Genomic and Proteomic Approaches in the Diagnosis of Male Infertility

17

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Abbreviations

AZF	Azoospermia factor
CGH	Comparative genomic hybridization
CNV	Copy number variation
HBPs	Heparin-binding proteins
NOA	Nonobstructive azoospermia
OA	Obstructive azoospermia
SNP	Single nucleotide polymorphisms

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Introduction

Despite rapid growth in the understanding of the genetic basis for male infertility, much remains poorly defined. Current estimates indicate that genetic abnormalities contribute to 15–30 % of male infertility [1, 2]. Many men have their conditions uncharacterized and are subsequently diagnosed as idiopathic infertility. It has been postulated that these men actually have unrecognized genetic aberrations [1, 2]. Unfortunately, even with the current genetic tools at a clinician's disposal (i.e., karyotype, Y-chromosome microdeletion assay, cystic fibrosis testing), there are many genetic causes that remain unrecognized.

Men with oligospermia and non-obstructive azoospermia (NOA) have a known predisposition to genetic abnormalities and comprise 40–50 % of all infertile men [3]. Current guidelines recommend genetic testing when either sperm density is <5 million/mL, NOA is present, or there are clinical signs of an abnormality [4]. The limitations of contemporary testing are reflected in the growth of recognized genomic and proteomic contributors towards male infertility [2]. Indeed, while much work needs to be done, it appears that future utilization of genetic evaluations will be determined with more direct delineation. As such, the current chapter aims to provide a background regarding the genetic tests that are currently available to clinicians investigating male infertility. Furthermore, advanced methodologies are discussed with recent advances in genomics and proteomics highlighted.

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Genomics: Chromosomal Abnormalities

Karyotype

The advent of modern genetic techniques has led to a rapid proliferation of tests and technologies that have the potential to alter the treatment of male infertility. However, in spite of large numbers of individuals affected by male infertility, genetic analysis has been slow to identify causes. The ability to identify genetic causes of male infertility is beneficial several reasons. First, by understanding the genetic basis of disease, one can hope to develop novel treatments for the future. Second, determination of signal transduction pathways underlying male infertility may yield better comprehension of mechanisms of disease. Importantly, investigators today believe that the majority of male infertility has a genetic basis [5]. Lastly, when using in vitro fertilization, natural selection is bypassed, thus opening up the possibility of transmitting unknown diseases to offspring. If clinicians can identify and control for these changes, risks for transmission would be decreased.

With respect to genetic testing, the karyotype was one of the earliest techniques developed for assessing human chromosomes. Using light microscopy, the number and appearance of chromosomes as well as variations in DNA composition of >4 megabases (Mb) in size became possible [6]. The technique documented the basics of human disease with the identification of numerical defects such as Down's syndrome (extra chromosome 21) and Turner's syndrome (XO) identified in the early 1950s [7, 8]. With regards to male subfertility, karyotypic chromosomal abnormalities was shown to occur at 5 times greater rates compared to the normal population [9]. In men with NOA, the prevalence numerical and structural chromosomal abnormalities is ~10–15 % [10] whereas in men with severe oligospermia (defined as <5 million sperm/mL), this rate correspondingly decreases and approaches ~5 % [11, 12]. Over the years, as the technology and accuracy has expanded, these numbers have increased. Most recently, Yatsenko and colleagues recorded that >11 % of men with NOA had abnormalities identified on karyotype [10]. Interestingly, men with normal sperm concentrations demonstrated <1 % prevalence of karyotype-associated abnormalities [11, 12] while the frequency of karyotypic abnormalities amongst infertile men is ~12.6 % [13].

Karyotype is currently recommended in men with NOA or severe oligospermia (<5 million/mL) [4]. In azoospermic men, sex chromosome abnormalities predominate, whereas in oligospermic men, autosomal anomalies (i.e., Robertsonian and reciprocal translocations) are more frequent [11]. Chromosomal inversions in autosomes 1, 3, 4, 6, 9, 10, and 21 are also more common in infertile men [14].

