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Multi-omics and male infertility: status, integration and future prospects

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1. ABSTRACT

Within the cell, gene expression analysis is the key to gain information about different cellular and physiological events. The multifaceted route of fertilization is a combination of different processes, which include production, maturation and ejaculation of the sperm, its travel through the female genital tract, followed by the ultimate fusion of the fertile sperm with the egg. Early embryogenesis and gametogenesis as well as gene expression at tissue level and global gene silencing are under different levels of stringent epigenetic checks. Moreover, transcriptome (expressed segment of the genome in form of RNA) and the proteome (expressed set of genomic proteins) contribute uniformly to the overall cellular gene expression. In both normal and pathophysiological environments, this gene expression is altered across various levels viz., genome variations, post-transcriptional modifications, protein expression and post translational modifications. Consequently, more informative conclusions can be drawn through a new 'omics' approach of system biology, which takes into account all the genomics, epigenomics, proteomics, and metabolomics findings under one roof, thus computing the alterations in all the entities (genes, proteins, metabolites) concurrently.

2. INTRODUCTION

The efficacy of fertilization is mainly influenced by the functional status of the sperm. Prior to fertilization, the mammalian spermatozoa undergo the process of maturation (capacitation) that renders it competent to fertilize the oocyte. The course of maturation accompanies a cascade of biochemical events which occur in the female genital tract immediately after ejaculation. Capacitation is the hyperactive state of the sperm which allows it to step forward to another physiological maturation; precondition of acrosome reaction. Acrosome reaction is the advanced step to fertilization and involves alterations to the acrosomal cap region of the sperm. Thus, the significance of the sperm is evaluated on the basis of capacitation and acrosome reaction. Different events occurring in the male reproductive system contribute to the composite route of fertilization leading to early embryonic development (1). Mature spermatozoa are specialized vehicles, which enable the transport of chromatin made up of DNA and its associated proteins to the oocyte. Sperm simultaneously protect the competent paternal DNA and ensure its successful delivery, thereby, making sure that the DNA is informative to the future embryo. The early embryogenesis and gametogenesis junctures as well as gene expression at tissue

level and global gene silencing are under different levels of stringent epigenetic checks, viz, histone modification and DNA methylation. The composite process of spermatogenesis is the consequence of tightly regulated interplay between numerous genes. Complete functionality of the sperm relies on the fidelity of the genome, epigenome, transcriptome, and proteome of the spermatogonial cells and the sperm cells (2).

In the growing modern society, infertility concerns about 15–20% couples of the reproductive age and in the Indian scenario, about 15–20 million cases are seen yearly. About one-third of these cases involve male infertility (3, 4, 5). Infertility is a state of heterogeneous etiology comprised of irregularities in multiple genes and their interactions with each other. Nevertheless, ample research in this arena is unable to answer for the reason behind the underlying cause of the male infertility. The exact cause is still unexplained, but reports in the literature reveal alterations in both transcriptome and proteome (2). Cytogenetic abnormalities (eg, Klinefelter syndrome), Y chromosome deletions and monogenic disorders like cystic fibrosis account for only up to 30% of cases (6, 7). Besides this, male factor infertility has been found to be linked with some rare X-linked copy number variations (CNVs), autosomal deletions, Y-linked syndromes, DNA repair mechanism defects, and some single nucleotide polymorphisms (SNPs) (8, 9, 10, 11). The decisive role of epigenetics in fertile sperm production, embryo development and assisted reproduction is evident in a number of studies (12). Similarly, alterations at the protein level have also been discussed in numerous reports in the literature. The ‘Omics’ revolution modernized biomedical research by its forte of studying whole genome, transcriptome, proteome, metabolome simultaneously. The switch from ‘molecular biology’ to ‘modular biology’ with different biological processes as the defined modules has enabled the study of them as complex systems of functionally interacting macromolecules which are interconnected. Simultaneous valuation and analysis of these multi-omics datasets delivers a holistic outline of biological systems in both physiological and clinical states. The reduced cost and time affiliated with this high-throughput integrated approach has enhanced its applicability in varied arena of biological sciences, ranging from cancer to reproductive biology. All this necessitates the requirement of an integrative method which responds to this dynamically changing transcriptome and proteome, thus providing information about the role of individual components of the biological system (13, 14). These ‘omics’ techniques have revolutionized the means of probing the different ‘omes’, thus providing essential information about the grounds of male infertility. The importance of these independent multi-omics datasets has been upgraded by the integration of these datasets. Association of epigenetic mechanisms, viz., abnormal DNA methylation, various histone tail modifications and the role of short non-coding RNAs with male infertility condition is supported by several studies in the literature. Collectively, the epigenetic and genetic modifications, which regulate the transcriptome and proteome, and phenotypic alterations, altogether provide an opportunity for a systems biology approach to evaluate the normal and abnormal physiology. Therefore, in the present review we intend to compile the information revealing the current status of ‘omics’ data with special reference to male infertility and integration of this multi-omics data with other molecular attributes related to male infertility, so as to gain more insights into its fundamental explanation.

3. EPIGENOMICS

3.1. Outline of epigenetic mechanisms and their regulation

The genetic information of an individual programmed within the nucleotide sequence of the organism’s DNA describes the genome of that entity. On the contrary, the term “epigenome” denotes the amendments in gene expression due to modifications in DNA and histone structure without altering the DNA sequence. Epigenetics is defined as ‘‘the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence’’ (15). It includes the cellular and physiological changes which are independent of the alterations in the DNA sequence. Gene expression is reliant on the mode in which DNA is enfolded around the histones as transcriptionally active regions bound loosely; conversely, tightly compacted regions of DNA behave as transcriptionally silent areas. Thus, the crosstalk between the histones and the DNA, scientifically defined as epigenetics, is imperative to decide the time specific and cell specific expression of genes (16). The genetic code is thus inert, and does not change in every cell for an organism’s entire life hence defining the stable phenotypic characters of an individual. Alternatively, the fluidity in the phenotype relates to the dynamics associated with the epigenetic code as it modifies according to the environment and other factors. Owing to the dynamics, these epigenetic modifications can be discovered as remedy objects for a number of diseases (16). The phenotype of an organism is governed by the epigenetic signatures present on the cell, which in turn reflects their developmental history and also explains that it is gene action and expression that give rise to the phenotype (17). The genome-wide approach to studying epigenetics is defined as epigenomics. The accessibility of the DNA is regulated epigenetically, since a definite set of genes are active at a particular stage of

development. Several biological processes are under strict epigenetic regulations, and spermatogenesis is one of them.

Epigenetic mechanisms of regulation include histone tail modifications at some specific amino acid residues, DNA methylation at the CpG site, small noncoding RNAs (nc RNAs) and chromatin remodeling. Histones, viz., H2A, H2B, H3 and H4 are the basic proteins packaging the nuclear DNA into the fundamental unit of nucleosomes. A post-translational modification of these histone proteins serves to be an epigenetic check in the male sperm cell which regulates the gene expression. The major alterations among the list include acetylation, methylation, phosphorylation, and ubiquitinylation at lysine, threonine, arginine and serine sites (18, 19). All these changes alone or in concert take part in maintaining the structural integrity and function of the chromatin by regulating its conversion from active to inactive state (18). The impact of these modifications on the transcriptional status of genes depends on the kind of modification and the type of amino acids involved (20).

Another means of controlling gene function epigenetically is methylation of cytosine in the CpG dinucleotide clusters, well known as CpG islands. Within the CpG islands the number of this methylated cytosine is erratic. CpG islands are defined as a DNA sequence of > 500 bp with G+C content > 55% and CpG frequency of >0.6. (observed to expected ratio) (21, 22). They play a significant role in controlling the transcriptional status of a gene. CpG methylation results in altered chromatin condensation which causes the loss of access of transcription factors to the DNA, thereby resulting in stable heritable silencing of the gene expression (23). Another means of epigenetic regulation is chromatin remodeling, which is an ATP dependent process in contrast to the former mechanisms centred on covalent interactions. The energy from the hydrolyzing ATP modifies the structure of the nucleosomes changing the availability of genes for transcription, thus switching on or off the gene expression (24). The remodeling of the chromatin can cause the DNA to twist, spool or bulge accordingly, thus allowing the transcription factors to act accordingly causing the gene to express or remain silent (25).

Small ncRNAs are present in the sperm nucleus and represent another mechanism of epigenetic check (26). The ncRNAs of 20–24 nucleotides are typically characterized by two distinct features viz., presence of high number of stop codons and absence of open reading frames thus not coding for any protein (26). These ncRNAs include the microRNAs (miRNAs), which act by base pairing with the complementary sequences within the mRNA resulting in the silencing of that gene (27).

3.2. Epigenetic transformations in sperm chromatin during spermatogenesis

In order to maintain this status of being a specialized cell, the spermatozoa undergoes a sequence of epigenetic modifications which check that the delivered DNA is not fragmented and is informative to the future embryo. Spermiogenesis is the haploid phase of spermatogenesis, which involves the differentiation and maturation of the meiotically separated spermatids into round flagellated cells containing compact nucleus without nuclear activity. In order to attain normal fertilization potential, the sperm DNA undergoes a sequence of dramatic alterations in its structure throughout spermatogenesis. DNA condensation is the most prominent and imperative event in the male germ cell and is also highly evolutionarily conserved (28). The compaction of the chromatin is a crucial event as it guards the sperm from the oxidizing environment of the female reproductive tract and also serves a vital role in relation to sperm motility (29). The reorganization of the sperm DNA into protamine rich chromatin renders it highly condensed character, where 90–95% of the histones are replaced by small basic nonhistone protamines (29). Initial alterations include the substitution of histones with histone variants (including testis-specific histone 2B), the most abundant histone variant found in mature sperm (30). Parallel to this, other histones undergo acetylation, thus eliciting successive cascade of events of protamine replacement (31). In the next step of chromatin condensation the hyperacetylated chromatin loosens and histones are replaced by more basic transition proteins (TP1 and TP2). The final event for chromatin compaction includes the substitution of these transition proteins by highly basic proteins called protamines (P1 and P2). The nuclear packaging proteins protamines, which are expressed during spermatogenesis have high arginine content and form intermolecular disulfide linkages between the cysteines, thus making the sperm chromatin 6–20 times more tightly packed than the somatic cell chromatin (32, 33). Protamination serves to be a form of epigenetic regulation in the sperm cell as after DNA compaction no more nuclear activity occurs in the cell. Although, 90% of DNA is packaged in toroidal structures, still 5–10% of it remains histone bound (34) and the post translational histone modifications store the epigenetic information needed to regulate the transcription in the silent nucleus of the sperm (35).

