Review Article
Male reproductive health and infertility

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Towards a Multi-Omics of Male Infertility

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Infertility is a common problem affecting one in six couples and in 30% of infertile couples, the male factor is a major cause. A large number of genes are involved in spermatogenesis and a significant proportion of male infertility phenotypes are of genetic origin. Studies on infertility have so far primarily focused on chromosomal abnormalities and sequence variants in protein-coding genes and have identified a large number of disease-associated genes. However, it has been shown that a multitude of factors across various omics levels also contribute to infertility phenotypes. The complexity of male infertility has led to the understanding that an integrated, multi-omics analysis may be optimal for unravelling this disease. While there is a vast array of different factors across omics levels associated with infertility, the present review focuses on known factors from the genomics, epigenomics, transcriptomics, proteomics, metabolomics, glycomics, lipidomics, miRNomics, and integrated omics levels. These include: repeat expansions in *AR, POLG, ATXN1, DMPK,* and *SHBG*, multiple SNPs, copy number variants in the AZF region, disregulated miRNAs, altered H3K9 methylation, differential *MTHFR, MEG3, PEG1,* and *LIT1* methylation, altered protamine ratios and protein hypo/hyperphosphorylation. This integrative review presents a step towards a multi-omics approach to understanding the complex etiology of male infertility. Currently only a few genetic factors, namely chromosomal abnormalities and Y chromosome microdeletions, are routinely tested in infertile men undergoing intracytoplasmic sperm injection. A multi-omics approach to understanding infertility phenotypes may yield a more holistic view of the disease and contribute to the development of improved screening methods and treatment options. Therefore, beside discovering as of yet unknown genetic causes of infertility, integrating multiple fields of study could yield valuable contributions to the understanding of disease development. Future multi-omics studies will enable to synthesise fragmented information and facilitate biomarker discovery and treatments in male infertility.

Keywords: DNA methylation; Epigenetics; Expression; Male infertility; Multi-omics; Non-coding RNAs (ncRNAs)

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INTRODUCTION

Infertility is a problem affecting one in six couples, which is approximately 15% of the population wishing to start a family. In 30% of infertile couples, the male factor is a major cause [1,2]. A man is considered infertile if he is not able to induce a pregnancy after at least 12 months of regular intercourse without contraception [3]. In 30% to 45% of infertile men, the cause of the abnormal semen parameters is not identified (idiopathic male infertility) [4]. The term idiopathic is used only if defined causes of male infertility can be excluded, such as: sperm autoimmunity, sexual and/or ejaculatory dysfunction, cryptorchidism, acquired testicular

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damage, drug-related issues, radiation-induced damage, varicocele, male accessory gland infection, endocrine causes, congenital bilateral absence of the vas deferens, hypogonadotrophic hypogonadism, cystic fibrosis, myotonic dystrophy, hypospadias, testis atrophy or abnormal karyotype. The following descriptive categories (or combinations thereof) can be given: idiopathic oligospermia (sperm concentration of <20 million per mL), idiopathic asthenospermia (sperm concentration above 20 million per mL but low motility), idiopathic teratospermia (normal sperm concentration and motility but the ejaculate contains a substantial fraction of morphologically abnormal sperm), idiopathic azoospermia (no spermatozoa in the ejaculate) [3].

In the past decade significant improvements have been made in our understanding of sperm cell biology and ability to diagnose and treat male infertility. This is the result of combined approaches of advanced proteomics, biochemistry, and functional genomics. Given the complexity of the process of spermatogenesis and the large number of genes involved, it is likely that a significant portion of male infertility phenotypes are of genetic origin [5,6]. So far over 800 genetic loci have been found to be implicated in male reproduction in different mammalian species [7], however, only a few genetic factors, such as chromosomal abnormalities and Y chromosome microdeletions are now tested routinely in infertile men [7-10]. Several genetic factors associated with infertility have been identified, but are not part of routine testing. These include mitochondrial DNA (mtDNA) anomalies [11], genes controlled by the cAMP-response-element modulator [12], histone deacetylase-dependent transcriptional repressor ZMYND15 [13] and a plethora of others [14].

However, research approaches focusing on single genes or omics levels have so far yielded limited results. While many contributing factors have been identified with these methods, the complex pathomechanism of male infertility remains unclear. For this purpose, an integrated multi-omics systems biology approach may be more beneficial in unravelling the underlying disease mechanism. Additionally, such an approach may allow for the development of novel diagnostic and treatment options.

In this review, we obtained the literature describing factors associated with male infertility on different omics levels, including genomics, epigenomics, transcriptomics, proteomics, metabolomics, glycomics, lipido-

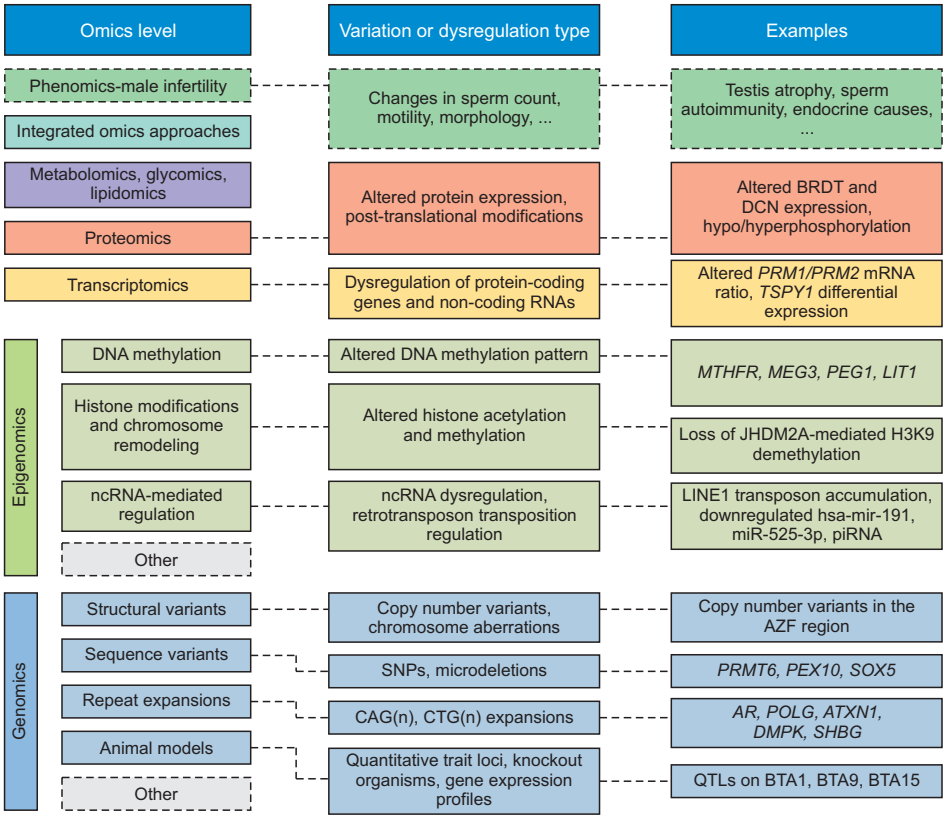

Fig. 1. Overview of omics levels covered in the present review.

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mics, miRNomics, and integrated omics (Fig. 1). In order to better understand male infertility, we included data for humans, large animal models (cattle, horse, pig, and sheep), and rodents (mouse and rat).

GENOMICS

Male infertility has been extensively studied on the DNA level and a large number of associated genetic factors have been identified [15]. These factors include: single nucleotide polymorphisms (SNPs), copy number variants (CNVs), chromosome aberrations, microdeletions, repeat expansions and others.

1. Structural variants

Copy number variation of genes involved in spermatogenesis might be among the underlying causes of male fertility problems. Feuk et al [16] defined CNVs as DNA segments of 1 kb or larger and present at variable copy number in comparison with a reference genome. DNA copy numbers could be one of the causes for gene expression variations; concordance between copy number and changes in mRNA expression levels has been observed in several genes located in CNV regions. CNVs could influence the activity of individual genes important for fertility, however increased number or specific distribution of CNVs might also result in defective recombination, meiotic failure and loss of germ cells [17].

Since the first definition of the azoospermia factor (AZF) regions, the Y chromosome has become an important target for studies aimed to identify genetic factors involved in male infertility. Due to its structure, which includes repeated homologous sequences, the Y chromosome is predisposed to structural rearrangements, especially deletions and duplications. AZF deletions including AZFa (P5/proximal-P1), AZFb (P5/distal-P1) and AZFc deletions are in a cause-effect relationship with spermatogenic failure and gr/gr deletion represents an example of a proven genetic risk factor in andrology [6,8,18,19]. CNV studies in male infertility provide evidence that CNVs contribute to the complex origin of male infertility and present a number of candidate genes possibly causing or being risk factors for spermatogenic failure [17].