Klinefelter's syndrome (KS) represents the most common genetic cause and karyotypic abnormality found in infertile men (47, XXY). Present in 11 % of men with azoospermia, KS occurs in 1 of 500 live births [11, 15, 16]. The majority (95 %) of affected males present in adulthood with infertility [17]. Most will have normal libido and erectile function with only 25 % demonstrating characteristic KS features of gynecomastia, tall stature, and small firm testes (8–10 cm^3) [18, 19].

KS results from a meiotic nondisjunction event in most cases; however, up to 3 % of men with KS are mosaic 46,XX/47,XXY [15, 18]. Mosaic males tend to have less severe phenotypic changes and many may be fertile. Spermatogenesis is typically profoundly affected in non-mosaic KS resulting in azoospermia in most with ~8.4 % of men may having sperm in the ejaculate [20–22]. In addition, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels are markedly increased. FSH is increased in response to abnormal spermatogenesis with an increase in LH reflecting maximal simulation of Leydig cells to produce androgen [20–23].

Karyotypic diagnosis is essential when KS is suspected since these patients are at increased risk for breast cancer, non-Hodgkin lymphoma, extragonadal germ cell tumors, and likely lung cancer [24–26]. Spermatogenic potential declines with advancing age in KS patients; however, the best approach to the adolescent with KS and adequate

virilization is currently unclear [19, 27–29]. Some have suggested testicular sperm extraction with cryopreservation of sperm or testicular tissue [30] while others have argued in favor of waiting for extraction in coordination with IVF-ICSI when paternity is desired [31]. Another concern in men with KS is the high rates of sperm aneuploidy [27, 31–33]. Despite these issues, many 46, XX and 46, XY live births have been reported in the literature [34–36]. Micro-TESE, coupled with ICSI, has proven to be a successful strategy for the majority of patients with azoospermia and KS [15].

There are no universally agreed-upon clinical or laboratory findings that predict successful sperm retrieval in KS; however, testis volume, testosterone level and age <35 are generally thought to be positive indices [19, 29, 37, 38]. Unfortunately, the primary difficulty with karyotypic analysis is that baseline resolution of the technique is unable to detect small DNA aberrations and as personalized medicine comes to the forefront, newer techniques are supplementing the karyotype.

Fluorescence In Situ Hybridization

A more advanced test compared to the karyotype focuses on fluorescent probes that are able to detect and localize specific DNA sequences on chromosomes [39]. This technique, termed Fluorescence in situ Hybridization (FISH), was developed to detect sperm aneuploidy as well as to determine the presence/absence of specific DNA sequences [40]. Sperm FISH is unaffected by functional deficiencies [39] and while it assesses defects in men with normal karyotypes (described above), it is limited by the cost of commercially available probes. Specifically, chromosomes X, Y, 13, 18, and 21 are the main probes used in sperm FISH since alterations in these chromosomes results in viable offspring [6]. The test is thus unable to detect aberrations in other chromosomes beyond these limited few, because of cost constraints.

As a method of further clarifying genetic abnormalities, FISH is used clinically as an adjunct to the karyotype. Some have proposed that FISH should be used to more accurately identify men with mosaic Klinefelter's syndrome [41]. Indeed, retrospective and prospective studies have noted that elevated aneuploidy obtained via sperm FISH correlated to fetal aneuploidy and IVF failure [39, 42, 43]. At the present time, sperm FISH is used as a screening tool as well as for patient counseling and clinical decision making. In certain situations and depending upon the clinical diagnosis, preimplantation genetic diagnosis and ICSI could be used to select genetically unaffected embryos.

Genomics: Gene Mutations

Cystic Fibrosis

Congenital bilateral absence of the vas deferens (CBAVD) is found in ~1 % of infertile males and up to 6 % of those with obstructive azoospermia [1, 19]. CBAVD is due to a mutation in the *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) gene located on chromosome 7 [44, 45] and results from gene mutations that cause cystic fibrosis (CF) or alterations in the genetic mechanisms controlling mesonephric duct differentiation [19]. CF is an autosomal recessive disease, affecting 1 in 1,600 people of Northern European background. It occurs with variable frequency in different geographic and ethnic populations. Genetic testing typically accounts for ethnicity and recognizes >850 genetic variants associated with CF [23, 46–50]. Most cases of CBAVD result from mutations in both the maternal and paternal copies of the genes that encode for the CFTR. Eighty percent of azoospermic men with CBAVD and one-third of men with unexplained obstruction will have CFTR mutations [51, 52]. The prevalence of CFTR mutations is increased in men with azoospermia related to congenital bilateral obstruction of the epididymis and those with unilateral vasal agenesis [4].