3.3. DNA methylation and male infertility

In humans, there are two copies of the genome in the autosomes inherited from either of the parents. The genetic information of the parents is inherited completely whereas the epigenome experiences thorough reprogramming at the primordial germ cell (PGC) stage (36). Genomic imprinting is

an epigenetic regulatory mechanism which guides the expression of certain parent specific genes. This reprogramming function via DNA methylation which tend to 'imprint' or 'silence' one allele, thus causing the expression of only one allele in an embryo in a parent-of-origin–dependent manner (37). The episode of genomic imprinting occurs before fertilization during the gametogenesis phase. Methylation status of the paternal and the maternal alleles differ as majority of the imprinted genes contain differentially methylated regions (DMRs) (38). As an example, in the insulin-like growth factor 2 (*IGF2/Igf2*) gene only the allele inherited from the father is expressed as the maternal allele is imprinted (silenced) by methylation. The expression of the imprinted genes is strictly an epigenetic mechanism as the activity of the transcription factors present in the nucleus cannot be judged since both silent and active promoters are present in a single nucleus (37). The phenotypes of the imprinted genes are vulnerable to both genetic and epigenetic alterations owing to the parent-of-origin dependent gene expression and are frequently found to be associated with several diseases related to development, cell growth and behaviour (37).

Both sperm function and post fertilization embryo development are dependent on the appropriate functioning of the epigenetic processes, and any aberration in these result in altered fertility status and also embryo development. DNA methylation at the CpG sites is one of such epigenetic mechanism. Although hypomethylation in the gene regulatory regions is linked to transcriptional activation, in testis hypomethylation is accounted in nonrepetitive, non-CpG sequences outside gene promoters (39). Unique patterns of DNA methylation are revealed in the testicular tissue and also genome-wide studies report that testis DNA is eight times more hypomethylated than the somatic tissue (39). Several studies from the literature, both global or target specific, hint towards a possible association of improper DNA methylation and atypical semen profiles as well as pregnancy failure (40).

Whether abnormal spermatogenesis is the outcome of imprinting errors was judged in a study involving two oppositely imprinted spermatozoal genes, H19 and MEST in men with low sperm counts (41). The findings illustrated that oligozoospermic males are susceptible to these imprinting errors (41). Since then several reports in the literature have come into being signifying the role of impaired methylation in the DMRs of imprinted loci for men with unexplained infertility (42, 43). The practicability of the whole genome DNA methylation or of larger regions lays in the development of large-scale DNA methylation detection technologies. The global sperm DNA methylation level was first of all analysed by Houshdran *et al* using genome wide array analysis (44). The study revealed that poor sperm quality, decreased sperm concentration and low sperm motility were associated with increase in DNA methylation levels at several sites. It was inferred that broad epigenetic defects in these abnormal semen parameters were the outcome of errors in the erasure machinery during epigenetic programming in the male germ cell line (44).

3.4. Global DNA methylation status in Oligozoospermic and Asthenozoospermic (AS) infertile males

A recent case control study investigated genome-wide discrepancies in the CpG promoter methylation regions among the low and high motile sperm cells from AS infertile patients and normal control individuals (45). The authors assessed both inter as well as intra-individual methylation inconsistency and confirmed that DNA methylation (both global and targeted) was relatively stable between and within the normal fertile and AS infertile individuals (45). Moreover, comparison of DNA methylation status revealed 34 differentially variable CpGs (DVCs), 134 differentially methylated CpGs (DMCs) and 41 DMRs linked with AS. Lastly, a catalog of 16 genes, which potentially affect the functions of the sperm via variations in DNA methylation, was proposed by the authors. Of these, seven genes were related to sperm motility and spermatogenesis, whereas the others were expressed in the testis (45).

In another recent genome-wide study, Urdinguio and co-authors discovered 2752 CpGs showing anomalous DNA methylation profiles in genomic sperm DNA of individuals with idiopathic infertility. Importantly, these DMCs were appreciably allied with CpG sites which are exclusively methylated in sperm in contrast to the somatic cells (46). Additional findings of the study suggested a statistically significant ($P < 0.0.01$) integration between hypomethylation and corresponding regions in the somatic cells, which are supplemented with suppressive histone mark H3K9me3. Moreover, contrastingly the DNA regions enriched in H3K4me1 and CTCF were hypermethtylated, thus concluding for a locus-dependent relationship between chromatin context and abnormal sperm DNA methylation in infertile men. Furthermore, the DNA methylation patterns at numerous repetitive sequences (LINE-1, D4Z4, NBL2, Alu Yb8) was found lesser in sperm cells than in the somatic cells (46).

Sperm global DNA methylation, along with other parameters of DNA fragmentation and chromatin integrity were assessed in a cohort of oligoasthenozoospermic patients with defects in sperm count and motility (47). Global sperm DNA methylation levels were measured by quantifying 5methylcytosine levels and were compared with the different sperm

parameters. The results of the study elucidated a direct correlation between DNA methylation and sperm concentration: low level of methylation in oligozoospermic males than normal. Also, low levels of global DNA methylation were seen in men with severe AS (<20% progressive motility) followed by no significant connection between sperm morphology and methylation (40). Furthermore, the investigators assessed methylation at DMRs of two imprinted loci H19 and *Peg1/Mest*. The results indicated hypomethylation at 16.7.% of the oligozoospermic patients at the H19 loci with no major changes in the DMRs of *Peg1/Mest* loci. Lastly, effect of global DNA methylation on the chromosome integrity and DNA fragmentation was looked into and a reverse association between these parameters was found. Overall outcome of the study emphasize that both locus specific and global sperm DNA methylation are important tools to mark testicular function and spermatogenesis (47). Therefore, unexplained male infertility could be revealed by analyzing global methylation profiling with array based and next generation sequencing based technologies.

4. GENOMICS AND MALE INFERTILITY

In the post genomic era when the whole genome has been sequenced, analyses of thousands of biological molecules in one go is the award of the present day 'omics' technology. The universal line of approach of these 'omics' technologies aims at the non-targeted and nonbiased detection of the gene products (transcripts, proteins and metabolites) (48). Exploitation of the genomics tools for in depth knowledge of male infertility related events is solely rational as later is addressed by faults in different processes like spermatogenesis, sperm motility and morphology. Since all these developments in the male gamete are tightly regulated, genomics can be helpful in unraveling the molecular targets and the networks sequentially involved in these episodes (49). Genomic techniques are broadly classified into array based and sequencing based methods, which are extensively used to demarcate the genetic associations and the variations in the complex diseases.

Aston and Carrell did the first genome wide association (GWA) study in Caucasian individuals, largely of Northern European descent and cross examined about 370,000 single nucleotide polymorphisms (SNPs) in men with azoospermia and severe oligozoospermia phenotype. The authors documented an association of 17 SNPs with azoospermia and 4 SNPs with oligozoospermia phenotype. SNPs rs10848911, rs10841496, rs2290870, rs1545125, rs4484160 and rs3105782, were established in the intronic region and in close proximity to the gene (50). The genes linked with these SNPs were EF-hand calcium binding domain 4B (EFCAB4B), phosphodiesterase 3A (PDE3A), ATPase, aminophospholipid transporter, class I, type 8A, member 1 (ATP8A1); cordon bleu homolog (COBL); prokineticin 2 (PROK2), and mannan-binding lectin serine peptidase 1 (MASP1) respectively (50). The investigators further extended this pilot study along with other SNPs associated with male infertility. In this follow up investigation the authors used the Illumina Bead Xpress platform and finally scrutinized 172 SNPs in men with abnormal sperm count, viz., oligozoospermia or idiopathic azoospermia. Significant SNPs in testis expressed 15 (TEX15), lysine-specific demethylase 3A (JMJD1A), bromodomain testis-specific (BRDT), and fas ligand (FASLG) genes related to spermatogenic failure were found in the study. Consequently, SNPs in TEX15, BRDT and JMJD1A resulted in conservative amino acid substitutions, whereas the SNP in FASLG is a non-coding SNP positioned in the promoter region. Notably, this follow-up study signifies that spermatogenic failure in the Caucasian men is not the outcome of single common SNP, rather interplay between a number of SNPs in significant proportion of cases (51).

A GWA study revealed that variants within the HLA region are allied with susceptibility for non-obstructive azoospermia (NOA) in Han Chinese males (52). Another three stage GWA study done in Han Chinese men with NOA discloses associations with common variants near PEX10 (rs2477686 at 1p36.3.2), SOX5 (rs10842262 at 12p12.1) and PRMT6 (rs12097821 at 1p13.3.). The study has suggested the role of genetic variants at 1p36.3.2, 12p12.1 and 1p13.3 in the etiology of NOA in the Han Chinese population (53). The same group of investigators further identified susceptibility loci at 6p21.3.2 (rs7194), 10q25.3 (rs7099208), 6p12.2 (rs13206743) and 1q42.1.3 (rs3000811) in Han Chinese men with NOA (54). The above mentioned three risk loci (rs12097821, rs2477686, and rs10842262) in NOA of Han Chinese population were later repeated in a follow-up study in Japanese population by another group of researchers (55). Any noteworthy involvement of the two SNPs rs12097821 and rs2477686 in PRMT6 and PEX10 genes in NOA was not established from the results, although rs10842262 in the SOX5 gene was found to be meaningfully related with the infertility phenotype (55). An interesting GWA observation published by Kosova *et al* discovered the genes that manipulate normal fertility in humans. Authors examined two fertility behaviours (family size and birth rate) in a population of 269 married men of European descent that disallows contraception and has large family sizes (56). In another GWA study nearly 250,000 autosomal SNPs were inspected to evaluate their relation with the discussed fertility traits and among them a total of 41 SNPs for either trait were selected for a validation study. A total of 123 ethnically unlike men from Chicago who had undergone semen analyses were recruited for the validation of the GWA results. Amongst them 9 SNPs were found associated with reduced fertility, which are reported to be coupled with one or more

measures of reduced sperm function and/or quantity. The candidate genes corresponding to one or more SNPs among the nine associated loci for male fertility included UBD and EPSTI1, which have probable role in innate immunity; USP8, a crucial deubiquitinating enzyme having function in acrosome assembly and LRRC32, encoding for a latent transforming growth factor β (TGF-β) receptor on regulatory T cells (56). The same candidate loci (rs7867029, rs7174015, rs12870438 and rs724078) as addressed by Kosava *et al* were challenged later in a study done in Japanese population by Sato and colleagues. (57). However, authors found noteworthy association of only three SNPs (rs7174015, rs7867029 and rs12870438) which could probably be a susceptibility factor for male infertility in Japanese population (57). SNPs association is a population specific phenomenon as reported by Chihara and co-investigators. Authors reported no association of four SNPs (rs5911500, rs10246939, rs2059807, and rs11204546) with male infertility in Japanese patients as previously found associated with European population (58).

