Check for updates

A systematic review identifying fertility biomarkers in semen: a clinical approach through Omics to diagnose male infertility

Marc Llavanera, M.Sc.,[a,b] Ariadna Delgado-Bermúdez, M.Sc.,[a,b] Jordi Ribas-Maynou, Ph.D.,[a,b] Albert Salas-Huetos, Ph.D.,[a,b,c,d] and Marc Yeste, Ph.D.[a,b]

[a] Unit of Cell Biology, Department of Biology, Faculty of Sciences, University of Girona, Girona, Spain; [b] Biotechnology of Animal and Human Reproduction (TechnoSperm), Institute of Food and Agricultural Technology, University of Girona, Girona, Spain; [c] Department of Nutrition, Harvard T.H. Chan School of Public Health, Harvard University, Boston, Massachusetts; and [d] Consorcio CIBER, M.P., Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

Objective: To identify the most robust molecular biomarkers in sperm and seminal plasma for the diagnosis of male infertility, and to evaluate their clinical use.
Design: Systematic review.
Setting: Not applicable.
Patient(s): Accessible studies reporting well-defined (in)fertile populations and semen molecular biomarkers were included in this review.
Intervention(s): A systematic search of the literature published in MEDLINE-PubMed and EMBASE databases was performed, following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.
Main outcome measure(s): The primary outcome was the content, expression, or activity of molecular biomarkers in human semen samples. Only studies reporting a receiver-operating characteristic (ROC) analysis values were included.
Result(s): Eighty-nine studies were included. Direct evaluation of sperm DNA damage has high potential as a diagnostic biomarker of fertility and assisted reproductive technology outcomes (area under the curve [AUCs] median = 0.67). Regarding strand break-associated chromatin modifications, γH2AX levels show good predictive value for the diagnosis of male infertility (AUCs median = 0.93). Some noncoding ribonucleic acid (RNA) exhibit excellent predictive values; miR-34c-5p in semen is the most well-characterized and robust transcriptomic biomarker (AUCs median = 0.78). While many proteins in semen show fair diagnostic value for sperm quality and fertilizing capacity, the levels of some, such as TEX101, in seminal plasma have an excellent diagnostic potential (AUCs median = 0.69). Although individual metabolites and metabolomic profiles in seminal plasma present good predictive value, the latter seem to be better than the former when inferring sperm quality and fertilizing capacity.
Conclusion(s): The current review supports that some Omics (e.g., DNA structure and integrity, genomics and epigenomics, transcriptomics, metabolomics, and proteomics) could be considered relevant molecular biomarkers that may help identify infertility etiologies and fertilization prognosis with cost-effective, simple, and accurate diagnosis. (Fertil Steril® 2022;118:291–313. ©2022 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Sperm, seminal plasma, infertility, molecular biomarkers, Omics

DIALOG: You can discuss this article with its authors and other readers at https://www.fertstertdialog.com/posts/34614

Received January 17, 2022; revised and accepted April 27, 2022; published online June 17, 2022.
M.L. and A.D-B. should be considered similar in author order. A.S-H. and M.Y. should be considered similar in author order.
M.L. has nothing to disclose. A.D-B. has nothing to disclose. J.R.-M. has nothing to disclose. A.S-H. has nothing to disclose. M.Y. has nothing to disclose.
Supported by grants (IJC2019-039615-I to A.S-H.; PRE2018-083488 to A.D-B.; FPU18/00666 to M.L.) from the Ministry of Science and Innovation, Spain, the European Union's Horizon 2020 research and innovation scheme under the Marie Skłodowska-Curie grant agreement No. 801342 to J.R-M. (Tecniospring INDUSTRY; TECSPR-19-1-0003), and the Regional Government of Catalonia (2017-SGR-1229 to M.Y.).
Correspondence: Albert Salas-Huetos, Ph.D., Harvard University T H Chan School of Public Health, Nutrition 677 Huntington Avenue, Boston, MA 02115, USA / Biotechnology of Animal and Human Reproduction (TechnoSperm), 17003, Spain (E-mail: albert.salas@udg.edu).

Fertility and Sterility® Vol. 118, No. 2, August 2022 0015-0282/$36.00
Copyright ©2022 American Society for Reproductive Medicine, Published by Elsevier Inc.
https://doi.org/10.1016/j.fertnstert.2022.04.028

In humans, infertility affects 8%–12% of couples worldwide. Because a male factor is involved in 50% of cases (1), prediction of male fertility is very important (1, 2). Despite the growing interest in reproductive health, there remains a lack of biomarkers able to predict male fertility with high accuracy and sensitivity. Traditionally, the prognosis of male fertility has been achieved through conventional semen analysis, which provides general information on quantitative parameters, such as ejaculate volume; sperm morphology, count, and concentration; and motility. Although the seminogram is a relatively simple, fast, informative, and economical assessment, it does not provide information about sperm physiology, because it leaves aside essential molecular aspects, such as DNA integrity, sperm oxidative status, and the presence of sperm-oocyte binding proteins, among others (3, 4). The application of conventional semen analysis for the prognosis and diagnosis of male fertility has been under debate for many years (3, 5). In this regard, exploring new and robust molecular biomarkers in sperm providing additional information on their functional status is of great interest for assisted human reproduction.

Omics are high-throughput measurements of specific molecular groups, such as proteins, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or metabolites among others, which are studied increasingly in the andrology field. Omics technologies and their derivatives are in constant development, allowing for the characterization of proteins, genes, metabolites, and epigenetic traits associated with male infertility (6). Along these lines, the advent of Omics has uncovered relevant molecular biomarkers that may help identify infertility etiologies and fertilization prognosis with cost-effective, simple, and accurate diagnosis. Therefore, the aim of this systematic review is to identify the most robust molecular biomarkers in sperm and seminal plasma for the diagnosis of male infertility, and to evaluate their potential clinical use. A comprehensive review of high-quality studies published to date investigating reliable molecular biomarkers in semen may assist physicians in the diagnosis of the conditions causing sperm quality impairments and male fertility disorders. To the best of our knowledge, this is the first well-designed systematic review of observational studies, on the basis of the receiver-operating characteristic (ROC) analysis outcomes, that critically appraises the quality of the current body of literature on relevant molecular biomarkers in human semen.

MATERIAL AND METHODS

Systematic Review Registration

The protocol of the present systematic review has been registered in the international database for the prospective registration of systematic reviews (PROSPERO 2020: CRD42020176417).

Data Sources and Search Strategy

A systematic search of the literature published in MEDLINE-PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and EMBASE databases (https://www.embase.com/#search) was performed, in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (7, 8). The strategy performed for literature search combined keywords and medical subject heading (MeSH) terms. The search was focused on "semen", "male (in)fertility," and "biomarkers" related words. The full search strategy and applied filters are available in Supplemental File 1 (available online).

Study Selection, Eligibility, and Data Extraction

Titles and abstracts were screened by 2 expert investigators in the field of male fertility (A.D.-B. and M.L.), and discrepancies were reevaluated together with 2 additional investigators (A.S.-H. and M.Y.). Moreover, a Patient, Intervention, Comparator, Outcome, Study (PICOS) design structure was set to develop the study questions and the inclusion/exclusion criteria (Supplemental Table 1, available online). The accessible studies reporting well-defined (in)fertile populations and semen molecular biomarkers were included in this review. The primary outcome of the present article was the content, expression, or activity of molecular biomarkers in human semen samples; only studies reporting a ROC analysis were considered. We excluded animal studies, review articles, editorial/opinions, case reports, and studies measuring (in)fertility biomarkers in blood or samples other than semen. After primary screening (assessing the scope of study) and evaluating the quality in accordance with inclusion/exclusion criteria, the full text of selected articles was obtained. The following information was extracted from each selected study: author/s, year of publication, study design, infertility status, sample size, sample type, measured biomarkers, measurement method, area under the curve (AUC), sensitivity, specificity, *P* value, and main conclusion.

Quality Assessment

The quality of selected observational studies was evaluated and scored using the quality assessment tool of the National Heart, Lung, and Blood Institute-National Institutes of Health for case-control studies to assess the quality of each study (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools). The quality scores were assessed in parallel by 2 investigators (A.D.-B. and M.L.), and discrepancies were reevaluated altogether to reach a consensus. Studies with a score between 0 and 5 points were considered of low quality (excluded) and those with a score >5 were considered of moderate to high quality (included for subsequent analysis).

RESULTS

Identification and Selection of the Articles

The primary search by MEDLINE-PubMed and EMBASE identified a total of 37,854 articles. We screened the titles and abstracts, and excluded a total of 36,979 studies for duplication or not meeting the scope of the study; 875 articles were selected for full text evaluation of the inclusion/exclusion criteria and quality assessment. Of these articles, 786 were excluded because they did not meet the inclusion/exclusion criteria or because of deficient quality scoring. After applying eligibility parameters, 89 studies were included for qualitative synthesis. The 89 selected articles were case-control studies

reporting ROC analysis of fertility molecular biomarkers in sperm and seminal plasma. These studies were classified in 5 groups: DNA structure and integrity ($n = 41$), genomics and epigenomics ($n = 6$), transcriptomics ($n = 8$), proteomics ($n = 17$), and metabolomics ($n = 20$). Three reports belonged to more than one group. The PRISMA Flow Diagram is shown in Figure 1.

Primary Outcomes of Interest

DNA structure and integrity. A total of 41 studies reporting 9 different DNA structure and integrity biomarkers to predict fertilizing capacity were selected (Table 1 and Supplemental Table 2, available online). Diagnostic potential of all biomarkers was evaluated through ROC curve analysis. Structure and integrity were evaluated exclusively in sperm DNA but not in cell-free DNA.

The terminal deoxytransferase-mediated deoxyuridine triphosphate (dUTP) nick end labeling assay (TUNEL) uses a terminal Terminal deoxynucleotidyl transferase to label the 3' free DNA ends. Therefore, sperm with fragmented DNA become highly labeled (9). The TUNEL assay measures DNA damage directly without an initial denaturation step and is coupled with flow cytometry, which makes this test reliable and reproducible (10). The TUNEL assay was analyzed as a potential fertility biomarker in 14 different works. The potential of the TUNEL assay as a discriminator of infertile patients from men with proven fertility has been demonstrated extensively with AUCs of 0.930 (11), 0.903 (12), 0.757 (13), and 0.608 (14). In addition, Sharma et al. (15,16) and Kabartan et al. (17) explored its potential as a diagnostic biomarker to identify idiopathic infertility owing to male factor (with AUCs of 0.820, 0.556, and 0.892, respectively. Hichri et al. (18) confirmed its potential to differentiate between samples from patients that conceived after an intrauterine insemination (IUI) and samples of patients with oligoasthenoteratozoospermia that ended up being enrolled in in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles. The results of the TUNEL test, therefore, would be a good prognostic biomarker to choose the best assisted reproductive technology (ART) strategy (AUC = 0.790). When considering the potential of the TUNEL test as a prognostic biomarker of pregnancy, results were found to be variable. On one hand, Vončina et al. (19) proved its good potential as a prognostic biomarker of natural conception in normospermic patients who had not been able to conceive after a year of unprotected intercourse (AUC = 0.700), and Avendaño et al. (20) determined that it should be useful as a moderate prognostic biomarker of clinical pregnancy in ICSI cycles (AUC = 0.700). On the other hand, Jin et al. (21) reported poor predictive power of TUNEL as a prognostic biomarker of successful clinical pregnancy in couples with a reduced ovarian reserve (AUC = 0.594). Moreover, the TUNEL test showed no predictive power as a predictor of clinical pregnancy in IUI cycles (AUC = 0.675) (22). In addition, Jin et al. (21), Thomson et al. (22), Benchaib et al. (23), and Esbert et al. (24) concluded this test does not have a significant power as a prognostic biomarker of clinical pregnancy in ICSI cycles (AUCs of 0.510, 0.530, 0.500, and 0.552, respectively). Similarly, Esbert et al. (24) explored the potential of the TUNEL test as a prognostic biomarker of successful clinical pregnancy in IVF/ICSI cycles with couples' or donor oocytes, and concluded that this assay does not have a significant power for this purpose (AUCs of 0.559 and 0.528, respectively). Finally, Esbert et al. (24) found that TUNEL has no significant power to predict successful delivery following IVF/ICSI cycles, regardless of whether all cases are considered together (AUC = 0.666) or cycles with own and donor oocytes are envisaged separately (AUCs of 0.652 and 0.670, respectively).

The molecular marker H2AX is a histone variant that has different posttranslational modifications. Phosphorylation of Ser-139 from H2AX, a variant known as histone γ-H2AX, is involved in the cell response to the induction of DNA double-strand breaks (DSBs) during spermatogenesis, which occurs to allow recombination and chromatin remodelling (25). This posttranslational modification spreads several bases on the DNA strand from the break, acting as an epigenetic mark to start DNA repair (26). Some DSBs, nevertheless, might remain unrepaired in mature sperm until the male pronucleus meets the DNA repair molecules provided by the oocyte after fertilization. Therefore, levels of γ-H2AX in mature sperm are proportional to the number of DSBs. Remarkably, Zhong et al. (27) confirmed the excellent potential of γ-H2AX levels as a biomarker capable to differentiate between fertile and infertile men (AUC = 0.930).

Oxidative DNA damage was investigated previously in 7 studies. The Comet assay can be performed under alkaline or neutral conditions, which allows for the differentiation between single and double-strand DNA breaks (ssSDF and dsSDF, respectively) induced by oxidative damage (28). The alkaline Comet assay was used in 4 studies to evaluate oxidative DNA damage in terms of ssSDF. Its power as a diagnostic biomarker of idiopathic male infertility was proven to be excellent by Simon et al. (29), Ribas-Maynou et al. (12) and Fernandez-Encinas et al. (30) with AUCs of 0.970, 0.937, and 0.994, respectively. It also was confirmed as a predictor of clinical pregnancy in IVF cycles with AUCs of 0.648 and 0.905 (29,31), but not in ICSI cycles (AUC = 0.601) (31). The predictive power of dsSDF evaluated through the neutral Comet assay also was assessed in 2 different studies; neither Fernandez-Encinas et al. (30) nor Ribas-Maynou et al. (12) were able to confirm its power as a good fertility biomarker, with AUCs of 0.373 and 0.516, respectively. Finally, oxidative DNA damage also can be determined through the measurement of 7,8-dihydro-8-oxo-2′-deoxoguanosine (8-OHdG), which is a modified DNA base that is prone to become a DSB and, thus, is a marker of latent DNA damage (32,33). Its potential as a fertility biomarker was investigated in 2 different studies, and the potential of 8-OHdG as a prognostic biomarker of clinical pregnancy was good in IUI, but failed in ICSI cycles (22) (AUCs of 0.794 and 0.496, respectively). Considering that this biomarker is indicative of latent DNA damage, Simon et al. (31) evaluated whether the treatment of sperm samples with formamidopyrimidine DNA glycosylase, which converts 8-OHdG into strand breaks before the alkaline Comet assay, improved the power of this technique. Indeed, this before treatment of samples increased the power of the alkaline Comet assay as a prognostic biomarker of clinical pregnancy in IVF cycles (AUC = 0.776) (31).