CBAVD is reliably diagnosed on physical exam with vasa absent bilaterally and seminal vesicles classically absent or atrophic.

Occasionally, the seminal vesicles can be large and cystic [19]. Testis size is also preserved and correspondingly, spermatogenesis is unaltered. The efferent ductules and caput of the epididymis are present and full with fluid from the testis. Transrectal ultrasound may reveal absence of the ampullae of the vas deferens or seminal vesicle abnormalities [53].

CFTR encodes an ion channel that maintains the viscosity of epithelial secretions via regulation of the sodium/chloride balance [19]. Analysis of the ejaculate will reveal thin, watery, low volume (<1.5 mL), and acidic (pH 6.5–7.0) fluid, as it is comprised primarily of prostatic secretions [19]. Pulmonary and pancreatic function in patients with CBAVD is unaltered [44]. Nearly all men with clinically detected CF demonstrate CBAVD [23, 54].

Significant genotypic differences are seen in CF and CBAVD. In males with CBAVD, the majority (~88 %) has a severe mutation resulting in absent CFTR function in combination with an allelic mutation that preserves some CFTR function [23, 55–57]. A three-base-pair deletion of CFTR, termed ΔF508, is the most common mutation found in both CF and CBAVD [19, 57]. When the patient is ΔF508 homozygous, clinical CF is apparent whereas CBAVD commonly results from a polymorphism within intron 8, sometimes termed the 5T allele, coupled with a ΔF508 mutation [19, 57, 58]. Several studies have demonstrated variable penetrance of the 5T allele, which results in a lowered efficiency of splicing that subsequently lowers levels of CFTR mRNA and protein required for maintenance of normal function [23, 55–57].

Failure of appropriate mesonephric duct differentiation before week 7 of gestation may underlie a second genetic etiology of CBAVD [19, 59]. If an isolated, unilateral injury occurs to one of the developing mesonephric ducts, unilateral renal and vasal agenesis may be present. In contrast, the presence of a genetic aberration that compromises mesonephric duct differentiation would affect both renal and reproductive ductal units, as in Potter's syndrome [19, 59]. Indeed, some patients may have unilateral vasal agenesis due to a non-cystic-fibrosis mediated embryologic defect, which is associated with unilateral absence of the kidney. A renal ultrasound is therefore indicated in these patients [60]. Unilateral renal atrophy/dysgenesis can also be associated with ipsilateral hydroureter and ectopic insertion into other genitourinary structures such as the seminal vesicles [61].

In patients found to have an abnormality on CFTR testing, the partner should similarly be screened. Microsurgical or percutaneous sperm retrieval in coordination with in vitro fertilization and intracytoplasmic sperm injection (IVF-ICSI) remains an option for these couples. If the partner is a carrier of a CF mutation, preimplantation genetic diagnosis can be employed to prevent the transfer of any embryos that will be predicted to have CF or CBAVD. Failure to detect a CFTR mutation in either partner does not exclude the presence of a mutation, which is not identifiable by routine analysis performed by most clinical genetics laboratories for diagnosing CF and not CBAVD, and therefore the progeny of the couple remains at some risk unless the entire gene is sequenced. Patients demonstrating CFTR mutations should therefore be referred for genetic counseling prior to IVF [62, 63].