In addition to the genetic polymorphisms studied by the genetic tools, rare copy number variations (CNV) have been identified across the genome. The clinical importance of these CNVs is in native stage and a lot of replication studies are needed to be done to pay impetus to their role in male infertility related issues. Recently, CNVs have been identified in 11p11.1.2, 14q11.2., 16p12.2., 15q11.1.1, 21q22.3., 6p21.3.2, and 13q11 genomic locations, which may involve gene mutations or modifications in genetic structure and function. The deviations may lead to spermatogenic failure by altering the gene expression in testicular tissues. The associated genes include EDDM3A, EDDM3B, NPHP1, NRG1, HLA-DRB1, COX10, MAK, HLA-DQA1, POTE B, GOLGA8C, RID2, ADAMTS20, DNMT3L, ALF, TWF1, and DNEL1 (59). CNVs in the PAK3, TRCP5, H2BFWT, PLEC, TSPAN7 loci have been found not only in men with Sertoli-cell-only syndrome (SCOS), but also in either patients with XY gonadal dysgenesis or premature ovarian failure (POF). Also the authors suggested that both spermatogonial loss in the male as well as loss of oogonia in the female have a common genetic origin (60). Recently, connection between CNVs and meiotic arrest and azoospermic phenotype was studied in Australian men (61). Two CNVs containing the MYRIP, LRRC4C genes and the long noncoding RNA LOC100507205 were found associated with azoospermic men having meiotic arrest. All the three genes were reported to be transcribed in the human testis, and MYRIP and LRRC4C localized to meiotic cells (61). This data pays emphasis to the use of CNVs as diagnostic tool for genome profiling.

With the help of high resolution X chromosome specific array-CGH, Krausz and co-investigators examined X-linked genetic factors in men with variable sperm count in male infertility followed by the analysis of selected, patient-specific deletions in large groups of controls and cases. The investigators reported 12 patient-specific deletions with probable clinical inference, and found that the patient phenotype had significantly higher deletions as compared to controls (62). Additionally, cancer testis antigen gene family members, which are the new genetic targets for altered spermatogenesis, were found as most frequently affected genes in men with variable sperm count (62). A different report suggested that rare Y-linked duplications alter a man's susceptibility for idiopathic spermatogenic impairment by 88%, rare X-linked CNVs by 29%, and rare autosomal deletions by 10%. Functional mutations in the DMRT1gene were also suggested as a risk factor and potential genetic cause for human spermatogenic failure (63).

Various researchers have also inspected the sperm transcriptome using these genomic tools, thus reporting a number of genes and the various pathways affected in infertile patients (64, 65). The differential transcripts were related to testis specific genes (TCP11, TESK1, TSPYL1, ADAD1), heat shock proteins (DNAJB4, DNAJB14), and Y-chromosome genes (DAZ1, TSPYL1) in low sperm motility patients (57). In oligozoospermia, genes involved in DNA repair, oxidative stress, histone modification, and spermatogenesis were found to have reduced expression (65). An integrome analysis of DNA methylation and gene expression in low motility sperm revealed nearly 20 differentially expressed transcripts viz., SIRT3, DNMT3A, HDAC1and also that 80% of the loci were hypomethylated (66). Transcriptome study of the sperm has screened a number of RNAs expressed in a particular stage of spermatogenesis, which can be further selected to study differences in their abundance in the infertility condition (67). Comparative examination of miRNA expression profile from three different infertile population viz., AS, teratozoospermia and oligozoospermia explored the differential status of the miRNAs (68). The hsa-miR-629–3p was found correlated with sperm motility, hsa-miR-34b-3p with age, and hsa-miR-335–5p, hsa-miR-885–5p, and hsa-miR-152–3p with sperm concentration (68). The differential miRNAs were predominantly localized in the introns, affecting the genes pertinent to spermatogenesis, thus proving the hypothesis that miRNAs have a role in spermatogenesis (68). Abhari and colleagues in two successive studies reported that the expression of estrogen receptor α and β subunits is regulated by different miRNAs, and it is impaired in the oligozoospermic state (69). In another report five miRNAs (hsa-miR-34c-5p, hsa-miR-34b*, hsa-miR-34b, hsa-miR-429, and hsa-miR-122) were confirmed and validated in different sets of patients with different forms of spermatogenic impairments

(subfertile and NOA) and control subjects (70). The same group previously scrutinized variance in different miRNAs in AS and oligoasthenozoospermic patients (70).

5. PROTEOMICS AND MALE INFERTILITY

As the cellular functions are entirely delineated by the proteins, the information revealed by the genomic experiments is not the mirror image of the exact situation. Also, it is not mandatory that changes at the mRNA level are entirely replicated at the protein levels as proteins up or down regulate without corresponding changes in the mRNA abundance. Proteins also exhibit post translational modifications, which allows them to interact with their partners. As many proteins and peptides undergo disease specific changes the major domain of these 'omics' technologies revolve around the biomarker discovery to understand the pathophysiology of the disease. Downstream to the biomarker identification the functional and structural information revealed by proteomics can be used to identify preventive therapeutic targets (48). The precise compositional, morphological and functional aspects allied with the human spermatozoa imparts it the quality of being an extremely specialized cell in comparison to other somatic and germinal cells. Being the end product of spermatogenesis, sperm exhibits huge variations in its genetic, cellular, and chromatin structures. These variations permit them to control fertility/infertility, embryo development, and heredity (71). Mature spermatozoa are incapable of protein synthesis as they are transcriptionally and translationally silent. Additionally, relatively higher amounts of membrane proteins are present in spermatozoa as compared to other cells. Both the genetic and epigenetic information of the paternal parent is carried to the next generation through the sperm. Last but not the least, the easy accessibility of the spermatozoa aids in purifying it in higher concentrations in both native and functional states (capacitated, noncapacitated, and acrosome reacted). All these traits make sperm particularly acquiescent for proteomic studies. Assessment of post testicular alterations and understanding of different physiological and pathological aspects of sperm is still inadequate due to the restricted capability for biosynthesis in the spermatozoa. Moreover, the readily available genome sequence, which is supposed to be preliminary for understanding the cellular and molecular functions in health and disease conditions, does not answer to the queries at the protein expression level. Consequently, transcriptome analysis as a method of choice is restricted and for that reason the discovery of the biological display of the human genome, as well as the physiological functions of each protein, should be understood first (72). The structural and functional aspects of proteins can be studied by proteomics. Recent studies of spermatozoa from the proteomics viewpoint have elucidated different sperm proteins accountable for the regulation of normal/defective sperm functions. Latest reports reveal several sperm-specific proteins involved in a plethora of sperm processes. Additionally, sperm proteome analysis via proteomics tools has established the importance of spermatozoal post-translational modifications and their ability to induce physiological changes responsible for fertilization (71).

The relevance of anti-sperm antibodies (ASA) in the fertility status has been reported in the literature. But very few studies have revealed the targets of these antibodies. Considering the fact that obstructive azoospermia (OA) patients have more ASA than other infertile group, Zangbar *et al* recently targeted OA patients to screen the targets of these ASA (73). Mass spectrometric analyses revealed tektin-2 (TEK2) and triose phosphate isomerase (TPI) as the two protein targets for the ASA. ASA against TEK2, a flagellar protein and TPI, an acrosomal protein, affects sperm motility as well as acrosome reaction, thus leading to infertility (73). Anomalous semen parameters are the most common cause of male infertility as reported in the literature. Abnormal morphology (globozoospermia), reduced motility (AS) and reduced number of sperms (oligo/azoospermia) are other probable causes of male infertility. Most commonly the sperm proteomics studies are patient centric dealing with aberrant semen analysis with low sperm motility (AS) condition majorly studied. Several investigators have analyzed through proteomics a number of proteins deranged in low sperm motility condition. Comparative studies using shotgun proteomics approaches have evaluated pathological conditions at the proteome level and displayed a whole lot of proteins that are differentially expressed in these infertile conditions (74, 75, 76, 77, 78, 79). Gel based approaches rely on the separation of proteins followed by mass spectrometry based identification of the altered proteins. Relative human sperm tail proteome study has revealed unique proteins which can be useful to set targets for male contraceptive development, to diagnose the sperm dysfunctions, and to predict embryo quality (74). Identification of prostate and testis expressed 1 (PATE1), a protein common in aging men and young AS concluded that the molecular basis for the declivity of the semen quality in both the groups is the same (75). Varicocele, a common cause of male infertility was the subject of study in a recent proteomic analysis to understand the underlying pathophysiology of the condition. As a result the gel based proteomic analysis revealed proteins related to spermatogenesis, sperm maturation, capacitation, acrosome reaction and sperm motility. Functional annotation analysis confirmed the involvement of apoptotic and signal transduction pathways in the varicocele group. Cysteine-rich secretory protein 2 precursor (CRISP2) and arginase-2 (ARG2) were the exclusively expressed proteins in the varicocele

patients, thus proving the utility of the mentioned pathways in the varicocele related male infertility condition (76). Several studies are reported in the literature reviewing the status of expression of proteins related to low sperm motility condition (77, 80, 81).

In modern day proteomics high throughput peptide quantification has replaced the gel based methods by employing both labelled and label free peptide quantification (2, 80, 82, 83, 84, 85). Using modern-day label free peptide quantification, Liu *et al* studied obesity induced changes in low motile spermatozoa isolated from AS men (82). Actin-binding-related protein T2 (ACTRT2) and endoplasmic reticulum protein 57 (ERp57) expressions were down regulated as assessed by immunofluorescence, western blot, and flow cytometry analyses (82). TMT-labeling of the peptides obtained by protein digestion was done in a recent report so as to identify the proteins related to low sperm motility state (84). The results concluded that the proteins involved in metabolic pathways viz., mitochondrial, cytoskeletal and vesicular trafficking proteins regulate the motility of the sperm (84). Saraswat *et al* performed shotgun proteomic analysis (label free-LC-MS) of the sperm cells and seminal plasma proteins in normal and AS samples and included 667 proteins for quantification in sperm samples and 429 proteins in seminal plasma samples. By using suitable statistical methods the authors inferred that sperm motility pathway defects are reflected in sperm proteomic signatures and the seminal plasma data set does not imitate any of these defective pathways (85). In another sperm proteome analysis testis-enriched missing proteins were identified (86). Protein phosphorylation is the most commonly occurring post translational modification. Sperm motility, an imperative requisite for fertilization, depends on cyclic AMP activated protein kinase A phosphorylation of flagellar proteins like axonemal dynein which further increase motility. Keeping in view the importance of phosphorylation in sperm motility, Parte and colleagues did a comparative analysis of the phosphoencriched sperm fractions of low motility patients by ultra-performance liquid chromatography (LC-MS ^{E}) so as to examine the altered proteins and the key pathways in the respective condition (80). A total of 66 differentially regulated phosphoproteins related to low sperm motility condition were found. Functional annotations of these proteins revealed the involvement of cAMP mediated PKA signaling, PI3K/AKT signaling, carbohydrate and energy metabolism pathways, and pathways regulating actin based motility by Rho, thus concluding that motility is regulated through the combined action of these pathways (80).