Another interesting parameter is sperm toroid integrity, whose evaluation allows determining the compaction of sperm DNA in toroids. It can detect latent DNA damage and abnormal or unstable chromatin structures that might not be detected through other tests only sensible to DNA strand breaks (34). It was evaluated as a fertility biomarker by Chan et al. (34), who confirmed it as a good predictor of miscarriage (AUC = 0.710), but not of pregnancy, after an ICSI treatment.

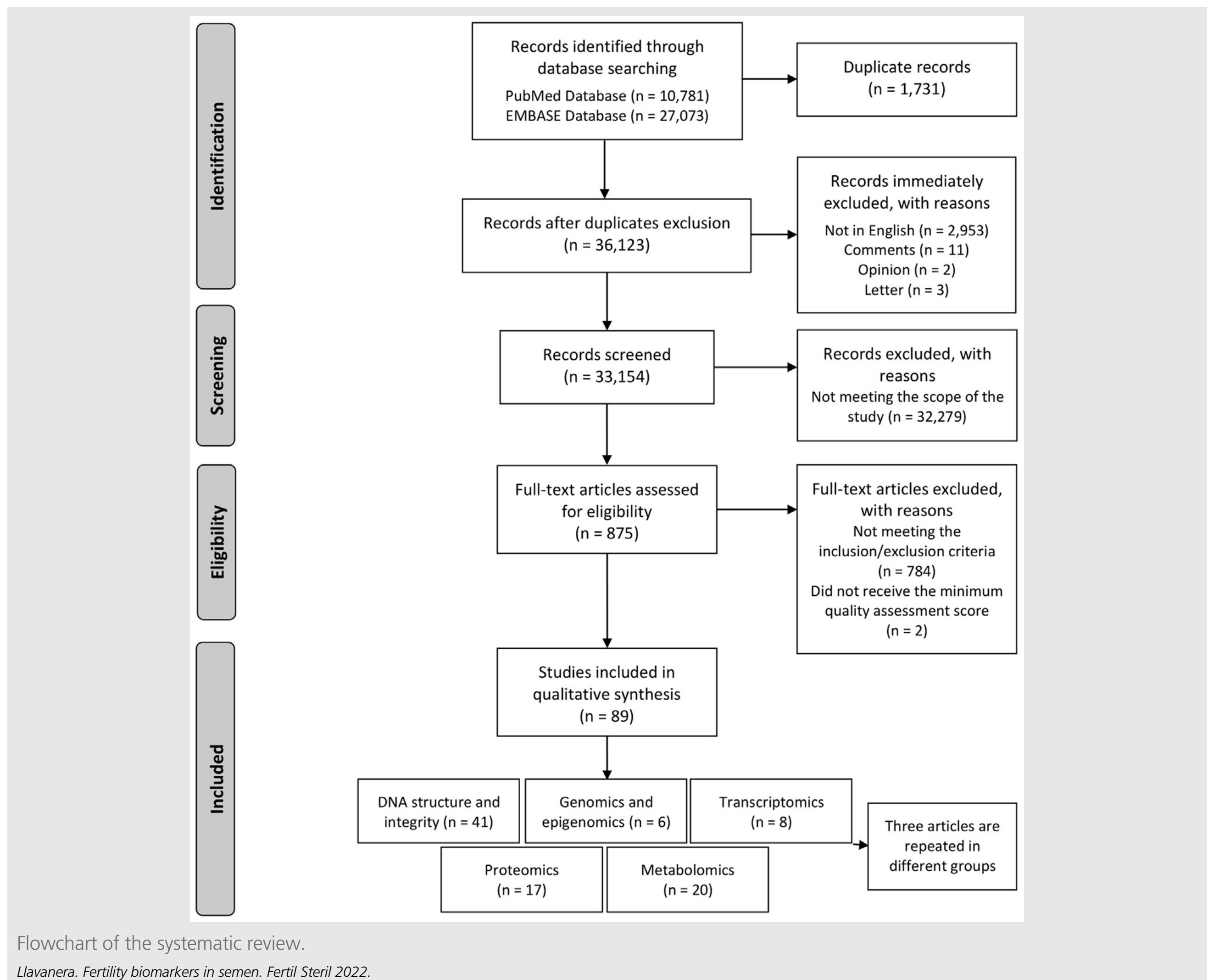
Other methods base the detection of SDF on the denaturing capacity of sperm chromatin. The sperm chromatin structure assay (SCSA) is based on the use of acridine orange (AO) to stain dsDNA and ssDNA in different colors (35), whereas sperm with intact DNA present a halo that is absent from those with fragmented DNA in the sperm chromatin dispersion (SCD) test (36). Jiang et al. (37) showed that the percentage of AO-stained sperm evaluated through fluorescence microscopy was a good predictor of clinical pregnancy in ICSI cycles (AUC = 0.750). Six studies analyzed AO staining through flow cytometry. Chromatin decondensation evaluated through SCSA was proven to be an excellent diagnostic biomarker of infertility by Venkatesh et al. (38), and its potential was confirmed to be good by Ribas-Maynou et al. (12), with AUCs of 0.919 and 0.792, respectively. In addition, this assay was found to provide a robust diagnostic and prognostic biomarker in idiopathic recurrent pregnancy loss (RPL) following spontaneous conception (39, 40) or IVF treatment (41), with AUCs of 0.830, 0.752 and 0.713, respectively. Its power as a predictor of spontaneous clinical pregnancy in patients with varicocele also was good with an AUC of 0.762 (42).

The potential of the SCD test as a fertility biomarker was evaluated through Halosperm G2 in 12 different studies. The SCD test exhibited a good potential to distinguish between samples presenting normal sperm characteristics according to World Health Organization criteria and samples not meeting these quality standards with an AUC = 0.753 (43). Ribas-Maynou et al. (12) confirmed SCD also was an excellent diagnostic biomarker of fertility with an AUC of 0.869. In addition, Esteves et al. (44) evaluated the diagnostic potential of SCD in infertile men with varicocele, which was confirmed to be excellent (AUC = 0.942). The SCD test also proved its ability to predict fertilization rates $\geq$50% in conventional IVF cycles (45) and in ICSI cycles (46) (AUCs of 0.664 and 0.680, respectively). Breznik et al. (45) and Tandara et al. (47) found that the percentage of sperm presenting chromatin dispersion had a good predictive value for embryo quality after conventional IVF cycles (AUCs of 0.771 and 0.710, respectively); Tandara et al. (47) also reported the percentage of sperm with undamaged DNA as an excellent predictor of embryo quality (AUC = 0.830). Finally, 7 studies evaluated the potential of SCD as a predictor of pregnancy after conventional IVF and ICSI cycles, but the conclusions of these studies were not consistent. The percentage of sperm with chromatin dispersion was a good prognostic biomarker for clinical pregnancy after IVF cycles by Tandara et al. (47), Bounartzi et al. (48), and Comhaire et al. (49) (with AUCs of 0.670, 0.700 and 0.830, respectively), similarly to the percentage of sperm with undamaged DNA (47) (AUC = 0.750). Yet, Sun et al. (50) did not confirm this parameter as a good predictor for the achievement of clinical pregnancy after IVF (AUC = 0.543). When considering ICSI cycles alone, Gosálvez et al. (51) reported SCD as a good prognostic biomarker of clinical pregnancy when using donor oocytes (AUC = 0.711), but again the results reported by Sun et al. (50) were in disagreement (AUC = 0.477). When IVF and ICSI cycles were considered together, López et al. (52) found SCD was a poor predictor of clinical pregnancy with an AUC = 0.546, and the results reported by Muriel et al. (53) evidenced it did not have a significant power to predict clinical pregnancy (AUC = 0.597).

Chromatin maturity can be assessed through the evaluation of the successful replacement of histones by protamines. Whereas CMA3 binds to the DNA's minor groove similarly to protamines and allows for the detection of deficient protamination (54), aniline blue (AB) stains persistent histones in the sperm nucleus (55). Chromatin protamination assessed through the CMA3 assay was evaluated as a fertility biomarker in 4 different works. Esterhuizen et al. (56, 57) determined the good potential of CMA3 to predict a fertilization rate >50% or >60% in IVF cycles (AUCs of 0.740 and 0.760, respectively), and Tarozzi et al. (58) evaluated the good potential of CMA3 as a predictor of successful fertilization after IVF (AUC = 0.769). Finally, Marchiani et al. (59) evaluated the potential of CMA3 as a predictor of embryo quality, and demonstrated its good potential as a biomarker (AUC = 0.778). Histone persistence measured through AB staining as a fertility biomarker was evaluated in 2 different studies. Marchiani et al. (59) determined the fair prognostic value of AB as a predictor of good fertilization rate in males of infertile couples with an AUC of 0.611. In addition, AB staining proved its fair potential as a prognostic biomarker of successful clinical pregnancy in couples with male factor infertility after IUI cycles with an AUC of 0.653 (60).

In summary, whereas the TUNEL test and the levels of histone variant γ-H2AX were confirmed to identify infertile patients, the oxidative damage measured through the alkaline Comet assay showed slightly higher sensitivity and specificity. The TUNEL assay showed a highly variable potential to identify the etiology of infertility and also was observed to predict the success of IUI. While the TUNEL assay also was useful to predict clinical pregnancy in natural conceptions, almost all studies concurred that this test did not have a good prognostic power in IVF and ICSI treatments. On the contrary, the alkaline Comet assay exhibited a variable prognostic value to predict clinical pregnancy after IVF. In addition, the measurement of 8-OHdG, when used alone, had a good diagnostic power for infertility but, when combined with the alkaline Comet assay, it also exhibited a good prognostic power of clinical pregnancy after IVF and ICSI cycles. The SCSA and SCD tests are excellent diagnostic biomarkers for infertility. In patients with varicocele, both might be excellent as a noninvasive diagnostic tool and a good prognostic biomarker of spontaneous pregnancy, respectively. These 2 techniques, therefore, could be used to assess sperm quality in fertility clinics and establish a proper treatment to solve this potential cause of infertility, thus helping decide which ART strategy is better for each patient. The SCD test showed a fair power as a predictor of fertilization rates following IVF and ICSI, and a good to excellent power as a

FIGURE 1



Flowchart of the systematic review.

Llavanera. Fertility biomarkers in semen. Fertil Steril 2022.

biomarker of embryo quality. As there is evidence for and against the prognostic value of SCD as a clinical pregnancy biomarker in IVF and ICSI cycles, it could be important for each laboratory to establish their own cutoff values; therefore, the standardization of this technique would be more difficult than others. Regarding SCSA, there is evidence supporting its potential as a good pregnancy biomarker in ICSI cycles. Finally, CMA3 and AB have proven their power as prognostic biomarkers of the outcome of different ARTs, both being accurate to predict successful fertilization after IVF and ICSI. In addition, CMA3 has good potential as an embryo quality biomarker after IVF, and AB is a fair prognostic biomarker of clinical pregnancy after IUI. In the light of the aforementioned, it is evident that reproductive clinics would benefit from the use of these techniques during sperm quality assessment, thus contributing to the election of the best ART strategy.

Gene expression and epigenomics. Regulation of gene expression can be evaluated through the detection of epigenetic marks or through the measurement of the products of gene expression. Nevertheless, few studies have evaluated this type of biomarkers in semen samples. A total of 6 studies reporting 9 different gene expression and epigenomics biomarkers for predicting fertilizing ability were selected (Table 1 and Supplemental Table 2). ROC curve analysis was used to assess the diagnostic potential of all these biomarkers. Gene expression and epigenomics features were evaluated in sperm DNA and cell-free DNA as potential biomarkers.

In terms of sperm DNA, Bonache et al. (61) defined an expression signature that was an excellent biomarker of clinical pregnancy rate after IUI (AUC = 0.910), which included the evaluation of the expression of *EIF5A*, *RPL13*, *RPL23A*, and *RPS27A*. This panel includes genes that encode a translation factor and 3 ribosomal proteins and, thus, they are essential for the basic cell function (62, 63). The other 3 studies evaluating sperm DNA identified different methylation patterns as fertility biomarkers. On one hand, global methylation patterns showed fair power to differentiate between fertile and infertile men, as well as

to predict embryo quality after IVF (64) (AUCs of 0.670 and 0.640, respectively). Furthermore, the top 500 most differentially methylated CpGs identified in the aforementioned study showed an excellent power as a diagnostic biomarker of male infertility (64) (AUC = 0.930). These results evidence that the analysis of differential methylation in site-specific CpG has higher diagnostic power as a biomarker of infertility than the global methylation pattern. Finally, whereas sperm methylation status of the *MLH1* promoter was proposed as a fair candidate biomarker for oligozoospermia, this was not the case for *MSH2* (65) (AUCs of 0.610 and 0.600, respectively). In fact, *MLH1* and *MSH2* are involved in DNA mismatch repair system, but only *MLH1* is related to a decrease in sperm production in mice, which is in agreement with the relationship with these results (66, 67). Finally, the methylation status of the *MTHFR* promoter was confirmed as a good diagnostic biomarker of infertility (68) (AUC = 0.730). This gene encodes the enzyme methylenetetrahydrofolate reductase, which is essential in folate metabolism and is crucial for methionine synthesis, which, in turn, is the donor of the methyl group in DNA methylation (69).

With regard to cell-free DNA, 2 different studies evaluated the potential of cell-free DNA in semen as a fertility biomarker. Whereas cell-free DNA concentration in semen did not have a significant predictive value as a clinical pregnancy success biomarker in IVF cycles (48) (AUC = 0.600), the methylation pattern of the *CCNA1* promoter from cell-free DNA showed a fair power to predict successful sperm retrieval in nonobstructive azoospermia (NOA) patients (70) (AUC = 0.670). This gene encodes cyclin A1, which is essential for the passage of spermatocytes into meiosis I and, in fact, null mice for this gene are healthy but infertile owing to cell cycle arrest in spermatogenesis (71). This last finding has a potential application in IVF clinics, because this determination could provide a noninvasive establishment of NOA etiology.