Genomics: Y-Chromosome Microdeletions

The Y chromosome contains 60 million base pairs and is composed of a short arm (Yp) and a long arm (Yq). The *SRY* gene is located on Yp and is essential to sex-specific embryogenesis and determination of the bipotential gonad [19, 64–66]. The male-specific region of the Y-chromosome (MSY) is the chromosomal material bridging the two polar pseudoautosomal regions, located at the tips of Yp and Yq, and comprises 95 % of the entirety of the Y chromosome [64, 65]. Many of the genes in this MSY region are poorly characterized but are involved in spermatogenesis. Included in the MSY region are three important zones that influence spermatogenesis. These Azoospermia Factor (AZF) regions are recognized as AZFa, (proximal), AZFb (central), and AZFc (distal). Known spermatogenesis genes

within these confines include *USP9Y* and *DBY* in AZFa and *DAZ*, *RBMY1*, and *BPY2* in AZFb and AZFc [19, 64–66].

There are eight palindromic sequences throughout the length of the Yq and, as the MSY region has no genetic partner sequence to pair or repair, it is postulated that this organization helps to maintain the genetic integrity of the Y chromosome [19, 64–67]. Sub-segments within these palindromic sequences, known as amplicons, can occasionally fuse resulting in loss of all intervening chromosomal material [19, 64, 65, 67]. When this occurs, it is termed a microdeletion, as despite the loss of a large magnitude of genetic material, it is undetectable on a karyotypic analysis [19, 67]. The subsequent genes within this sequence are lost, resulting in impaired spermatogenesis and possibly other undefined consequences.

The overall prevalence of Y chromosome microdeletions in patients with sperm counts greater than 5×10^6/mL is low (~0.7 %) [68]. A rate that increases to 4 % in oligospermic men and 11 % in azoospermic men [68]. Other studies have identified microdeletions in 6–12 % of men with impaired spermatogenesis—a value that can increase to 16 % in men with azoospermia [69]. Within the AZF regions, AZFc deletions are the most common, being seen in 13 % of men with NOA and 6 % of severely oligospermic men [19, 70, 71]. The *DAZ* (Deleted in Azoospermia) gene, which encodes a transcription factor present in men with normal fertility, resides in the AZFc region. In contrast, microdeletions within the AZFa region occur in approximately 1 % of NOA men and do not involve any of the aforementioned palindromic sequences [19].

The location of AZF deletions impacts the likelihood of spermatogenesis and is prognostic in regards to the success of micro-TESE. Men with AZFc microdeletions have quantitatively impaired spermatogenesis with either severe oligospermia or azoospermia. The quality of sperm produced is typically normal in terms of fertilization, embryo development, and live birth [19, 72]. The level of spermatogenesis is typically stable among individuals, and micro-TESE with ICSI remains a therapeutic option [4, 19, 73]. In contrast, deletions of the AZFa or AZFb regions portend a very poor prognosis for sperm retrieval [19, 74, 75]. In a study by Hopps et al. [76], a total of 78 men with AZF deletions were analyzed with respect to the ability to identify sperm following diagnostic testes biopsies or TESE. Men with an isolated AZFc deletion had sperm identified in 56 % of cases [76].

With regards to heredity, men with Y-chromosome microdeletions will pass the abnormality to their sons who consequently may also be infertile. Although limited data exists, microdeletions of the Y-chromosome are known to have minor somatic health consequences (i.e., permanent tooth size [77] and short stature [78]) or testicular abnormalities [19]. It is possible however, that transmission of AZF microdeletions may have unrecognized consequences to offspring. Couples may elect to forgo use of the partner's sperm, utilize the ejaculated or testicular sperm for IVF-ICSI or elect for preimplantation genetic screening to transfer only female embryos. Therefore, men exhibiting NOA or severe oligospermia should be offered a Y-chromosome microdeletion assay and genetic counseling prior to pursuing micro-TESE for IVF-ICSI [19]. Indeed, molecular studies of patients with Y-chromosome microdeletions have shown previously unknown Y structural variations in NOA men [79].

