Molecular Diagnosis & Therapy
https://doi.org/10.1007/s40291-021-00524-8

REVIEW ARTICLE

Omics in Seminal Plasma: An Effective Strategy for Predicting Sperm Retrieval Outcome in Non-obstructive Azoospermia

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Accepted: 17 March 2021

Abstract
Non-obstructive azoospermia (NOA) is a severe form of male factor infertility resulting from the impairment of sperm production. Surgical sperm retrieval followed by intracytoplasmic sperm injection (ICSI) is the only alternative for NOA patients to have their own genetic children. Nevertheless, due to an approximately 50% chance of success, harvesting sperm from these patients remains challenging. Thus, discovering noninvasive biomarkers, which are able to reliably predict the probability of sperm acquisition, not only can eliminate the risk of surgery but also can lower the costs of NOA diagnosis and treatment. Seminal plasma is the non-cellular and liquid portion of the ejaculate that consists of the secretions originating from testes and male accessory glands. In past years, a wide range of biomolecules including DNAs, RNAs, proteins, and metabolic intermediates have been identified by omics techniques in human seminal plasma. The current review aimed to briefly describe genomic, transcriptomic, proteomic, and metabolomic profiles of human seminal plasma in an attempt to introduce potential candidate noninvasive biomarkers for sperm-retrieval success in men with NOA.

1 Introduction

Azoospermia is a disorder related to male fertility that is characterized by the absence of sperm in two successive semen samples. Approximately 1% of the total male population and 10–15% of cases with male infertility suffer from this disorder. According to the impairment in the production and delivery of sperm, azoospermia is generally divided into non-obstructive azoospermia (NOA) and obstructive azoospermia (OA) categories, respectively [1].

From the prevalence point of view, NOA, which comprises 60% of patients with azoospermia, is more frequent

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Published online: 15 April 2021

Key Points

In contrast to mRNAs, seminal plasma miRNAs and especially lncRNAs might be useful noninvasive biomarkers for predicting the outcome of surgical sperm extraction in non-obstructive azoospermia (NOA) patients.

Protein biomarkers identified by proteomic analyses of seminal plasma have so far demonstrated moderate predictive efficiency for the outcome of surgical sperm extraction in NOA patients.

than OA. Regardless of the idiopathic causes, the etiologies of NOA can be split into two main categories: (1) hypogonadotropic hypogonadism and (2) spermatogenesis impairment. The former condition has pre-testicular causes and arises from hypothalamic-pituitary-gonadal axis derangements, leading to inadequate testicular stimulation by pituitary gonadotropins. Conversely, spermatogenesis impairment has different testicular causes including genetic abnormalities (Klinefelter syndrome and Y chromosome microdeletions), congenital malformations (cryptorchidism), or environmental factors (radio/chemotherapy, genital trauma, and mumps orchitis). In this condition, interventional sperm retrieval followed by intracytoplasmic sperm injection (ICSI) is the only alternative for NOA patients to father their genetic children [2].

In NOA cases, sperm is frequently present only in testicular tissue and is generally hard to acquire. Numerous approaches have been introduced to retrieve sperm from NOA patients surgically, of which conventional testicular sperm extraction (cTESE), microdissection testicular sperm extraction (micro-TESE), and fine-needle aspiration (FNA) map-guided TESE are three common methods. However, clinically utilizable sperm can currently be obtained from almost 50% of patients with NOA [3].

With regard to the restricted success rate of sperm-retrieval approaches in patients with NOA, discovering noninvasive parameters, which are able to reliably predict the probability of sperm acquisition, not only can eliminate the risk of surgery but also can lower the costs of NOA diagnosis and treatment. To date, a variety of clinical, laboratory, and histopathological parameters have been considered as possible predictors for sperm-retrieval rate in NOA men [4]. Nevertheless, none of these parameters, alone or in combination, have presented a reliable and satisfactory estimation for the likelihood of successful sperm acquisition, other than the detection of Y chromosome microdeletions in AZFa/b sequences, which is mostly associated with failure in sperm recovery. However, a small proportion (5–10%) of azoospermia cases result from Y chromosome microdeletions [5], limiting their utility to a subpopulation of NOA patients. The histopathological pattern of testes also contains useful predictive information regarding sperm-retrieval rate, as the prognosis of hypospermatogenesis, followed by maturation arrest, for a successful outcome is better than Sertoli cell-only syndrome [6]. Nevertheless, taking a testicular biopsy with the aim of identifying histopathological characteristics endangers the success of subsequent sperm-retrieval attempt [6]. Indeed, the comparison of sperm recovery outcomes between NOA patients with and without a history of testicular biopsies revealed that successful sperm extraction was more probable in individuals with no previous biopsy and significantly attenuated with increasing frequency of performing the biopsy [7]. Therefore, in clinical practice, there is still a need for comprehensive and noninvasive marker(s) to distinguish the subgroup of NOA patients with testicular spots of complete spermatogenesis.

Seminal plasma in combination with spermatozoa are the two major constituents of normal semen. It is the non-cellular and liquid portion of the ejaculate that consists of the secretions originating from testes and male accessory glands, i.e., epididymis, prostate, and seminal vesicles. In the past years, a wide range of biomolecules including DNAs, RNAs, proteins, and metabolic intermediates have been identified in human seminal plasma [8]. With the significant advances in omics technologies, the analyses of seminal plasma via these techniques may hold the key to finding accurate and noninvasive predictors for the sperm-retrieval outcome in NOA patients (Fig. 1). The current review aimed to briefly describe genomic, transcriptomic, proteomic, and metabolomic profiles of human seminal plasma in an attempt to introduce potential candidate noninvasive biomarkers for sperm-retrieval success in men with NOA. For this purpose, PubMed was extensively searched using combination of the subject terms “azoospermia,” “seminal plasma,” “TESE,” “biomarkers,” “DNA,” “RNA,” “proteomics,” and “metabolomics.” After screening titles and abstracts, studies investigating omics-based biomarkers in NOA patients with a particular emphasis on the prediction of sperm-retrieval outcome by TESE were found to be eligible for inclusion.