Cell-cell interactions are pivotal for the fertilization process as the contact between the sperm and the egg is the decisive step for the future embryo. In a latest report the investigators drafted a testis/sperm-enriched protein interaction network. Also, potential target of the interactome was validated in human spermatozoa for pharmacological intervention (87). The study unravelled A-kinase anchor protein 4 (AKAP4), a testis-specific protein to be the interacting partner of phosphoprotein phosphatase 1 catalytic subunit gamma 2 (PPP1CC2), a protein crucial for spermatogenesis and spermatozoa motility and also revealed the potential of the complex as contraceptive target (87). Glycosyaminoglycans (GAGs) are imperative for the cellular communications and reports from the literature suggest the role of heparin in crucial processes of capacitation and acrosome reaction. Certain heparin binding proteins (HBPs) interact with these GAGs present in the female reproductive tract thus facilitating zona pellucida induction. Our group identified and characterized for the first time seven HBPs in the normal seminal fluid using affinity chromatography followed by matrix assisted laser desorption ionization-time of flight/mass spectrometry (MALDI-TOF/MS) identification (88). Additionally, our group also studied concavalin-A binding glycoproteins and subsequently identified them by MALDI-TOF/MS with prostatic acid phosphatase (PAP), lactoferrin, aminopeptidase N, prostate specific antigen (PSA), zinc-alpha-2-glycoprotein, prolactin inducible protein (PIP), Izumo sperm-egg fusion protein, progestagen-associated endometrial protein as the major proteins (89). Recently, Glycosylation sites, glycan compositions and structures for 243 glycopeptides belonging to 73 N-glycosylation sites on 50 glycoproteins have been elucidated by Saraswat *et al* (90).

6. METABOLOMICS AND MALE INFERTILITY

Amongst all the 'omics' approaches defined till date, 'metabolomics' is recognized as the most recent and is in its infancy. Despite of being in its naive state, the scientific relevance of metabolomics is gaining elevation in the current 'omics' field. Apart from genes, transcripts and proteins being the marker of any metabolic process of a cell, metabolites also demarcate between the physiological and pathophysiological state. Within a cell, there are definite sets of metabolites, and the information corresponding to the absolute outcome and measurement of these endpoints defines the term 'metabolomics'. Accurately, 'metabolomics' is the estimation of both endogenous and exogenous metabolites such as drugs, food, etc., which correspond to small molecules of ≤ 1500Da. As said these metabolites can be both cellular (those generated via the internal cellular processes) and acellular (produced as a result of exogenously administered drugs) (91). Since metabolites are downstream of the genes and proteins in the metabolic hierarchy of the cell, they more closely replicate the exact phenotypic state of the cell than the transcriptome and the proteome (91).

Metabolomics inclined towards male infertility in search of biomarkers for the evaluation of the

different infertility phenotypes is reported in recent literature. More recently, using the sperm proteome data a proteome-scale metabolic network model of the sperm cell, namely SpermNet has been constructed which consists of 2,968 reactions, 2,034 metabolites and 1,242 genes (92). The model depicts that non-glycolytic pathways, like oxidative phosphorylation, fatty acid oxidation are responsible for deficient energy metabolism in AS condition (92). In a recent finding, (1)H NMR based metabolomic profiling of males with different infertility parameters (oligozoospermia, AS, azoospermia, teratozoospermia) discovered considerable differences between the fertile and infertile group (94). The de-regulation (up or down) of small molecules/compounds like arginine, lysine, citrate, tyrosine, fructose and proline was found to be linked with the infertile phenotypes (94). Using the same technique the metabolome of males with low sperm motility was studied and alterations in metabolic pathways pertaining to lipid, cholesterol, nucleoside, and phospholipids were established (95).

The most recent urinary metabolome of the oligozoospermia studied by Zang and co-workers identified reduced aspartic acid, leucylproline, and acylcarnitines and increased methylxanthine and adenine to be strongly associated with the risk of decrease in the number of sperms (oligozoospermia). The outcome of the study paid impetus to the fact that antioxidant defences in spermatogenesis regulated the oligozoospermic phenotype (93). Spinal cord injury is reported to be a cause of male infertility as reports suggest that the sperm motility is reduced in such patients with no change on the sperm concentration. In order to trace the mechanism leading to this trait, a group of investigators used MALDI-TOF-MS to screen the lipid profile of the men with spinal cord injury induced male infertility (96). The results exhibited increased concentration of various lipids which were related to CTP, UTP and GTP biosynthesis, arachidonic acid metabolism, and sterol biosynthesis, thus inferring that signal transduction is probably altered in these males (96).

Oxidative stress induced as a result of imbalanced production of reactive oxygen species (ROS) and weakened antioxidant defense mechanism is the most widely studied aspect in metabolomics related to male infertility. Latest reports on metabolomics fingerprinting as determined by Raman spectroscopy suggest an imbalanced production of ROS due to oxidative stress in men with an idiopathic origin of infertility (97). Using (1)H NMR spectroscopy Zhang and colleagues reported an elevation of oxysterols such as 5α-cholesterol and 7-ketocholesterol in the seminal plasma of patients with AS, signifying the importance of oxidative stress induced mechanisms for reduction in the semen quality (95). Likewise, variations in the metabolome of low sperm motility males were accounted by Gilany and co-workers where chemometrics was used on the patterns of Raman spectra obtained by Raman spectroscopy to analyze the metabolome (98).

7. INTEGRATIVE LINKS AMONG MULTI-OMICS APPROACHES FOR MALE INFERTILITY

Several studies related to male infertility based on different 'omics' technologies are already reported in the literature. To move further in the field, it is necessary to discover something relevant in the biology of male infertility with the help of existing literature. Some of the studies revealed that combination, or integrative analysis, of two different but biologically related 'omics' datasets is a more practical approach to deal with multifactorial male infertility (66). As an extension to this, Pacheco and colleagues used multi-omics genome wide approaches to study the DNA methylation status and the mRNA expression in males with low sperm motility (66). The authors performed GWA of sperm DNA methylation and mRNA content and through linear models of microarray analysis (LIMMA) 9,189 CpG loci were identified having appreciably altered methylation (Q<0.0.5) in the sperm samples with low motility. 80% of the disrupted CpGs were hypomethylated and mostly associated with the imprinted genes. mRNA expression was analyzed using Human Gene 1.0. ST Affymetrix Gene Chip Array and nearly 20 candidate gene transcripts were categorized as differentially present in low motility sperm, including SIRT3 (NCBI 23410), DNMT3A and HDAC1 (NCBI 3065) (66).

In another integrome analysis, genital tract markers in human seminal plasma were discovered using an integrated genomics approach. Rolland *et al* identified 699 seminal plasma proteins by executing proteomic analysis and further compared their findings with other previous proteomic data sets. The authors finally reported 2545 unique proteins in the seminal plasma. Transcriptomic gene expression analysis classified 17 prostate, 7 seminal vesicle, 42 epididymis, and 83 testis candidate protein markers among those proteins. Finally, the authors validated the expression of testis-specific candidate proteins LDHC, TKTL1, and PGK2 in the germ cells and conclusively reported that these markers can suitably distinguish between the semen of fertile and infertile men (99). In another integration analysis with respect to the men with NOA, promoter DNA methylation and mRNA gene expression was studied by comparing fibroblasts cultured from testicular biopsies using a high resolution Infinium 450K methylation array (100). Differentially methylated CpG sites and nearly 20 genes with aberrant DNA methylation were identified in men with NOA. Of these, discoidin domain receptor 1(DDR1), a hypermethylated and testis expressed

gene, was selected for further validation and the abnormal gene expression pattern was exhibited (100). Moreover, quantitative analysis by bisulfite clonal sequencing showed that one of the CpG sites (cg13329862) of DDR1 promoter was hypermethylated in NOA patients compared with fertile controls (53% vs. 15%). Furthermore, immunohistochemical analysis suggests presence of DDR1 within the cytoplasm of germ cells and peritubular connective tissue (in men with hypospermatogenesis) and decreased expression of the protein in men with Sertoli-cell only syndrome (100). As integrative data analysis reports are very limited in case of male infertility, more and more integrative biology approaches combining genome wide association, global methylation, trascriptomics, global miRNA profiling and proteomics should be done. Table 1 illustrates the different 'omics' studies related to male infertility (Table 1).

8. FUTURE OF INTEGRATION OF MULTI-OMICS DATA IN MALE INFERTILITY

The central dogma of a cell signifies that the genetic information flows in a unidirectional and irreversible mode from DNA to RNA and finally to protein. The information thus carried by the DNA dictates the ultimate end product, the protein. In modern day biology the genetic and protein compliment expressed by a cell at a particular time is addressed as the 'genome', 'transcriptome' and the 'proteome' and the true biological state of the cell relies on the transcriptome and the proteome profile of the cell. In both normal and diseased states the gene expression status is subjected to alterations in response to the different milieus existing in the cell. Moreover, both post-transcriptional and post-translational regulation of gene expression adds another level in the hierarchy of dynamicity of the cellular gene expression (12). Functional approaches to study the transcriptome/genome and proteome have modified the status of 'molecular biology' to 'modular biology' where biological processes of interest are the modules to be studied. Both clinical and cellular conditions of the cell are the outcome of interplay between these individual components of the biological system. The misleading information obtained due to the false positives allied with the 'omics' approach modifies the end result. Also, the study of single annotation does not delineate the precise sketch of the gene function (101). Thus, investigating the data obtained from 'omics' module independently does not decipher the true information as the molecular differences occur across multiple layers. All these shortcomings can be curtailed under one roof by coupling the different 'omics' datasets together so as to frame a biological hypothesis which may aid in drawing more informative conclusions.

Intergration analysis serves to bridge the gap between the 'omics' information and systems biology and evaluates the functional annotations of genes by protein-protein interactions. The directionality of the different biological networks in the module can also be judged with the help of data integration. A summary of the different 'omics' techniques is illustrated in figure 1 (Figure 1). In other pathological manifestations, such as cancer, there are reports available that discover the new horizons by cross omics data integration, rather than with a single set of data (102, 103). The study highlights the integration of genome wide methylation and mRNA expression in esophageal cancer thereby exploring the complex interactions involved (102). Nowadays, plant biologists are also exploiting the intergrome data to analyze the biosynthetic pathways of plant-based medicinal metabolites (104). Also, the cellular mechanisms imparting CHO (Chinese Hamster ovarian) cell lines their special properties for the production of biopharmaceutical molecules were elucidated by integrating the DNA methylation and gene expression data (105). Male infertility is a complex life style related disorder and has a compound etiology resulting from multiple epistatic gene interactions. A systems biology approach integrating the high throughput 'omics' data across multiple layers, viz., genome, transcriptome, epigenome, proteome, metabolome is the instant compulsion of the hour. Implementation of this systems biology approach in understanding the process of spermatogenesis would allow a deeper insight into the disease mechanism. As large amounts of 'omics' data keep on pouring in day by day for male infertility from different worldwide populations, one can identify the common molecular alterations associated with the disorder by doing integrative analysis within or outside of the specific population. Integration analysis of the transcriptome and proteome hold immense potential in figuring out the life style related and environmental factors associated with male infertility. With the arrival of new sequencing approaches, such as next generation sequencing technology, one can easily generate different 'omics' data from the same available biological samples in a timely fashion. The major lacuna present in the field of integrative biology is the scarcity of the available bioinformatics tools. Integration of the datasets in male infertility is in its native state, as currently very few mathematical algorithms are available for cross omics data integration. The development in this field depends on the availability of interactive databases with improved functionality and also of skilled researchers who can analyze the data. Applicability of this multi-omics approach would be in the accurate identification of panel of biomarkers which would help in understanding the cause of idiopathic male infertility. Also, integromics approach may be exploited by clinicians to diagnose and treat infertile patients and take significant decisions related to assisted reproductive technique (ART).

