*; *2019*). Furthermore, more insight into the mechanism should be gained as well as confirmation of other well-designed studies before these cytokines can be considered as biomarkers.

Several biomarkers have only been reported once, but, in some cases, these findings can be strengthened by findings in animal or in-vitro studies. A significant positive correlation between IVF fertilization rate and galectin-3 bound to SP-derived EVs was found by *Mei et al. (2019)*. Interestingly, a study in a domestic cat model showed that the inhibition of galectin-3 before IVF resulted in poor fertilization capacity (*Rowlison and Comizzoli, 2023*). Another potential biomarker verified in an animal model is urea, where SP urea levels negatively affected the development of bovine oocyte and blastocyst after IVF (*Kowsar et al., 2021*), confirming the negative correlation between SP urea levels and fertilization rates described by *Allahkarami et al. (2017)*. Prosaposin was found to positively correlate with IVF fertilization rates (*Xu et al., 2019*), which has been described in sperm–oocyte binding in chickens (*Amann et al., 1999*) and mice (*Magargee et al., 2000*). Contrary to prosaposin, an inhibitory effect on the sperm–oocyte conjugation by glycodelin-A was reported by *Koistinen et al. (2003)*, substantiating their previous study reporting higher SP glycodelin-A levels in couples with lower fertilization rates (*Koistinen et al., 2000*).

In many studies, complementary results are lacking, which may be explained by substantial heterogeneity among studies concerning study population, sample size, ART procedure, methodology, and, most importantly, analysis method. One of the main analysis discrepancies is the outcome assessment: the different outcomes are analysed as either continuous or categorical variables. For instance, fertilization rates were either analysed based on a cut of value, e.g. more or less than 63% fertilization rate, or as continuous correlation. It is known that categorizing variables increases the chance for statistical significance (*Naggara et al., 2011*), as can be seen in the studies of *Matorras et al. (1995)* and *Seshadri et al. (2011*; *2012*). All three studies reported no significant correlation between the biomarker and IVF fertilization rate as continuous variable; however, significance was established when the fertilization rate was transformed from continuous variable to categorial variable. In addition, statistical significance was established for V, Zn, Ca, K, and Mg based on categorizing fertilization rate (*Rodríguez-Díaz et al., 2022*), whereas, as continuous analysis, no significant difference was established (*Zhou et al., 2021*). This phenomenon is also seen with PSA (*Koistinen et al., 2002*; *Kanannejad and Gharesi-Fard, 2019*), AMH (*El-Halawaty et al., 2011*; *Li et al., 2021*) and reactive oxygen species (*Zorn et al., 2003*; *Hammadeh et al., 2008*). Categorizing is indispensable for dichotomous outcomes, such as pregnancy: either successful or unsuccessful. Categorizing outcomes such as fertilization percentages may lead to misinterpretation of the results as the percentage is presented in a certain context. In contrast, when fertilization rates are analysed as continuous variable, the association between the percentage and potential biomarker can be analysed free of any established structure (*Lazic, 2008*; *Naggara et al., 2011*).

In addition to the analysis method, variability in biomarker measurement was observed. For example, two independent studies evaluated the biomarker MDA using the same ART programme and outcome analysis (*Jędrzejczak et al., 2005*; *Al-Saleh et al., 2021*), yet only *Jędrzejczak et al. (2005)* reported a significant relationship between MDA and fertilization rate. Significance could be biased (seven times smaller in the sample size of the study by *Jędrzejczak et al. [2005]*), and the discrepancy could also be caused by the methods of MDA measurement. *Jędrzejczak et al. (2005)* assessed MDA via colorimetric assay, whereas *Al-Saleh et al. (2021)* measured MDA via HPLC. A study compared MDA measurement using colorimetric assay and HPLC in urinary samples, revealed that colorimetric is more sensitive compared with HPLC, and a better choice as MDA assay (*Yalcin, 2010*). This emphasizes the necessity of uniformly designed studies.