Infertile men can have other Y chromosome structural abnormalities including, ring Y, truncated Y, isodicentric Y and various other mosaic states which may be present on karyotype analysis [10, 11, 19, 80, 81]. Early work hypothesized that the Y-chromosome contained a region that was initially thought to contain no X-Y crossing over; however, it has recently been shown to have extensive recombination and is termed the male-specific region (MSY) [64]. This area is flanked by pseudoautosomal regions (PAR) where X-Y crossing over is normal [64]. Indeed, Y-chromosome microdeletions can also include PAR defects causing genetic disorders such as SHOX [82]. The sequencing of the MSY region has been conducted [64] and further studies have found that high mutation rates resulting in structural polymorphisms in the human Y-chromosome exist with selective constraints possible [83].

In all cases, a Y-chromosome microdeletion assay is a necessary complementary test to determine the presence of the AFZ regions and direct counseling [10, 11, 19, 80].

Genomics: Advanced Techniques

Given the limits of detection and resolution of the above-mentioned techniques (karyotype, FISH, etc.), new approaches are being developed that test the current limits of genomic resolution. One of these involves detection of Copy number variations (CNVs). CNVs are defined as small (~1 kb) pieces of DNA that vary between individuals. Affecting ~20 % of the human genome, CNVs are either additions/duplications or deletions within the genome [84] that are critical sources of genetic diversity. Given that they lie within regions that are potentially invisible to karyotype analysis, novel techniques were developed to assess the impact of CNVs on human disease.

Array comparative genomic hybridization (aCGH) is one approach that focuses on single nucleotides in the human genome. It has the capacity to identify both small and large-scale changes by examining the relative quantities of DNA between samples. Gene copy number are optimally analyzed and depicted as a function of chromosome location with fluorescence identifying copy number gain or loss [85]. In the context of the microarray platform, resolution of aCGH has improved to <1 kb [86] with the ability to scale the testing in order to perform thousands of experiments in a single run [6]. Indeed, genome-wide assays are gradually replacing karyotyping for prenatal genetic diagnoses [87].

While aCGH has been applied to numerous malignancies including those of the breast, nasopharynx, ovary, stomach and bladder, others are using the technology to probe for alterations in infertile men [88]. Array CGH has already been used in the context of male infertility to identify Y-chromosome microdeletions in infertile males [82]. An earlier study ascertained whether CNVs were involved in patients with oligospermia/azoospermia compared to controls [89]. Several genes and genomic regions were identified on autosomes and sex-chromosomes that were theorized to be involved in spermatogenesis [89]. While the authors could not identify any large CNV (>1 Mb) variants between men with infertility; 11 CNVs in severe oligospermia and 4 CNVs in men with azoospermia (i.e., *EPHA3*, *PLES*, *DDX11*, *ANKS1B*) were identified in more than one patient suggesting that these regions were potential candidates for infertility genes [89]. Defects in the pseudoautosomal regions (PARs) of the Y-chromosome cause genomic disorders such as SHOX that can be affiliated with infertility, mental and stature disorders and subsequently transmitted to offspring [82].

Another technique that has recently benefitted from significant technological improvement is gene-expression DNA microarray. The primary advantage of DNA microarray technology is the ability to perform simultaneous analysis of thousands of genes at the same time [90]. By generating a large amount of data, DNA-microarrays require modern computational and statistical bioanalytic and bioinformatics approaches. The power of the technique lies in the ability to provide a snapshot of all transcriptional activity in a sample.

Preliminary studies by Sha et al. [91] utilizing cDNA microarrays identified 101 candidate fertility genes. Lin et al. [92] expanded on these early findings by pooling cDNA from testicular biopsy samples grouped by pathology. More recently, Malcher et al. [93] utilized testicular biopsy samples from controls and men with NOA. Gene expression found 4,946 differentially expressed genes with SPACA4 and CAPN11 significantly downregulated in infertile patients [93]. Interestingly, SPACA4 (or SAMP14) has been found in the sperm acrosome and postulated to be involved with sperm–egg interactions [94] with CAPN11 potentially involved in cytoskeletal remodeling during spermatogenesis [93, 95].