Table 1. Male infertility types studied by various OMICS technologies and their major outcomes

Male Infertility Type	Technique Used	Major Outcomes of the study	Reference
EPIGENOMICS (DNA Methylation)			
AS vs Normozoospermic	liquid hybridization capture-based bisulfite sequencing	134 differentially methylated CpGs, 41 differentially methylated regions and 134 differentially variable CpGs were identified. Also a catalogue of 16 differentially methylated genes that are required for spermatogenesis and sperm motility or dominantly expressed in testis were also found	45
Fertile vs men with unexplained infertility	Microarray analysis	2752 CpGs showing anomalous DNA methylation profiles were identified in genomic sperm DNA of individuals with idiopathic infertility. Also, DNA methylation patterns at repetitive sequences (LINE-1, D4Z4, NBL2, Alu Yb8) was found lesser in sperm cells than in the somatic cells	46
Oligoasthenizoospermic	ELISA like method	DNA methylation and sperm concentration and motility were found to be directly correlated with no significant connection between sperm morphology and methylation	47
GENOMICS			
Azoospermia and severe oligozoospermia	Microarray	Identified 17 SNPs associated with azoospermia and 4 SNPs with oligozoospermia phenotype	50
Azoospermia and severe oligozoospermia	Microarray	Significant SNPs in testis expressed 15 (TEX15), lysine-specific demethylase 3A (JMJD1A), bromodomain testis-specific (BRDT), and fas ligand (FASLG) genes related to spermatogenic failure were found in the study	51
NOA	Microarray	GWA study revealed that variants within the HLA region are allied with susceptibility for non-obstructive azoospermia (NOA) in Han Chinese males	52
NOA	Microarray	The study suggested the role of genetic variants at 1p36.3.2, 12p12.1.and 1p13.3. in the etiology of NOA in the Han Chinese population	53
NOA	Microarray	Identified susceptibility loci at 6p21.3.2, 10q25.3., 6p12.2. and 1q42.1.3 in Han Chinese men with NOA	54
NOA	DNA sequencing	Two SNPs rs12097821 and rs2477686 in PRMT6 and PEX10 genes were not associated with NOA although rs10842262 in the SOX5 gene was found to be related with the infertility phenotype	55
Normal men of European descent	Microarray	41 SNPs were significantly correlated with family size or birth rate four SNPs (rs7867029, rs7174015, rs12870438 and rs724078) were found to be associated with semen parameters in ethnically diverse men from Chicago	56
Azoospermia and Oligiozoospermia	(RFLP)-PCR	Candidate loci (rs7867029, rs7174015, rs12870438) for human male fertility traits were significantly associated with the risk of male infertility in Japanese population	57
Azoospermia and Oligiozoospermia	Real Time PCR	No significant association of four SNPs (rs5911500, rs10246939, rs2059807, and rs11204546) with infertility in Japanese population was found	58
Chromosomal abnormality and azoospernmia	Genome sequencing	CNVs were identified in 11p11.1.2, 14q11.2., 16p12.2., 15q11.1.1, 21q22.3., 6p21.3.2, and 13q11 genomic locations	59
Oligozoospermia and Sertoli-cell only syndrome (SCOS)	Microarray	Ten recurring CNVs were found in patients with severe oligozoospermia, three only in SCOS and one CNV in both groups with spermatogenic failure	60
Azoospermia and Meiotic arrest	Microarray	Two CNVs unique to meiosis arrest patients were identified	61
Azoospermic, Oligozoospermic	Microarray	73 X-linked CNVs were identified in relation to spermatogenesis. Cancer Testis Antigen gene family members were the most frequently affected genes	62
Spermatogenic impairment	Microarray	Functional mutations in the DMRT1gene were found as a risk factor for human spermatogenic failure	63
AS and idiopathic infertility	Microarray	A number of differential transcripts were identified	64
Oligozoospermia	Microarray	Significant reduction in expression of genes involved in spermatogenesis, sperm motility, DNA repair, oxidative stress regulation and histone modification genes was found	65
AS	Microarray	20 differentially expressed transcripts related to epigenetic regulatory genes with 80% hypomethylation were identified	66
AS, teratozoospermia and oligozoospermia	Microarray	hsa-miR-629–3p was found correlated with sperm motility, hsa-miR-34b-3p with age, and hsa-miR-335–5p, hsa-miR-885–5p, and hsa-miR-152–3p with sperm concentration	68
Oligozoospermic	Real Time PCR	Expression of estrogen receptor α and β subunits is regulated by different miRNAs, and it is impaired in the oligozoospermic state	68
Subfertile and nonobstructive azoospermia	Real Time PCR	Expression of hsa-miR-429 was significantly increased and hsa-miR-34c-5p, hsa-miR-34b*, hsa-miR-34b and hsa-miR-122 were decreased in both tested groups	70

PROTEOMICS			
Obstructive azoospermia	MS	Tektin-2 (TEK2) and triose phosphate isomerase (TPI) as the two protein targets for the ASA.	73
AS vs normozoospermic	2D-PAGE, MALDI-TOF/MS	14 differential proteins were identified	74
Aged men and young AS patients	2D-PAGE, MALDI-TOF/MS	22 differential proteins were identified	75
Unilateral Varicocele and normal fertile males	SDS-PAGE, MS	Cysteine-rich secretory protein 2 precursor (CRISP2) and arginase-2 (ARG2) were the exclusively expressed in the varicocele patients	76
AS vs normozoospermic	2D-PAGE, MALDI-TOF/MS	16 differential proteins were identified	77
Globozoospermic vs normozoospermic	2D-DIGE, MALDI-TOF/MS	35 differential proteins having roles in spermatogenesis, cell skeleton, metabolism and spermatozoa motility were identified	78
Oligoastheno zoospermic vs Normozoospermic	2D-PAGE, MALDI-TOF/MS	4 differential proteins were identified	79
AS vs Normozoospermic	Nano UPLC-MS	66 differential phospho proteins were identified	80
AS vs Normozoospermic	2D-PAGE, MALDI-TOF/MS	8 differential proteins were identified	81
AS vs Normozoospermic	Label free LC-MS	127 differential proteins were identified	82
Normozoospermic sperm samples with different IVF outcomes (pregnancy versus no pregnancy)	TMT labelling, SDS-PAGE, LC-MS	66 differential proteins were identified	83
AS vs Normozoospermic	TMT labelling, LC-MS/MS	80 differential proteins were identified	84
AS vs Normozoospermic	Label free LC-MS	667 proteins for quantification in sperm samples and 429 proteins in seminal plasma samples were found	85
Normal sperm	Mass Spectrometry and Bioinformatics	A-kinase anchor protein 4 (AKAP4), a testis-specific protein to be the interacting partner of phosphoprotein phosphatase 1 catalytic subunit gamma 2 (PPP1CC2), a protein crucial for spermatogenesis and spermatozoa motility	87
Normozoospermic	Affinity chromatography and MALDI-TOF	Major HBPs were semenogelin I fragment, semenogelin II, lactoferrin and its fragments, prostate specific antigen (PSA), homolog of bovine seminal plasma-proteins (BSP), zinc finger protein (Znf 169) and fibronectin fragments	88
Normozoospermic	Affinity chromatography and MALDI-TOF	The major proteins identified in this study included Aminopeptidase N, PSA, prostatic acid phosphatase (PAP) zinc-alpha-2-glycoprotein, lactoferrin, Izumo sperm-egg fusion protein, progestagen-associated endometrial protein, and prolactin inducible protein (PIP)	89
SP glycoproteome	CID-MS/MS	glycosylation sites, glycan compositions and structures for 243 glycopeptides belonging to 73 N-glycosylation sites on 50 glycoproteins have been elucidated	90
METABOLOMICS			
Spermatozoa	Bioinformatics	Metabolic network model of the sperm cell, namely SpermNet was been constructed	92
oligozoospermia, AS, azoospermia and teratozoospermia	NMR spectroscopy	De-regulation (up or down) of small molecules/compounds like arginine, lysine, citrate, tyrosine, fructose and proline was found to be linked with the infertile phenotypes	94
AS	NMR spectroscopy	Various metabolites were found to be deregulated	95
Spinal cord injury induce male infertility		Increased concentration of various lipids related to CTP, UTP and GTP biosynthesis, arachidonic acid metabolism, and sterol biosynthesis indicating altered signal transduction	96
Unexplained infertility	Raman Spectroscopy	An imbalanced production of ROS due to oxidative stress in men with an idiopathic origin of infertility	97
AS	Raman Spectroscopy	Altered metabolite profiles	98

AS: Asthenozoospermia, NOA: Non Obstructive Azoospermia, RFLP-PCR: Restriction Fragment Length Polymorphism-Polymerase Chain Reaction, MS: Mass Spectrometry, 2D-PAGE: 2 Dimensional Polyacrylamide Gel Electrophoresis, MALDI-TOF: Matrix Assisted Laser Desorption Ionization-Time of Flight, UPLC: Ultra Performance Liquid Chromatography, CID: Collision Induced Dissociation, NMR: Nuclear Magnetic Resonance