2. Sequence variants

Many studies have associated SNPs and small insertions or deletions in different genes and in non-coding areas with male infertility [20-23]. Polymorphisms in genes involved in spermatogenesis are considered potential risk factors, as polymorphisms in them could lead to infertility [24].

Genome-wide association studies (GWAS) have emerged as a powerful novel approach for identification of susceptibility loci. Using this approach, the entire genomes of fertile and infertile men were compared to identify differences in the nucleotide sequences. The ultimate goal of these genetic studies is the assessment of an individual's risk, leading to specific preventive measures (model "predict and prevent") [25]. Array-based testing platforms revolutionized our understanding of the molecular genetic basis of infertility. Taking a cue from gene arrays, the hope is that panels of tens to hundreds of protein and peptide markers can transcend the heterogeneity to generate a higher level of diagnostic specificity [26]. In this way Aston and Carrell [5] employed genotyping microarray technology to investigate over 370,000 SNPs in men with azoospermia and severe oligozoospermia, along with normozoospermic controls. They found 20 SNPs significantly associated with azoospermia or oligozoospermia [5].

In order to identify common variants contributing to non-obstructive azoospermia (NOA) Hu et al [27] performed GWAS and identified significant associations between NOA risk and common variants near *PRMT6*, *PEX10* and *SOX5* gene. SNP-based microarrays applied to preimplantation genetic diagnosis for chromosomal rearrangements can differentiate between normal and balanced (carrier) embryos. This may allow patients that carry chromosomal rearrangements the ability to choose to transfer chromosomally normal embryos preferentially over embryos that are balanced carriers of the parental translocation [2]. However, future GWAS studies which will evaluate identified SNPs on additional samples are necessary. They will further expand our understanding of the causes of male infertility and eventually provide specialized diagnostic tests and new targets for treatment [5]. Genes with sequence variants and their associated phenotype of male infertility are summarized in Table 1.

3. Repeat expansions

Nucleotide repeat expansions are a consequence of slipped strand mispairing during DNA replication

Table 1. Genes with sequence variants and their associated phenotype of male infertility

Gene symbol	Associated phenotype	Reference
ADGRG2	Congenital bilateral absence of vas deferens, X-linked	Houston et al, 2021 [15]
AMH	Persistent Mullerian duct syndrome, type I	Houston et al, 2021 [15]
AMHR2	Persistent Mullerian duct syndrome, type II	Houston et al, 2021 [15]
ANOS1	Hypogonadotropic hypogonadism 1 with or without anosmia (Kallmann syndrome 1)	Houston et al, 2021 [15]
AURKC	Spermatogenic failure 5	Houston et al, 2021 [15]
CFAP43	Spermatogenic failure 19	Houston et al, 2021 [15]
CFAP44	Spermatogenic failure 20	Houston et al, 2021 [15]
CFTR	Congenital bilateral absence of vas deferens	Houston et al, 2021 [15]
CHD7	Hypogonadotropic hypogonadism 5 with or without anosmia	Houston et al, 2021 [15]
CYP11A1	Adrenal insufficiency, congenital, with 46XY sex reversal, partial or complete	Houston et al, 2021 [15]
CYP11B1	Adrenal hyperplasia, congenital, due to 11-beta-hydroxylase deficiency	Houston et al, 2021 [15]
CYP17A1	Adrenal hyperplasia, congenital, due to 17-alpha-hydroxylase deficiency	Houston et al, 2021 [15]
PRMT6	Non-obstructive azoospermia	Hu et al, 2012 [27]
PEX10	Non-obstructive azoospermia	Hu et al, 2012 [27]
SOX5	Non-obstructive azoospermia	Hu et al, 2012 [27]

Table 2. Genes with repeat expansions and their associated phenotype of male infertilty

Gene	Phenotype	Reference
AR	Idiopathic infertility	Metin Mahmutoglu et al, 2022 [28]
AR	Idiopathic azoospermia	Pan et al, 2002 [31]
ATXN1	Idiopathic oligozoospermia	Lai et al, 2009 [30]
ATXN3	Idiopathic azoospermia	Pan et al, 2002 [31]
DMPK	Idiopathic azoospermia	Pan et al, 2002 [31]
POLG	Idiopathic subfertility/infertility	Jensen et al, 2004 [29]
SHBG	Idiopathic infertility	Safarinejad et al, 2011 [32]

and are responsible for various disorders. A number of repeat expansions in genes have been associated with male infertility, including *AR, POLG, ATXN1, DMPK, ATXN3,* and *SHBG*; their associated phenotypes are presented in Table 2 [28-32]. However, these associations are often contradictory. A review by Peterlin et al [33] showed that ten studies associated the (CAG)n repeat polymorphism in the *AR* gene with male infertility, however 20 studies failed to do so. The latter indicates that the results are often inconsistent and replication studies often fail to validate initial findings. Additionally, a correlation has also been established between the length of CAG repeats in mitochondrial polymerase (DNA directed) gamma (*POLG*) and male infertility [29,34], although this finding was inconsistent with other studies [35-38].

Kunej et al [39] reported that the number of CTG repeats of the *DMPK* gene was not associated with idiopathic male subfertility nor with clinical characteristic of male subfertility in Slovene population. The variability of the results by various research groups might be due to different ethnic origins and, hence, different genetic modifiers of the populations studied [5]. Therefore, additional studies on larger cohorts of patients and meta-analyses are encouraged and only this will make the interpretation of data obtained from patient studies more reliable.

4. Animal models

Animal models have been used in the study of many human diseases with genetic components, and male infertility is no exception. In the Mouse Genome Informatics (MGI) database, as well as in literature, are a number of knockout strains of mice that are male-sterile when homozygous for a particular null allele. The pathology of such model animals provides a mechanism to begin comparisons with similar problems in humans [40]. For example, Vamp4 protein expression was sup-

pressed in mice by RNAi-mediated knockdown method, which was the first direct evidence for the important role of SNARE proteins in acrosome formation. In mice with suppressed Vamp4 protein, abnormalities of acrosomal vesicle fusion and of the nucleus were seen in spermatids and sperm [41]. As soon as a convergence of phenotypes is observed between a specific mutated mouse gene and a human phenotype, mutations in the human ortholog gene can be screened for [42]. Due to the fact that such phenotype driven strategies do not rely on previous knowledge of gene identity, they are almost wholly unbiased and allow capture of genes whose function in reproduction is unanticipated [43].

A quantitative trait locus (QTL) is a statistically identified genomic region hypothetically responsible for genetic variation in a trait. Experimentally, QTL estimation is usually carried out within a given resource population with a specific mating scheme designed for detecting genetic segregations useful for the test [44]. In mammals, male fertility is a quantitative feature determined by numerous genes acting at different levels, therefore the underlying genes are very difficult to identify [45]. Defining wide chromosomal regions involved in male fertility in mammals is the first step towards fine-mapping of causal genes. To date, several QTL for male fertility traits have been reported in mouse, cattle, sheep, pig, and rat [45-51]. The latest release of AnimalQTL database includes entries for several reproductive traits, such as QTLs for sperm concentration in bulls, which were mapped to *BTA1, BTA9,* and *BTA15* [52]. Le Roy et al [48] used inbred strains of laboratory mice to identify five chromosomal regions associated with testis weight and one associated with associated with seminal vesicle weight. Genetic regions associated with male infertility identified with animal models are summarized in Table 3. Identification of genes linked with reproduction traits in animals facilitate the discovery of corresponding human genes via the use of comparative maps and subsequently developing animal models for sterility.