2 Genomic Profile of Seminal Plasma

Body fluids are known to contain a large number of degraded DNA fragments, called cell-free DNA [9]. Although the mechanism of their presence in body fluids is not fully understood yet, it is speculated that cell-free DNA results from apoptosis, necrosis, and active secretion. Due to the

size resemblance between apoptotic DNA and cell-free DNA fragments, apoptosis is considered as one of the origins of cell-free DNA. Furthermore, the lengthy fragments of cell-free DNA (over 1000 bp) are attributed to necrotic events. In addition, the DNA content of exosomes or DNA-lipoprotein complexes is hypothesized to be released by cells as a result of active secretion [10]. At present, genomic profiling of cell-free DNA is attracting a lot of interest in the diagnosis and prognosis of various pathological conditions [9]. With regard to the high rate of apoptosis (up to 75% of potential spermatozoa) among testicular germ cells during spermatogenesis, semen is thought to have high amounts of cell-free DNA [11].

To determine the general characteristics of seminal cell-free DNA, Li et al. [12] analyzed the quantity and size distribution of cell-free DNA in semen samples from normozoospermic ($n = 11$) and NOA ($n = 9$) individuals. According to the authors, remarkably larger amounts of cell-free DNA were present in seminal plasma compared to other body fluids, with an average concentration of 1.34 and 2.56 µg/mL in normozoospermic and NOA subjects, respectively, indicating significantly higher concentrations of cell-free DNA in seminal plasma from NOA patients. Furthermore, and in the case of size distribution, a range of multiples of 180 bp DNA fragments were obtained from each seminal plasma specimen. Interestingly, this pattern is the characteristic of genomic DNA, which is degraded during apoptosis by caspase-activated DNase [13]. Given that the high levels of seminal cell-free DNA in NOA men and the apoptotic origin of 180 bp DNA multiples, the authors hypothesized that elevated levels of cell-free DNA in seminal plasma from azoospermic patients may stem from massive pathological apoptosis of spermatogenic germ cells in testes, implying an association between the characteristics of seminal cell-free DNA and spermatogenesis status [12]. However, as far as we know, no study has confirmed this hypothesis to date. Moreover, regarding the high concentration and wide range of size distribution, seminal cell-free DNA can be appropriate for many molecular biology techniques such as DNA mutation, methylation, and oxidation. Overall, cell-free DNA in seminal plasma may offer the opportunity to develop possible noninvasive biomarkers for predicting the presence of residual spermatogenesis in NOA patients intending to undergo TESE operation.

3 Transcriptomic Profile of Seminal Plasma

3.1 Germ Cell-Specific mRNAs

Human seminal plasma contains large quantities of cell-free messenger RNAs (mRNAs) with sufficient durability that originate from the various tissues of the male reproductive system. Of these divergent transcripts, the presence of germ cell-exclusive mRNAs in semen can be the hallmark of germ cell existence within the testes [14]. Thus, the identification of transcripts in seminal plasma, which are specifically expressed at certain stages of meiotic maturation, can potentially serve as sensitive and specific biomarkers for the existence of germ cells with respective maturation stages in the testes. In this regard, Aslani et al. [15] evaluated semen samples from 203 azoospermic men in terms of the expression of stage-exclusive genes including deleted in azoospermia (*DAZ*), A-kinase anchoring protein-4 (*AKAP4*), protamine-1 (*PRM1*), and protamine-2 (*PRM2*). According to the authors, there was no significant correlation between the seminal expression of relevant genes and sperm recovery outcomes using the TESE procedure. However, the expression of *DAZ* and *PRM2* was associated with the presence of either spermatozoa or spermatids in the testicular tissue specimens, suggesting the potential applicability of *DAZ* and *PRM2* transcripts as noninvasive predictors of late-stage germ cell existence in the testes. By contrast, Eghbali et al. [16] observed that not only was *DAZ* mRNA absent in 60% and 38.5% of semen samples from men with hypospermatogenesis and maturation arrest, respectively, but that it was present in the semen of 16.5% of patients with germ cell aplasia. These results indicate that seminal DAZ expression is incapable of reliably predicting germ cell presence in the testes. Similar disagreement with testicular histopathological findings was also seen for seminal expression of other germ cell-exclusive mRNAs including testis-specific Y-encoded protein 1 (*TSPY1*), spermatocyte/spermatid-specific thioredoxin-3 (*SPTRX3*), and spermatid-specific thioredoxin-1 (*SPTRX1*), implying that these transcripts are of little predictive value as biomarkers for spermatogenesis status. In a similar study, Pansa et al. [17] examined whether the presence of extraembryonic spermatogenesis homeobox 1 (*ESX1*) mRNA in the seminal fluid can serve as a useful indicator of residual spermatogenesis and positive sperm recovery in a population of 56 NOA patients. According to the authors, spermatozoa were retrieved from 26 out of 44 (59%) and five out of 12 (42%) patients with positive and negative seminal *ESX1* expression, respectively; which corresponds to a predictive sensitivity of 84% for successful sperm extraction. However, and despite this good sensitivity, the seminal expression of *ESX1* displayed a poor prognostic specificity (28%) for positive TESE outcome, which means that it would not be able to prevent potentially unsuccessful surgical interventions. Furthermore, the level of *ESX1* expression in the semen did not show a significant association with the severity of spermatogenesis deficiency, as evidenced by histopathological examination. In a more recent study, Hashemi et al. [18] assessed germ cell-specific mRNAs in seminal fluid, including *ESX1*, zinc finger MYND-containing protein 15 (*ZMYND15*), transition