The elevated cost of these techniques and the difficult translation into the clinical environment might be a reason for the lack of exhaustive studies regarding the potential of these biomarkers in semen samples.

Transcriptomics. Noncoding RNAs are known to be essential regulatory elements in biological systems, and reproductive biology processes do not escape from their regulation (72). Specifically, microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs) are short and single-stranded noncoding RNAs that act as regulatory elements. miRNA regulate gene expression at the transcriptional level in many cell types, whereas piRNA represses transposons at the transcriptional or posttranscriptional level in the germline and gonadal somatic cells (73, 74). In this regard, noncoding RNAs present in semen could evidence dysregulations in spermatogenesis leading to semen quality and fertility disorders. Some studies, indeed, suggested the potential of noncoding RNAs as biomarkers for reproductive diseases or for reproduction success (75). Herein, we compiled robust miRNAs and piRNAs biomarkers present in semen samples showing high diagnostic value for sperm quality and fertility disorders. A total of 8 studies reporting 31 high-quality noncoding RNA biomarkers (22 miRNAs and 9 piRNAs) to predict semen quality and/or fertility were selected (Table 1 and Supplemental Table 2). All RNAs were validated by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). To date, miRNAs and piRNAs are the only types of noncoding RNAs showing robust diagnostic value in semen samples. Wheras miRNAs exhibited high-quality prediction results in sperm and seminal plasma, piRNAs were reported to perform exclusively as good quality biomarkers in seminal plasma but not in sperm.

Few studies in the literature reported robust transcriptomic biomarkers for male idiopathic infertility. A panel of 5 miRNA (hsa-miR-34b-5p, 34b-3p, 34c-5p, 122-5p, and 429) (76) and 5 independent sperm miRNAs (hsa-miR-122-5p, 34b-3p, 34b-5p, 34c-5p, and 429) (76,77) were strong predictors of semen quality and ICSI pregnancy rates. Specifically, a study by Cui et al. (77) tested sperm hsa-miR-34c-5p and hsa-miR-34b-3p in 162 patients with idiopathic male infertility undergoing ICSI cycles, and established hsa-miR-34c-5p, but not hsa-miR-34b-3p, as an indicator of embryo quality, implantation, clinical pregnancy, and live birth following ICSI. Thus, sperm hsa-miR-34c-5p is the only noncoding RNA in semen showing a robust predictive value for male idiopathic infertility undergoing ICSI cycles, being associated with embryo quality, implantation, clinical pregnancy, and live birth in ICSI treatment outcomes. On the other hand, Abu-Halima et al. (76) validated a set of 5 microRNAs (hsa-miR-34b-5p, 34b-3p, 34c-5p, 122-5p, and 429) using qRT-PCR in a cohort of 226 men attending an infertility clinic and reported these miRNAs to show good diagnostic value for oligozoospermia. The potential application of these noncoding RNAs as noninvasive semen biomarkers could minimize or avoid surgical sampling for the diagnosis of this type of sperm disorders.

On the other hand, a greater number of miRNA predictors of semen quality have been described in seminal plasma. A total of 10 seminal plasma miRNAs (hsa-miR-122-5p, 146-5p, 181a-5p, 205-5p, 210-3p, 31-5p, 34c-5p, 374b-5p, 509-5p, and 513a-5p) and a panel of 3 miRNA (hsa-miR-141, 429, and 7-1-3p) were reported to have good to excellent diagnostic potential for sperm quality disorders (60–62). Good diagnostic power was reported for azoospermia (hsa-miR-122-5p, 146-5p, 181a-5p, 34c-5p, 374b-5p, 509-5p, and 513a-5p) (78), nonobstructive azoospermia (panel of 3-miRNA [hsa-miR-141, 429, and 7-1-3p]) (79), asthenozoospermia (hsa-miR-122-5p, 146-5p, 181a-5p, 34c-5p, 374b-5p, 509-5p, and 513a-5p) (78), and varicocele-induced dyszoospermia (hsa-miR-210-3p) (80). Furthermore, seminal plasma miRNAs showed predictive value for discriminating among different types of sperm quality conditions, such as asthenozoospermia and azoospermia (hsa-miR-122-5p, 146-5p, 181a-5p, 34c-5p, 374b-5p, 509-5p, and 513a-5p) (78), as well as nonobstructive and obstructive azoospermia (hsa-miR-31-5p, hsa-miR-205-5p) (81). The same study, however, reported 4 miRNAs (hsa-miR-182-3p, 449a, 539-5p, 941) not to be sufficient to discriminate between nonobstructive and obstructive azoospermia.

Other research tested the diagnostic potential of sperm and seminal plasma piRNAs. Seminal plasma piRNAs showed

good diagnostic potential for azoospermia and asthenozoospermia. Hong et al. (82) performed high-throughput sequencing technology in a cohort of 302 men and identified a panel of 61 piRNAs differentially expressed between normozoospemic donors and infertile patients. After qRT-PCR validation, 5 individual piRNAs (piR-30198, 31068, 31925, 43771, and 43773) were significantly downregulated in seminal plasma of azoospermia and asthenozoospermia patients, when compared with normozoospermic men. The same investigators also tested the diagnostic potential of these piRNAs combined in a panel of 5 piRNAs (piR-31068, 31925, 43771, 43773, and 30198) and another of 4 piRNAs (piR-31068, 31925, 43771, and 43773). They found increased diagnostic value for azoospermia in the panel of 5 piRNAs. Furthermore, these investigators reported piR-30198 to be able to discriminate between asthenozoospermia and azoospermia patients, whereas Barceló et al. (81) showed piR-58527 as a useful biomarker to differentiate nonobstructive from obstructive azoospermia. On the contrary, although piRNAs from sperm correlated with sperm concentration and fertilization rates after ICSI, they did not show a good diagnostic value either for sperm quality or male fertility (83).

Along these lines, several noncoding RNA (miRNAs and piRNAs) in semen have shown excellent predictive values, showing good to excellent diagnostic value for sperm quality disorders, such as azoospermia, oligozoospermia, asthenozoospermia, oligoasthenozoospermia, and varicocele-induced dyszoospermia. The potential application of these semen biomarkers in fertility clinics could lead to faster and reliable diagnostics for male reproductive disorders, as they show high sensitivity and specificity (AUCs ranging from 0.730 to 0.990). However, hsa-miR-34c-5p in sperm and seminal plasma is the most well-characterized and robust transcriptomic biomarker for the diagnosis of sperm quality disorders and male factor infertility. In this regard, the relatively cheap and simple quantification methods for noncoding RNAs make these molecular biomarkers suitable candidates for their implementation in fertility clinics and should be tested before assisted reproduction procedures to predict their success. Further validation and implantation strategies, however, should be envisaged before their clinical use in fertility clinics.

Proteomics. The role of semen proteins as a diagnostic tool for male reproduction disorders also was reviewed in the present study. Contrary to other molecular biomarkers, proteins are the most time-saving, inexpensive, and simpler diagnostic tools since they can be measured using simple, and quick techniques, such as rapid colloidal gold immunochromatography, enzyme-linked immunosorbent assay or spectrophotometry. In the present study, 17 studies reporting a total of 32 semen protein biomarkers for different parameters predicting sperm quality, functionality, and/or fertilizing capacity were selected (Table 1 and Supplemental Table 2). Protein concentration was measured using highly sensitive and specific techniques, such as enzyme-linked immunosorbent assay, LC-MS/MS, Western blot, and flow cytometry, whereas their activity was quantified through spectrophotometry.

The concentration or activity of sperm or seminal plasma candidate biomarkers were characterized to explore their putative diagnostic value. Seminal plasma levels of testis-expressed protein 101 (TEX101) (84) (AUC = 0.990) and enzymatic activity of N-acetyl-b-D-hexosaminidase (85) (AUC = 0.800) were identified as robust biomarkers for the diagnosis of azoospermia. In addition, the concentration of NT-proCNP (86) (AUC = 0.733) and PELP1 (87) (AUC = 0.781) in seminal plasma was established as a putative diagnostic tool for asthenozoospermia and oligozoospermia, respectively. Furthermore, Intasqui et al. (88) conducted a proteomic analysis using a cohort of 156 normozoospermic men and revealed some seminal plasma proteins that were able to identify ejaculated sperm showing low mitochondrial activity (proteomic profile of Annexin A7 and CD63; AUC = 0.993), altered acrosome integrity (proteomic profile of PLTP and COL12A1; AUC = 0.972), and high DNA fragmentation (CRISPLD1; AUC = 0.882). In this regard, the previously mentioned protein biomarker candidates showed high diagnostic value for sperm disorders, such as azoospermia, asthenozoospermia, and oligozoospermia, as well as for sperm physiology alterations, such as mitochondrial activity, acrosome stability and DNA integrity, thus being promising candidate biomarkers for clinical diagnosis of male reproduction disorders.

Other sperm and seminal plasma proteins have been demonstrated to predict ejaculate fertility. Concentration of sperm BAG6 and HIST1H2BA (89), the nuclease activity corrected by sperm count (30) and PON-1 activity in seminal plasma (90) were able to discriminate between fertile and subfertile/infertile men showing abnormal semen analysis, with AUCs of 0.921, 0.935, 0.705, and 0.950, respectively. Similarly, PON-1 activity in seminal plasma also showed an excellent predictive value for subfertility diagnosis (90) (AUC = 0.950). Marsillach et al. (91), however, explored the subfertility diagnostic power of PON-1 concentration and activity in a cohort of 93 men attending an infertility clinics, but found no significant diagnostic value. Moreover, Korbakis et al. (84) explored the potential of TEX101 as a diagnostic tool for idiopathic male infertility, although they did not show a significant predictive value. Along these lines, although lacking conclusive results, PON-1, BAG6, and HIST1H2BA are putative protein biomarker candidates to predict male (in)fertility, although further studies with larger cohorts would be warranted.

Similarly, some proteins in semen did show robust results as predictors of the success of ART. Martinez-Soto et al. (92) reported that total but not active uPA in seminal plasma was able to predict ART outcomes with an AUC of 0.720. Similarly, Bøllehuus Hansen et al. (93) disclosed the percentage of CYP24A1-positive sperm as a good biomarker of the IUI-clinical pregnancy success (AUC = 0.710). Moreover, Ovayolu et al. (94) identified the levels of Presepsin (a soluble CD14 subtype) in seminal plasma as a significant biomarker for live birth and chemical pregnancy after ICSI, although it showed limited robustness (AUCs of 0.634 and 0.677, respectively). Finally, CATSPER1 expression in sperm measured by fluorescence intensity using flow cytometry showed predictive value for ART success, being able to discriminate between poor quality and good quality embryos (59).

TABLE 1

Summary table of the statistically significant biomarkers identified in the systematic review.

Biomarker	Type	Case	Control	Methods	AUC	Reference
A. DNA structure and integrity Sperm						
SDF - 3' free ends	SDF	Infertile ($n = 348$)	Fertile (normo) ($n = 86$)	TUNEL	0.757	(13)
		Infertile (no clinical pregnancy after 1 y) ($n = 427$)	Fertile (proven fertility) ($n = 40$)		0.608	(14)
		Infertile ($n = 72$)	Fertile (proven fertility) ($n = 21$)		0.903	(12)
		Infertile (no pregnancy after 1 y) ($n = 66$)	Fertile (proven fertility) ($n = 47$)		0.930	(11)
		Infertile, seeking for treatment ($n = 194$)	Fertile (normo) ($n = 25$)		0.820	(15)
		Infertile, seeking for treatment ($n = 261$)	Fertile (normo) ($n = 95$)		0.556	(16)
		Infertile (no pregnancy after 1 y), no clinical pregnancy within observation period ($n = 59$)	Infertile (no pregnancy after 1 y), clinical pregnancy within observation period ($n = 26$)		0.700	(19)
		Infertile, no clinical pregnancy after ICSI ($n = 21$)	Infertile, clinical pregnancy after ICSI ($n = 15$)		0.700	(20)
		Infertile, undergoing IVF/ICSI cycles ($n = 65$)	Clinical pregnancy through natural conception or IUI ($n = 57$)		0.790	(18)
		Partners with reduced ovarian reserve, no clinical pregnancy after ICSI ($n = 255$)	Partners with reduced ovarian reserve, clinical pregnancy after ICSI ($n = 72$)		0.594	(21)
		Infertile, idiopathic oligoasthenoteratozoospermia ($n = 73$)	Fertile (normo) ($n = 20$)		0.892	(17)
γ-H2AX levels	SDF	Infertile ($n = 100$)	Fertile (proven fertility) ($n = 100$)	Flow cytometry with FITC–conjugated anti-γH2AX	0.927	(27)
Single strand DNA breaks (ssSDF)	SDF - oxidative DNA damage	Normo idiopathic infertile, astheno, terato, asthenoteratozoospermic, azoospermic ($n = 83$)	Fertile (normo) ($n = 11$)	Alkaline Comet	0.994	(30)
		Infertile, no clinical pregnancy after IVF ($n = 180$)	Infertile, clinical pregnancy after IVF ($n = 39$)		0.648	(31)
		Infertile, enrolled in IVF ($n = 70$)	Fertile (normo) ($n = 28$)		0.970	(29)
		Infertile, no clinical pregnancy after IVF ($n = 50$)	Infertile, clinical pregnancy after IVF ($n = 20$)		0.905	(29)
		Infertile ($n = 133$)	Fertile (proven fertility) ($n = 50$)		0.937	(12)
8-OHdG bases	SDF - oxidative DNA damage	Infertile, no clinical pregnancy after IUI (total $n = 53$)	Infertile, clinical pregnancy after IUI (total $n = 53$)	Immunohistochemistry using FITC-labeled 8-OHdG-specific protein	0.794	(22)
		Infertile, no clinical pregnancy after IVF ($n = 63$)	Infertile, clinical pregnancy after IVF ($n = 10$)		0.776	(31)

Llavanera. Fertility biomarkers in semen. Fertil Steril 2022.

TABLE 1

Continued.