Table 3. Sequence variants in genetic regions and their associated phenotype in animal models associated with male infertility

Affected genetic region (chromosome)	Animal model	Associated phenotype	Reference
Region on chr. 1 (BTA1)	Bull	Reduced sperm concentration	Huo et al, 2019 [52]
Region on chr. 9 (BTA9)	Bull	Reduced sperm concentration	Huo et al, 2019 [52]
Region on chr. 15 (BTA15)	Bull	Reduced sperm concentration	Huo et al, 2019 [52]
DSS1 (MMU6)	Mouse	Decreased sperm survival	L'Hôte et al, 2007 [45]
LTW1 (MMU11)	Mouse	Low testis weight	L'Hôte et al, 2007 [45]
LTW2 (MMU6)	Mouse	Low testis weight	L'Hôte et al, 2007 [45]
LPW1 (MMU19)	Mouse	High prostate weight	L'Hôte et al, 2007 [45]
SH1 (MMU6)	Mouse	Altered sperm nucleus shape	L'Hôte et al, 2007 [45]
SH2 (MMU3)	Mouse	Altered sperm nucleus shape	L'Hôte et al, 2007 [45]
SH3 (MMU12)	Mouse	Altered sperm nucleus shape	L'Hôte et al, 2007 [45]
SH4 (MMU11)	Mouse	Altered sperm nucleus shape	L'Hôte et al, 2007 [45]
Vamp4 (MMU1)	Mouse	Abnormalities in acrosomal vesicle fusion	Guo et al, 2010 [41]
Region on chr. X (MMUX)	Mouse	Low testis weight	Ford et al, 2001 [46]
Region on chr. 4 (MMU4)	Mouse	Low testis weight	Ford et al, 2001 [46]
Region on chr. 10 (MMU8)	Mouse	Low testis weight	Ford et al, 2001 [46]
Region on chr. 13 (MMU13)	Mouse	Low testis weight	Ford et al, 2001 [46]
Region on chr. 18 (MMU18)	Mouse	Low testis weight	Ford et al, 2001 [46]
Region on chr. X (SSCX)	Pig	Testis weight	Sato et al, 2003 [50]
Region on chr. 3 (SSC3)	Pig	Elevated FSH levels	Rohrer et al, 2001 [49]
Region on chr. 3 (SSC3)	Pig	Testis weight	Sato et al, 2003 [50]
Region on chr. 8 (SSC8)	Pig	Elevated FSH levels	Rohrer et al, 2001 [49]
Region on chr. 10 (SSC10)	Pig	Elevated FSH levels	Rohrer et al, 2001 [49]
Xq (SSCX)	Pig	Altered FSH secretion and testis weight	Ford et al, 2001 [46]
Region near *D8Cebr204S21* (RNO8)	Rat	Seminal vesicle mass	Zídek et al, 1999 [51]

BTA: *Bos taurus*, FSH: follicle-stimulating hormone, MMU: *Mus musculus*, SSC: *Sus scrofa*, RNO: *Rattus norvegicus*.

EPIGENOMICS

Several studies reported that epigenetic mechanisms like DNA methylation, residual histone modifications, chromatin remodelling, and non-coding RNA-mediated regulation could have effect on male infertility, embryonic development and assisted reproductive techniques outcome [53].

1. DNA methylation

A common epigenetic regulatory mechanism is DNA methylation. This is done by DNA methyltransferases and achieves gene repression by altering the 3D structure of DNA, making the binding of transcription factors more difficult [54]. In humans, fetal spermatogonia seem to be mostly unmethylated at the *H19* (H19 imprinted maternally expressed transcript) differentially methylated region (DMR), although spermatogonia of adult testis are significantly methylated in this region. Epigenetic perturbations of the 6th CTCF (CCCTC-binding factor) site of the *H19* DMR have been proposed as a relevant biomarker for quantitative defects of spermatogenesis - teratozoospermia and/or oligo-astheno-teratozoospermia (OAT) in humans [55]. Furthermore, Marques et al [56] studied the methylation patterns of *H19* and *MEST* imprinted genes and suggested that abnormal methylation of these genes in human sperm is associated with oligozoospermia. It is worth considering abnormal paternal DNA methylation which has been demonstrated at *H19* and *MEG3* (synonym *GTL2*) and abnormalities of maternal DMRs at *MEST* (synonym *PEG1*), *KCNQ1OT1* (synonym *LIT1*), *PLAGL1* (synonym *ZAC*), *PEG3* and *SNRPN* [57].

Studies also revealed that sperm methylation abnormalities may involve large numbers of genes that also affect non-imprinted genes. Wu et al [58] reported that hypermethylation of the promoter of *MTHFR* gene in sperm is associated with idiopathic male infertility. Additionally, Dhillon et al [59] have found that *GSTM1* epigenetic silencing is associated with male infertility. Analysis of DNA methylation status of CpGs in human sperm revealed a loss of methylation in the teratozoospermia group compared to the control group. Loss of methylation appeared in CpG positions either in the IGF2 DMR2 and/or the sixth CTCF binding site of the *H19* DMR [55]. In the OAT group, a severe loss of methylation of the 6th CTCF were presented and was associated with sperm concentration [55]. Furthermore, Nanassy and Carrell [60] discovered that patients with an abnormal protamine 1/protamine 2 (P1/P2) ratio or oligozoospermia display an increased abnormal methylation of *CREM* compared with control subjects. Differentially methylated genetic regions and their associated phenotypes are summarized in Table 4.

2. Histone modifications

Successful spermiogenesis requires a sequence of histone modifications, as well as a shift from histones to transition proteins and testis-specific histone variants, and later to protamines [61]. Differential post-translational modifications (PTMs) of histones have been

Table 4. Differentially methylated genetic regions and their associated phenotypes

Affected genes	Phenotype	Reference
CREM	Oligozoospermia	Nanassy et al, 2011 [60]
GSTM1	OAT	Dhillon et al, 2007 [59]
GTL	Oligozoospermia	Kobayashi et al, 2007 [57]
H19	Oligozoospermia	Kobayashi et al, 2007 [57]
H19	Oligozoospermia	Marques et al, 2008 [56]
H19	Teratozoospermia/OAT	Boissonnas et al, 2010 [55]
IGF2	Teratozoospermia/OAT	Boissonnas et al, 2010 [55]
KCNQ1OT1	Oligozoospermia	Kobayashi et al, 2007 [57]
MEST	Oligozoospermia	Marques et al, 2008 [56]
MEST	Oligozoospermia	Kobayashi et al, 2007 [57]
PEG3	Oligozoospermia	Kobayashi et al, 2007 [57]
PLAGL1	Oligozoospermia	Kobayashi et al, 2007 [57]
SNRPN	Oligozoospermia	Kobayashi et al, 2007 [57]

OAT: oligo-astheno-teratozoospermia.

identified in males with abnormal semen parameters. These altered PTMs include altered H4 acetylation as well as altered H4K20 and H3K9 methylation compared to normozoospermic samples [62]. In a genome-wide analysis of histone locations and modifications, Hammoud et al noted that five of the seven males with reproductive dysfunction had non-programmatic histone retention genome-wide. However, they note that localization of methylation at H3 Lysine 4 (H3K4me) and H3 Lysine 27 (H3K27me) was similar between males with reproductive dysfunction and the control group [63].

Asthenozoospermia (AS) sperm was found to have an increased presence of histone modifications at H3K4Me1, H3K9Me2, H3K4Me3, H3K79Me2 and H3K36Me3 [64]. Following this model, Yuen et al created a line of *H3f3b* knockout mice, which could not produce the H3.3 histone variant. This resulted in abnormal spermatozoa, a decrease in germ cell types and testis atrophy, leading to male infertility [65]. The loss of JHDM2A-mediated H3K9 demethylation in mice was shown to impair post-meiotic chromatin condensation, leading to infertility [66]. Differentially modified histone positions and their phenotypes are summarized in Table 5. It is therefore apparent that histone modifications are an integral part of spermiogenesis and that the dysregulation of this process may be a contributing factor or core cause of some infertility cases.

3. Non-coding RNAs

In epigenetics, non-coding RNAs (ncRNAs) are RNA molecules which, depending on their type, regulate gene expression in different ways. NcRNAs are divided into long ncRNAs and short ncRNAs, and each also has several distinct classes. MicroRNAs (miRNAs) are a class of short ncRNAs that act as post-transcriptional repressors and have been associated with multiple diseases, including male infertility [67,68]. Due to their importance in gene expression regulation, the field of miRNomics is dedicated to studying this class of short ncRNAs. MiRNA targets, their functions and miRNAs are summarized in Table 6.

MiR-471 has been shown to target *DSC1* and *FOXD1*, which are highly expressed in Sertoli cells [69]. MiRNAs also often have multiple mRNA targets. MiR-34/449 family members have a large array of targets, including cyclin-dependents kinases, *NOTCH1*, *BCL2* and *CASP3*, which play a role in the generation of mature spermatozoa by regulating the cell cycle [70]. Other ncRNAs have also been associated with

Table 5. Histone positions with altered post-translational modifications and their associated phenotypes

Affected histone position	Phenotype	Species	Reference
H3K4	Asthenozoospermia	Human	La Spina et al, 2014 [64]
H3K4	Infertility	Human	Hammoud et al, 2011 [63]
H3K9	Asthenoteratozoospermia	Human	Schon et al, 2019 [62]
H3K9	Asthenozoospermia	Human	La Spina et al, 2014 [64]
H3K27	Infertility	Human	Hammoud et al, 2011 [63]
H3K36	Asthenozoospermia	Human	La Spina et al, 2014 [64]
H3K79	Asthenozoospermia	Human	La Spina et al, 2014 [64]
H4K20	Asthenoteratozoospermia	Human	Schon et al, 2019 [62]
H3K9	Infertility	Mouse	Okada et al, 2010 [66]

Table 6. MiRNAs, their regulatory targets and their functions or phenotypes associated with male infertility

miRNA	Target	Target function or phenotype	Reference
miR-34/449 family	*BCL2*	Generation of mature spermatozoa	Pantos et al, 2021 [70]
miR-34/449 family	*CASP3*	Generation of mature spermatozoa	Pantos et al, 2021 [70]
miR-34/449 family	Cyclin-dependent kinases	Generation of mature spermatozoa	Pantos et al, 2021 [70]
miR-34/449 family	*NOTCH1*	Generation of mature spermatozoa	Pantos et al, 2021 [70]
miR-471	*DSC1*	Highly expressed in Sertoli cells	Panneerdoss et al, 2012 [69]
miR-471	*FOXD1*	Highly expressed in Sertoli cells	Panneerdoss et al, 2012 [69]

spermiogenesis and male infertility, such as long non-coding RNAs (lncRNAs) [71]. Some lncRNAs are known to be necessary for meiosis and spermatogenesis, such as lncRNA-Tsx, whose absence results in apoptosis of spermatocytes during pachytene [72]. NcRNAs include a large number of RNA types and affect fertility on multiple omics levels. Dysregulations in ncRNA expression fall under the purview of transcriptomics and are thus described in section 3.