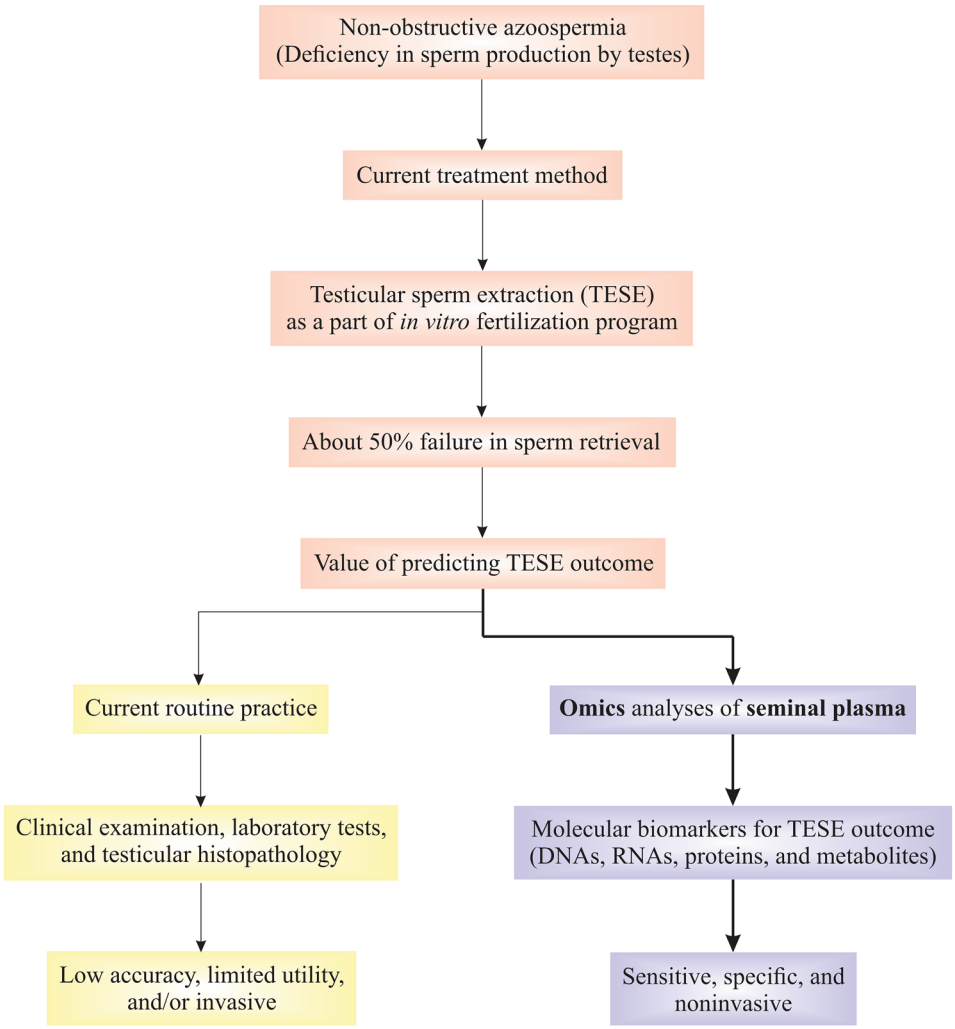

Fig. 1 Flowchart representing the study idea. Testicular sperm extraction (TESE) followed by in vitro fertilization is the sole therapeutic option for men with non-obstructive azoospermia (NOA), a pathologic condition associated with spermatogenic impairment, to father their biologic children. Since TESE is a surgical intervention and fails to retrieve spermatozoa in ~ 50% of cases, pre-surgical prediction of its outcome is of clinical importance. Clinical examination, laboratory tests (e.g., Y chromosome deletions), and testicular histopathology are currently used as sperm-retrieval predictors, but they have several shortcomings including low accuracy, limited utility, and/or invasiveness. Given that seminal plasma provides a non-invasive and enriched source of molecular biomarkers (including DNAs, RNAs, proteins, and metabolites) derived from testicular germ cells, its analysis by omics technologies can lead to the identification of sensitive, specific, and noninvasive biomarkers for the chance of sperm retrieval from by TESE in NOA patients

protein 1 (*TNP1*), and *PRM1* to predict sperm-retrieval rate in 19 NOA men. Except for ESX1, the expression levels of other mRNAs were significantly lower in NOA patients than in normozoospermic men. Importantly, all of the mRNAs were present in higher quantities in the semen of men with successful sperm recovery compared to men with failed sperm recovery, but statistical significance was only seen in the cases of *TNP1* and *PRM1*. Of these two mRNAs, *PRM1* presented better sensitivity (89%), specificity (90%), and overall prognostic accuracy (0.89) for positive sperm retrieval, suggesting its potential to be verified as an efficient biomarker for predicting TESE outcome preoperatively in future studies.

Several studies have examined the potential utilization of seminal *DDX4* (DEAD [Asp-Glu-Ala-Asp] box polypeptide 4; also recognized as VASA) mRNA as a noninvasive distinguishing biomarker for the existence of germ cells, including spermatozoa, in the testes. For instance, by analyzing a total of 145 NOA patients with certain biopsy histopathology, Li and colleagues [19, 20] found that *DDX4* transcript existed in the semen of all patients with hypospermatogenesis, maturation arrest, and incomplete Sertoli cell-only histopathological phenotypes. However, while it was expected to be absent in the semen of men with complete Sertoli cell-only, around 25–50% of them were positive for seminal *DDX4* mRNA. Regarding the heterogeneity of spermatogenesis and the specificity of *DDX4* to germ cells, the authors claim that patients with Sertoli cell-only histopathology and positive seminal *DDX4* do not actually suffer from Sertoli cell-only syndrome and have germ cells in their testes. In addition, Abdallah et al. [21] saw further inconsistencies between the results of seminal *DDX4* expression and testicular histopathology in a cohort of 39 NOA patients. Accordingly, not only were 41.2% of patients with complete Sertoli cell-only diagnosis positive for seminal *DDX4* transcript but 65% and 77% of men with maturation arrest and incomplete Sertoli cell-only diagnoses did not express seminal *DDX4*, questioning the predictive accuracy of seminal *DDX4* mRNA as a biomarker for germ cell existence in the testes. Regardless of germ cell prediction, seminal DDX4 also displayed

a poor prognostic value for the mTESE outcome, as of 19 NOA patients expressing *DDX4* in their semen, spermatozoa were recovered from six subjects (almost 42%) [20]. This most probably stems from the fact that *DDX4* transcription is not necessarily exclusive to mature spermatozoa, but to spermatogenic cells in general [22].

Taken together, evidence on the potential of germ cell-specific mRNAs as predictive biomarkers for the sperm-retrieval rate by TESE operation is highly conflicting or insufficient. Moreover, there is evidence of amplification failure in a considerable portion (around 50%) of semen samples from NOA men, possibly due to the degradation of mRNAs from 3′ terminus [16, 18], which can further hinder the applicability of seminal mRNAs in predicting TESE outcome. Therefore, there is a need for additional prospective, randomized, and multicenter studies with large sample sizes to demonstrate the exact potential of seminal mRNAs for predicting the presence of spermatozoa in the testes as well as to determine the efficiency of RNA extraction and cDNA synthesis from seminal plasma. We speculate that the selection of candidate mRNAs in such studies should be made based on their expression timeline during spermatogenesis, as mRNAs with specific expression at the final stages of spermatogenesis can more accurately predict sperm-retrieval rate whereas mRNAs with ubiquitous expression throughout the spermatogenic stages can be more appropriate predictive biomarkers for the existence of germ cells in general.