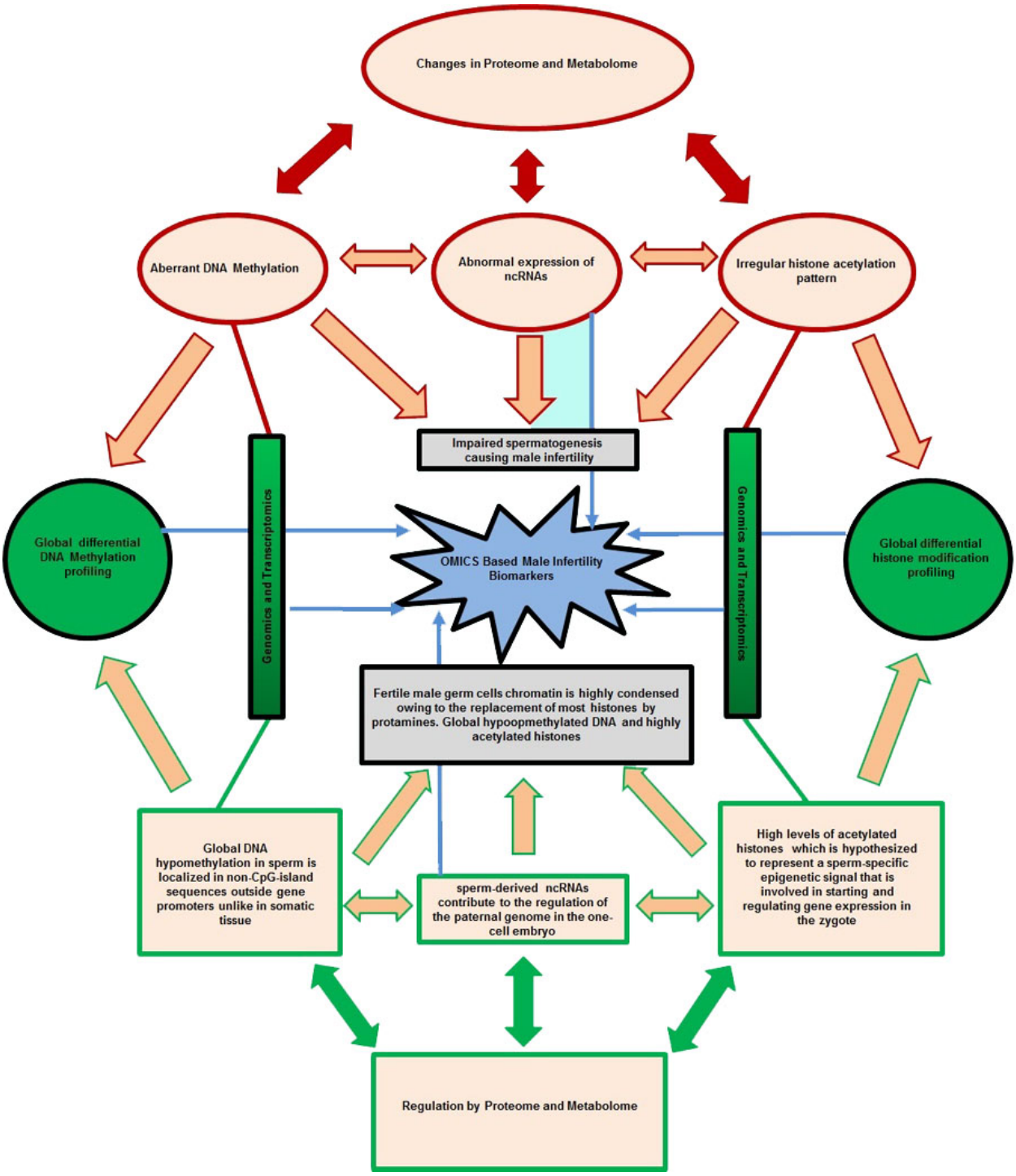

Figure 1. Male infertility and OMICS technologies: Infertility in males is suggested to be associated with aberrant DNA methylation, abnormal expression of ncRNAs and irregular histone acetylation discovered by various 'omic' technology-based studies (40). The three parameters are represented in figure by redlined ovals and these are also interconnected as represented by bidirectional arrows. Accurate global hypomethylation of DNA, expression of specific set of ncRNAs and high level of acetylated histone at correct places in the genome leads to fertile male germ cells as represented by green lined rectangles. Solid green colored circles and rectangles represent different OMICS technologies used for differential profiling that leads to OMICS based male infertility markers. As different OMICS technologies have only selected information of a particular plane of condition, they all need to be integrated to deduce a multidimensional view on male infertility.

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10. REFERENCES

1. MR Maduro, KC Lo, WW Chuang, DJ Lamb: Genes and male infertility: what can go wrong? *J Androl* 24, 485–493 (2003) DOI: 10.1002/j.1939-4640.2003.tb02697.x

2. DT Carrell, KI Aston, R Oliva, BR Emery, CJ De Jonge: The "omics" of human male infertility: integrating big data in a systems biology approach. *Cell Tissue Res* 363, 295–312 (2016) DOI: 10.1007/s00441-015-2320-7

3. P Thonneau, S Marchand, A Tallec, ML Ferial, B Ducot, J Lansac, P Lopes, JM Tabaste, A Spira: Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988–1989). *Hum Reprod* 6, 811–816 (1991) DOI: 10.1093/oxfordjournals.humrep.a137433

4. J Boivin, L Bunting, JA Collins, KG Nygren: International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Hum Reprod* 22, 1506–1512 (2007) DOI: 10.1093/humrep/dem046

5. DM de Kretser: Male infertility. *Lancet* 349, 787–790 (1997) DOI: 10.1016/S0140-6736(96)08341-9

6. KI Aston, DF Conrad: A review of genome-wide approaches to study the genetic basis for spermatogenic defects. *Methods Mol Biol* 927, 397–410 (2013) DOI: 10.1007/978-1-62703-038-0_34

7. JM Hotaling: Genetics of male infertility. *Urol Clin North Am* 41, 1–17 (2014) DOI: 10.1016/j.ucl.2013.08.009

8. N Gupta, S Gupta, M Dama, A David, G Khanna, A Khanna, S Rajender: Strong association of 677 C > T substitution in the MTHFR gene with male infertility—a study on an Indian population and a meta-analysis. *PLoS One* 6, 22277 (2011) DOI: 10.1371/journal.pone.0022277

9. YN Teng, YP Chang, JT Tseng, PH Kuo, IW Lee, MS Lee, PL Kuo: A single-nucleotide polymorphism of the DAZL gene promoter confers susceptibility to spermatogenic failure in the Taiwanese Han. *Hum Reprod* 27, 2857–2865 (2012) DOI: 10.1093/humrep/des227

10. W Wu, J Lu, Q Tang, S Zhang, B Yuan, J Li, WU Di, H Sun, C Lu, Y Xia, D Chen, J Sha, X Wang: GSTM1 and GSTT1 null polymorphisms and male infertility risk: an updated meta-analysis encompassing 6934 subjects. *Sci Rep* 3, 2258 (2013)

11. S Gunes, M Al-Sadaan, A Agarwal: Spermatogenesis, DNA damage and DNA repair mechanisms in male infertility. *Reprod Biomed Online* 31, 309–319 (2015) DOI: 10.1016/j.rbmo.2015.06.010

12. L Stuppia, M Franzago, P Ballerini, V Gatta, I Antonucci: Epigenetics and male reproduction: the consequences of paternal lifestyle on fertility, embryo development, and children lifetime health. *Clin Epigenetics* 7, 120 (2015) DOI: 10.1186/s13148-015-0155-4

13. D Kumar, G Bansal, A Narang, T Basak, T Abbas, D Dash: Integrating transcriptome and proteome profiling: Strategies and applications. *Proteomics* 16, 2533–2544 (2016) DOI: 10.1002/pmic.201600140

14. AR Lima, L Bastos Mde, M Carballo, P Guedes de Pinho: Biomarker Discovery in Human Prostate Cancer: an Update in Metabolomics Studies. *Transl Oncol* 9, 357–370 (2016) DOI: 10.1016/j.tranon.2016.05.004

15. AD Riggs, RA Martinssen, VEA Russo: Epigenetic mechanisms of gene regulation. In:. Cold Spring Harbor, NY: Cold Spring Harbor Press; 1996

16. GI Egger, G Liang, A Aparicio, PA Jones: Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 429, 457–463 (2004) DOI: 10.1038/nature02625

17. CH Waddington: Canalization of development and the inheritance of acquired characters. *Nature* 150, 563–565 (1942) DOI: 10.1038/150563a0

18. A Santenard, ME Torres-Padilla: Epigenetic reprogramming in mammalian reproduction: contribution from histone variants. *Epigenetics* 4, 80–84 (2009) DOI: 10.4161/epi.4.2.7838

19. W An: Histone acetylation and methylation: combinatorial players for transcriptional regulation. *Subcell Biochem* 41, 351–369 (2007) DOI: 10.1007/1-4020-5466-1_16

20. T Kouzarides: Chromatin modifications and their function. *Cell* 128, 693–705 (2007) DOI: 10.1016/j.cell.2007.02.005

21. RS Illingworth, AP Bird: CpG islands—''a rough guide''. *FEBS Lett* 83, 1713–1720 (2009) DOI: 10.1016/j.febslet.2009.04.012

22. D Takai, PA Jones: Comprehensive analysis of CpG islands in human chromosomes 21 and 22. *Proc Natl Acad Sci U S A* 99, 3740–3745 (2002) DOI: 10.1073/pnas.052410099

23. R Dada, M Kumar, R Jesudasan, JL Fernández, J Gosálvez, A Agarwal: Epigenetics and its role in male infertility. *J Assist Reprod Genet* 29, 213–223 (2012) DOI: 10.1007/s10815-012-9715-0

24. PD Partensky, GJ Parrikar: Chromatin remodelers act globally sequence positions nucleosomes locally. *J Mol Biol* 391, 12–25 (2009) DOI: 10.1016/j.jmb.2009.04.085

25. GJ Narlikar, HY Fan, RE Kingston: Cooperation between complexes that regulate chromatin structure and transcription. *Cell* 108, 475–487 (2002) DOI: 10.1016/S0092-8674(02)00654-2

26. FF Costa: Non-coding RNA: new players in eukaryotic biology. *Gene* 357, 83–94 (2005) DOI: 10.1016/j.gene.2005.06.019

27. T Hamatani: Human spermatozoal RNAs. *Fertil Steril* 97, 275–281 (2012) DOI: 10.1016/j.fertnstert.2011.12.035

28. J Ausio, JM Eirin-Lopez, LJ Frehlick: Evolution of vertebrate chromosomal sperm proteins: implications for fertility and sperm competition. *Soc Re-prod Fertil Suppl* 65, 63–79 (2007)

29. R Oliva: Protamines and male infertility. *Hum Reprod Update* 12, 417–35 (2006) DOI: 10.1093/humupd/dml009

30. HJ van Roijen, MP Ooms, MC Spaargaren, WM Baarends, RF Weber, JA Grootegoed, JT Vreeburg: Immunoexpression of testis-specific histone 2B in human spermatozoa and testis tissue. *Hum Reprod* 3, 1559–1566 (1998) DOI: 10.1093/humrep/13.6.1559

31. S Rousseaux, J Gaucher, J Thevenon, C Caron, ALVitte, S Curtet, C Derobertis, AK Faure, R Levy, I Aknin-Seifer, C Ravel, JP Siffroi, K Mc Elreavey, H Lejeune, C Jimenez, S Hennebicq, S Khochbin: Spermiogenesis: histone acetylation triggers male genome reprogramming. *Gynecol Obstet Fertil* 37, 519–522 (2009) DOI: 10.1016/j.gyobfe.2009.04.003

32. WS Ward, DS Coffey: DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. *Biol Reprod* 44, 569–574 (1991) DOI: 10.1095/biolreprod44.4.569

33. SM Wykes, SA Krawetz: The structural organization of sperm chromatin. *J Biol Chem* 278, 29471–29477 (2003) DOI: 10.1074/jbc.M304545200

34. SS Hammoud, DA Nix, H Zhang, J Purwar, DT Carrell, BR Cairns: Distinctive chromatin in human sperm packages genes for embryo development. *Nature* 460, 473–478 (2009) DOI: 10.1038/nature08162

35. TF Wu, DS Chu: Epigenetic processes implemented during spermatogenesis distinguish the paternal pronucleus in the embryo. *Reprod Biomed Online* 16, 13–22 (2008) DOI: 10.1016/S1472-6483(10)60552-4

36. F von Meyenn, W Reik : Forget the Parents: Epigenetic Reprogramming in Human Germ Cells. Cell 161, 1248–1251 (2015) DOI: 10.1016/j.cell.2015.05.039

37. K Pfeifer: Mechanisms of Genomic Imprinting. *Am J Hum Genet* 672, 777–787 (2000) DOI: 10.1086/303101

38. X Cui, X Jing, X Wu, M Yan, Q Li, Y Shen, Z Wang: DNA methylation in spermatogenesis and male infertility. *Exp Ther Med* 12, 1973–1979 (2016) DOI: 10.3892/etm.2016.3569