Unfortunately, previous studies examining gene expression have been mostly conducted in cellular homogenates obtained from testicular biopsy specimens. As such, the comparisons between patients with SCO and controls are essentially classifying cellular heterogeneity. Indeed, given that spermatocytes and spermatids have high rates of RNA synthesis [96], their

presence in the control population affects all genetic outputs analyzed [97]. Interestingly, Yatsenko et al. [98] previously assessed genes involved in meiosis for mutations using the long-living residual RNA found in mature sperm from semen ejaculate. If examining whole-system alterations, an alternative approach would be to examine tissue fibroblasts. This method allows determination of conserved pathways to be more thoroughly examined while not being affected by the presence, or absence of germ cells [97]. Future studies using DNA microarrays are currently being conducted with results poised to highlight signal transduction pathways unique to human male infertility.

Genomics: Epigenetics

Epigenetics, the study of genetic alterations due to indirect modifications of the DNA sequence, is gaining prominence as a mechanism to regulate male fertility. Since it is crucial for sperm to be correctly arranged and programmed, epigenetic modifications have the potential to evoke system-wide changes. For example, DNA-binding proteins as well as DNA methylation are just two of the epigenetic variations that have the potential to alter genetic code without directly affecting the DNA sequence. In this context, the regulation of transcription and gene expression can be appropriately, or inappropriately, modified.

The most well described epigenetic factors in the realm of male infertility has so far focused on protamines and packaging of the sperm genome [99–101]. Indeed, a critical component of spermatogenesis involves chromatin packaging during which ~85 % of the histones are replaced with protamines [102]. Alterations in protamine [103] may thus result in improper post-translational processing and subsequently decreased sperm counts, motility, morphology and increased DNA fragmentation [100, 101, 103]. Two types of human protamines (PRM), PRM-1 and PRM-2, have been identified [103] with alterations in the timing or ratio of expression resulting in arrested spermatogenesis and infertility [100, 101]. Indeed, men with asthenospermia have been shown to have lower levels of PRM-1 and PRM-2 messenger RNA [104] with altered protamination inversely associated functionally and fertilization ability [105]. Histones that are not replaced by protamine during chromatin packaging are termed "retained histones" and have been found to contain both activating and silencing epigenetic influences making them ready for rapid gene activation or inhibition. DNA to histone binding is also affected by the methylation of genomic DNA with several genes, including IGF2 and MEST affected in oligozoospermic men [106].

Maternal or paternal imprinting is the result of DNA methylation that subsequently regulates embryonic gene expression. Methylation is another important source of epigenetic modification. Occurring by the addition of a methyl ($–CH_3$) group to a cytosine to a CpG site within DNA, the ability to alter genetic profiles with DNA methylation may hold the key to epigenetic control of male infertility [107]. Indeed, aberrant patterns of methylation in differentially methylated regions (DMRs) of DNA have been found in men with moderate to severe oligospermia [108]. Abnormal germ-line epigenetic reprogramming was proposed as a possible mechanism affecting spermatogenesis [109]. Wide-ranging erasure of DNA methylation followed by sex-specific patterns of de-novo DNA methylation with subsequent incomplete reprogramming of male germ cells was found to alter sperm DNA methylation; thus worsening spermatogenesis outcomes [109]. More recently, DNA methylation profiling using a Methylation array identified 471 CpG sites encompassing 287 genes that were differentially methylated between men with infertility and fertile controls [110]. The fact that sperm DNA methylation profiles are consistent over time and highly reproducible [111] makes this an interesting and promising avenue of future research.

Proteomics

The study of the human proteome lies in the interface between genes and their protein products. By examining the function of proteins in the

context of the expressed complement of the human genome, an indication of active cellular protein content can be ascertained. This is important in the context that while distinct genes are expressed in a cell-dependent manner, protein expression can vary under different times, physiological states and environmental conditions [2]. Alternative splicing of a gene transcript can also yield unique isoforms of a given gene [112]. Moreover, given that messenger RNA is not always translated to protein, proteomic analysis of specific products in exact disease states has the potential to provide accurate biomarkers; especially in the realm of male infertility.