TRANSCRIPTOMICS

Transcriptomics is the study of the transcriptome - the entire set of coding and non-coding RNAs that are transcribed in a cell at a specific point in time. The transcriptome thus differs based on cell types and their developmental stages and, when dysregulated, can be indicative of pathology. In the case of male infertility, transcriptomic analyses have revealed differential expression and regulation of multiple disease-associated genes.

1. Protein-coding genes

The global analysis of spermatozoa messenger RNAs (mRNAs) allows us to measure the expression of thousands of differentially expressed genes (DEG) in a single sample and in this way explore clinical markers for male infertility. Molecular signatures obtained by comparing gene expression profiles from fertile and infertile groups are used to identify genes and gene networks critical to spermatogenesis and then potentially serves as a diagnostic platform and suggest gene therapy targets of male infertility [40,73,74]. Differentially expressed protein-coding genes and their associated phenotypes are presented in Table 7.

Microarray analysis in sperm from fertile and infertile men with normal semen parameters successfully demonstrated significant differences in spermatozoal mRNA profiles [75]. Therefore, spermatozoal mRNA may be useful as biomarkers for predicting male infertility [76]. Rockett et al compared gene expression profiles from normal and abnormal human testes with those from comparable infertile mouse models. Forty-seven mouse genes exhibited differential testicular gene expression and 19 human genes were differentially expressed between normal and abnormal samples [40]. Additionally, Montjean et. al. studied the sperm transcriptome profile in oligozoospermia and found that transcription profile in germ cells of men with idiopathic infertility is different from that of fertile individuals [77]. Furthermore, using complementary DNA (cDNA) microarray analysis, 10 novel sterility-related genes were identified [78]. Mitochondrial sirtuins (SIRT3, SIRT4 and SIRT5), responsible for regulating energy production, have been shown to be significantly downregulated in semen samples of infertile males [79].

It has also been found that the *PRM1/PRM2* mRNA ratio in testicular spermatids and ejaculated spermatozoa significantly differ between infertile men and fertile controls [80]. Furthermore, protamine mRNAs appeared to have a role in the formation of fully functional mature spermatozoa and as such, have potential as a diagnostic tool for male infertility [76]. In addition, glucose phosphate isomerase has also been reported to be differentially expressed in two species (infertile mice and humans) [40]. Furthermore, *RBMY1*, *DAZ1*, *TSPY1* and *DDX3Y* are differentially expressed in males with oligozoospermia or azoospermia in comparison to the normozoospermic control group [81]. However, discrepancies in the results of gene expression analyses have been observed and may result from the differences in experimental protocols and statistical approaches, or nonhomogeneous cohort characteristics.

Table 7. Differentially expressed proteins and their associated male infertility phenotypes

Protein symbol	Associated phenotype	Reference
DAZ1	Oligozoospermia/azoospermia	Lardone et al, 2007 [82]
DDX3Y	Oligozoospermia/azoospermia	Lardone et al, 2007 [82]
RBMY1	Oligozoospermia/azoospermia	Lardone et al, 2007 [82]
SIRT3	Oligoasthenospermia/asthenospermia	Bello et al, 2022 [79]
SIRT4	Oligoasthenospermia/asthenospermia	Bello et al, 2022 [79]
SIRT5	Oligoasthenospermia/asthenospermia	Bello et al, 2022 [79]
TSPY1	Oligozoospermia/azoospermia	Lardone et al, 2007 [82]

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2. Non-coding RNAs

Beside mRNA, non-coding RNAs have also been reported to be dysregulated in association with male infertility. For example, altered miRNA profiles of testicular biopsies from NOA patients have been observed [82]. Beside dysregulation, ncRNAs could also be studied for their interactions with downstream targets [83]. Dysregulated ncRNAs and their associated phenotypes are summarized in Table 8.

Nucleus of mature spermatozoa contains a complex population of RNAs that are transcriptionally inert, but they may serve as biomarkers for male infertility. Bioinformatics analysis performed by Krawetz et al revealed the presence of multiple classes of small RNAs in human spermatozoa [84]. These include miRNAs, Piwi-interacting RNAs (piRNAs), and repeat-associated small RNAs [84]. The importance of small non-coding RNAs as regulators of RNA transcription stability and translation is becoming increasingly evident [85]. MiRNAs are believed to be associated with male infertility due to their function as posttranscriptional suppressors through binding to their target mRNAs by base-pairing and subsequently inducing either translational repression or mRNA destabilization [86]. Therefore, high levels of gene expression do not always correspond to elevated of protein expression, because miRNAs repress protein synthesis from targeted mRNAs and control approximately 30% of human genes [87,88].

Molecular mechanisms of small RNA molecules regulating spermatogenesis have been reviewed by He et al [89]. Microarray analyses have revealed dynamic changes in small ncRNAs expression during spermatogenesis and in case of different types of infertility. In this way the miRNA expression profiles of testes of patients with NOA and normal controls were performed by using microarray technologies. Altered miRNA expression in NOA patients was found and findings have been confirmed by RT-qPCR assays which confirmed 19 up- and 154 down-regulated miRNAs in patients with NOA [90]. Additionally, hsa-mir-191 was found to be down-regulated in teratozoospermia [91]. Curry et al analysed differences in miRNA expression between normal porcine sperm samples and those exhibiting high percentages of morphological abnormalities or low motility. There were increases in the expression of four miRNAs in the abnormal group, whereas one was decreased compared to controls and two miRNAs were increased in the low motility group when compared to controls [92].

All of the above alterations suggest the importance of appropriate levels of ncRNAs for fertility. In this way Yan et al, with the aim to understand miRNA expression during mammalian spermatogenesis, used miRNA microarray to determine miRNA expression patterns of immature and mature rhesus monkey and mature human testis tissues [93]. Analysis indicated that 15 of the 26 miRNAs may be responsible for the difference between immature and mature testis miRNA expression. Additionally, Yan et al found 19 miRNAs differentially expressed between immature and mature mice testes (14 up regulated and five down regulated) [94], while Lian et al used RT-qPCR assay and found 122 miRNAs differentially expressed in the immature and mature porcine testes [95]. Furthermore, Ro et al reported cloning and expression profiling of 141 miRNAs expressed in mice testes of which 28 are preferentially or exclusively expressed in testes [96]. It is noteworthy that Torley et al used sheep as a model with the aim to identify the expression of miRNAs in mammalian fetal gonad and revealed significant differences between testes among 24 miRNAs at gestational day (GD) 42 and 43 miRNAs at GD75. In addition, their data indicate that miR-22 is involved in repressing estrogen signalling within fetal testes and in situ hybridization revealed miR-22 localization within fetal testicular cords [97].

Single-nucleotide polymorphisms (SNPs) also play an important role on the RNA level, because SNPs of miRNA precursors and their target sites, as well as the silencing machinery, interfere with miRNA function and are likely to affect phenotypic variation, including disease susceptibility [98]. Ogorevc et al discussed genetic variability of miRNA targets within male infertility genes, which can represent a source of novel molecular-genetic markers that can be used for the diagnosis of male infertility [99]. Genes with high levels of testis-specific expression, polymorphic 3'-UTRs, and/or conserved miRNA target sites represent promising

Table 8. Differentially expressed ncRNAs and their associated phenotypes

ncRNA	Phenotype	Reference
hsa-mir-191	Teratozoospermia	Grinchuk et al, 2010 [92]
hsa-miR-525-3p	AS	Zhou et al, 2019 [101]
lncRNA Gm2044	NOA	Liang et al, 2019 [105]

candidates for targets involved in miRNA-regulated pathogenesis of male infertility. Additionally, miRNA-target interactions have also been experimentally validated; reduced expression of miR-525-3p and elevated expression of its target *SEMG1* have been shown to be associated with AS and male infertility [100].