39. CC Oakes, S La Salle, DJ Smiraglia, B Robaire, JM Trasler: A unique configuration of genome-wide DNA methylation patterns in the testis. *Proc Natl Acad Sci U S A* 104, 228–233 (2007)
DOI: 10.1073/pnas.0607521104

40. U Schagdarsurengin, A Paradowska, K Steger: Analysing the sperm epigenome: roles in early embryogenesis and assisted reproduction. *Nat Rev Urol* 9, 609–619 (2012)

41. CJ Marques, F Carvalho, M Sousa, A Barros: Genomic imprinting in disruptive spermatogenesis. *Lancet* 363, 1700–1702 (2004)
DOI: 10.1016/S0140-6736(04)16256-9

42. H Kobayashi, A Sato, E Otsu, H Hiura, C Tomatsu, H Sasaki, N Yaegashi, T Arima: Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. *Hum Mol Genet* 16, 2542–2551 (2007)
DOI: 10.1093/hmg/ddm187

43. N El Hajj, U Zechner, E Schneider, A Tresch, J Gromoll, T Hahn, M Schorsch, T Haaf: Methylation status of imprinted genes and repetitive elements in sperm DNA from infertile males. *Sex Dev* 5, 60– 69 (2011)
DOI: 10.1159/000323806

44. S Houshdaran, VK Cortessis, K Siegmund, A Yang, PW Laird, R Z Sokol: Widespread epigenetic abnormalities suggest a broad DNA methylation erasure defect in abnormal human sperm. *PLoS One* 2, (2007)
DOI: 10.1371/journal.pone.0001289

45. Y Du, M Li, J Chen, Y Duan, X Wang, Y Qiu, Z Cai, Y Gui, H Jiang: Promoter targeted bisulfite sequencing reveals DNA methylation profiles associated with low sperm motility in asthenozoospermia. *Hum Reprod* 31, 24–33 (2016)
DOI: 10.1093/humrep/dev283

46. RG Urdinguio, GF Bayón, M Dmitrijeva, EG Toraño, C Bravo, MF Fraga, L Bassas, S Larriba, AF Fernández: Aberrant DNA methylation patterns of spermatozoa in men with unexplained infertility. *Hum Reprod* 30, 1014–1028 (2015)
DOI: 10.1093/humrep/dev053

47. D Montjean, A Zini, C Ravel, S Belloc, A Dalleac, H Copin, P Boyer, K Mc Elreavey, M Benkhalifa: Sperm global DNA methylation level: association with semen parameters and genome integrity. *Andrology* 3, 235–240 (2015)
DOI: 10.1111/andr.12001

48. RP Horgan, OH Clancy, JE Myers, PN Baker: An overview of proteomic and metabolomic technologies and their application to pregnancy research. *BJOG* 116, 173–181 (2009)
DOI: 10.1111/j.1471-0528.2008.01997.x

49. E Com, N Melaine, F Chalmel, C Pineau: Proteomics and integrative genomics for unraveling the mysteries of spermatogenesis: the strategies of a team. *J Proteomic* 31, 128–143 (2014)
DOI: 10.1016/j.jprot.2014.04.013

50. KI Aston, DT Carrell: Genome-Wide Study of Single-Nucleotide Polymorphisms Associated With Azoospermia and Severe Oligozoospermia. *J Androl* 30, 711–725 (2009)
DOI: 10.2164/jandrol.109.007971

51. KI Aston, C Krausz, I Laface, E Ruiz-Castané, DT Carrell: Evaluation of 172 candidate polymorphisms for association with oligozoospermia or azoospermia in a large cohort of men of European descent. *Hum Reprod* 25, 1383–1397 (2010)
DOI: 10.1093/humrep/deq081

52. H Zhao, J Xu, H Zhang, J Sun, Y Sun, Z Wang, J Liu, Q Ding, S Lu, R Shi, L You, Y Qin, X Zhao, X Lin, X Li, J Feng, L Wang, JM Trent, C Xu, Y Gao, B Zhang, X Gao, J Hu, H Chen, G Li, J Zhao, S Zou, H Jiang, C Hao, Y Zhao, J Ma, SL Zheng, ZJ Chen. A genome-wide association study reveals that variants within the HLA region are associated with risk for nonobstructive azoospermia. *Am J Hum Genet* 90, 900–906 (2012)
DOI: 10.1016/j.ajhg.2012.04.001

53. Z Hu, Y Xia, X Guo, J Dai, H Li, H Hu, Y Jiang, F Lu, Y Wu, X Yang, H Li, B Yao, C Lu, C Xiong, Z Li, Y Gui, J Liu, Z Zhou, H Shen, X Wang, J Sha: A genome-wide association study in Chinese men identifies three risk loci for non-obstructive Azoospermia. *Nat Genet* 44, 183–186 (2011)
DOI: 10.1038/ng.1040

54. Z Hu, Z Li, J Yu, C Tong, Y Lin, X Guo, F Lu, J Dong, Y Xia, Y Wen, H Wu, H Li, Y Zhu, P Ping, X Chen, J Dai, Y Jiang, S Pan, P Xu, K Luo, Q Du, B Yao, M Liang, Y Gui, N Weng, H Lu, Z Wang, F Zhang, X Zhu, X Yang, Z Zhang, H Zhao, C Xiong, H Ma, G

Jin, F Chen, J Xu, X Wang, Z Zhou, Z Chen, J Liu, H Shen, J Sha: Association analysis identifies new risk loci for non-obstructive zoospermia in Chinese men. *Nat Commun* 5, 3857(2014)
DOI: 10.1038/ncomms4857

55. S Zou, Z Li, Y Wang, T Chen, P Song, J Chen, X He, P Xu, M Liang, K Luo, X Zhu, E Tian, Q Du, Z Wen, Z Li, M Wang, Y Sha, Y Cao, Y Shi, H Hu: Association study between polymorphisms of PRMT6, PEX10, SOX5, and nonobstructive azoospermia in the Han Chinese population. *Biol Reprod* 90, 96 (2014)
DOI: 10.1095/biolreprod.113.116541

56. G Kosova, NM Scott, C Niederberger, GS Prins, C Ober: Genome-wide association study identifies candidate genes for male fertility traits in humans. *Am J Hum Genet* 90, 950–961 (2012)
DOI: 10.1016/j.ajhg.2012.04.016

57. Y Sato, A Tajima, K Tsunematsu, S Nozawa, M Yoshiike, E Koh, J Kanaya, M Namiki, K Matsumiya, A Tsujimura, K Komatsu, N Itoh, J Eguchi, I Imoto, A Yamauchi, T Iwamoto: An association study of four candidate loci for human male fertility traits with male infertility. *Hum Reprod* 30, 1510–1514 (2015)
DOI: 10.1093/humrep/dev088

58. M Chihara, K Yoshihara, T Ishiguro, Y Yokota, S Adachi, H Okada, K Kashima, T Sato, A Tanaka, K Tanaka, T Enomoto: Susceptibility to male infertility: replication study in Japanese men looking for an association with four GWAS-derived loci identified in European men. *J Assist Reprod Genet* 32, 903–908 (2015)
DOI: 10.1007/s10815-015-0468-4

59. Y Dong, Y Pan, R Wang, Z Zhang, Q Xi, RZ Liu: Copy number variations in spermatogenic failure patients with chromosomal abnormalities and unexplained azoospermia. *Genet Mol Res* 14, 16041–16049 (2015)
DOI: 10.4238/2015.December.7.17

60. F Tüttelmann, M Simoni, S Kliesch, S Ledig, B Dworniczak, P Wieacker, A Röpke: Copy number variants in patients with severe oligozoospermia and Sertoli-cell-only syndrome. *PLoS One* 6, (2011)
DOI: 10.1371/journal.pone.0019426

61. S Eggers, KD De Boer, J van den Bergen, L Gordon, SJ White, D Jamsai, RI McLachlan, AH Sinclair, MK O'Bryan: Copy number variation associated with meiotic arrest in idiopathic male infertility. *Fertil Steril* 103, 214–219 (2015)
DOI: 10.1016/j.fertnstert.2014.09.030

62. C Krausz, C Giachini, D Lo Giacco, F Daguin, C Chianese, E Ars, E Ruiz- Castane, G Forti, E Rossi: High resolution X chromosome-specific array-CGH detects new CNVs in infertile males. *PLoS One* 7, (2012)

63. AM Lopes, KI Aston, E Thompson, F Carvalho, J Gonçalves, N Huang, R Matthiesen, MJ Noordam, I Quintela, A Ramu, C Seabra, AB Wilfert, J Dai, JM Downie, S Fernandes, X Guo, J Sha, A Amorim, A Barros, A Carracedo, Z Hu, ME Hurles, S Moskovtsev, C Ober, DA Paduch, JD Schiffman, PN Schlegel, M Sousa, DT Carrell, DF Conrad: Human spermatogenic failure purges deleterious mutation load from the autosomes and both sex chromosomes including the gene DMRT1. *PLoS Genet* 9, (2013)
DOI: 10.1371/journal.pgen.1003349

64. SK Bansal, N Gupta, SN Sankhwar, S Rajender: Differential Genes Expression between Fertile and Infertile Spermatozoa Revealed by Transcriptome Analysis. *PLoS One* 10, (2015)
DOI: 10.1371/journal.pone.0127007

65. D Montjean, P De La Grange, D Gentien, A Rapinat, S Belloc, P Cohen-Bacrie, Y Menezo, M Benkhalifa: Sperm transcriptome profiling in oligozoospermia. *J Assist Reprod Genet* 29, 3–10 (2012)
DOI: 10.1007/s10815-011-9644-3

66. SE Pacheco, EA Houseman, BC Christensen, CJ Marsit, KT Kelsey, M Sigman, K Boekelheide: Integrative DNA methylation and gene expression analyses identify DNA packaging and epigenetic regulatory genes associated with low motility sperm. *PLoS One* 6, (2011)
DOI: 10.1371/journal.pone.0020280

67. SS Hammoud, DH Low, C Yi, DT Carrell, E Guccione, BR Cairns: Chromatin and transcription transitions of mammalian adult germline stem cells and spermatogenesis. *Cell Stem Cell* 14, 239–253 (2014)
DOI: 10.1016/j.stem.2014.04.006

68. A Salas-Huetos, J Blanco, F Vidal, A Godo, M Grossmann, MC Pons, S F-Fernández, N Garrido, E Anton: Spermatozoa from patients with seminal alterations exhibit a differential micro-ribonucleic acid profile. *Fertil Steril* 104, 591–601 (2015) DOI: 10.1016/j.fertnstert.2015.06.015

69. A Abhari, N Zarghami, V Shahnazi, A Barzegar, L Farzadi, H Karami, S Zununi Vahed, M Nouri: Significance of microRNA targeted estrogen receptor in male fertility. *Iran J Basic Med Sci* 17, 81–86 (2014)