Biomarker	Type	Case	Control	Methods	AUC	Reference
		Infertile, no clinical pregnancy after ICSI treatment ($n = 38$)	Infertile, clinical pregnancy after ICSI ($n = 15$)	Alkaline comet+formamido pyrimidine DNA glycosylase treatment	0.704	(31)
STI	Chromatin structure	Infertile, miscarriage after ICSI ($n = 6$)	Infertile, pregnancy with livebirth after ICSI ($n = 15$)	STI	0.710	(34)
Chromatin decondensation	SDF	Infertile, idiopathic RPL ($n = 45$)	Fertile (normo) ($n = 20$)	SCSA	0.830	(39)
	SDF	Infertile with varicocele, no clinical pregnancy ($n = 66$)	Varicocele, spontaneous pregnancy ($n = 22$)		0.762	(42)
	SDF	Infertile ($n = 74$)	Fertile (proven fertility) ($n = 49$)		0.792	(12)
	SDF	Idiopathic infertility ($n = 100$)	Fertile (recently proven fertility, 2 y) ($n = 100$)		0.919	(38)
	SDF	Infertile, recurrent spontaneous abortion ($n = 139$)	Fertile (recently proven fertility, 2 y) ($n = 200$)		0.752	(40)
	SDF	Infertile, RPL after IVF ($n = 461$)	Infertile, clinical pregnancy after IVF ($n = 411$)		0.713	(41)
	SDF	Infertile, no clinical pregnancy after ICSI ($n = 39$)	Infertile, clinical pregnancy after ICSI ($n = 24$)	Acridine orange	0.750	(37)
	SDF	Infertile, no clinical pregnancy ($n = 88$)	Fertile (proven fertility) ($n = 58$)	Halosperm (SCD)	0.546	(52)
	SDF	Infertile ($n = 74$)	Fertile (proven fertility) ($n = 49$)		0.869	(12)
	SDF	Infertile, embryos of low quality (CES 1, 2 or 3) after IVF ($n = 43$)	Infertile, embryos of high quality (CES 4 or 5) after IVF ($n = 37$)		0.710	(47)
	Undamaged DNA (big halo)	Infertile, embryos of low quality (CES 1, 2 or 3) after IVF ($n = 43$)	Infertile, embryos of high quality (CES 4 or 5) after IVF ($n = 37$)		0.830	(47)
	SDF	Infertile, no clinical pregnancy after IVF ($n = 61$)	Infertile, clinical pregnancy after IVF ($n = 27$)		0.670	(47)
	Undamaged DNA (big halo)	Infertile, no clinical pregnancy after IVF ($n = 61$)	Infertile, clinical pregnancy after IVF ($n = 27$)		0.750	(47)
	SDF	Infertile, poor fertilization rates after ICSI (total $n = 135$)	Infertile, good fertilization rates after ICSI (total $n = 135$)		0.680	(46)
	SDF	Infertile, no clinical pregnancy after IVF ($n = 44$)	Infertile, clinical pregnancy after IVF ($n = 11$)		0.700	(48)
	SDF	Infertile, fertilization rate $<50\%$ after conventional IVF ($n = 29$)	Infertile, fertilization rate $\geq 50\%$ after conventional IVF ($n = 104$)		0.664	(45)
	SDF	Cycles with total embryo development arrest ($n = 15$)	Cycles of at least one developed blastocyst ($n = 97$)		0.771	(45)
	SDF	Infertile with varicocele ($n = 98$)	Fertile ($n = 80$)		0.942	(44)
	SDF	Non-normo ($n = 434$)	Fertile (normo) ($n = 234$)		0.753	(43)
	SDF	Infertile, no clinical pregnancy after ICSI ($n = 32$)	Infertile, clinical pregnancy after ICSI ($n = 49$)		0.711	(51)
	SDF	Infertile, no pregnancy after IVF (total $n = 38$)	Infertile, pregnancy after IVF (total $n = 38$)		0.830	(49)

Llavanera. Fertility biomarkers in semen. Fertil Steril 2022.

TABLE 1

Continued.

Biomarker	Type	Case	Control	Methods	AUC	Reference
Deficient protamination	Chromatin maturity	Infertile, fertilization rate ≤60% after IVF (total n = 140)	Infertile, fertilization rate >60% after IVF (total n = 140)	CMA3 staining	0.760	(57)
		Infertile, fertilization rate ≤50% after IVF (n = 28)	Infertile, fertilization rate >50% after IVF (n = 43)		0.740	(57)
		Infertile, <50% embryos of A quality after IVF/ICSI (n = 133)	Infertile, ≥50% embryos of A quality after IVF/ICSI (n = 9)		0.778	(59)
		Infertile, no fertilization after IVF (n = 37 non-fertilized oocytes of a total of 235 inseminated oocytes from 82 different patients, each in a single IVF cycle)	Infertile, fertilization after IVF (n = 198 fertilized oocytes of a total of 235 inseminated oocytes from 82 different patients, each in a single IVF cycle)		0.769	(58)
Histone persistence	Chromatin maturity	Infertile with mild male factor or unexplained infertility, no clinical pregnancy after IUI (n = 221)	Infertile with mild male factor or unexplained infertility, clinical pregnancy after IUI (n = 22)	Aniline blue staining	0.653	(60)
		Infertile, fertilization rate <80% after IVF/ICSI (n = 89)	Infertile, fertilization rate ≥80% after IVF/ICSI (n = 117)		0.611	(59)
B. Gene expression and epigenomics						
Sperm						
Expression model (EIF5A + RPL13 + RPL23A + RPS27A)	Expression signature	Low IUI-clinical pregnancy rate (<13.6%) (n = 17)	High IUI-clinical pregnancy rate (>13.6%) (n = 51)	TaqMan Arrays	0.910	(61)
Sperm global DNA methylation pattern	Methlylation pattern	All IVF patients (female factor previously excluded) (n = 127)	Normo, fertile donors (n = 54)	GW sperm DNA methylation	0.670	(64)
		IVF patients (female factor previously excluded) with poor embryogenesis (n = 72); either with positive (n = 42) or negative (n = 30) chemical pregnancies after IVF	IVF patients (female factor previously excluded) with good embryogenesis and positive chemical pregnancy (n = 55)		0.640	(64)
Sperm site-specific DNA methylation pattern	Methlylation pattern	All IVF patients (female factor previously excluded) (n = 127)	Normo fertile donors (n = 54)	Bisulphite conversion and methylation microarray	0.930	(64)
Methylation of *MLH1*promoter	Methylation pattern	Oligo men (n = 10)	Samples from normo men (n = 29)	Bisulphite modification and methylation-specific polymerase chain reaction	0.611	(65)
Hypermethylation of MTHFR promoter region	Methylation pattern	Idiopathic infertile men (n = 40)	Samples from donors with proven fertility (n = 40)	Bisulfite Pyrosequencing	0.734	(68)

Llavanera. Fertility biomarkers in semen. Fertil Steril 2022.

TABLE 1

Continued.

Biomarker	Type	Case	Control	Methods	AUC	Reference
Seminal plasma						
CCNA1 promoter methylation from cell-free DNA	Cell-free DNA methylation	NOA patients with SCO and with round spermatid MA ($n = 42$)	NOA patients with hypospermatogenesis (HO) ($n = 26$)	Bisulfite treatment of DNA and MethyLight analysis	0.668	(70)
C. Transcriptomics						
miRNA in sperm						
hsa-miR-122-5p	miRNA	Astheno/Oligo ($n = 80$)	Normo ($n = 90$)	qRT-PCR	0.777	(76)
hsa-miR-34b-3p	miRNA	Astheno/Oligo ($n = 80$)	Normo ($n = 90$)	qRT-PCR	0.944	(76)
hsa-miR-34b-5p	miRNA	Astheno/Oligo ($n = 80$)	Normo ($n = 90$)	qRT-PCR	0.798	(76)
hsa-miR34c-5p	miRNA	Astheno/Oligo ($n = 80$)	Normo ($n = 90$)	qRT-PCR	0.776	(76)
		No clinical pregnancy after ICSI ($n = 97$)	Clinical pregnancy after ICSI ($n = 65$)		0.750	(77)
hsa-miR-429	miRNA	Astheno/Oligo ($n = 80$)	Normo ($n = 90$)	qRT-PCR	0.980	(76)
hsa-miR-34b-5p, 34b-3p, 34c-5p, 122-5p, 429	miRNA profile	Astheno/Oligo ($n = 80$)	Normo ($n = 90$)	qRT-PCR	0.987	(76)
miRNA in seminal plasma						
hsa-miR-122-5p	miRNA	Azoo ($n = 73$)	Normo fertile ($n = 68$)	Seq and qRT-PCR	0.921	(78)
		Astheno ($n = 73$)	Normo fertile ($n = 68$)		0.733	(78)
		Astheno ($n = 73$)	Azoo ($n = 73$)		0.967	(78)
hsa-miR-146-5p	miRNA	Azoo ($n = 73$)	Normo fertile ($n = 68$)	Seq and qRT-PCR	0.825	(78)
		Astheno ($n = 73$)	Normo fertile ($n = 68$)		0.789	(78)
		Astheno ($n = 73$)	Azoo ($n = 73$)		0.963	(78)
hsa-miR-181a-5p	miRNA	Azoo ($n = 73$)	Normo fertile ($n = 68$)	Seq and qRT-PCR	0.875	(78)
		Astheno ($n = 73$)	Normo fertile ($n = 68$)		0.781	(78)
		Astheno ($n = 73$)	Azoo ($n = 73$)		0.976	(78)
hsa-miR-205-5p	miRNA	Secretory azoo ($n = 14$)	OA ($n = 13$)	miRCURY LNA™ array and qRT-PCR	0.838	(81)
hsa-miR-210-3p	miRNA	Varicocele (dyszoospermia) ($n = 204$)	Normo ($n = 30$)	qRT-PCR	0.940	(79)
hsa-miR-31-5p	miRNA	Secretory azoo ($n = 14$)	OA ($n = 13$)	miRCURY LNA™ array and qRT-PCR	0.963	(81)
hsa-miR-34c-5p	miRNA	Azoo ($n = 73$)	Normo fertile ($n = 68$)	Seq and qRT-PCR	0.894	(78)
		Astheno ($n = 73$)	Normo fertile ($n = 68$)		0.783	(78)
		Astheno ($n = 73$)	Azoo ($n = 73$)		0.990	(78)
hsa-miR-374b-5p	miRNA	Azoo ($n = 73$)	Normo fertile ($n = 68$)	Seq and qRT-PCR	0.839	(78)
		Astheno ($n = 73$)	Normo fertile ($n = 68$)		0.813	(78)
		Astheno ($n = 73$)	Azoo ($n = 73$)		0.984	(78)
hsa-miR-509-5p	miRNA	Azoo ($n = 73$)	Normo fertile ($n = 68$)	Seq and qRT-PCR	0.822	(78)
		Astheno ($n = 73$)	Normo fertile ($n = 68$)		0.836	(78)
		Astheno ($n = 73$)	Azoo ($n = 73$)		0.983	(78)
hsa-miR-513a-5p	miRNA	Azoo ($n = 73$)	Normo fertile ($n = 68$)	Seq and qRT-PCR	0.825	(78)

Llavanera. Fertility biomarkers in semen. Fertil Steril 2022.

TABLE 1

Continued.

Biomarker	Type	Case	Control	Methods	AUC	Reference
		Astheno (n = 73)	Normo fertile (n = 68)		0.806	(78)
		Astheno (n = 73)	Azoo (n = 73)		0.966	(78)
hsa-miR-141, 429, 7-1-3p	miRNA profile	NOA (n = 96)	Normo fertile (n = 96)	TLDA and qRT-PCR	0.833	(80)
piRNA in seminal plasma						
piR-30198	piRNA	Azoo (n = 52)	Normo fertile (n = 58)	Seq and qRT-PCR	0.986	(82)
		Astheno (n = 74)	Azoo (n = 52)		0.955	(82)
piR-31068	piRNA	Astheno (n = 74)	Normo fertile (n = 58)	Seq and qRT-PCR	0.985	(82)
		Azoo (n = 52)	Normo fertile (n = 58)		0.996	(82)
piR-31068, 31925, 43771, 43773	piRNA profile	Astheno (n = 74)	Normo fertile (n = 58)	Seq and qRT-PCR	0.894	(82)
piR-31068, 31925, 43771, 43773, 30198	piRNA profile	Azoo (n = 52)	Normo fertile (n = 58)	Seq and qRT-PCR	0.991	(82)
piR-31925	piRNA	Astheno (n = 74)	Normo fertile (n = 58)	Seq and qRT-PCR	0.932	(82)
		Azoo (n = 52)	Normo fertile (n = 58)		0.967	(82)
piR-43771	piRNA	Astheno (n = 74)	Normo fertile (n = 58)	Seq and qRT-PCR	0.903	(82)
		Azoo (n = 52)	Normo fertile (n = 58)		0.954	(82)
piR-43773	piRNA	Astheno (n = 74)	Normo fertile (n = 58)	Seq and qRT-PCR	0.796	(82)
		Azoo (n = 52)	Normo fertile (n = 58)		0.880	(82)
piR-58527	piRNA	Secretory azoo (n = 14)	OA (n = 13)	miRCURY LNA™ array and qRT-PCR	0.744	(81)
D. Metabolomics						
Sperm						
ROS (AU)	ROS	Infertile patients (n = 133)	Normo (n = 91)	Chemiluminescence (luminol)	0.789	(103)
Cer(d18:1/24:0)	Ceramide	No clinical pregnancy after ICSI (n = 16)	Clinical pregnancy after ICSI (n = 22)	UHPLC-time-of-flight (TOF)-MS	0.700	(119)
Cer(d18:1/22:0)	Ceramide	No clinical pregnancy after ICSI (n = 16)	Clinical pregnancy after ICSI (n = 22)	UHPLC-time-of-flight (TOF)-MS	0.710	(119)
Cer(d18:1/23:0)	Ceramide	No clinical pregnancy after ICSI (n = 16)	Clinical pregnancy after ICSI (n = 22)	UHPLC-time-of-flight (TOF)-MS	0.700	(119)
Phosphatidylcholines (O-22:0/20:4)	Phosphatidylcholines	No clinical pregnancy after ICSI (n = 16)	Clinical pregnancy after ICSI (n = 22)	UHPLC-time-of-flight (TOF)-MS	0.770	(119)
Phosphatidyleth anolamines (P-16:0/18:2)	Phosphatidyleth anolamines	No clinical pregnancy after ICSI (n = 16)	Clinical pregnancy after ICSI (n = 22)	UHPLC-time-of-flight (TOF)-MS	0.700	(119)
	Sphingomyelins			UHPLC-time-of-flight (TOF)-MS	0.700	(119)

Llavanera. Fertility biomarkers in semen. Fertil Steril 2022.

TABLE 1

Continued.