3.2 MicroRNAs

MicroRNAs (miRNAs) are small (18–25 nucleotides) non-coding RNA molecules, which are implicated in the post-translational regulation of gene expression by triggering translation inhibition or mRNA degradation. Investigations have indicated that miRNAs perform crucial functions in various types of biological activities such as cellular multiplication, differentiation, and programmed death [23, 24]. As expected, miRNAs substantially contribute to germ cell development and spermatogenesis [25, 26]. In addition to the intracellular milieu, miRNAs are found in extracellular spaces, particularly in various biofluids such as seminal plasma [27]. It has been shown that seminal cell-free miRNAs exist in the form of protein-bonded complexes in particular, but also as microvesicular bodies [28]. This distribution results in the protection of seminal miRNAs against degradation of existent RNase enzymes, allowing them to serve as potential noninvasive indicators of spermatogenesis status. Analyzing the presence of two miRNAs, *miR-19b* and *let-7a*, in the semen of 192 infertile men showed that the expression levels of both miRNAs, particularly *miR-19b* (6.3-fold over-expression), were significantly elevated in patients with NOA, but not with oligozoospermia, compared to fertile men [29]. An additional study by the same group also revealed significantly higher expression levels of *miR-141*, *miR-429*, and *miR-7-1-3p* in seminal plasma from NOA individuals compared to fertile controls [30]. These results suggest that the profile of seminal plasma miRNAs differs between NOA and fertile men, and can be of value as a noninvasive sensitive and specific biomarker for the diagnosis of impaired spermatogenesis.

To find useful biomarkers for differential diagnosis of azoospermia as well as the presence of intra-testicular spermatozoa, Barceló et al. [31] recently determined the seminal plasma-derived exosomal miRNA fingerprint in men with normozoospermia, secretory azoospermia (SA), and OA. The authors identified 393 miRNAs in seminal plasma, among which 60 miRNAs had distinctive levels of expression in azoospermic patients compared to normozoospermic ones. The comparison of miRNA fingerprints between OA and SA disclosed two miRNAs, *miR-205-5p* and *miR-31-5p*, as well as a germ cell-exclusive *piR-58527* with a marked under-expression in OA subjects compared to SA ones. Importantly, individual expression levels of these three small noncoding RNAs were associated with the diagnosis of OA with a robust predictive efficiency, attaining superior diagnostic accuracy (~ 0.96) in the case of *miR-31-5p*. Accordingly, *miR-31-5p* could successfully discriminate the incidence of OA from SA with a sensitivity and specificity of 92.9% and 80%, respectively, implying the potential applicability of *miR-31-5p* as a distinguishing biomarker for etiology of azoospermia. Moreover, although seminal expression levels of neither miRNAs nor the piRNA was single-handedly associated with sperm recovery rate from SA patient, concomitant expression values of *miR-539-5p* and the *miR-941* were capable of predicting the existence of intra-testicular spermatozoa with outstanding sensitivity and specificity (both 100%). However, it should be noted that aside from a very small sample size (12 individuals), sperm positive samples were obtained from men with either NOA or cryptozoospermia (a condition of existing very few spermatozoa in the ejaculate) rather than only NOA patients. In addition, and in some ways interestingly, neither of the aforementioned miRNA biomarker candidates were specifically expressed in testicular tissue, as the expression of *miR-205-5p* in epididymis and prostate and *miR-31-5p*, *miR-539-5p*, and *miR-941* in the three reproductive organs (testis, epididymis, and prostate) was evidenced. Furthermore, a recent study found that *miR-539-5p* not only was devoid of predictive value for the recovery of testicular spermatozoa from NOA patients but also had a poor diagnostic efficacy for discriminating normozoospermic, oligozoospermic, and azoospermic men from each other [32]. Altogether, though seminal plasma miRNAs have been hypothesized to be useful predictive biomarkers for TESE outcome in NOA men, their predictive efficacy is currently a matter of debate and needs to be corroborated by prospective, randomized, and

multicenter studies including a large population of NOA men undergoing TESE.

3.3 Long Noncoding RNAs

Long noncoding RNAs (lncRNAs) are a subset of gene transcripts composed of more than 200 nucleotides that do not code proteins. LncRNAs significantly participate in various physiological and pathological processes by regulating gene expression at the transcriptional, post-transcriptional, and translation levels. Although little evidence exists about the role of lncRNAs in human spermatogenesis, studies on other species demonstrated testis-specific and dynamic expression of numerous lncRNAs during the process of male gametogenesis, suggesting their substantial contribution to both typical and atypical spermatogenesis [33]. Recently, Bo et al. [34] analyzed the expression profile of lncRNAs and their competing endogenous RNA network in testicular tissues derived from azoospermic men in order to elucidate the actions and modes of action of lncRNAs on human spermatogenesis and NOA pathology. According to the authors, lncRNAs were in association with multiple biological activities and signaling cascades implicating in spermatogenesis process such as "male gamete generation," "meiotic cell cycle," and "resolution of sister chromatid cohesion." Additionally, and importantly, the copy numbers of some lncRNAs exhibited a significant correlation with Johnsen score, a grading system for spermatogenesis capability based on testicular histopathology, proposing that a panel of particular testicular lncRNAs can serve as biomarkers for the existence and stage of spermatogenesis in patients with NOA. Concerning the presence of lncRNAs in seminal plasma and their differential expression profile under different spermatogenic situations [35], profiling the expression pattern of lncRNAs in seminal plasma in preference to testicular tissue can be considered as a noninvasive prognostic approach for the sites of focal spermatogenesis in testes.

Recently, Xie et al. [32] tested this hypothesis that exosomal seminal plasma lncRNAs could be useful for predicting the presence of spermatozoa in testicular biopsies of NOA patients. They identified a total of 88 differentially expressed-exosomal lncRNAs (including 62 downregulated and 26 upregulated lncRNAs) in NOA men compared to normozoospermic individuals using RNA sequencing, of which 19 lncRNAs are specifically expressed in testes. Based on the prognostic accuracy of individual lncRNAs in terms of sperm-retrieval outcome, the authors constructed and verified a predictive panel consisting of nine lncRNAs including *LOC100505685*, *SPATA42*, *CCDC37-DT*, *GABRG3-AS1*, *LOC440934*, *LOC101929088* (XR-927561.2), *LOC101929088* (XR-001745218.1), *LINC00343*, and *LINC00301* in training ($n = 30$) and validation ($n = 66$) sets of NOA men. Accordingly, by using a cutoff value of 0.532, they obtained prognostic accuracies of 0.986 and 0.960, sensitivities of 88.9% and 93.5%, and specificities of 100.0% and 90.0% for the panel in training and validation sets, respectively. Moreover, the panel showed the ability to accurately predict successful sperm recovery in 95.238% of NOA patients ($n = 96$) whose scores of exosomal lncRNAs panel were higher than 0.532, suggesting that at scores exceeding cutoff value, the performance of mTESE might be recommended, while at scores lower than 0.532 surgical intervention could be avoided. Overall, these results indicated the reliability of exosomal seminal plasma lncRNAs as predictors of sperm-retrieval rate by mTESE in NOA patients and their potential applicability in assisting clinicians with clinical decision-making. However, and similar to previous transcriptomic biomarkers, the predictive efficiency of seminal plasma lncRNAs for the presence of testicular spermatozoa in NOA men needs to be addressed in future prospective, randomized, and multicenter studies.