70. M Abu-Halima, M Hammadeh, C Backes, U Fischer, P Leidinger, AM Lubbad, A Keller, E Meese: Panel of five microRNAs as potential biomarkers for the diagnosis and assessment of male infertility. *Fertil Steril* 102, 989–997 (2014) DOI: 10.1016/j.fertnstert.2014.07.001

71. MS Rahman, JS Lee, WS Kwon, MG Pang: Sperm proteomics: road to male fertility and contraception. *Int J Endocrinol* 2013 (2013) DOI: 10.1155/2013/360986

72. NL Anderson, NG Anderson: Proteome and proteomics: new technologies, new concepts, and new words. *Electrophoresis* 19, 1853–1861 (1998) DOI: 10.1002/elps.1150191103

73. MS Zangbar, S Keshtgar, J Zolghadri, B Gharesi-Fard: Antisperm protein targets in azoospermia men. *J Hum Reprod Sci* 9, 47–52 (2016) DOI: 10.4103/0974-1208.178629

74. MI Hashemitabar, S Sabbagh, M Orazizadeh, A Ghadiri, MA Bahmanzadeh: A proteomic analysis on human sperm tail: comparison between normozoospermia and asthenozoospermia. *J Assist Reprod Genet* 32, 853–863 (2015) DOI: 10.1007/s10815-015-0465-7

75. FJ Liu, X Liu, JL Han, YW Wang, SH Jin, XX Liu, J Liu, WT Wang, WJ Wang: Aged men share the sperm protein PATE1 defect with young asthenozoospermia patients. *Hum Reprod* 30, 861–869 (2015) DOI: 10.1093/humrep/dev003

76. A Agarwal, R Sharma, D Durairajanayagam, A Ayaz, Z Cui, B Willard, B Gopalan, E Sabanegh: Major protein alterations in spermatozoa from infertile men with unilateral varicocele. *Reprod Biol Endocrinol* 13, 8 (2015) DOI: 10.1186/s12958-015-0007-2

77. SI Shen, J Wang, J Liang, D He: Comparative proteomic study between human normal motility sperm and idiopathic asthenozoospermia. *World J Urol* 31, 1395–1401 (2013) DOI: 10.1007/s00345-013-1023-5

78. TT Liao, Z Xiang, WB Zhu, LQ Fan: Proteome analysis of round-headed and normal spermatozoa by 2-D fluorescence difference gel electrophoresis and mass spectrometry. *Asian J Androl* 11, 683–693 (2009) DOI: 10.1038/aja.2009.59

79. S Thacker, SP Yadav, RK Sharma, A Kashou, B Willard, D Zhang, A Agarwal: Evaluation of sperm proteins in infertile men: a proteomic approach. *Fertil Steril* 95, 2745–2748 (2011) DOI: 10.1016/j.fertnstert.2011.03.112

80. PP Parte, P Rao, S Redij, V Lobo, SJ D'Souza, R Gajbhiye, V Kulkarni: Sperm phosphoproteome profiling by ultra-performance liquid chromatography followed by data independent analysis (LC-MS (E)) reveals altered proteomic signatures in asthenozoospermia. *J Proteomics* 75, 5861–5867 (2012) DOI: 10.1016/j.jprot.2012.07.003

81. AB Siva, DB Kameshwari, V Singh, K Pavani, CS Sundaram, N Rangaraj, M Deenadayal, S Shivaji. Proteomics-based study on asthenozoospermia: differential expression of proteasome alpha complex. *Mol Hum Reprod* 16, 452–462 (2010) DOI: 10.1093/molehr/gaq009

82. YI Liu, Y Guo, N Song, Y Fan, K Li, X Teng, Q Guo, Z Ding: Proteomic pattern changes with obesity-induced asthenozoospermia. *Andrology* 3, 247–259 (2015) DOI: 10.1111/andr.289

83. R Azpiazu, A Amaral, J Castillo, JM Estanyol, M Guimerà, JL Ballescà, J Balasch, R Oliva: High-throughput sperm differential proteomics suggests that epigenetic alterations contribute to failed assisted reproduction. *Hum Reprod* 29, 1225–1237 (2014) DOI: 10.1093/humrep/deu073

84. A Amaral, C Paiva, C Attardo Parrinello, JM Estanyol, JL Ballescà, J Ramalho-Santos, R

Oliva: Identification of proteins involved in human sperm motility using high-throughput differential proteomics. *J Proteome R*es 13, 5670–1584 (2014)
DOI: 10.1021/pr500652y

85. M Saraswat, S Joenväärä, T Jain, AK Tomar, A Sinha, S Singh, S Yadav, R Renkonen: Human Spermatozoa Quantitative Proteomic Signature Classifies Normo- and Asthenozoospermia. *Mol Cell Proteomics* 16, 57–72 (2017)
DOI: 10.1074/mcp.M116.061028

86. Y Vandenbrouck, L Lane, C Carapito, P Duek, K Rondel, C Bruley, C Macron, A Gonzalez de Peredo, Y Couté, K Chaoui, E Com, A Gateau, AM Hesse, M Marcellin, L Méar, E Mouton-Barbosa, T Robin, O Burlet-Schiltz, S Cianferani, M Ferro, T Fréour, C Lindskog, J Garin, C Pineau: Looking for Missing Proteins in the Proteome of Human Spermatozoa: An Update. *J Proteome Res* 15, 3998–4019 (2016)
DOI: 10.1021/acs.jproteome.6b00400

87. JV Silva, S Yoon, PJ De Bock, AV Goltsev, K Gevaert, JF Mendes, M Fardilha: Construction and analysis of a human testis/sperm-enriched interaction network: Unraveling the PPP1CC2 interactome. *Biochim Biophys Acta* 1861, 375–385 (2017)
DOI: 10.1016/j.bbagen.2016.11.041

88. V Kumar, MI Hassan, T Kashav, TP Singh, S Yadav: Heparin-binding proteins of human seminal plasma: purification and characterization. *Mol Reprod Dev* 75, 1767–1774 (2008)
DOI: 10.1002/mrd.20910

89. AK Tomar, BS Sooch, I Raj, S Singh, TP Singh, S Yadav: Isolation and identification of Concanavalin A binding glycoproteins from human seminal plasma: a step towards identification of male infertility marker proteins. *Dis Markers* 31, 379–386 (2011)
DOI: 10.1155/2011/798072

90. M Saraswat, S Joenväärä, AK Tomar, S Singh, S Yadav, R Renkonen: N-Glycoproteomics of Human Seminal Plasma Glycoproteins. *J Proteome Res* 15, 991–1001 (2016)
DOI: 10.1021/acs.jproteome.5b01069

91. A Minai-Tehrani, N Jafarzadeh, K Gilany: Metabolomics: a state-of-the-art technology for better understanding of male infertility. *Andrologia* 48, 609–616 (2016)
DOI: 10.1111/and.12496

92. A Asghari, SA Marashi, N Ansari-Pour: A sperm-specific proteome-scale metabolic network model identifies non-glycolytic genes for energy deficiency in asthenozoospermia. *Syst Biol Reprod Med,* 1–13(2017)
DOI: 10.1080/19396368.2016.1263367

93. J Zhang, Z Huang, M Chen, Y Xia, FL Martin, W Hang, H Shen: Urinary metabolome identifies signatures of oligozoospermic infertile men. *Fertil Steril* 102, 44–53 (2014)
DOI: 10.1016/j.fertnstert.2014.03.033

94. V Jayaraman, S Ghosh, A Sengupta, S Srivastava, HM Sonawat, PK Narayan: Identification of biochemical differences between different forms of male infertility by nuclear magnetic resonance (NMR) spectroscopy. *J Assist Reprod Genet* 31, 1195–1204 (2014)
DOI: 10.1007/s10815-014-0282-4

95. X Zhang, R Diao, X Zhu, Z Li, Z Cai: Metabolic characterization of asthenozoospermia using non targeted seminal plasma metabolomics. *Clin Chim Acta* 450, 254–261 (2015)
DOI: 10.1016/j.cca.2015.09.001

96. BF da Silva, PT Del Giudice, DM Spaine, FC Gozzo, EG Lo Turco, RP Bertolla: Metabolomics of male infertility: characterization of seminal plasma lipid fingerprints in men with spinal cord injury. *Fertil Steril* 96, S233-S233 (2011)
DOI: 10.1016/j.fertnstert.2011.07.893

97. N Jafarzadeh, A Mani-Varnosfaderani, A Minai-Tehrani, E Savadi-Shiraz, MR Sadeghi, K Gilany: Metabolomics fingerprinting of seminal plasma from unexplained infertile men: a need for novel diagnostic biomarkers. *Mol Reprod Dev* 82, 150 (2015)
DOI: 10.1002/mrd.22457

98. K Gilany, RS Moazeni-Pourasil, N Jafarzadeh, E Savadi-Shiraz: Metabolomics fingerprinting of the human seminal plasma of asthenozoospermic patients. *Mol Reprod Dev* 81, 84–86 (2014)
DOI: 10.1002/mrd.22284

99. AD Rolland, R Lavigne, C Dauly, P Calvel, C Kervarrec, T Freour, B Evrard, N Rioux-Leclercq, J Auger, C Pineau: Identification of

genital tract markers in the human seminal plasma using an integrative genomics approach. *Hum Reprod* 28, 199–209 (2016) DOI: 10.1093/humrep/des360

100. R Ramasamy, A Ridgeway, LI Lipshultz, DJ Lamb: Integrative DNA methylation and gene expression analysis identifies discoidin domain receptor 1 association with idiopathic nonobstructive azoospermia. *Fertil Steril* 102, 968–973 (2014) DOI: 10.1016/j.fertnstert.2014.06.028

101. H Ge, AJ Walhout, M Vidal: Integrating 'omic' information: a bridge between genomics and systems biology. *Trends Genet* 19, 551–560 (2003) DOI: 10.1016/j.tig.2003.08.009

102. V Singh, LC Singh, M Vasudevan, I Chattopadhyay, BB Borthakar, AK Rai, RK Phukan, J Sharma, J Mahanta, AC Kataki, S Kapur, S Saxena: Esophageal Cancer Epigenomics and Integrome Analysis of Genome-Wide Methylation and Expression in High Risk Northeast Indian Population. *OMICS* 19, 688–699 (2015) DOI: 10.1089/omi.2015.0121

103. S Pineda, P Gomez-Rubio, A Picornell, K Bessonov, M Márquez, M Kogevinas, FX Real, K Van Steen, N Malats: Framework for the Integration of Genomics, Epigenomics and Transcriptomics in Complex Diseases. *Hum Hered* 79, 124–36 (2015) DOI: 10.1159/000381184

104. A Rai, K Saito, M Yamazaki: Integrated omics analysis of specialized metabolism in medicinal plants. *Plant J* (2017) DOI: 10.1111/tpj.13485

105. A Wippermann, O. Rupp, K. Brinkrolf, R. Hoffrogge, T. Noll: Integrative analysis of DNA methylation and gene expression in butyrate-treated CHO cells. *J Biotechnol* (2016) DOI: 10.1016/j.jbiotec.2016.11.020

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