Biomarker	Type	Case	Control	Methods	AUC	Reference
Sphingomyelins (38:1)		No clinical pregnancy after ICSI ($n = 16$)	Clinical pregnancy after ICSI ($n = 22$)			
Sphingomyelins (d18:1/22:0)	Sphingomyelins	No clinical pregnancy after ICSI ($n = 16$)	Clinical pregnancy after ICSI ($n = 22$)	UHPLC-time-of-flight (TOF)-MS	0.720	(119)
Sphingomyelins (42:1)	Sphingomyelins	No clinical pregnancy after ICSI ($n = 16$)	Clinical pregnancy after ICSI ($n = 22$)	UHPLC-time-of-flight (TOF)-MS	0.720	(119)
Sphingomyelins (d18:1/25:0)	Sphingomyelins	No clinical pregnancy after ICSI ($n = 16$)	Clinical pregnancy after ICSI ($n = 22$)	UHPLC-time-of-flight (TOF)-MS	0.740	(119)
Phosphatidylcholines (0:0/20:0)	Phosphatidylcholines	No clinical pregnancy after ICSI ($n = 16$)	Clinical pregnancy after ICSI ($n = 22$)	UHPLC-time-of-flight (TOF)-MS	0.710	(119)
Seminal plasma						
ROS (AU)	ROS	Idiopathic infertility ($n = 28$)	Normo fertile ($n = 24$)	Chemiluminescence	0.743	(107)
		Infertile varicocele ($n = 55$)	Normo fertile ($n = 24$)		0.689	(107)
		Infertile varicocele with prostatitis ($n = 8$)	Normo fertile ($n = 24$)		0.948	(107)
TAC (AU)	TAC	Idiopathic infertility ($n = 28$)	Normo fertile ($n = 24$)	Chemiluminescence	0.818	(107)
		Infertile varicocele ($n = 55$)	Normo fertile ($n = 24$)		0.802	(107)
		Infertile varicocele with prostatitis ($n = 8$)	Normo fertile ($n = 24$)		0.828	(107)
TAC (uM Trolox equivalent)	TAC	Infertile ($n = 279$)	Normo fertile ($n = 46$)	TAC assay kit	0.608	(108)
ROS-TAC (AU)	ROS/TAC	Idiopathic infertility ($n = 28$)	Normo fertile ($n = 24$)	Chemiluminescence	0.845	(107)
		Infertile varicocele ($n = 55$)	Normo fertile ($n = 24$)		0.808	(107)
		Infertile varicocele with prostatitis ($n = 8$)	Normo fertile ($n = 24$)		0.932	(107)
Lipid fingerprint	Metabolomic profile	High lipid peroxidation levels (spectrophotometry) ($n = 29$)	Low lipid peroxidation levels (spectrophotometry) ($n = 27$)	MALDI-TOF MS	0.974	(118)
Lipid fingerprint	Metabolomic profile	High SDF (Comet Assay) ($n = 15$)	Low SDF (Comet Assay) ($n = 17$)	MALDI-TOF MS	0.925	(118)
Lactate, alanine, choline, citrate, glycero phosphocholine, glutamine, tyrosine, histidine, phenylalanine, and uridine	Metabolomic profile	Infertile normo ($n = 65$)	Normo fertile ($n = 60$)	H-NMR	0.994	(116)
Lactate, alanine, choline, citrate, glycero phosphocholine, glutamine,	Metabolomic profile	Infertile oligo ($n = 60$)	Normo fertile ($n = 60$)	H-NMR	0.993	(116)

Llavanera. Fertility biomarkers in semen. Fertil Steril 2022.

TABLE 1

Continued.

Biomarker	Type	Case	Control	Methods	AUC	Reference
tyrosine, histidine, phenylalanine, and uridine						
Cholesterol, 5a-cholesterol, 7-ketocholesterol, lipids, citrate, a-ketoglutaric acid, creatine, choline, phosphocholine, glycero phosphocholine, uridine, cytidine, cysteine, glutamine, glutamate, phenylalanine, tyrosine, histidine, taurine	Metabolomic profile	Astheno ($n = 10$)	Normo ($n = 11$)	H-NMR	0.927	(117)
L-Valine	Aminoacid	Astheno ($n = 30$)	Normo ($n = 30$)	GC/MS	0.801	(120)
Cholecalciferol	Vitamin D	Astheno ($n = 30$)	Normo ($n = 30$)	GC/MS	0.762	(120)
D-Pinitol	Cyclic polyol	Astheno ($n = 30$)	Normo ($n = 30$)	GC/MS	0.804	(120)
Hexadecanoic acid	LCFA	Astheno ($n = 30$)	Normo ($n = 30$)	GC/MS	0.813	(120)
Oleic acid	LCFA	Astheno ($n = 30$)	Normo ($n = 30$)	GC/MS	0.729	(120)
Nonadecanoic acid	Straight-chain fatty acid	Astheno ($n = 30$)	Normo ($n = 30$)	GC/MS	0.820	(120)
Benzoic acid	Aromatic carboxylic acids	Astheno ($n = 30$)	Normo ($n = 30$)	GC/MS	0.862	(120)
Testosterone (nM)	Hormone	NOA patients without sperm ($n = 26$)	NOA patients with sperm ($n = 30$)	Competitive immunoassay	0.771	(121)
Testosterone/E2 ratio	Hormone	NOA patients without sperm ($n = 26$)	NOA patients with sperm ($n = 30$)	Competitive immunoassay	0.886	(121)
Ejaculate						
ROS (mv/30min/million sperm)	ROS	Infertile ($n = 48$)	Normo fertile ($n = 41$)	Chemiluminescence	0.833	(102)
ROS (RLU/s/10^6 sperm)	ROS	Infertile (normo and abnormal sperm) ($n = 318$)	Normo fertile ($n = 56$)	Chemiluminescence	0.683	(101)
		Partners of women with RPL ($n = 50$)	Normo ($n = 33$)		0.630	(104)

Llavanera. Fertility biomarkers in semen. Fertil Steril 2022.

TABLE 1

Continued.

Biomarker	Type	Case	Control	Methods	AUC	Reference
		Terato ($n = 79$)	Normo ($n = 56$)		0.614	(105)
		High number of PMN ($n = 41$)	Normo ($n = 94$)		0.888	(106)
ORP (mV/10^6 sperm/mL)	ORP	Primary or secondary infertility ($n = 106$)	Normo ($n = 51$)	MiOXSYSTM System	0.770	(114)
		Abnormal motility (<40%) ($n = 15$)	Normal motility (>40%) ($n = 44$)		0.648	(111)
		Abnormal sperm (at least 1 abnormal WHO semen parameter; $n = 152$)	Normo ($n = 42$)		0.809	(110)
		Oligo ($n = 92$)	Normo ($n = 42$)		0.754	(110)
		Astheno ($n = 102$)	Normo ($n = 42$)		0.751	(110)
		Terato ($n = 95$)	Normo ($n = 42$)		0.693	(110)
		Abnormal sperm ($n = 292$)	Normo fertile ($n = 15$)		0.596	(113)
		Abnormal sperm (at least 1 abnormal semen parameter; $n = 1893$)	Normo ($n = 199$)		0.765	(112)
NBT (μg formazan/10^7 sperm)	NBT	Abnormal sperm (at least 1 abnormal semen parameter; $n = 36$)	Normo fertile ($n = 21$)	Photometry (colorimetric nitro blue tetrazolium)	0.880	(122)
E. Proteomics						
Sperm						
BAG6	Regulatory protein	Infertile ($n = 16$)	Normo fertile ($n = 7$)	LC-MS/MS and Western blot	0.921	(89)
CatSper1	Calcium channel	Bad quality embryos (EQA < 50%) ($n = 16$)	Good quality embryos (EQA > 50%) ($n = 120$)	Flow cytometry (fluorescence intensity)	0.682	(59)
CYP24A1	Enzyme	No clinical pregnancy after IUI ($n = 68$)	Clinical pregnancy after IUI ($n = 15$)	Immunocytochemistry (% positive cells)	0.710	(93)
HIST1H2BA	Histone	Infertile ($n = 16$)	Normo fertile ($n = 7$)	LC-MS/MS and Western blot	0.935	(89)
Nuclease activity corrected by sperm count	Enzimatic activity	Idiopathic infertile, astheno, terato, asthenoteratozoospermic, azoo ($n = 83$)	Normo fertile ($n = 11$)	SRED method	0.705	(30)
PELP1	Scaffolding protein	Oligospermic ($n = 26$)	Normo ($n = 17$)	ICC + FACS analysis	0.781	(87)
Seminal plasma						
CRISP1/PAP	Glycoprotein	NOA ($n = 14$)	OA or prevasectomy ($n = 14$)	Western blot	0.929	(95)
Inhibin B	Hormone	Failed TESE ($n = 45$)	Successful TESE ($n = 17$)	Two-site ELISA	0.910	(96)
PON1 activity (U/L)	Antioxidant enzyme	Subfertile with abnormal semen parameters ($n = 32$)	Normo fertile ($n = 30$)	Spectrophotometry (colorimetric test)	0.950	(90)
Presepsin	CD24 subunit (inflammatory response)	Non-live birth ($n = 86$)	Live birth ($n = 28$)	PATHFAST chemiluminescence immunoassay analyzer	0.634	(94)
		No chemical pregnancy after ICSI ($n = 81$)	Chemical pregnancy after ICSI ($n = 33$)		0.677	(94)
Annexin A7 i CD63	Proteomic profile	Low mitochondrial activity ($n = 12$)	Normal mitochondrial activity ($n = 12$)	LC-MS/MS	0.993	(88)
CRISPLD1	Protein	High SDF (Comet Assay) ($n = 12$)	Low SDF (Comet Assay) ($n = 12$)	LC-MS/MS	0.882	(88)
PLTP and COL12A1	Proteomic profile	Altered acrosome integrity (PNA) ($n = 12$)	Normal acrosome integrity (PNA) ($n = 12$)	LC-MS/MS	0.972	(88)
TEX101	Glycoprotein	NOA ($n = 81$)	OA or prevasectomy ($n = 93$)	ELISA	0.670	(84)
		Azoo ($n = 137$)	Normo ($n = 64$)		0.990	(84)
		Fertile prevasectomy ($n = 64$)	Fertile postvasectomy ($n = 57$)		1.000	(84)
		No sperm retrieval in NOA ($n = 11$)	Sperm retrieval in NOA ($n = 15$)		0.690	(84)

Llavanera. Fertility biomarkers in semen. Fertil Steril 2022.

TABLE 1

Continued.

Biomarker	Type	Case	Control	Methods	AUC	Reference
Total uPA	Enzyme	No clinical pregnancy after AI (AIH or ICSI) ($n = 23$)	Clinical pregnancy after AI (AIH or ICSI) ($n = 23$)	ELISA	0.720	(92)
Transferrin receptors	Protein receptors	Azoo ($n = 25$)	Postvasectomy ($n = 40$)	ELISA	0.630	(98)
α-glucosidase	Enzymatic activity	Azoo (vasectomy) ($n = 27$)	Azoo (testicular defects) ($n = 33$)	Spectrophotometry	0.760	(97)
		Successful vasectomy ($n = 27$)	Unsuccessful vasectomy ($n = 11$)		0.830	(97)
α-glucosidase	Enzyme concentration	NOA ($n = 14$)	OA or prevasectomy ($n = 14$)	Immunoblotting	0.610	(95)

8-OHdG, 8-hydroxydeoxyguanosine; γ-H2AX, gamma-H2A.X; AI, artificial insemination; AIH, artificial insemination by husband; Astheno, asthenozoospermic; Azoo, azoospermia; BAG6, BAG Cochaperone 6; CMA3, chromomycin A3; CRISP1, cysteine-rich secretory protein 1; CYP24A1, vitamin D inactivating enzyme; E2, estradiol; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; GW, genome wide; HIST1H2BA; histone H2B type 1-A; H-NMR, proton nuclear magnetic resonance; HO, hypo-spermatogenesis; ICC, immunocytochemistry; ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, in vitro fertilization; LCFA, long-chain fatty acids; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MA, maturation arrest; miRNA, MicroRNA; NBT, nitro blue tetrazolium; NOA, nonobstructive azoospermia; Normo, normozoospermic; OA, obstructive azoospermia; Oligo, oligozoospermic; ORP, oxidation-reduction potential; PELP1, proline, glutamate, and leucine-rich protein 1; piRNA, piwi-interacting RNA; PMN, polymorphonuclear leukocytes; PNA, peanut agglutinin; PON1, Paraoxonase 1; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; ROS, reactive oxygen species; RPL, recurrent pregnancy loss; SCD, stearoyl coenzyme A desaturase; SCO, Sertoli cell only; SDF, sperm DNA fragmentation; Seq, sequencing; STI, sperm toroid intergrity; T, testosterone; TAC, total antioxidant capacity; Terato, teratozoospermic; TESE, testicular sperm extraction; TEX101, testis-expressed protein 101; TLDA, TaqMan low density arrays; total n, n was not reported for case and control groups separately and a global sample size is indicated; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling assay; UHPLC, ultra-high-performance liquid chromatography; uPA, urokinase-type plasminogen activator.

Llavanera. Fertility biomarkers in semen. Fertil Steril 2022.

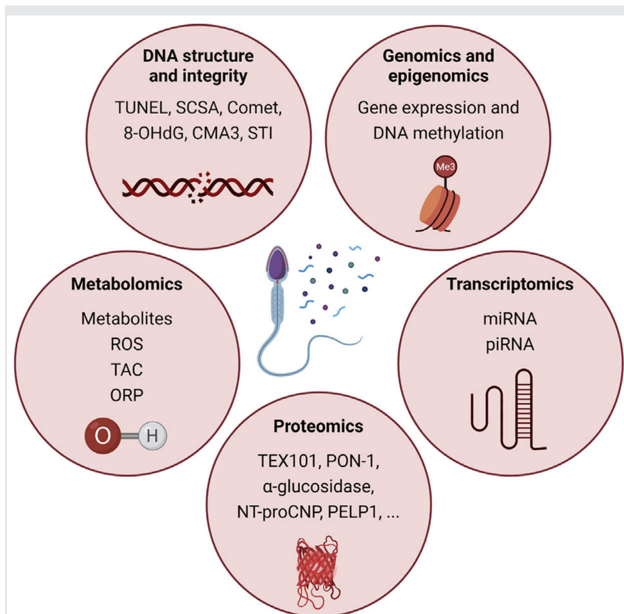
Additionally, protein biomarkers in semen were observed to be able to predict the etiology of male fertility disorders, discriminating between nonobstructive and obstructive azoospermia as well as predicting the etiology of azoospermic men (vasectomy or testicular defects). In effect, seminal plasma levels of TEX101 (84), CRISP1/PAP (95), and α-glucosidase (95) were reported to be able to discriminate between nonobstructive and obstructive azoospermia, showing AUCs of 0.670, 0.929, and 0.609, respectively. Moreover, seminal plasma proteins showed the ability to predict the success of surgical procedures, such as testicular sperm extraction and to verify the success of vasectomy. Concentrations of TEX101 (84) and inhibin B (96) in seminal plasma were found to predict the success of testicular sperm extraction, showing AUCs of 0.690 and 0.910, respectively. Similarly, TEX101 concentration (84) and α-glucosidase activity (97) were reported to be significant validators of successful vasectomy procedures, showing AUCs of 1 and 0.730, respectively. Finally, the activity of α-glucosidase (97) and concentration of transferrin receptors (98) in seminal plasma were related to the etiology of azoospermic men (vasectomy or testicular defects), with AUCs of 0.760 and 0.630, respectively.