4 Proteomic Profile of Seminal Plasma

Over the past few years, considerable progress in genome sequencing and bioinformatics with concomitant development of accurate, precise, and reliable measurement techniques has profited proteomics, making it a robust tool for testing complicated physiological fluids and exploring novel biomarkers relevant to special pathological conditions [36]. In this regard, and considering that seminal plasma can open up a possibility for noninvasive diagnosis, the proteomic profile of seminal plasma has been suggested as an advantageous biomarker with superior diagnostic value for male reproductive disorders. Accordingly, Drabovich et al. [37] proposed a proteomic signature of 16, 3, and 11 candidate biomarkers with the ability to discriminate between three pathophysiological situations, normozoospermia, NOA, and OA. In a similar work, Légaré et al. [38] examined if the measurement of cysteine-rich secretory protein-1 (CRISP1) in seminal plasma would differentiate between OA and NOA in a cohort of 80 azoospermic patients. They found that at a cutoff value of 0.655 for relative intensity, CRISP1 was capable of differentiating OA patients from NOA ones with a sensitivity of 92%, specificity of 85%, and cumulative diagnostic power of 0.9286, highlighting the great discriminative value of seminal CRISP1 between OA and NOA patients and its potential to be a possible biomarker for differential diagnosis of azoospermia. However, despite the significant difference between OA and NOA patients, the authors did not observe markedly different levels of CRISP1 when comparing normozoospermic individuals with NOA ones, showing that seminal plasma CRISP1 can be an indicator of obstruction in the genital tract rather than active testicular spermatogenesis.

Regarding the association of seminal plasma proteins with sperm-retrieval outcome by TESE, Roshdy and Mostafa [39] observed that while modest levels of survivin were present in seminal plasma of NOA men with a positive TESE outcome, it was undetectable in specimens of NOA patients with a negative outcome. Likewise, it has been reported that seminal plasma clusterin levels were associated with the outcome of mTESE in 28 men with NOA and thus could serve as a possible biomarker for sperm-retrieval rate [40]. With the intent of identifying predictive biomarkers for focal spermatogenesis in men suffering from NOA, Freour et al. [36] determined the proteomic signature of seminal plasma from 40 patients. The authors found 12 proteins with significantly differential patterns of expression between individuals with dissimilar sperm retrieval outcome. Of these differentially expressed candidates, they selected lectin galactoside-binding, soluble 3 binding protein (LGALS3BP) as a biomarker of choice for further analyses. Accordingly, the amounts of seminal LGALS3BP were significantly higher in men with desirable TESE outcomes than in those with unfavorable results, and at a cutoff point of 153 ng/mL were predictive of successful sperm retrieval with great sensitivity (100%), but low specificity (45%). Similarly, screening of 18 proteins as potential predictor candidates in seminal plasma from 77 azoospermic men resulted in the identification of two proteins, epididymis-specific Extracellular matrix protein 1 (ECM1) and testis-specific testis expressed 101 (TEX101), with the capability of discriminating the etiology of azoospermia and predicting the chance of sperm recovery [41]. In detail, ECM1 values less than 2.3 µg/mL yielded a sensitivity, specificity, and cumulative diagnostic power of 100%, 73%, and 0.94, respectively, for discriminating OA patients from NOA ones. Furthermore, seminal plasma TEX101 could differentiate between Sertoli cell-only subjects and those with either hypospermatogenesis or maturation arrest histopathology, which could be helpful for predicting patients with poor odds of successful sperm recovery. Although the predictive value of ECM1 for sperm-retrieval rate in NOA patients remains to be elucidated, a more recent study by Korbakis et al. [42] evaluated whether seminal plasma TEX101 could be a biomarker for the presence of sperm or spermatid in the testes of NOA men (n = 26). Accordingly, with a cutoff point of 0.6 ng/mL, TEX101 exhibited a predictive accuracy, sensitivity, and specificity of 0.69, 73%, and 64%, respectively, which corresponds to a moderate prognostic efficacy and has no superiority over conventional biomarkers such as serum FSH. In another study regarding the proteomic profile of seminal plasma, Cui et al. [43] provided a comparison between NOA patients with normal serum FSH and a group of NOA men with elevated serum FSH. According to the authors, there were 12 proteins with differential expression levels between the groups, predominantly belonging to extracellular regions. However, glutathione S-transferase M3 (GSTM3) and phosphoglycerate kinase 2 (PGK2), as intracellular proteins, displayed over-expression in the normal FSH group compared to the elevated FSH counterpart, suggesting that these proteins may be potential candidates for discriminating NOA subjects with a differential panel of serum FSH and predicting the chance of sperm retrieval by TESE procedure. Collectively, although numerous protein candidates have been proposed as biomarkers for screening the patients with azoospermia, the prognostic power of most of them in terms of testicular sperm recovery is so far undetermined and remains to be established in future studies.

5 Metabolomic Profile of Seminal Plasma

Metabolomics is a set of techniques that assess a wide range of metabolism-produced small intermediate compounds, namely metabolites, in biological specimens for comprehensive biological fingerprinting of metabolic status [44]. Metabolomics is an ultimate reflection of gene and protein expression and a better representative of the genuine phenotype. The characterization of male sterility by using metabolomics methods has been fascinating in recent years [45]. In this respect, Zhang et al. [46] have successfully differentiated the sufferers of NOA from healthy individuals by profiling serum metabolomics. They screened and detected a total of 24 metabolites as possible biomarkers, which primarily are implicated in energy production, redox status, and programmed cell death during spermatogenesis. Moreover, the authors found that patients with NOA have disruptions in numerous metabolic pathways including the citric acid cycle, pyruvate metabolism, and alanine, aspartate, and glutamate metabolism. These findings support the potential of the metabolomic approaches for predicting spermatogenesis status in the testes.