Currently, semen analysis is the best tool physicians have to assess male fertility potential; however, many cases of male infertility remain undiagnosed. Proteomics has made rapid progress over the years and by understanding the types and amounts of proteins as well as their modifications (i.e., acetylaction, glycosylation), the potential for the field are enormous. While it is challenging to sort through the vast amounts of data collected in proteomic analyses to select the handful of genes, several novel biomarkers have already been proposed.

In the context of male fertility, the most difficult challenges lay in the composition of the biological fluid itself and the variability of the possible changes. Semen is made up of sperm and seminal plasma and contains products from multiple different organs including the prostate, seminal vesicles, and bulbourethral glands [113]. The fact that variations in semen occur seasonally and with age makes analysis difficult. Post-ejaculation, variable proteins are activated during coagulation and liquefaction making the generation of a proteomic profile distinct to men with NOA exceptionally challenging.

Research on protein products contained in the seminal plasma began early in the 1940s. Advancements in the field eventually came following the identification of a germ cell binding, Sertoli cell secreted protein, transferrin [114]. Proteolytic breakdown of seminal plasma proteins was examined by two-dimensional (2D) electrophoresis followed by silver staining and found to be accelerated in oligospermic men compared to azoospermic and normospermic cohorts [115]. The development and use of mass-spectrometric techniques allowed more thorough investigations of complex body fluids. Using this technology, in combination with 2D gel electrophoresis, a more detailed characterization of the proteins involved in male infertility was conducted [116]. Differences were identified between men with Sertoli Cell Only (SCO) Syndrome and vasectomized men [116]. Further studies on a single individual using this technology found 923 unique proteins in seminal plasma and provided an accurate and in-depth inventory of proteins in this biological substance [117]. While only 10 % of the reported proteins were known as originating from the male reproductive tract, they encompassed nearly all the proteins identified by two previous studies [118, 119]. Investigators then assessed the seminal proteins of fertile men, and found ~919–1,487 unique proteins in each individual with 83 common in all fertile men [120]. Of these, human cationic microbial protein (hCAP18) was present in the human epididymis and the seminal plasma while spindlin1 was also implicated given its localization to the tails of murine sperm and previously known involvement with spermatogenesis [120, 121].

Batruch [122] expanded this work by examining the constituents of seminal plasma from control men compared to those men who had vasectomies. In post-vasectomy (PV) men, the testicular and epididymal secretions were physically blocked from reaching the ejaculate and as such, the investigators were able to assess proteins originating from different areas of the reproductive tract. These authors identified 32 proteins unique to controls and 4 unique to PV patients [122]. From these, TEX101, the “testis expressed 101” gene located at chromosome 19q13.31 was noted to be one of the leading biomarker candidates. TEX101, a glycosylphosphatidylinositol (GPI)-anchored protein is essential for the production of fertile mouse spermatozoa [123]. Indeed, via interaction with ADAM3 (A disintegrin and metallopeptidase domain 3), a sperm membrane protein critical for both sperm migration into the oviduct [124] and sperm binding to the zona pellucida [125] TEX101 has the potential to be a regulator of male fertility.

Further work from the same authors compared the proteome of NOA men [126] to their previously published results [122] finding several proteins that were elevated (Control vs. NOA, $n=34$; NOA vs. PV, $n=59$) and others that were decreased (Control vs. NOA, $n=18$; NOA vs. PV, $n=16$). Given that several of these proteins were from the male reproductive tract and have previously been linked to fertility, it is tempting to speculate that many of these proteins play important roles in male infertility.

Several other proteins that are of interest as potential biomarkers of male fertility include Heparin binding proteins (HBPs) and prolactin inducible protein (PIP). HBPs are glycosaminoglycans that are potent enhances of sperm capacitation in animals [127]. Purification of seven HBPs from human seminal plasma identified them as semenogelin 1 and 2 as well as PSA and zinc finger protein. PIP, a 17-kDa glycoprotein, is also increased in azoospermic men and, as an abundant seminal plasma protein, it also has a role in capacitation and acts to improve sperm motility [128].

In summary, proteomic analysis of seminal plasma, while at its infancy, is currently expanding the scope of potential male infertility biomarkers. While much work still needs to be conducted, the premise of the research is exciting.

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