In short, protein biomarkers for the prediction of male fertility and sperm quality should be tested further in clinical trials, as they could facilitate and improve diagnosis and prognosis of male fertility disorders.

Metabolomics. Recently, the sperm metabolome has been studied increasingly in humans and farm animals. Anabolic and catabolic reactions are known to be essential processes in sperm, and their substrates, products, and byproducts could be used as indicators of sperm metabolic status. In this regard, the application of metabolomics as a promising tool to uncover biomarkers of sperm quality and fertilizing capacity is of great interest for the field of andrology (99). Twenty studies exploring the predictive potential of metabolites for sperm quality and/or fertility disorders were selected (Table 1 and Supplemental Table 2). All biomarkers were analyzed by specific techniques, such as chemiluminescence or complex and sensitive analytical procedures, such as MALDI-TOF MS and ^{1}H-NMR.

Oxidative stress (OS) reflects the imbalance between the production of reactive oxygen species and the antioxidant capacity of sperm (100). The diagnosis of male OS with highly sensitive metabolomic biomarkers is essential to achieve better clinical outcomes. In this study, 3 methods for the detection of sperm OS – reactive oxygen species (ROS), total antioxidant capacity (TAC) and oxidation-reduction potential (ORP) – were reviewed. Physiologic ROS levels are known to be essential for an adequate sperm function. In the present review, 6 studies reported ROS to show high diagnostic potential for male infertility. ROS levels in the entire ejaculate measured by chemiluminescence exhibited fair to excellent diagnostic value for infertility, reporting AUCs of 0.683 (101) and 0.833 (102). When measured in sperm cells, ROS also exhibited a good predictive value with an AUC of 0.789 (103). Regarding the diagnostic potential for idiopathic infertility, ROS levels in the entire ejaculate, despite exhibiting limited robustness, were reported to be able to predict RPL, with an AUC of 0.630 (104),

FIGURE 2



Schematic summary of proposed well-characterized molecular fertility biomarkers in semen with a potential clinical application. 8-OHdG, 8-hydroxy-2' -deoxyguanosine; CMA3, chromomycin A3; Me3, tri-methylation; miRNA, micro ribonucleic acid; NT-proCNP, N-terminal C-type natriuretic propeptide; ORP, oxidation-reduction potential; PELP1, proline-, glutamic acid- and leucine-rich protein 1; piRNA, piwi ribonucleic acid; PON-1, Paraoxonase 1; ROS, reactive oxygen species; SCSA, sperm chromatin structure assay; STI, sperm toroid integrity; TAC, total antioxidant capacity; TEX101, testis-expressed protein 101; TUNEL, terminal deoxy-transferase-mediated deoxy-uridine triphosphate (dUTP) nick end labeling.

Llavanera. Fertility biomarkers in semen. Fertil Steril 2022.

and to diagnose teratozoospermia, with an AUC of 0.614 (105). An excellent diagnostic potential, however, was observed to discriminate between ejaculates with high and low number of polymorphonuclear leukocytes (PMN; AUC of 0.888) (106). Finally, a fair diagnostic power was seen for seminal plasma ROS to differentially diagnose varicocele (AUC of 0.689), and an excellent diagnostic power in the diagnosis of varicocele with prostatitis (AUC of 0.948) (107). On the other hand, TAC is known to be a parameter that indicates sperm oxidative status. Total antioxidant capacity in seminal plasma was studied by Sharma et al. (107) to explore its diagnostic value in male reproduction. They reported seminal plasma TAC to differentially diagnose between varicocele with prostatitis (AUC = 0.828) and varicocele (AUC = 0.802), as well as to predict normozoospermic idiopathic infertility (AUC = 0.818). Yet, another study from Roychoudhury et al. (108), although significant, reported fair diagnostic power for seminal plasma TAC in the prediction of male infertility (AUC = 0.608). Furthermore, because of the relationship between ROS and TAC, both indicating sperm oxidative status, as well as their good diagnostic power when assessed individually, Sharma et al. (107) explored their male fertility diagnostic potential when combined. They reported an excellent diagnostic value for ROS-TAC in seminal plasma to predict idiopathic infertility (AUC = 0.845) and to differentially diagnose varicocele with prostatitis (AUC = 0.932) and varicocele (AUC = 0.808) from normozoospermic men. Finally, ORP is a measure reflecting the balance between ROS and TAC of sperm, which is determined by the MiOXSYS system on the basis of a galvanostatic measure of electrons (109). Oxidation-reduction potential in the entire ejaculate has been explored as a new diagnostic tool by others (109–113). Semen ORP, evaluated through a MiOXSYS system, has been established as a good diagnostic tool for oligozoospermia (110) (AUC = 0.754), asthenozoospermia (AUCs of 0.751 [109], and 0.648 [110]) and teratozoospermia (110) (AUC = 0.693). The differentiation between normozoospermic and abnormal sperm using ORP in seminal plasma also was explored in different studies, reporting AUCs of 0.765 (112), 0.596 (113), and 0.809 (110). Finally, they also explored the potential of seminal plasma ORP to diagnose primary or secondary male infertility and found a good predictive AUC value (114) (AUC = 0.770). Along these lines, increased ROS formation and/or decreased TAC were found to lead to an unbalanced oxidation-reduction and subsequent sperm quality and fertility disorders (115). On the basis of the reviewed literature, ROS and TAC in the ejaculate exhibit good to excellent diagnostic value for male factor infertility and varicocele. Interestingly, the combination of both biomarkers shows better predictive values for both reproductive disorders. Notwithstanding, ROS levels in the ejaculate are able to diagnose the presence of PMN in semen. Similarly, the reviewed literature supports that ORP is a robust biomarker for oligozoospermia, asthenozoospermia, teratozoospermia, and primary or secondary male factor infertility. Remarkably, the prediction of male infertility using ORP shows similar results than the combination of ROS and TAC. Along these lines, OS biomarkers, such as ROS, TAC, and ORP, seem to be strong predictors of sperm quality and fertility disorders, as they are indicators of sperm metabolic status.

On the other hand, metabolomic fingerprints in seminal plasma evaluated by MALDI-TOF Ms. and ^{1}H-NMR were established as excellent biomarkers for male infertility disorders, such as idiopathic infertility (116) (AUC = 0.994), oligozoospermia (116) (AUC = 0.993), and asthenozoospermia (117) (AUC = 0.927), as well as indicators of sperm physiologic status, such as high sperm DNA fragmentation (118) (AUC = 0.925) and lipid peroxidation (118) (AUC = 0.974). The study of Rivera-Egea et al. (119) tested sperm lipids as biomarkers for pregnancy rates following ICSI through Ultra-High-Performance Liquid Chromatography -time-of-flight (TOF) MS. Some ceramides (e.g., Cer[d18:1/24:0]), phosphatidylcholines, phosphatidylethanolamines, and sphingomyelins showed a good predictive value for ICSI clinical pregnancy, with AUCs ranging from 0.700 to 0.770. Another study by Tang et al. (120) used gas chromatography mass spectrometry (GC/MS) to determine the potential of fatty acids in seminal plasma (L-valine, cholecalciferol, D-pinitol, hexadecanoic acid, oleic acid, nonadecanoic acid, and benzoic acid) to diagnose asthenozoospermia, with AUCs ranging from 0.729 to 0.862. Lipid hormones, such as testosterone or estradiol, in seminal plasma showed a good predictive value for sperm recovery in nonobstructive azoospermia patients (121). Whereas

testosterone showed an AUC of 0.771 for discriminating between NOA patients with and without successful sperm recovery, the relationship of testosterone/estradiol AUC was 0.886. Finally, Tunc et al. (122) reported nitro blue tetrazolium in the entire ejaculate normalized by sperm concentration to be able to predict sperm quality with high sensitivity and specificity, showing an AUC of 0.880. Thus, sperm metabolomic analysis for predicting male fertility disorders has been proven as a robust technique, as it provides relevant information for the diagnosis of male factor infertility.

In short, the available literature supports the high diagnostic value of ROS, TAC, ORP, metabolite profiles, and individual lipids in sperm and seminal plasma for male sperm quality and fertility disorders. Moreover, the quantification of metabolite profiles in seminal plasma by ^{1}H-NMR or MALDI-TOF MS has been shown to be better than that of individual metabolites when predicting sperm quality and fertility. Nevertheless, the need of highly specialized technicians and expensive instruments required for the analysis of metabolomic biomarkers may hinder its implementation in fertility clinics.

DISCUSSION

The current review supports that some Omics (e.g., DNA structure and integrity, genomics and epigenomics, transcriptomics, metabolomics, and proteomics) could be considered relevant molecular biomarkers that may help identify infertility etiologies and fertilization prognosis with cost-effective, simple, and accurate diagnosis.

The identification of reliable molecular biomarkers in semen could help in the diagnosis and treatment of conditions causing semen quality and male fertility disorders. In this sense, exploring semen biomarkers showing high diagnostic value is of great interest to determine their potential to become clinical tools for the diagnosis of sperm quality dysfunctions and predict male fertility and ART success. Implementation of these novel tools in fertility clinics may be translated into noninvasive, time-saving, inexpensive, and robust diagnostics for male reproductive disorders. In the present study, many molecular biomarkers of sperm and seminal plasma showing high diagnostic power have been evaluated. To the best of our knowledge, the present study is the first in compiling semen robust biomarkers that show high diagnostic value for male sperm quality and fertility disorders (Fig. 2).

The differential diagnosis and/or etiology of male factor infertility by conventional semen analysis often remains elusive, which makes the prognosis and treatment of infertile patients difficult. The advent of Omics techniques, nevertheless, has uncovered relevant molecular biomarkers that may help identify infertility etiologies with cost-effective, simple, and accurate diagnosis. Although highly specific and robust (in)fertility biomarkers have been unearthed using Omics techniques, an accurate prediction of sperm physiologic status and/or (in)fertility should envisage the integration of data from each Omics field. The combination of independent biomarkers to reach a system biology approach currently is in its infancy in the andrology field. The advent of diagnostic tools incorporating independent molecular biomarkers, however, could be of great interest for spermatology because it would improve the identification of male fertility disorders through highly accurate and robust diagnostics.

The use of these novel biomarkers in IVF clinics could be useful since their implementation might be translated into cost-effective, noninvasive, time-saving, and accurate diagnosis of male reproductive disorders. Yet, although Omics techniques have uncovered a wide range of highly accurate molecular biomarkers, the translation of such results into a clinical setting often is challenging. The high costs of some Omics techniques and the need for highly specialized technicians are 2 major limitations for the implementation of such diagnostic tools. Despite this, the continuous innovation, simplification, and cost-reduction of some Omics can make them become a routine tool for the diagnosis of male fertility disorders.

There are limitations and strengths of our study. The observational studies revised in the present review reported ROC analysis of the molecular biomarker candidates. The ROC analysis provides an objective statistical method to assess the diagnostic accuracy of biomarkers and indicate the diagnostic power for the assessment of male (in)fertility (123). Nonetheless, some of the studies reported herein had limited sample size, which could generate biases in ROC analysis. Furthermore, some of the articles evaluated for inclusion did not report essential information regarding the studied population and ROC analysis and, consequently, could not be included, although corresponding investigators were contacted by e-mail to ask for those data. Moreover, there is a very limited number of studies linking biomarkers with clinical outcomes using ROC and OR/RR and, therefore, a diagnostic test accuracy, unfortunately, is not possible. On the other hand, we also detected a disparity in the analytical techniques and methodology, thus hindering the comparison between molecular biomarkers. Finally, because of the heterogeneity of studies, approaches, and methodologies, it was not possible, despite intended, to undertake a meta-analysis. Further studies and clinical trials with a larger number of samples, standardized methodology, and well-characterized cohorts are required before these molecular diagnostic tools may be implemented in IVF clinics.

CONCLUSIONS

The current review presents the largest evidence to date supporting that some Omics have revealed relevant molecular biomarkers that may help identify infertility etiologies and fertilization prognosis with cost-effective, simple, and accurate diagnosis.

Acknowledgments: A.S-H. and M.Y. initiated the idea and designed the review. A.D-B. and M.L. collected and selected the data, assessed the articles, and wrote the manuscript. A.S-H. and M.Y. contributed to the drafting and critically reviewed the article for important intellectual content. J.R-M.

critically revised the manuscript. All the authors approved the final manuscript.