By employing untargeted metabolomic profiling, Gilany et al. [47] identified 36 distinct metabolites in the seminal plasma of a cohort of 20 NOA men between TESE negative and positive groups and claimed that these metabolites can potentially be applied as specific indicators for various categories of NOA patients. For instance, owing to their significantly higher concentrations in TESE negative groups compared with both TESE positive and fertile men, tartaric acid, 4,5-Dimethoxy-1,2-benzenedicarboxylic acid (a derivative of phthalic acid), and three dietary flavors, namely 2,2,4,4,6,6-hexamethyl-1,3,5-trithiane, 6,6-dimethyl-(1S)-bicyclo(3.1.1)hept-2-ene-2-methanol, and 2-pyrrolidine acetic acid, emerged as discriminative metabolites for the TESE negative group. However, the clinical value of these metabolites is questionable since they have not been validated by reference standards and do not exist in the Human Metabolome Database. In another recent study

Table 1 Omics-based biomarkers of seminal plasma with respect to the prediction of sperm retrieval outcome in men with NOA

References	Study population	Technique	Results
Genomics			
[12]	Nine men with NOA	qPCR	Due to appropriate concentration and size distribution, cell-free seminal DNA offers a possibility of sperm retrieval prediction based on DNA mutation, methylation, and oxidation analyses
Transcriptomics			
[15]	203 men with azoospermia	RT-PCR	Concomitant expression of DAZ and PRM2 in seminal plasma predicted the presence of late-stage (spermatid or later) germ cells in testicular biopsies
[17]	56 men with NOA	RT-qPCR	Seminal ESX1 expression resulted in a sensitivity and specificity of 84% and 28%, respectively, for successful sperm retrieval, respectively
[18]	19 men with NOA	RT-qPCR	TNP1 and PRM1 displayed AUCs of 0.87 and 0.89, sensitivities of 87%, and specificities of 54% and 90% for successful sperm retrieval, respectively
[20]	106 men with NOA	RT-PCR	Seminal DDX4 expression acted as an indicator of germ cell existence in the testes, thereby abolished incorrect diagnosis of Sertoli cell-only syndrome
[31]	12 men with secretory azoospermia	RT-qPCR	Concomitant expression of miR-539-5p and the miR-941 predicted the presence of spermatozoa in testicular biopsies with a sensitivity and specificity of 100%
[32]	96 men with NOA	RNA sequencing and RT-qPCR	A predictive panel consisting of nine exosomal lncRNAs demonstrated an AUC of 0.986. At a cutoff value of 0.532, sensitivity and specificity were 88.9% and 100%, respectively. The panel accurately predicted sperm retrieval rate by TESE in 95.238% of the study population
Proteomics			
[36]	40 men with NOA	ICPL, ELISA	Seminal LGALS3BP levels higher than 153 ng/mL were predictive of successful sperm retrieval with a sensitivity and specificity of 100% and 45%, respectively
[39]	37 men with NOA	ELISA	Survivin was present in seminal plasma of NOA men with positive sperm recovery, whereas it was not detected in samples of patients with a negative outcome
[40]	28 men with NOA	ELISA	Seminal plasma clusterin appeared as a predictor of sperm retrieval by mTESE in univariate analysis, but its significance did not persist after multivariate analysis
[41]	27 men with NOA	MS	Seminal TEX101 levels lower than 5 ng/mL discriminated Sertoli-cell only syndrome from other histopathological categories
[42]	26 men with NOA	ELISA	With a cutoff point of 0.6 ng/mL, seminal TEX101 was predictive of TESE outcome with AUC, sensitivity, and specificity of 0.69, 73%, and 64%, respectively
Metabolomics			
[47]	20 men with NOA	GC-MS	36 differentiating metabolites were identified as prognostic biomarkers for positive and negative sperm-retrieval outcomes
[48]	20 men with NOA	Raman spectrometry	Metabolic fingerprint, especially oxidative status, significantly differed between TESE positive and negative men

AUC area under the curve, *ELISA* enzyme-linked immunosorbent assay, *GC-MS* gas chromatography-mass spectrometry, *ICPL* isotope-coded protein label, *MS* mass spectrometry, *NOA* non-obstructive azoospermia, *qPCR* quantitative polymerase chain reaction, *RT-PCR* reverse transcription polymerase chain reaction, *RT-qPCR* quantitative reverse transcription polymerase chain reaction, *TESE* testicular sperm extraction

by the same group, 20 NOA men were dichotomized into negative and positive sperm-retrieval groups according to metabolomic fingerprinting of seminal plasma via Raman spectroscopy [48]. Interestingly, metabolomic fingerprints of sperm-positive NOA men displayed an overlap with those of fertile individuals. Furthermore, there was a possibility to subdivide the sperm-negative group into three distinct classes, which might be attributed to hypospermatogenesis,

maturation arrest, and Sertoli cell-only syndrome in order of proximity to metabolic fingerprints of sperm-positive and fertile groups. In comparison with TESE positive groups, an imbalance in oxidative status was also noted in TESE negative patients. Overall, these findings suggest that metabolomic fingerprinting of seminal plasma not only can serve as a noninvasive procedure for realizing testicular spermatogenesis before the operation but can also yield histopathological information about negative sperm-retrieval cases. However, these results should be interpreted cautiously since they have been acquired by examining a small cohort of the population with NOA.

Fructose is an essential metabolite of seminal plasma that is implicated in the energy provision of mature spermatozoa [49]. According to Lei et al.'s [50] findings, the concentration of seminal plasma fructose is related to histologic patterns of testes in patients with azoospermia and hence may be beneficial to make predictions about the pathologic cause of male sterility. Furthermore, seminal plasma fructose has been demonstrated to potentially act as a biomarker for differentiating non-obstructive azoospermic subjects from obstructive ones, as NOA patients exhibited higher levels of fructose in their seminal plasma compared to OA men [51]. However, from the sperm-retrieval viewpoint, the difference did not achieve statistical significance for seminal plasma fructose upon the classification of azoospermic individuals based on the existence or lack of spermatozoa in their testicular biopsies [52].

6 Conclusion and Future Prospects

The lack of accurate markers to pre-surgically estimate the sperm-retrieval rate in men with NOA imposes health and financial burdens on these patients, especially on some in whom TESE approaches fail to harvest spermatozoa. Thus, developing sensitive and specific markers for the success of sperm retrieval in NOA men is of particular clinical importance. The application of basic sciences to fill clinical needs (currently known as translational medicine) may provide a good opportunity to find such markers. In this context, the current review collected evidence on the potential capability of seminal plasma genome, transcriptome, proteome, and metabolome to predict the sperm recovery outcome in NOA (Table 1).