Another class of sncRNAs are Piwi-interacting RNAs (piRNAs), which have multiple functions, such as retrotransposon transposition repression, mRNA stability and epigenetic regulation [101,102]. Yan et al identified piRNA like RNAs (pilRNAs) from mouse testes and suggest that the testis is the organ with the highest expression of pilRNAs both in number and in abundance and that pilRNAs probably play an important role in the regulation of spermatogenesis [94]. Further functional studies are required to identify the exact mode of action of piRNAs.

Beside short ncRNA also long non-coding RNAs (lncRNAs) have also been shown to have a potential for new biomarkers and therapeutic strategies as they have been shown to be dysregulated in association with low sperm count in a mouse model [103]. LncRNA Gm2044 has been shown to play a role in NOA [104]. While the number of publications related with circular RNAs (circRNA) is increasing, our literature review did not reveal any publication describing their role in male infertility.

PROTEOMICS

Proteomics is the study of the proteome, which is a set of proteins produced by an organism. The field has evolved as a major area of research in biology and medicine, its main objective is to identify and validate potential targets, at the molecular level, for development of more sensitive diagnostic tools [105]. Proteomic approaches are used to study, among others, protein-protein interactions (PPI), PTMs and differential protein expression [106]. Proteins and their associated functions in fertility/infertility are presented in Table 9.

Milardi et al [107] identified at least 919 unique proteins per individual seminal plasma sample and some of these proteins might be involved in male fertility. One of the candidates is sperm surface protein fertilin (heterodimer composed of α and β subunits) which may promote sperm-egg binding, fusion, and egg activation [108]. The latter was confirmed by protein immunoblot analysis of spermatogenic cells and sperm which revealed that sperm from mice lacking fertilin β is deficient in sperm-egg membrane adhesion, sperm-egg fusion, migration from the uterus into the oviduct, and binding to the egg zona pellucida [109]. Kumar et al [110] first reported about native human serum albumin (HSA)–prolactin inducible protein (PIP) complex formation in seminal plasma. As HSA has been known to preserve the motility of sperm, native HSA–PIP complex formation may point towards an important role of PIP, which can directly be correlated with male fertility/infertility [111]. Studies showed that mean P1/P2 ratio is approximately 1 and that abnormal expression of protamines is found in a significant percentage of men presenting for infertility analysis. Aoki et al [112] reported that human sperm protamine content is significantly related with DNA fragmentation.

Coştur et al [113] suggested that iNOS (NOS2) has an essential role in spermatogenesis. They performed histologic evaluation and immunostaining of testicular sperm samples from spermatozoa-absent men and fertile controls. In the spermatozoa-absent groups of

Table 9. Proteins and their associated traits or functions in male infertility

Protein	Associated trait or function	Reference
BRDT	Impaired spermatogenesis	Barda et al, 2012 [117]
DCN	Impaired spermatogenesis	Adam et al, 2011 [115]
Fertilin	Infertility/fertility	Cho et al, 1998 [121]
GSK3A	Sperm motility	Vijayaraghavan et al, 2000 [119]
HSA-PIP complex	Infertility/fertility	Harrison et al, 1982 [112]
NOS2	Spermatogenesis	Coştur et al, 2012 [114]
PARK7	AS	Wang et al, 2009 [116]
TUBGCP2	Sperm motility	Chan et al, 2009 [118]

AS: asthenozoospermia.

azoospermic human testis complete germ cell loss and intense expression of iNOS in the Sertoli and Leydig cells was found, conversely iNOS expression was very weak in the control group. Additionally, it has also been proposed that the increase in extracellular matrix protein decorin (DCN) produced by myofibroblastic, peritubular cells in the walls of seminiferous tubules, may imbalance the paracrine signalling pathways in human testis and therefore have possible role in male infertility. This hypothesis was confirmed by immunohistochemical analysis which revealed DCN deposits in the walls of tubules with impaired spermatogenesis [114].

Patients with AS showed differential regulation of proteins; 45 proteins were upregulated and 56 proteins were downregulated in comparison with the control. PARK7 (formerly DJ-1) was one of the down regulated proteins in AS seminal plasma. PARK7 concentration was lower by approximately half compared to control samples. Additionally, levels of reactive oxygen species (ROS) were 3.3-fold higher in AS samples [115]. Barda et al [116] characterized the *BET* gene family expression in human testis with spermatogenetic impairments what revealed that *BRDT* is the only *BET* family gene expressed exclusively in testicular germ cells and *BRDT* gene was not expressed in testicular tissue from patients with Sertoli cells only. It is worth considering that high low motility sperm show differential protein phosphorylation. Chan et al [117] identified that γ-tubulin complex associated protein 2 (TUBGCP2) was hypophosphorylated in low motility sperm.

The importance of protein phosphorylation was also reported by Vijayaraghavan et al [118] who showed that tyrosine phosphorylation of a GSK3A 55 kDa protein varied in direct proportion to motility what suggest that regulation by phosphorylation, could be a key element underlying motility initiation in the epididymis and regulation of mature sperm function. In order to identify protein candidates and to develop the diagnostic markers for AS, the expression of 101 sperm protein spots was compared between 20 AS samples and 10 semen donor control samples. Using a two-dimensional proteomic analysis 17 protein spots were identified at different amounts in the AS samples compared with controls [65]. Protein biomarkers may help us toward better understanding of unknown cases of male infertility that, in turn, can guide us to find better therapeutic solutions [119].

METABOLOMICS, GLYCOMICS AND LIPIDOMICS

Several other omics types are expected to enable identification of unknown genetic factors and diagnostics of male fertility in the near future. For example, metabolomics presents a potential new tool for diagnostics tests [120]. Changes in oxidative stress levels, as well as altered levels of glycerylphosphorylcholine, citrate and lactate have been associated with male infertility through metabolomic studies [121]. Subsections of metabolomics, such as glycomics and lipidomics, also hold the potential to identify diagnostic targets. For example, increased levels of arachidonic acid and other fatty acids have been reported in AS spermatozoa [122]. Furthermore, it has been suggested that the seminal plasma glycome profile may be associated with male reproductive potential [123]. This approach could be extended using other omics data, for example, following the proposed taxonomy of multi-omics science [124,125].

INTEGRATED OMICS APPROACHES

While an analysis on a single omics level already yields a large amount of data, it does not necessarily accurately represent the complex nature of certain pathologies. Integrated omics approaches have been successfully used in the studies of several diseases, including cancer, infections and age-related diseases [126]. An integrated omics approach has been used to define the human sperm microtubulome - combining proteomics, transcriptomics and interactomics data highlight several novel factors potentially associated with fertility [127]. Additionally, the results of this analysis suggest that CUL3 and DCDC2C play a role in the functioning of the sperm flagellum [127]. Talluri et al [128] conducted an extensive integrated multi-omics study on bull (*Bos taurus*) fertility, identifying the dysregulation of 4,766 mRNA, 785 proteins and 33 metabolites between bulls with high and low fertility. Relatively few integrated omics studies on male infertility currently exist, despite its promising applications. In regards to male infertility, this field is still in its infancy [129].

FUTURE DIRECTIONS

A systems biology approach may prove useful in unravelling complex diseases, however it requires a

large data set. Therefore, international collaboration on whole-genome or whole-exome studies may be required in order to obtain additional insight into the pathology of male infertility. The cooperation of multiple medical and research centres would be of great benefit to the development of this field.

Such an effort could also keep in mind the varying genetic backgrounds of different populations in regards to male infertility. Candidate loci with associations below the threshold for statistical significance are not necessarily irrelevant for the disease, but should rather be tested for association in other populations.

In combination with an integrative, comparative genomic approach, animal models could help identify additional male infertility loci. Animal models could also be used to identify additional disease-associated ncRNAs as well as the interactions between infertility factors. This would expand the data set of potential factors in humans and assist future international research.

CONCLUSIONS

Male infertility is a complex disease with a large number of associated risk factors. These appear on several omics levels, including those outside of the scope of the present review. Thus far, studies have predominantly focused on a singular contributing factor or omics level. However, given the multi-faceted nature of the disease, limiting research to a single disease-associated structural variant, sequence variant or dysregulation may not be optimal. Instead, studies on male infertility phenotypes may be more successful with the application of an integrative, multi-omics approach. A shift to an integrative study of infertility on multiple omics levels would contribute to the understanding of the underlying pathomechanism and allow for the development of novel diagnostic and treatment options through systems biology methods.

Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: TK. Data curation: AOW, AT. Investigation: AOW, AT, TK. Visualization: AT. Writing – original draft: AOW, AT. Writing – review & editing: TK.