DIALOG: You can discuss this article with its authors and other readers at https://www.fertstertdialog.com/posts/34614

REFERENCES

1. Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, et al. Male infertility. Lancet 2021;397:319–33.
2. Khatun A, Rahman MS, Pang MG. Clinical assessment of the male fertility. Obstet Gynecol Sci 2018;61:179–91.
3. Lewis SEM. Is sperm evaluation useful in predicting human fertility? Reproduction 2007;134:31–40.
4. Altmäe S, Salumets A. A novel genomic diagnostic tool for sperm quality? Reprod Biomed Online 2011;22:405–7.
5. Kwon W-S, Rahman MS, Pang MG. Diagnosis and prognosis of male infertility in mammal: the focusing of tyrosine phosphorylation and phosphotyrosine proteins. J Proteome Res 2014;13:4505–17.
6. Egea RR, Puchalt NG, Escrivá MM, Varghese AC. OMICS: current and future perspectives in reproductive medicine and technology. J Hum Reprod Sci 2014;7:73–92.
7. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009;339.
8. Page MJ, Mckenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. PLoS Med 2021;18:e1003583.
9. Gorczyca W, Traganos F, Jesionowska H, Darzynkiewicz Z. Presence of DNA strand breaks and increased sensitivity of DNA in situ to denaturation in abnormal human sperm cells: analogy to apoptosis of somatic cells. Exp Cell Res 1993;207:202–5.
10. Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril 2010;93:1027–36.
11. Sergerie M, Laforest G, Bujan L, Bissonnette F, Bleau G. Sperm DNA fragmentation: threshold value in male fertility. Hum Reprod 2005;20:3446–51.
12. Ribas-Maynou J, García-Peiró A, Fernández-Encinas A, Abad C, Amengual MJ, Prada E, et al. Comprehensive analysis of sperm DNA fragmentation by five different assays: TUNEL assay, SCSA, SCD test and alkaline and neutral Comet assay. Andrology 2013;1:715–22.
13. Muratori M, Marchiani S, Tamburrino L, Cambi M, Lotti F, Natali I, et al. DNA fragmentation in brighter sperm predicts male fertility independently from age and semen parameters. Fertil Steril 2015;104:582–90.e4.
14. Punjabi U, Van Mulders H, Goovaerts I, Peeters K, Roelant E, De Neubourg D. DNA fragmentation in concert with the simultaneous assessment of cell viability in a subfertile population: establishing thresholds of normality both before and after density gradient centrifugation. J Assist Reprod Genet 2019;36:1413–21.
15. Sharma RK, Sabanegh E, Mahfouz R, Gupta S, Thiyagarajan A, Agarwal A. TUNEL as a test for sperm DNA damage in the evaluation of male infertility. Urology 2010;76:1380–6.
16. Sharma R, Ahmad G, Esteves SC, Agarwal A. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using bench top flow cytometer for evaluation of sperm DNA fragmentation in fertility laboratories: protocol, reference values, and quality control. J Assist Reprod Genet 2016;33:291–300.
17. Kabartan E, Gunes S, Arslan MA, Asci R. Investigating the relationship between BRCA1 and BRCA2 genes methylation profile and sperm DNA fragmentation in infertile men. Andrologia 2019;51:e13308.
18. Hichri R, Amor H, Khammari M, Harzallah M, El Fekih S, Saad A, et al. Apoptotic sperm biomarkers and the correlation between conventional sperm parameters and clinical characteristics. Andrologia 2018;50:1–9.
19. Vončina SM, Golob B, Ihan A, Kopitar AN, Kolbezen M, Zorn B. Sperm DNA fragmentation and mitochondrial membrane potential combined are better for predicting natural conception than standard sperm parameters. Fertil Steril 2016;105:637–44.e1.
20. Avendaño C, Franchi A, Duran H, Oehninger S. DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. Fertil Steril 2010;94:549–57.
21. Jin J, Pan C, Fei Q, Ni W, Yang X, Zhang L, et al. Effect of sperm DNA fragmentation on the clinical outcomes for in vitro fertilization and intracytoplasmic sperm injection in women with different ovarian reserves. Fertil Steril 2015;103:910–6.
22. Thomson LK, Zieschang JA, Clark AM. Oxidative deoxyribonucleic acid damage in sperm has a negative impact on clinical pregnancy rate in intrauterine insemination but not intracytoplasmic sperm injection cycles. Fertil Steril 2011;96:843–7.
23. Benchaib M, Braun V, Lornage J, Hadj S, Salle B, Lejeune H, et al. Sperm DNA fragentation decreases the pregnancy rate in an assisted reproductive technique. Hum Reprod 2003;18:1023–8.
24. Esbert M, Pacheco A, Vidal F, Florensa M, Riqueros M, Ballesteros A, et al. Impact of sperm DNA fragmentation on the outcome of IVF with own or donated oocytes. Reprod Biomed Online 2011;23:704–10.
25. Redon C, Pilch D, Rogakou E, Sedelnikova O, Newrock K, Bonner W. Histone H2A variants H2AX and H2AZ. Curr Opin Genet Dev 2002;12:162–9.
26. Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 directly binds phosphorylated histone H2AX to Regulate cellular responses to DNA Double-strand breaks. Cell 2005;123:1213–26.
27. Zhong HZ, Lv FT, Deng XL, Hu Y, Xie DN, Lin B, et al. Evaluating γh2AX in spermatozoa from male infertility patients. Fertil Steril 2015;104:574–81.
28. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184–91.
29. Simon L, Lutton D, McManus J, Lewis SEM. Sperm DNA damage measured by the alkaline Comet assay as an independent predictor of male infertility and in vitro fertilization success. Fertil Steril 2011;95:652–7.
30. Fernandez-Encinas A, García-Peiró A, Ribas-Maynou J, Abad C, Amengual MJ, Navarro J, et al. Characterization of nuclease activity in human seminal plasma and its relationship to semen parameters, sperm DNA fragmentation and male infertility. J Urol 2016;195:213–9.
31. Simon L, Brunborg G, Stevenson M, Lutton D, McManus J, Lewis SEM. Clinical significance of sperm DNA damage in assisted reproduction outcome. Hum Reprod 2010;25:1594–608.
32. De Iuliis GN, Thomson LK, Mitchell LA, Finnie JM, Koppers AJ, Hedges A, et al. DNA damage in Human spermatozoa is highly correlated with the efficiency of chromatin remodeling and the formation of 8-hydroxy-2′-deoxyguanosine, a marker of oxidative stress. Biol Reprod 2009;81:517–24.
33. Croteau DL, Bohr VA. Repair of oxidative damage to nuclear and mitochondrial DNA in mammalian cells. J Biol Chem 1997;272:25409–12.
34. Chan PJ, Orzylowska EM, Corselli JU, Jacobson JD, Wei AK. A simple sperm DNA toroid integrity test and risk of miscarriage. Biomed Res Int 2015;2015:780983.
35. Golan R, Shochat L, Weissenberg R, Soffer Y, Marcus Z, Oschry Y, et al. Evaluation of chromatin condensation in human spermatozoa: a flow cytometric assay using acridine orange staining. Mol Hum Reprod 1997;3:47–54.
36. Fernández JL, Muriel L, Goyanes V, Segrelles E, Gosálvez J, Enciso M, et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertil Steril 2005;84:833–42.
37. Jiang H, He RB, Wang CL, Zhu J. The relationship of sperm DNA fragmentation index with the outcomes of in-vitro fertilisation-embryo transfer and intracytoplasmic sperm injection. J Obstet Gynaecol 2011;31:636–9.
38. Venkatesh S, Singh A, Shamsi MB, Thilagavathi J, Kumar R, Mitra DK, et al. Clinical significance of sperm DNA damage threshold value in the assessment of male infertility. Reprod Sci 2011;18:1005–13.
39. Kumar K, Deka D, Singh A, Mitra DK, Vanitha BR, Dada R. Predictive value of DNA integrity analysis in idiopathic recurrent pregnancy loss following spontaneous conception. J Assist Reprod Genet 2012;29:861–7.
40. Yuan M, Huang L, Leung WT, Wang M, Meng Y, Huang Z, et al. Sperm DNA fragmentation valued by SCSA and its correlation with conventional

sperm parameters in male partner of recurrent spontaneous abortion couple. Bio Sci Trends 2019;13:152–9.

41. Zhu XB, Chen Q, Fan WM, Niu ZH, Xu BF, Zhang AJ. Sperm DNA fragmentation in Chinese couples with unexplained recurrent pregnancy loss. Asian J Androl 2020;22:296–301.
42. Ni K, Steger K, Yang H, Wang H, Hu K, Zhang T, et al. A comprehensive investigation of sperm DNA damage and oxidative stress injury in infertile patients with subclinical, normozoospermic, and astheno/oligozoospermic clinical varicocoele. Andrology 2016;4:816–24.
43. Gill K, Jakubik J, Rosiak-Gill A, Kups M, Lukaszuk M, Kurpisz M, et al. Utility and predictive value of human standard semen parameters and sperm DNA dispersion for fertility potential. Int J Environ Res Public Health 2019;16:2004.
44. Esteves SC, Gosálvez J, López-Fernández C, Núñez-Calonge R, Caballero P, Agarwal A, et al. Diagnostic accuracy of sperm DNA degradation index (DDSi) as a potential noninvasive biomarker to identify men with varicocele-associated infertility. Int Urol Nephrol 2015;47:1471–7.
45. Breznik BP, Kovačič B, Vlaisavljević V. Are sperm DNA fragmentation, hyperactivation, and hyaluronan-binding ability predictive for fertilization and embryo development in in vitro fertilization and intracytoplasmic sperm injection? Fertil Steril 2013;99:1233–41.
46. Xue LT, Wang RX, He B, Mo WY, Huang L, Wang SK, et al. Effect of sperm DNA fragmentation on clinical outcomes for Chinese couples undergoing in vitro fertilization or intracytoplasmic sperm injection. J Int Med Res 2016; 44:1283–91.
47. Tandara M, Bajić A, Tandara L, Bilić-Zulle L, Šunj M, Kozina V, et al. Sperm DNA integrity testing: big halo is a good predictor of embryo quality and pregnancy after conventional IVF. Andrology 2014;2:678–86.
48. Bounartzi T, Dafopoulos K, Anifandis G, Messini CI, Koutsonikou C, Kouris S, et al. Pregnancy prediction by free sperm DNA and sperm DNA fragmentation in semen specimens of IVF/ICSI-ET patients. Hum Fertil (Camb) 2016;19:56–62.
49. Comhaire F, Messiaen A, Decleer W. A mathematical model predicting the individual outcome of IVF through sperm-analysis: the role of the Halo-SpermG2® DNA fragmentation test. Med Hypotheses 2018;117:50–3.
50. Sun TC, Zhang Y, Li HT, Liu XM, Yi DX, Tian L, et al. Sperm DNA fragmentation index, as measured by sperm chromatin dispersion, might not predict assisted reproductive outcome. Taiwan J Obstet Gynecol 2018;57: 493–8.
51. Gosálvez J, Caballero P, López-Fernández C, Ortega L, Guijarro JA, Fernández JL, et al. Can DNA fragmentation of neat or swim-up spermatozoa be used to predict pregnancy following ICSI of fertile oocyte donors? Asian J Androl 2013;15:812–8.
52. López G, Lafuente R, Checa MA, Carreras R, Brassesco M. Diagnostic value of sperm DNA fragmentation and sperm high-magnification for predicting outcome of assisted reproduction treatment. Asian J Androl 2013;15:790–4.
53. Muriel L, Garrido N, Fernández JL, Remohí J, Pellicer A, De Los Santos MJ, et al. Value of the sperm deoxyribonucleic acid fragmentation level, as measured by the sperm chromatin dispersion test, in the outcome of in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril 2006;85:371–83.
54. Bianchi PG, Manicardi GC, Bizzaro D, Bianchi U, Sakkas D. Effect of deoxyribonucleic acid protamination on fluorochrome staining and in situ nick-translation of murine and human mature spermatozoa. Biol Reprod 1993; 49:1083–8.
55. Dadoune JP, Mayaux MJ, Guihard-Moscato ML. Correlation between defects in chromatin condensation of human spermatozoa stained by aniline blue and semen characteristics. Andrologia 1988;20:211–7.
56. Esterhuizen AD, Franken DR, Lourens JGH, Prinsloo E, Van Rooyen LH. Sperm chromatin packaging as an indicator of in-vitro fertilization rates. Hum Reprod 2000;15:657–61.
57. Esterhuizen AD, Franken DR, Lourens JGH, Van Zyl C, Müller II, Van Rooyen LH. Chromatin packaging as an indicator of human sperm dysfunction. J Assist Reprod Genet 2000;17:508–14.
58. Tarozzi N, Nadalini M, Stronati A, Bizzaro D, Dal Prato L, Coticchio G, et al. Anomalies in sperm chromatin packaging: implications for assisted reproduction techniques. Reprod Biomed Online 2009;18:486–95.
59. Marchiani S, Tamburrino L, Benini F, Fanfani L, Dolce R, Rastrelli G, et al. Chromatin protamination and catsper expression in spermatozoa predict clinical outcomes after assisted reproduction programs. Sci Rep 2017;7: 15122.
60. Irez T, Dayioglu N, Alagöz M, Karatas S, Güralp O. The use of aniline blue chromatin condensation test on prediction of pregnancy in mild male factor and unexplained male infertility. Andrologia 2018;50:1–7.
61. Bonache S, Mata A, Ramos MD, Bassas L, Larriba S. Sperm gene expression profile is related to pregnancy rate after insemination and is predictive of low fecundity in normozoospermic men. Hum Reprod 2012;27:1556–67.
62. Joe YA, Park MH. Structural features of the eIF-5A precursor required for posttranslational synthesis of deoxyhypusine. J Biol Chem 1994;269: 25916–21.
63. Kenmochi N, Kawaguchi T, Rozen S, Davis E, Goodman N, Hudson TJ, et al. A map of 75 human ribosomal protein genes. Genome Res 1998;8:509–23.
64. Aston KI, Uren PJ, Jenkins TG, Horsager A, Cairns BR, Smith AD, et al. Aberrant sperm DNA methylation predicts male fertility status and embryo quality. Fertil Steril 2015;104:1388–97.e5.
65. Gunes S, Agarwal A, Henkel R, Mahmutoglu AM, Sharma R, Esteves SC, et al. Association between promoter methylation of MLH1 and MSH2 and reactive oxygen species in oligozoospermic men—a pilot study. Andrologia 2018;50:2–7.
66. Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, et al. Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. Nat Genet 1996;13:336–42.
67. Paul C, Povey JE, Lawrence NJ, Selfridge J, Melton DW, Saunders PTK. Deletion of genes implicated in protecting the integrity of male germ cells has differential effects on the incidence of DNA breaks and germ cell loss. PLoS One 2007;2:e989.
68. Karaca MZ, Konac E, Yurteri B, Bozdag G, Sogutdelen E, Bilen CY. Association between methylenetetrahydrofolate reductase (MTHFR) gene promoter hypermethylation and the risk of idiopathic male infertility. Andrologia 2017;49:1–6.
69. Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr 2012;3:21–38.
70. Wu C, Ding X, Tan H, Li H, Xiong C. Alterations of testis-specific promoter methylation in cell-free seminal deoxyribonucleic acid of idiopathic nonobstructive azoospermic men with different testicular phenotypes. Fertil Steril 2016;106:1331–7.
71. Wolgemuth DJ, Lele KM, Jobanputra V, Salazar G. The A-type cyclins and the meiotic cell cycle in mammalian male germ cells. Int J Androl 2004;27: 192–9.
72. Holt JE, Stanger SJ, Nixon B, McLaughlin EA. Non-coding RNA in Spermatogenesis and epididymal maturation. In: Wilhelm D, Bernard P, editors. Non-coding RNA and the Reproductive System. Dordrecht: Springer Netherlands; 2016:95–120.
73. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136:215–33.
74. Siomi MC, Sato K, Pezic D, Aravin AA. PIWI-interacting small RNAs: the vanguard of genome defence. Nat Rev Mol Cell Biol 2011;12:246–58.
75. Robles V, Valcarce DG, Riesco MF. Non-coding RNA research non-coding rna regulation in reproduction: their potential use as biomarkers. Non-coding RNA Res 2019;4:54–62.
76. Abu-Halima M, Hammadeh M, Backes C, Fischer U, Leidinger P, Lubbad AM, et al. Panel of five microRNAs as potential biomarkers for the diagnosis and assessment of male infertility. Fertil Steril 2014;102: 989–97.
77. Cui L, Fang L, Shi B, Qiu S, Ye Y. Spermatozoa micro ribonucleic acid–34c level is correlated with intracytoplasmic sperm injection outcomes. Fertil Steril 2015;104:312–7.