Despite its appropriate concentration and stability, studies on the predictive value of seminal plasma cell-free DNA in terms of testicular sperm acquisition are yet to be carried out. With regard to seminal plasma transcriptome, current data suggest that germ cell-specific mRNAs have not been shown to be useful for the prediction of sperm retrieval rate, which at least in part could be due to their susceptibility to degradation. By contrast with mRNAs, seminal plasma miRNAs might accurately predict the presence of sperm in the testicular biopsies of NOA patients, among which miR-539-5p and the miR-941 showed relatively good predictive competence and deserve further investigation. In light of current evidence, exosomal seminal plasma lncRNAs demonstrated impressive superiority over other omics-based biomarkers in terms of predicting sperm-retrieval rate by mTESE and thus translation to clinical practice, but it should be kept in mind that their prognostic capability still needs to be confirmed. Evidence on the seminal plasma proteome also identified LGALS3BP and TEX101 as potential biomarkers, though additional large sample-sized studies are needed to directly compare them between men with positive and negative sperm retrieval and confirm their efficacy. Finally, the metabolomic fingerprint of seminal plasma emerged as a promising tool for the prognosis of active spermatogenesis foci in the testicular biopsies from NOA men, but there is limited evidence in this respect, and validation studies are still required. It is of interest to note that in addition to biomarkers described by individual omics techniques, integration of data obtained from genomics, transcriptomics, proteomics, and metabolomics studies can open a new window into the discovery of seminal plasma biomarkers predicting sperm-retrieval outcome. Importantly, integrative multi-omics analyses not only can identify single biomarkers but also can bring about the development of biomarker panels, which generally offer far superior diagnostic and prognostic efficiency in comparison with individual biomarkers. Overall, the continuation of omics studies on the seminal plasma in order to verify current potential predictors and/or identify new candidates with better predictive accuracy opens up an opportunity to establish reliable biomarkers for sperm-retrieval success and better counsel patients with NOA.

Declarations

Funding This work was not supported by any external funding.

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and material Not applicable.

Code availability Not applicable.

Authors' contributions RZ: investigation, writing—original draft. SN: writing—original draft. HO: investigation. YR: investigation. DJ: investigation. YA: writing—review and editing. MN: project administration. AF: conceptualization, writing—review and editing. RD: supervision.

References

1. Wosnitzer M, Goldstein M, Hardy MP. Review of azoospermia. Spermatogenesis. 2014;4(1):e28218.
2. Flannigan R, Bach PV, Schlegel PN. Microdissection testicular sperm extraction. Transl Androl Urol. 2017;6(4):745–52.
3. Shin DH, Turek PJ. Sperm retrieval techniques. Nat Rev Urol. 2013;10(12):723–30.
4. Bernie AM, Ramasamy R, Schlegel PN. Predictive factors of successful microdissection testicular sperm extraction. Basic Clin Androl. 2013;23(1):5.
5. Cerván-Martín C, Palomino-Morales C. Genetic landscape of nonobstructive Azoospermia and new perspectives for the clinic. J Clin Med. 2020;9(2):300.
6. Arshad MA, Majzoub A, Esteves SC. Predictors of surgical sperm retrieval in non-obstructive azoospermia: summary of current literature. Int Urol Nephrol. 2020;52(11):2015–38.
7. Ramasamy R, Schlegel PN. Microdissection testicular sperm extraction: effect of prior biopsy on success of sperm retrieval. J Urol. 2007;177(4):1447–9.
8. Cannarella R, Barbagallo F, Crafa A, La Vignera S, Condorelli RA, Calogero AE. Seminal plasma transcriptome and proteome: towards a molecular approach in the diagnosis of idiopathic male infertility. Int J Mol Sci. 2020;21(19):7308.
9. Stewart CM, Kothari PD, Mouliere F, Mair R, Somnay S, Benayed R, et al. The value of cell-free DNA for molecular pathology. J Pathol. 2018;244(5):616–27.
10. Chen Q, Zhang Z-H, Wang S, Lang J-H. Circulating cell-free DNA or circulating tumor DNA in the management of ovarian and endometrial cancer. Oncotargets Ther. 2019;12:11517–30.
11. Shaha C, Tripathi R, Mishra DP. Male germ cell apoptosis: regulation and biology. Philos Trans R Soc B Biol Sci. 2010;365(1546):1501–15.
12. Li H-G, Huang S-Y, Zhou H, Liao A-H, Xiong C-L. Quick recovery and characterization of cell-free DNA in seminal plasma of normozoospermia and azoospermia: implications for non-invasive genetic utilities. Asian J Androl. 2009;11(6):703–9.
13. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res. 2001;61(4):1659–65.
14. Huang S, Li H, Ding X, Xiong C. Presence and characterization of cell-free seminal RNA in healthy individuals: implications for noninvasive disease diagnosis and gene expression studies of the male reproductive system. Clin Chem. 2009;55(11):1967–76.
15. Aslani F, Modarresi MH, Soltanghoraee H, Akhondi MM, Shabani A, Lakpour N, et al. Seminal molecular markers as a non-invasive diagnostic tool for the evaluation of spermatogenesis in non-obstructive azoospermia. Syst Biol Reprod Med. 2011;57(4):190–6.
16. Eghbali M, Sadeghi MR, Lakpour N, Edalatkhah H, Zeraati H, Soltanghoraee H, et al. Molecular analysis of testis biopsy and semen pellet as complementary methods with histopathological analysis of testis in non-obstructive azoospermia. J Assist Reprod Genet. 2014;31(6):707–15.
17. Pansa A, Sirchia SM, Melis S, Giacchetta D, Castiglioni M, Colapietro P, et al. ESX1 mRNA expression in seminal fluid is an indicator of residual spermatogenesis in non-obstructive azoospermic men. Hum Reprod. 2014;29(12):2620–7.
18. Hashemi M-S, Mozdarani H, Ghaedi K, Nasr-Esfahani MH. Could analysis of testis-specific genes, as biomarkers in seminal plasma, predict presence of focal spermatogenesis in non-obstructive azoospermia? Andrologia. 2020;52(2):e13483.
19. Li H, Wu C, Gu X, Xiong C. A novel application of cell-free seminal mRNA: non-invasive identification of the presence of germ cells or complete obstruction in men with azoospermia. Hum Reprod. 2012;27(4):991–7.
20. Yu Q, Gu X, Shang X, Li H, Xiong C. Discrimination and characterization of Sertoli cell-only syndrome in non-obstructive azoospermia using cell-free seminal DDX4. Reprod Biomed Online. 2016;33(2):189–96.
21. Abdallah W, Hashad D, Abdelmaksoud R, Hashad MM. Does detection of DDX4 mRNA in cell-free seminal plasma represents a reliable noninvasive germ cell marker in patients with nonobstructive azoospermia? Andrologia. 2017;49(8):e12739.
22. Kossack N, Terwort N, Wistuba J, Ehmcke J, Schlatt S, Schöler H, et al. A combined approach facilitates the reliable detection of human spermatogonia in vitro. Hum Reprod. 2013;28(11):3012–25.
23. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281–97.
24. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet. 2004;5(7):522–31.
25. Hayashi K, de Sousa Lopes SMC, Kaneda M, Tang F, Hajkova P, Lao K, et al. MicroRNA biogenesis is required for mouse primordial germ cell development and spermatogenesis. PLoS ONE. 2008;3(3):e1738.
26. Bouhallier F, Allioli N, Lavial F, Chalmel F, Perrard M-H, Durand P, et al. Role of miR-34c microRNA in the late steps of spermatogenesis. RNA. 2010;16(4):720–31.
27. Weber JA, Baxter DH, Zhang S, Huang DY, How Huang K, Jen Lee M, et al. The MicroRNA spectrum in 12 body fluids. Clin Chem. 2010;56(11):1733–41.
28. Li H, Huang S, Guo C, Guan H, Xiong C. Cell-free seminal mRNA and MicroRNA exist in different forms. PLoS ONE. 2012;7(4):e34566.
29. Wu W, Hu Z, Qin Y, Dong J, Dai J, Lu C, et al. Seminal plasma microRNAs: potential biomarkers for spermatogenesis status. Mol Hum Reprod. 2012;18(10):489–97.
30. 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(7):1827–36.
31. 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(6):1087–98.
32. Xie Y, Yao J, Zhang X, Chen J, Gao Y, Zhang C, et al. A panel of extracellular vesicle long noncoding RNAs in seminal plasma for predicting testicular spermatozoa in nonobstructive azoospermia patients. Hum Reprod. 2020;35(11):2413–27.
33. Sahlu BW, Zhao S, Wang X, Umer S, Zou H, Huang J, et al. Long noncoding RNAs: new insights in modulating mammalian spermatogenesis. J Anim Sci Biotechnol. 2020;11(1):16.
34. Bo H, Liu Z, Zhu F, Zhou D, Tan Y, Zhu W, et al. Long noncoding RNAs expression profile and long noncoding RNA-mediated competing endogenous RNA network in nonobstructive azoospermia patients. Epigenomics. 2020;12(8):673–84.
35. Lu H, Xu D, Wang P, Sun W, Xue X, Hu Y, et al. RNA-sequencing and bioinformatics analysis of long noncoding RNAs and mRNAs in the asthenozoospermia. Biosci Rep. 2020. https://doi.org/10.1042/BSR20194041.
36. Freour T, Com E, Barriere P, Bouchot O, Jean M, Masson D, et al. Comparative proteomic analysis coupled with conventional protein assay as a strategy to identify predictors of successful testicular sperm extraction in patients with non-obstructive azoospermia. Andrology. 2013;1(3):414–20.
37. Drabovich AP, Jarvi K, Diamandis EP. Verification of male infertility biomarkers in seminal plasma by multiplex selected reaction monitoring assay. Mol Cell Proteom. 2011;10(12):M110.004127.