REFERENCES

1. Droździk M, Stefankiewicz J, Kurzawa R, Górnik W, Baczkowski T, Kurzawski M. Association of the MDR1 (ABCB1) gene 3435C>T polymorphism with male infertility. Pharmacol Rep 2009;61:690-6.
2. Harton GL, Tempest HG. Chromosomal disorders and male infertility. Asian J Androl 2012;14:32-9.
3. Hackstein JH, Hochstenbach R, Pearson PL. Towards an understanding of the genetics of human male infertility: lessons from flies. Trends Genet 2000; 16: 565-72.
4. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, et al; European Association of Urology Working Group on Male Infertility. European Association of Urology guidelines on male infertility: the 2012 update. Eur Urol 2012;62:324-32.
5. Aston KI, Carrell DT. Genome-wide study of single-nucleotide polymorphisms associated with azoospermia and severe oligozoospermia. J Androl 2009;30:711-25.
6. Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, et al. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet 1995;10:383-93.
7. Ogorevc J, Dovc P, Kunej T. Comparative genomics approach to identify candidate genetic loci for male fertility. Reprod Domest Anim 2011;46:229-39.
8. Krausz C. Male infertility: pathogenesis and clinical diagnosis. Best Pract Res Clin Endocrinol Metab 2011;25:271-85.
9. Peterlin B, Kunej T, Hristovski D. Diagnostic test for Y chromosome microdeletion screening in male infertility. Genet Test 2004;8:45-9.
10. Krausz C, Hoefsloot L, Simoni M, Tüttelmann F. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. Andrology 2014;2:5-19.
11. May-Panloup P, Chrétien MF, Savagner F, Vasseur C, Jean M, Malthièry Y, et al. Increased sperm mitochondrial DNA content in male infertility. Hum Reprod 2003;18:550-6.
12. Kimmins S, Kotaja N, Davidson I, Sassone-Corsi P. Testis-specific transcription mechanisms promoting male germ-cell differentiation. Reproduction 2004;128:5-12.
13. Yan W, Si Y, Slaymaker S, Li J, Zheng H, Young DL, et al.

Zmynd15 encodes a histone deacetylase-dependent transcriptional repressor essential for spermiogenesis and male fertility. J Biol Chem 2010;285:31418-26.
14. O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. Fertil Steril 2010;93:1-12.
15. Houston BJ, Riera-Escamilla A, Wyrwoll MJ, Salas-Huetos A, Xavier MJ, Nagirnaja L, et al. A systematic review of the validated monogenic causes of human male infertility: 2020 update and a discussion of emerging gene-disease relationships. Hum Reprod Update 2021;28:15-29.
16. Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. Nat Rev Genet 2006;7:85-97.
17. Tüttelmann F, Simoni M, Kliesch S, Ledig S, Dworniczak B, Wieacker P, et al. Copy number variants in patients with severe oligozoospermia and Sertoli-cell-only syndrome. PLoS One 2011;6:e19426.
18. Repping S, Skaletsky H, Lange J, Silber S, Van Der Veen F, Oates RD, et al. Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. Am J Hum Genet 2002;71:906-22.
19. Visser L, Repping S. Unravelling the genetics of spermatogenic failure. Reproduction 2010;139:303-7.
20. Hodžić A, Maver A, Plaseska-Karanfilska D, Ristanović M, Noveski P, Zorn B, et al. De novo mutations in idiopathic male infertility-a pilot study. Andrology 2021;9:212-20.
21. Carrell DT, Aston KI. The search for SNPs, CNVs, and epigenetic variants associated with the complex disease of male infertility. Syst Biol Reprod Med 2011;57:17-26.
22. La Gatta E, Zace D, Hoxhaj I, Beccia F, Di Pietro ML, Genuardi M. Single nucleotide polymorphisms and idiopathic male infertility in GWAS: a meta-analysis. Eur J Public Health 2021;31(Suppl 3):ckab164.855.
23. Tang Q, Chen Y, Wu W, Ding H, Xia Y, Chen D, et al. Idiopathic male infertility and polymorphisms in the DNA methyltransferase genes involved in epigenetic marking. Sci Rep 2017;7:11219.
24. Massart A, Lissens W, Tournaye H, Stouffs K. Genetic causes of spermatogenic failure. Asian J Androl 2012;14:40-8.
25. Libioulle C, Bours V. [Complex diseases: the importance of genetics]. Rev Med Liege 2012;67:220-5. French.
26. Petricoin EF, Belluco C, Araujo RP, Liotta LA. The blood peptidome: a higher dimension of information content for cancer biomarker discovery. Nat Rev Cancer 2006;6:961-7.
27. Hu Z, Xia Y, Guo X, Dai J, Li H, Hu H, et al. A genome-wide association study in Chinese men identifies three risk loci for non-obstructive azoospermia. Nat Genet 2012;44:183-6.
28. Metin Mahmutoglu A, Hurre Dirie S, Hekim N, Gunes S, Asci R, Henkel R. Polymorphisms of androgens-related genes and idiopathic male infertility in Turkish men. Andrologia 2022;54:e14270.
29. Jensen M, Leffers H, Petersen JH, Nyboe Andersen A, Jørgensen N, Carlsen E, et al. Frequent polymorphism of the mitochondrial DNA polymerase gamma gene (POLG) in patients with normal spermiograms and unexplained subfertility. Hum Reprod 2004;19:65-70.
30. Lai YC, Wang WC, Yang JJ, Li SY. Expansion of CAG repeats in the spinocerebellar ataxia type 1 (SCA1) gene in idiopathic oligozoospermia patients. J Assist Reprod Genet 2009;26:257-61.
31. Pan H, Li YY, Li TC, Tsai WT, Li SY, Hsiao KM. Increased (CTG/CAG)(n) lengths in myotonic dystrophy type 1 and Machado-Joseph disease genes in idiopathic azoospermia patients. Hum Reprod 2002;17:1578-83.
32. Safarinejad MR, Shafiei N, Safarinejad S. Association of the (TAAAA)n repeat and Asp327Asn polymorphisms in the sex hormone-binding globulin (SHBG) gene with idiopathic male infertility and relation to serum SHBG concentrations. J Steroid Biochem Mol Biol 2011;123:37-45.
33. Peterlin B, Zorn B, Teran N, Kunej T. Analysis of the CAG repeat number in exon 1 of the androgen receptor gene in Slovene men with idiopathic azoospermia and oligoasthenoteratozoospermia. Asian J Androl 2007;9:280-2.
34. Rovio AT, Marchington DR, Donat S, Schuppe HC, Abel J, Fritsche E, et al. Mutations at the mitochondrial DNA polymerase (POLG) locus associated with male infertility. Nat Genet 2001;29:261-2.
35. Aknin-Seifer IE, Touraine RL, Lejeune H, Jimenez C, Chouteau J, Siffroi JP, et al. Is the CAG repeat of mitochondrial DNA polymerase gamma (POLG) associated with male infertility? A multi-centre French study. Hum Reprod 2005;20:736-40.
36. Brusco A, Michielotto C, Gatta V, Foresta C, Matullo G, Zeviani M, et al. The polymorphic polyglutamine repeat in the mitochondrial DNA polymerase gamma gene is not associated with oligozoospermia. J Endocrinol Invest 2006;29:1-4.
37. Krausz C, Guarducci E, Becherini L, Degl'Innocenti S, Gerace L, Balercia G, et al. The clinical significance of the POLG gene polymorphism in male infertility. J Clin Endocrinol Metab 2004;89:4292-7.
38. Liu SY, Zhang CJ, Peng HY, Yao YF, Shi L, Chen JB, et al. CAG-repeat variant in the polymerase γ gene and male infertility in the Chinese population: a meta-analysis. Asian J Androl 2011;13:298-304.
39. Kunej T, Teran N, Zorn B, Peterlin B. CTG amplification in the DM1PK gene is not associated with idiopathic male sub-