78. Wang C, Yang C, Chen X, Yao B, Yang C, Zhu C, et al. Altered profile of seminal plasma microRNAs in the molecular diagnosis of male infertility. Clin Chem 2011;57:1722–31.
79. Wu W, Qin Y, Li Z, Dong J, Dai J, Lu C, et al. Genome-wide microRNA expression profiling in idiopathic non-obstructive azoospermia: significant up-regulation of miR-141, miR-429 and miR-7-1-3p. Hum Reprod 2013; 28:1827–36.
80. Xu Y, Zhang Y, Yang Y, Liu X, Chen Y. Seminal plasma miR-210-3p is a biomarker for screening dyszoospermia caused by varicocele. Andrologia 2019;51:1–8.
81. Barceló M, Mata A, Bassas L, Larriba S. Exosomal microRNAs in seminal plasma are markers of the origin of azoospermia and can predict the presence of sperm in testicular tissue. Hum Reprod 2018;33: 1087–98.
82. Hong Y, Wang C, Fu Z, Liang H, Zhang S, Lu M, et al. Systematic characterization of seminal plasma piRNAs as molecular biomarkers for male infertility. Sci Rep 2016;6:1–10.
83. Cui L, Fang L, Shi B, Qiu S, Ye Y. Spermatozoa expression of piR-31704, piR-39888, and piR-40349 and their correlation to sperm concentration and fertilization rate after ICSI. Reprod Sci 2018;25:733–9.
84. Korbakis D, Schiza C, Brinc D, Soosaipillai A, Karakosta TD, Légaré C, et al. Preclinical evaluation of a TEX101 protein ELISA test for the differential diagnosis of male infertility. BMC Med 2017;15:1–16.
85. Tassi C, Angelini A, Beccari T, Capodicasa E. Fluorimetric determination of activity and isoenzyme composition of N-acetyl-β-D-hexosaminidase in seminal plasma of fertile men and infertile patients with secretory azoospermia. Clin Chem Lab Med 2006;44:843–7.
86. Tomasiuk R, Faundez R, Cacko M, Mikaszewska-Sokolewicz M, Cacko A, Rabijewski M. NT-proCNP as a new indicator of asthenozoospermia. Adv Med Sci 2017;62:74–7.
87. Skibinska I, Andrusiewicz M, Soin M, Jendraszak M, Urbaniak P, Jedrzejczak P, et al. Increased expression of PELP1 in human sperm is correlated with decreased semen quality. Asian J Androl 2018;20: 425–31.
88. Intasqui P, Camargo M, Antoniassi MP, Cedenho AP, Carvalho VM, Cardozo KHM, et al. Association between the seminal plasma proteome and sperm functional traits. Fertil Steril 2016;105:617–28.
89. Intasqui P, Agarwal A, Sharma R, Samanta L, Bertolla RP. Towards the identification of reliable sperm biomarkers for male infertility: a sperm proteomic approach. Andrologia 2017;50:1–11.
90. Verit FF, Verit A, Ciftci H, Erel O, Çelik H. Paraoxonase-1 activity in subfertile men and relationship to sperm parameters. J Androl 2009;30:183–9.
91. Marsillach J, Lafuente R, Checa MA, Maestre-Martínez C, Fabián E, Brassesco M, et al. Paraoxonase-1 is only present in traceable amounts in seminal fluid and does not show any relationship with male subfertility. BJU Int 2011;108:566–70.
92. Martinez-Soto JC, Landeras J, Mollá M, Mondejar I, Nicolás M, Fernández-Olmedilla L, et al. Total urokinase-type plasminogen activator (uPA) levels in seminal plasma are associated with positive assisted reproductive technology outcomes. J Assist Reprod Genet 2018;35:1091–101.
93. Bøllehuus Hansen L, Lorenzen M, Bentin-Ley U, Nielsen JE, Krog H, Berg AH, et al. Presence of the vitamin D inactivating enzyme CYP24A1 in human sperm and prediction of the success of intrauterine insemination: A prospective study. J Steroid Biochem Mol Biol 2019;191:105353.
94. Ovayolu A, Arslanbuğa CY, Gun I, Devranoglu B, Ozdemir A, Cakar SE. Can blood and semen presepsin levels in males predict pregnancy in couples undergoing intra-cytoplasmic sperm injection? Pakistan J Med Sci 2016; 32:1116–20.
95. Légaré C, Cloutier F, Makosso-Kallyth S, Laflamme N, Jarvi K, Tremblay RR, et al. Cysteine-rich secretory protein 1 in seminal plasma: potential biomarker for the distinction between obstructive and nonobstructive azoospermia. Fertil Steril 2013;100:1253–60.
96. Nagata Y, Fujita K, Banzai J, Kojima Y, Kasima K, Suzuki M, et al. Seminal plasma inhibin-B level is a useful predictor of the success of conventional testicular sperm extraction in patients with non-obstructive azoospermia. J Obstet Gynaecol Res 2005;31:384–8.
97. Mahmoud AM, Geslevich J, Kint J, Depuydt C, Huysse L, Zalata A, et al. Seminal plasma α-glucosidase activity and male infertility. Hum Reprod 1998;13:591–5.
98. Zalata A, Hafez T, Schoonjans F, Comhaire F. The possible meaning of transferrin and its soluble receptors in seminal plasma as markers of the seminiferous epithelium. Hum Reprod 1996;11:761–4.
99. Panner Selvam MK, Finelli R, Agarwal A, Henkel R. Proteomics and metabolomics — current and future perspectives in clinical andrology. Andrologia 2020;53(2):1–18.
100. Betteridge DJ. What is oxidative stress? Metabolism 2000;49:3–8.
101. Agarwal A, Sharma RK, Sharma R, Assidi M, Abuzenadah AM, Alshahrani S, et al. Characterizing semen parameters and their association with reactive oxygen species in infertile men. Reprod Biol Endocrinol 2014; 12:1–9.
102. Yumura Y, Iwasaki A, Saito K, Ogawa T, Hirokawa M. Effect of reactive oxygen species in semen on the pregnancy of infertile couples. Int J Urol 2009;16:202–7.
103. Nallella KP, Sharma RK, Allamaneni SSR, Agarwal A. Identification of male factor infertility using a novel semen quality score and reactive oxygen species levels. Clinics (Sao Paulo) 2005;60:317–24.
104. Jayasena CN, Radia UK, Figueiredo M, Revill LF, Dimakopoulou A, Osagie M, et al. Reduced testicular steroidogenesis and increased semen oxidative stress in Male partners as novel markers of recurrent miscarriage. Clin Chem 2019;65:161–9.
105. Agarwal A, Tvrda E, Sharma R. Relationship amongst teratozoospermia, seminal oxidative stress and male infertility. Reprod Biol Endocrinol 2014; 12:2–9.
106. Homa ST, Vessey W, Perez-Miranda A, Riyait T, Agarwal A. Reactive oxygen species (ROS) in human semen: determination of a reference range. J Assist Reprod Genet 2015;32:757–64.
107. Sharma RK, Pasqualotto FF, Nelson DR, Thomas AJ, Agarwal A. The reactive oxygen species - Total antioxidant capacity score is a new measure of oxidative stress to predict male infertility. Hum Reprod 1999;14:2801–7.
108. Roychoudhury S, Sharma R, Sikka S, Agarwal A. Diagnostic application of total antioxidant capacity in seminal plasma to assess oxidative stress in male factor infertility. J Assist Reprod Genet 2016;33:627–35.
109. Agarwal A, Roychoudhury S, Bjugstad KB, Cho CL. Oxidation-reduction potential of semen: what is its role in the treatment of male infertility? Ther Adv Urol 2016;8:302–18.
110. Agarwal A, Wang SM. Clinical relevance of oxidation-reduction potential in the evaluation of male infertility. Urology 2017;104:84–9.
111. Agarwal A, Sharma R, Roychoudhury S, Du Plessis S, Sabanegh E. MiOXSYS: a novel method of measuring oxidation reduction potential in semen and seminal plasma. Fertil Steril 2016;106:566–73.e10.
112. Agarwal A, Selvam MKP, Arafa M, Okada H, Homa S, Killeen A, et al. Multicenter evaluation of oxidation-reduction potential by the MiOXSYS in males with abnormal semen. Asian J Androl 2019;21:565–9.
113. Agarwal A, Henkel R, Sharma R, Tadros NN, Sabanegh E. Determination of seminal oxidation–reduction potential (ORP) as an easy and cost-effective clinical marker of male infertility. Andrologia 2017;50:1–8.
114. Agarwal A, Roychoudhury S, Sharma R, Gupta S, Majzoub A, Sabanegh E. Diagnostic application of oxidation-reduction potential assay for measurement of oxidative stress: clinical utility in male factor infertility. Reprod Biomed Online 2017;34:48–57.
115. Chianese R, Pierantoni R. Mitochondrial reactive oxygen species (ROS) production alters sperm quality. Antioxidants 2021;10:1–19.
116. Gupta A, Mahdi AA, Ahmad MK, Shukla KK, Jaiswer SP, Shankhwar SN. 1H NMR spectroscopic studies on human seminal plasma: a probative discriminant function analysis classification model. J Pharm Biomed Anal 2011;54:106–13.
117. Zhang X, Diao R, Zhu X, Li Z, Cai Z. Metabolic characterization of asthenozoospermia using nontargeted seminal plasma metabolomics. Clin Chim Acta 2015;450:254–61.
118. Camargo M, Intasqui P, Bruna de Lima C, Montani DA, Nichi M, Pilau EJ, et al. Maldi-tof fingerprinting of seminal plasma lipids in the study of human male infertility. Lipids 2014;49:943–56.

119. Rivera-Egea R, Garrido N, Sota N, Meseguer M, Remohí J, Dominguez F. Sperm lipidic profiles differ significantly between ejaculates resulting in pregnancy or not following intracytoplasmic sperm injection. J Assist Reprod Genet 2018;35:1973–85.
120. Tang B, Shang X, Qi H, Li J, Ma B, An G, et al. Metabonomic analysis of fatty acids in seminal plasma between healthy and asthenozoospermic men based on gas chromatography mass spectrometry. Andrologia 2017;49:1–13.
121. Qiufang Z, Quan B, Yang Y, Ping L, Jie Q. Assessment of seminal estradiol and testosterone levels as predictors of human spermatogenesis. J Androl 2010;31:215–20.
122. Tunc O, Thompson J, Tremellen K. Development of the NBT assay as a marker of sperm oxidative stress. Int J Androl 2010;33:13–21.
123. Søreide K. Receiver-operating characteristic curve analysis in diagnostic, prognostic and predictive biomarker research: trade-off between sensitivity and specificity with change of test cut-offs. J Clin Pathol 2009;62:1051.

Revisión sistemática en la identificación de biomarcadores de fertilidad en el semen: un abordaje clínico a través de las ómicas para el diagnóstico de la infertilidad masculina.

Objetivo: Identificar los biomarcadores moleculares más robustos en el semen y plasma seminal para el diagnóstico de la infertilidad masculina y evaluar su utilidad clínica.

Diseño: Revisión sistemática.

Lugar: No aplica.

Paciente(s): Se incluyeron en esta revisión estudios accesibles que reportan poblaciones (in)fértiles bien definidas y biomarcadores moleculares seminales.

Intervención(es): Se realizó una búsqueda sistemática de la literatura publicada en las bases de datos de MEDLINE-PubMed y EMBASE, siguiendo las guías de Elementos de informe preferidos para revisiones sistemáticas y metanálisis (PRISMA).

Medida de resultado(s) principal(es): Los resultados primarios fueron el contenido, la expresión o actividad de los biomarcadores moleculares en las muestras de semen humano. Solo se incluyeron estudios que reportan valores de análisis de características operativas del receptor (ROC).

Resultado(s): Se incluyeron ochenta y nueve estudios. La evaluación directa del daño en el DNA de los espermatozoides tiene un alto potencial como biomarcador diagnóstico de fertilidad y los resultados de las tecnologías de reproducción asistida (área por debajo de la curva [AUCs] mediana = 0.67). Con respecto a la rotura de hebras asociadas a modificaciones de la cromatina, los niveles de H2AX muestran buenos valores predictivos para el diagnóstico de la infertilidad masculina (AUCs mediana = 0.93). Algunos ácidos ribonucleicos (ARN) no codificantes exhiben buenos valores predictivos; el miR-34c-5p en semen es el biomarcador transcriptómico mejor caracterizado y robusto (AUCs median = 0.78). Mientras que muchas proteínas en el semen muestran valores pobres para el diagnóstico de la calidad seminal y la capacidad de fecundación, tales como TEX101 en el plasma seminal tiene un excelente potencial de diagnóstico (AUCs mediana = 0.69). Aunque, metabolitos individuales y perfiles metabolómicos en el plasma seminal presentan buenos valores predictivos, los últimos parecen ser mejores que los primeros a la hora de inferir sobre la calidad seminal y la capacidad de fecundación.

Conclusión(es): La revisión actual apoya que algunas ómicas (por ejemplo, la integridad y estructura del ADN, genómica y epigenómica, transcriptómica, metabolómica y proteómica) podrían ser consideradas biomarcadores moleculares relevantes que ayuden a identificar etiologías de infertilidad y pronóstico de fecundación con diagnósticos costo-efectivos, simples y precisos.