The above-described limitations imply the difficulties of comparing distinct studies and to draw conclusions that also hinder the meta-analysis and subsequently sensitivity and certainty analysis. Regardless of the extensive literature search, a limited number of clinical studies linked factors in SP with ART outcomes. Accordingly, all records, regardless of the quality score, were included. Most of the studies lacked important quality assessment criteria, including inclusion and exclusion criteria and sample size justification. It needs to be mentioned that the assessment includes low- and high-quality scored research, but crucial data are missing. Some studies lacked concrete data such as *P*-values, e.g. *Al-Saleh et al. (2019)* scored nine out of 14 lacking *P*-values and *Ford et al., (1996)* scored eight out of 14 while lacking both concrete fertilization rate values and *P*-values. Interestingly, concrete data are not considered a criterion in the quality assessment.

Currently, conventional semen quality analysis does not provide sufficient

information to predict ART outcome. As SP factors can still execute their function on spermatozoa regardless of the removal of SP during ART procedures (*Martínez et al., 2011*; Rodríguez- *Szczykutowicz et al., 2019*; *Chen et al., 2021*), SP molecular biomarkers could potentially be incorporated in a non-invasive, cost-effective diagnostic tool. Moreover, a reliable SP biomarker can provide insight into factors that are fundamentally essential for fertilization or which factors negatively intervene with fertilization. This can subsequently be used as fertility enhancers to improve IVF/ICSI procedures. Therefore, a reliable SP biomarker is of great interest for the implementation in a clinical setting. For now, however, the translation of SP biomarkers to assisted reproduction clinics remains improbable, and well-defined future studies are required to determine the efficacy and clinical significance of these potential biomarkers. Broad spectrum analysis, such as omics analyses, e.g. genomics, transcriptomics, proteomics and metabolomics), using adequately powered sample sizes and homogenous methodologies should be used to reveal highly accurate biomarkers. As SP contains a high molecule concentration of diverse nature, which can interact with spermatozoa, oocyte, endometrium and more cell types of the female reproductive system, it is of great importance to define correct outcome measures for a reliable SP biomarker to be established. It is difficult to establish a direct correlation with SP biomarkers and complex events like implantation, pregnancy, and live birth that are affected by high variety of factors. For instance, increased levels of the SP components clusterin (*Moulton et al., 1996*; *Tapia et al., 2008*), IL-11 (*George et al., 2020*), and galectin-3 (*Yang et al., 2011*) have been associated with increased endometrial receptivity. This could influence the IVF/ICSI pregnancy and live birth outcome, but not fertilization rates.

The primary outcome of a future study should focus on the continuous correlation between fertilization rate and SP biomarkers; pregnancy, i.e. biochemical and clinical, and live birth can be included as a secondary outcome measurement. A few studies have already been conducted, showing conflicting results; some report beneficial effects of SP exposure (*Chicea et al., 2013*; *Friedler et al., 2013*), whereas another study detected no significant effect (*von Wolff et al., 2013*). This may be explained by patient-specific variation in SP composition and, therefore, should be included as factors that can influence the clinical outcome in future studies.

This review presents the most comprehensive overview of relevant SP biomarkers that may predict or explain ART outcome to date and might be of clinical interest in infertility investigation and assisted reproduction. Although a firm conclusion cannot be drawn and clearly the potential of SP in biomarker research is under-exploited, this review could serve as a starting point to design an all-encompassing study for biomarkers in SP and their predictive ability for ART outcomes and to develop a non-invasive diagnostic tool or new approaches for optimising ART. More insight into the effect of SP molecules on reproductive success would benefit fundamental knowledge on the role of SP and its components in reproduction and could ultimately lead to new fertility enhancing approaches to IVF/ICSI and natural conception.

DATA AVAILABILITY

No data was used for the research described in the article.

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AUTHOR'S ROLES

The study was initiated by GS, SA and BA GS and NMM drafted the study design, including search strategy. JSB and NMM conducted literature search, drafting reports, evaluated eligibility criteria, and quality assessment. JSB extracted data, interpreted results, and drafted the manuscript. GS, NMM, BA, and SA revised the manuscript critically. Final manuscript was approved by all authors.

SUPPLEMENTARY MATERIALS

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