fertility. Hum Reprod 2004;19:2084-7.
40. Rockett JC, Patrizio P, Schmid JE, Hecht NB, Dix DJ. Gene expression patterns associated with infertility in humans and rodent models. Mutat Res 2004;549:225-40.
41. Guo X, Shen J, Xia Z, Zhang R, Zhang P, Zhao C, et al. Proteomic analysis of proteins involved in spermiogenesis in mouse. J Proteome Res 2010;9:1246-56.
42. Jamsai D, O'Bryan MK. Mouse models in male fertility research. Asian J Androl 2011;13:139-51.
43. Handel MA, Lessard C, Reinholdt L, Schimenti J, Eppig JJ. Mutagenesis as an unbiased approach to identify novel contraceptive targets. Mol Cell Endocrinol 2006;250:201-5.
44. Hu ZL, Reecy JM, Wu XL. Design database for quantitative trait loci (QTL) data warehouse, data mining, and meta-analysis. Methods Mol Biol 2012;871:121-44.
45. L'Hôte D, Serres C, Laissue P, Oulmouden A, Rogel-Gaillard C, Montagutelli X, et al. Centimorgan-range one-step mapping of fertility traits using interspecific recombinant congenic mice. Genetics 2007;176:1907-21.
46. Ford JJ, Wise TH, Lunstra DD, Rohrer GA. Interrelationships of porcine X and Y chromosomes with pituitary gonadotropins and testicular size. Biol Reprod 2001;65:906-12.
47. Hu ZL, Park CA, Wu XL, Reecy JM. Animal QTLdb: an improved database tool for livestock animal QTL/association data dissemination in the post-genome era. Nucleic Acids Res 2013;41:D871-9.
48. Le Roy I, Tordjman S, Migliore-Samour D, Degrelle H, Roubertoux PL. Genetic architecture of testis and seminal vesicle weights in mice. Genetics 2001;158:333-40.
49. Rohrer GA, Wise TH, Lunstra DD, Ford JJ. Identification of genomic regions controlling plasma FSH concentrations in Meishan-White Composite boars. Physiol Genomics 2001;6:145-51.
50. Sato S, Oyamada Y, Atsuji K, Nade T, Sato S, Kobayashi E, et al. Quantitative trait loci analysis for growth and carcass traits in a Meishan x Duroc F2 resource population. J Anim Sci 2003;81:2938-49.
51. Zídek V, Pintír J, Musilová A, Bílá V, Kren V, Pravenec M. Mapping of quantitative trait loci for seminal vesicle mass and litter size to rat chromosome 8. J Reprod Fertil 1999;116:329-33.
52. Hu ZL, Park CA, Reecy JM. Building a livestock genetic and genomic information knowledgebase through integrative developments of Animal QTLdb and CorrDB. Nucleic Acids Res 2019;47(D1):D701-10.
53. Giacone F, Cannarella R, Mongioì LM, Alamo A, Condorelli RA, Calogero AE, et al. Epigenetics of male fertility: effects on assisted reproductive techniques. World J Mens Health 2019;37:148-56.
54. Moore LD, Le T, Fan G. DNA methylation and its basic function. Neuropsychopharmacology 2013;38:23-38.
55. Boissonnas CC, Abdalaoui HE, Haelewyn V, Fauque P, Dupont JM, Gut I, et al. Specific epigenetic alterations of IGF2-H19 locus in spermatozoa from infertile men. Eur J Hum Genet 2010;18:73-80.
56. Marques CJ, Costa P, Vaz B, Carvalho F, Fernandes S, Barros A, et al. Abnormal methylation of imprinted genes in human sperm is associated with oligozoospermia. Mol Hum Reprod 2008;14:67-74.
57. Kobayashi H, Sato A, Otsu E, Hiura H, Tomatsu C, Utsunomiya T, et al. Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. Hum Mol Genet 2007;16:2542-51.
58. Wu W, Shen O, Qin Y, Niu X, Lu C, Xia Y, et al. Idiopathic male infertility is strongly associated with aberrant promoter methylation of methylenetetrahydrofolate reductase (MTHFR). PLoS One 2010;5:e13884.
59. Dhillon VS, Shahid M, Husain SA. Associations of MTHFR DNMT3b 4977 bp deletion in mtDNA and GSTM1 deletion, and aberrant CpG island hypermethylation of GSTM1 in non-obstructive infertility in Indian men. Mol Hum Reprod 2007;13:213-22.
60. Nanassy L, Carrell DT. Abnormal methylation of the promoter of CREM is broadly associated with male factor infertility and poor sperm quality but is improved in sperm selected by density gradient centrifugation. Fertil Steril 2011;95:2310-4.
61. Wang T, Gao H, Li W, Liu C. Essential role of histone replacement and modifications in male fertility. Front Genet 2019;10:962.
62. Schon SB, Luense LJ, Wang X, Bartolomei MS, Coutifaris C, Garcia BA, et al. Histone modification signatures in human sperm distinguish clinical abnormalities. J Assist Reprod Genet 2019;36:267-75.
63. Hammoud SS, Nix DA, Hammoud AO, Gibson M, Cairns BR, Carrell DT. Genome-wide analysis identifies changes in histone retention and epigenetic modifications at developmental and imprinted gene loci in the sperm of infertile men. Hum Reprod 2011;26:2558-69.
64. La Spina FA, Romanato M, Brugo-Olmedo S, De Vincentiis S, Julianelli V, Rivera RM, et al. Heterogeneous distribution of histone methylation in mature human sperm. J Assist Reprod Genet 2014;31:45-9.
65. Yuen BT, Bush KM, Barrilleaux BL, Cotterman R, Knoepfler PS. Histone H3.3 regulates dynamic chromatin states during spermatogenesis. Development 2014;141:3483-94.
66. Okada Y, Tateishi K, Zhang Y. Histone demethylase JH-

DM2A is involved in male infertility and obesity. J Androl 2010;31:75-8.
67. Piletič K, Kunej T. MicroRNA epigenetic signatures in human disease. Arch Toxicol 2016;90:2405-19.
68. Barbu MG, Thompson DC, Suciu N, Voinea SC, Cretoiu D, Predescu DV. The roles of MicroRNAs in male infertility. Int J Mol Sci 2021;22:2910.
69. Panneerdoss S, Chang YF, Buddavarapu KC, Chen HI, Shetty G, Wang H, et al. Androgen-responsive microRNAs in mouse Sertoli cells. PLoS One 2012;7:e41146.
70. Pantos K, Grigoriadis S, Tomara P, Louka I, Maziotis E, Pantou A, et al. Investigating the role of the microRNA-34/449 family in male infertility: a critical analysis and review of the literature. Front Endocrinol (Lausanne) 2021;12:709943.
71. Zhao S, Heng N, Weldegebriall Sahlu B, Wang H, Zhu H. Long noncoding RNAs: recent insights into their role in male infertility and their potential as biomarkers and therapeutic targets. Int J Mol Sci 2021;22:13579.
72. Anguera MC, Ma W, Clift D, Namekawa S, Kelleher RJ 3rd, Lee JT. Tsx produces a long noncoding RNA and has general functions in the germline, stem cells, and brain. PLoS Genet 2011;7:e1002248.
73. He Z, Chan WY, Dym M. Microarray technology offers a novel tool for the diagnosis and identification of therapeutic targets for male infertility. Reproduction 2006;132:11-9.
74. Waclawska A, Kurpisz M. Key functional genes of spermatogenesis identified by microarray analysis. Syst Biol Reprod Med 2012;58:229-35.
75. Garrido N, Martínez-Conejero JA, Jauregui J, Horcajadas JA, Simón C, Remohí J, et al. Microarray analysis in sperm from fertile and infertile men without basic sperm analysis abnormalities reveals a significantly different transcriptome. Fertil Steril 2009;91(4 Suppl):1307-10.
76. Hamatani T. Human spermatozoal RNAs. Fertil Steril 2012;97:275-81.
77. Montjean D, De La Grange P, Gentien D, Rapinat A, Belloc S, Cohen-Bacrie P, et al. Sperm transcriptome profiling in oligozoospermia. J Assist Reprod Genet 2012;29:3-10.
78. Lin YH, Lin YM, Teng YN, Hsieh TY, Lin YS, Kuo PL. Identification of ten novel genes involved in human spermatogenesis by microarray analysis of testicular tissue. Fertil Steril 2006;86:1650-8.
79. Bello JH, Khan MJ, Amir S, Kakakhel HG, Tahir F, Sultan S, et al. Dysregulation of mitochondrial sirtuin genes is associated with human male infertility. Andrologia 2022;54:e14274.
80. Steger K, Wilhelm J, Konrad L, Stalf T, Greb R, Diemer T, et al. Both protamine-1 to protamine-2 mRNA ratio and Bcl2 mRNA content in testicular spermatids and ejaculated spermatozoa discriminate between fertile and infertile men. Hum Reprod 2008;23:11-6.
81. Lardone MC, Parodi DA, Valdevenito R, Ebensperger M, Piottante A, Madariaga M, et al. Quantification of DDX3Y, RBMY1, DAZ and TSPY mRNAs in testes of patients with severe impairment of spermatogenesis. Mol Hum Reprod 2007;13:705-12.
82. Zhang HT, Zhang Z, Hong K, Tang WH, Liu DF, Mao JM, et al. Altered microRNA profiles of testicular biopsies from patients with nonobstructive azoospermia. Asian J Androl 2020;22:100-5.
83. Hrovatin K, Kunej T. Classification of miRNA-related sequence variations. Epigenomics 2018;10:463-81.
84. Krawetz SA, Kruger A, Lalancette C, Tagett R, Anton E, Draghici S, et al. A survey of small RNAs in human sperm. Hum Reprod 2011;26:3401-12.
85. Plasterk RH. Micro RNAs in animal development. Cell 2006;124:877-81.
86. Kim VN, Nam JW. Genomics of microRNA. Trends Genet 2006;22:165-73.
87. McIver SC, Roman SD, Nixon B, McLaughlin EA. miRNA and mammalian male germ cells. Hum Reprod Update 2012;18:44-59.
88. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005;120:15-20.
89. He Z, Kokkinaki M, Pant D, Gallicano GI, Dym M. Small RNA molecules in the regulation of spermatogenesis. Reproduction 2009;137:901-11.
90. Lian J, Zhang X, Tian H, Liang N, Wang Y, Liang C, et al. Altered microRNA expression in patients with non-obstructive azoospermia. Reprod Biol Endocrinol 2009;7:13.
91. Grinchuk OV, Jenjaroenpun P, Orlov YL, Zhou J, Kuznetsov VA. Integrative analysis of the human cis-antisense gene pairs, miRNAs and their transcription regulation patterns. Nucleic Acids Res 2010;38:534-47.
92. Curry E, Safranski TJ, Pratt SL. Differential expression of porcine sperm microRNAs and their association with sperm morphology and motility. Theriogenology 2011;76:1532-9.
93. Yan N, Lu Y, Sun H, Qiu W, Tao D, Liu Y, et al. Microarray profiling of microRNAs expressed in testis tissues of developing primates. J Assist Reprod Genet 2009;26:179-86.
94. Yan N, Lu Y, Sun H, Tao D, Zhang S, Liu W, et al. A microarray for microRNA profiling in mouse testis tissues. Reproduction 2007;134:73-9.
95. Lian C, Sun B, Niu S, Yang R, Liu B, Lu C, et al. A comparative profile of the microRNA transcriptome in immature and mature porcine testes using Solexa deep sequencing. FEBS J