38. 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(5):1253–60.
39. Roshdy N, Mostafa T. Seminal plasma survivin in fertile and infertile males. J Urol. 2009;181(3):1269–72.
40. Fukuda T, Miyake H, Enatsu N, Matsushita K, Fujisawa M. Seminal level of clusterin in infertile men as a significant biomarker reflecting spermatogenesis. Andrologia. 2016;48(10):1188–94.
41. Drabovich AP, Dimitromanolakis A, Saraon P, Soosaipillai A, Batruch I, Mullen B, et al. Differential diagnosis of azoospermia with proteomic biomarkers ECM1 and TEX101 quantified in seminal plasma. Sci Transl Med. 2013;5(212):212ra160.
42. 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):60.
43. Cui Z, Agarwal A, da Silva BF, Sharma R, Sabanegh E. Evaluation of seminal plasma proteomics and relevance of FSH in identification of nonobstructive azoospermia: a preliminary study. Andrologia. 2018;50(5):e12999.
44. Courant F, Antignac J-P, Monteau F, Le Bizec B. Metabolomics as a potential new approach for investigating human reproductive disorders. J Proteome Res. 2013;12(6):2914–20.
45. Kovac JR, Pastuszak AW, Lamb DJ. The use of genomics, proteomics, and metabolomics in identifying biomarkers of male infertility. Fertil Steril. 2013;99(4):998–1007.
46. Zhang Z, Zhang Y, Liu C, Zhao M, Yang Y, Wu H, et al. Serum metabolomic profiling identifies characterization of non-obstructive azoospermic men. Int J Mol Sci. 2017;18(2):238.
47. Gilany K, Mani-Varnosfaderani A, Minai-Tehrani A, Mirzajani F, Ghassempour A, Sadeghi MR, et al. Untargeted metabolomic profiling of seminal plasma in nonobstructive azoospermia men: a noninvasive detection of spermatogenesis. Biomed Chromatogr. 2017;31(8):e3931.
48. Gilany K, Jafarzadeh N, Mani-Varnosfaderani A, Minai-Tehrani A, Sadeghi MR, Darbandi M, et al. Metabolic fingerprinting of seminal plasma from non-obstructive azoospermia patients: positive versus negative sperm retrieval. J Reprod Infertil. 2018;19(2):109–14.
49. Reisman L, Park L, Neri QV, Paniza T, Rosenwaks Z, Palermo GD. Seminal Fluid Biomarkers and IUI Clinical Outcome. Hum Reprod. 2015;30:146–7.
50. Lei B, Lv D, Zhou X, Zhang S, Shu F, Ding Y, et al. Biochemical hormone parameters in seminal and blood plasma samples correlate with histopathologic properties of testicular biopsy in azoospermic patients. Urology. 2015;85(5):1074–8.
51. Lei B, Xing R, Zhou X, Lv D, Wan B, Shu F, et al. Neutral alpha-1,4-glucosidase and fructose levels contribute to discriminating obstructive and nonobstructive azoospermia in Chinese men with azoospermia. Andrologia. 2016;48(6):670–5.
52. Soudabeh S, Ali MA, Mahshid H, Mohammad Mehdi A, Haleh S, Naser A, et al. Comparing seminal plasma biomarkers between normospermic and azoospermic men. J Reprod Infertil. 2010;11(1):39–46.