2012;279:964-75.
96. Ro S, Park C, Sanders KM, McCarrey JR, Yan W. Cloning and expression profiling of testis-expressed microRNAs. Dev Biol 2007;311:592-602.
97. Torley KJ, da Silveira JC, Smith P, Anthony RV, Veeramachaneni DN, Winger QA, et al. Expression of miRNAs in ovine fetal gonads: potential role in gonadal differentiation. Reprod Biol Endocrinol 2011;9:2.
98. Georges M, Coppieters W, Charlier C. Polymorphic miRNA-mediated gene regulation: contribution to phenotypic variation and disease. Curr Opin Genet Dev 2007;17:166-76.
99. Ogorevc J, Dovc P, Kunej T. Polymorphisms in microRNA targets: a source of new molecular markers for male reproduction. Asian J Androl 2011;13:505-8.
100. Zhou QZ, Guo XB, Zhang WS, Zhou JH, Yang C, Bian J, et al. Expressions of miR-525-3p and its target gene SEMG1 in the spermatozoa of patients with asthenozoospermia. Andrology 2019;7:220-7.
101. Grivna ST, Pyhtila B, Lin H. MIWI associates with translational machinery and PIWI-interacting RNAs (piRNAs) in regulating spermatogenesis. Proc Natl Acad Sci U S A 2006;103:13415-20.
102. Sai Lakshmi S, Agrawal S. piRNABank: a web resource on classified and clustered Piwi-interacting RNAs. Nucleic Acids Res 2008;36:D173-7.
103. Wichman L, Somasundaram S, Breindel C, Valerio DM, McCarrey JR, Hodges CA, et al. Dynamic expression of long noncoding RNAs reveals their potential roles in spermatogenesis and fertility. Biol Reprod 2017;97:313-23.
104. Liang M, Hu K, He C, Zhou J, Liao Y. Upregulated lncRNA Gm2044 inhibits male germ cell development by acting as miR-202 host gene. Anim Cells Syst (Seoul) 2019;23:128-34.
105. Tomar AK, Saraswat M, Chhikara N, Kumar S, Yadav VK, Sooch BS, et al. Differential proteomics of sperm: insights, challenges and future prospects. Biomark Med 2010;4:905-10.
106. Pandey A, Mann M. Proteomics to study genes and genomes. Nature 2000;405:837-46.
107. Milardi D, Grande G, Vincenzoni F, Messana I, Pontecorvi A, De Marinis L, et al. Proteomic approach in the identification of fertility pattern in seminal plasma of fertile men. Fertil Steril 2012;97:67-73.e1.
108. Bigler D, Chen M, Waters S, White JM. A model for sperm-egg binding and fusion based on ADAMs and integrins. Trends Cell Biol 1997;7:220-5.
109. Cho C, Bunch DO, Faure JE, Goulding EH, Eddy EM, Primakoff P, et al. Fertilization defects in sperm from mice lacking fertilin beta. Science 1998;281:1857-9.
110. Kumar S, Tomar AK, Singh S, Saraswat M, Singh S, Singh TP, et al. Human serum albumin as a new interacting partner of prolactin inducible protein in human seminal plasma. Int J Biol Macromol 2012;50:317-22.
111. Harrison RA, Dott HM, Foster GC. Bovine serum albumin, sperm motility, and the "dilution effect". J Exp Zool 1982;222:81-8.
112. Aoki VW, Liu L, Jones KP, Hatasaka HH, Gibson M, Peterson CM, et al. Sperm protamine 1/protamine 2 ratios are related to in vitro fertilization pregnancy rates and predictive of fertilization ability. Fertil Steril 2006;86:1408-15.
113. Coştur P, Filiz S, Gonca S, Çulha M, Gülecen T, Solakoğlu S, et al. Êxpression of inducible nitric oxide synthase (iNOS) in the azoospermic human testis. Andrologia 2012;44 Suppl 1:654-60.
114. Adam M, Schwarzer JU, Köhn FM, Strauss L, Poutanen M, Mayerhofer A. Mast cell tryptase stimulates production of decorin by human testicular peritubular cells: possible role of decorin in male infertility by interfering with growth factor signaling. Hum Reprod 2011;26:2613-25.
115. Wang J, Wang J, Zhang HR, Shi HJ, Ma D, Zhao HX, et al. Proteomic analysis of seminal plasma from asthenozoospermia patients reveals proteins that affect oxidative stress responses and semen quality. Asian J Androl 2009;11:484-91.
116. Barda S, Paz G, Yogev L, Yavetz H, Lehavi O, Hauser R, et al. Expression of BET genes in testis of men with different spermatogenic impairments. Fertil Steril 2012;97:46-52.e5.
117. Chan CC, Shui HA, Wu CH, Wang CY, Sun GH, Chen HM, et al. Motility and protein phosphorylation in healthy and asthenozoospermic sperm. J Proteome Res 2009;8:5382-6.
118. Vijayaraghavan S, Mohan J, Gray H, Khatra B, Carr DW. A role for phosphorylation of glycogen synthase kinase-3alpha in bovine sperm motility regulation. Biol Reprod 2000;62:1647-54.
119. Tomar AK, Sooch BS, Singh S, Yadav S. Differential proteomics of human seminal plasma: a potential target for searching male infertility marker proteins. Proteomics Clin Appl 2012;6:147-51.
120. Mehrparavar B, Minai-Tehrani A, Arjmand B, Gilany K. Metabolomics of male infertility: a new tool for diagnostic tests. J Reprod Infertil 2019;20:64-9.
121. Minai-Tehrani A, Jafarzadeh N, Gilany K. Metabolomics: a state-of-the-art technology for better understanding of male infertility. Andrologia 2016;48:609-16.
122. Walters JLH, Gadella BM, Sutherland JM, Nixon B, Bromfield EG. Male infertility: shining a light on lipids and lipid-modulating enzymes in the male germline. J Clin Med 2020;9:327.
123. Kołodziejczyk J, Blixt O, Olejnik B, Zimmer M, Ferens-Sieczkowska M. Application of lectin microarrays for the analysis

of seminal plasma glycome. Andrologia 2018;50:e13018.
124. Pirih N, Kunej T. Toward a taxonomy for multi-omics science? Terminology development for whole genome study approaches by omics technology and hierarchy. OMICS 2017;21:1-16.
125. Redenšek S, Dolžan V, Kunej T. From genomics to omics landscapes of Parkinson's disease: revealing the molecular mechanisms. OMICS 2018;22:1-16.
126. Misra BB, Langefeld CD, Olivier M, Cox LA. Integrated omics: tools, advances, and future approaches. J Mol Endocrinol 2019;62:R21-45.
127. Jumeau F, Chalmel F, Fernandez-Gomez FJ, Carpentier C, Obriot H, Tardivel M, et al. Defining the human sperm microtubulome: an integrated genomics approach. Biol Reprod 2017;96:93-106.
128. Talluri TR, Kumaresan A, Sinha MK, Paul N, Ebenezer Samuel King JP, Datta TK. Integrated multi-omics analyses reveals molecules governing sperm metabolism potentially influence bull fertility. Sci Rep 2022;12:10692.
129. Carrell DT, Aston KI, Oliva R, Emery BR, De Jonge CJ. The "omics" of human male infertility: integrating big data in a systems biology approach. Cell Tissue Res 2016;363:295-312.