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Review Article

An Insight into Male Infertility: A Narrative Review

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Abstract: Male infertility is a widespread health issue globally, frequently remaining untreated due to stigmatization and the challenges in diagnosis and treatment. This review presents a comprehensive update on the literature covering key aspects such as causes, diagnostic techniques, and treatment options for managing male factor infertility. It includes an in-depth analysis of global infertility data, using resources from the World Health Organization, Web of Science, Google Scholar, Elsevier, Medline, PubMed, and Scopus databases to gather relevant articles on male infertility. A total of 41 articles from 2000-2023 were reviewed. High throughput techniques, along with sophisticated assays, are being employed for accurate diagnosis. Surgical procedures such as testicular sperm extraction, vasovasostomy, vasoepididymostomy, sperm retrieval techniques, and non-surgical procedures including sclerotherapy, gonadotropin-releasing hormone therapy, and antiestrogens are available to treat infertile males. Additionally, in recent years, flavonoids have been extensively explored for their antioxidant, anti-inflammatory, immune-stimulating, anti-apoptotic, anticarcinogenic, anti-allergic, and antiviral activities. These properties of flavonoids are being investigated for their potential to address biological mechanisms underlying anomalies such as spermatogenesis disturbance and sperm quality decline. This review serves as a comprehensive guide to better understand the etiologies and treatment modalities of male factor infertility, ultimately facilitating affected individuals in making informed reproductive choices.

Keywords: Male Infertility, Genetics, Environmental Factors, Diagnosis, Treatment, Flavonoids.

1. INTRODUCTION

Male infertility is defined as the inability to produce a pregnancy despite regular intercourse without contraception for at least one year. This is least considerate disorder in many societies. Regardless of how clinical experts might characterize infertility, couples mostly don't characterize themselves as 'infertile' or present themselves for treatment unless they embrace parenthood as a desired social role [1]. Globally, male infertility is under-reported particularly in male dominant societies. Male infertility is a common problem that affects approximately 48.5 million couples worldwide and was predicted to be largely genetic in origin [2]. However, other studies reported involvement of epigenetics in disease manifestation including DNA methylation, histone tail modifications, and non-coding RNAs [3]. In addition to genetics and epigenetics factors, environmental and lifestyle elements significantly contribute to male infertility. Exposure to pollutants, occupational hazards, smoking, alcohol consumption, substance abuse, poor dietary habits, obesity, stress, infections and certain medications can adversely affect reproductive health.

Sperm production is a highly complex process and genetic variant/s may compromise normal sperm development and maturation. Various studies to investigate genetic basis in both humans and mice revealed the involvement of various crucial pathways for male infertility, including sexual differentiation, development of the genitourinary system and gametogenesis. According to Jackson Laboratory's Mouse Genome Informatics database

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more than 600 male infertility genes and 2300 testis-enriched in human have been reported. In addition to genetics and epigenetics, the causes of male infertility are numerous. Blockages in the reproductive tract and hormonal imbalances can cause infertility [4-7].

Despite the complex nature of male infertility, it can be treated in some cases through various surgical procedures such as testicular sperm extraction, vasovasostomy, vasoepididymostomy, and sperm retrieval techniques. Non-surgical methods like sclerotherapy and gonadotropin-releasing hormone therapy are also utilized. Currently, the global search for an inhibitor of male reproductive system dysfunction continues. Among many natural products, flavonoids have been extensively investigated as potential inhibitors for treating male infertility [8]. Flavonoids are the most prevalent and well-studied polyphenols in human diet. There are approximately 4000 flavonoid varieties reported so far commonly found in seeds, citrus fruit, olive oil, tea, and red wine [9]. Flavonoids are a broad group of natural antioxidant compounds with flavan nucleus and a benzo-γ-pyrone structure having a basic C6-C3-C6 phenyl-benzopyran backbone. Interestingly, these amphipathic molecules can penetrate the lipid bilayer of membranes, thus providing possible protection for the entire spermatozoa and acrosome membrane [10]. Hence, flavonoids can prevent oxidative damage and facilitating the acrosome reaction of spermatozoa necessary for fertilization.

This review discusses the causes of male infertility and outlines the diagnostic and treatment strategies available. Additionally, it explores the therapeutic effects of flavonoids on male reproductive system dysfunction.

2. METHODOLOGY

A literature survey was conducted for meta-analyses and systematic reviews related to male infertility, using Google Scholar (https://scholar.google.com/), MEDLINE (https://medlineplus.gov/), and PubMed (https://pubmed.ncbi.nlm.nih.gov/) to search for articles published in English in peer-reviewed journals. Keywords used in the search included "male infertility," "genetic causes," "environmental causes," "diagnosis," and "treatment".

Inclusion criteria: Initially, a total of 59 articles related to male infertility from 2000-2023 were selected. The titles and abstracts of these articles were screened to determine which studies met the inclusion criteria. The full texts of the shortlisted articles were then reviewed, and data were extracted and pooled. Ultimately, 41 articles were chosen based on their relevance to the topic or the availability of the full text.

Exclusion criteria: Studies containing ambiguous or duplicated data from the same author across different journal articles were also excluded. Additionally, articles that were not available in full-text format and were inaccessible through Sci-Hub (https://sci-hub.se/) were excluded from the review.

3. ETIOLOGY

3.1. Genetic

Over 2000 genes have been linked with spermatogenesis and spermiogenesis, any mutation in these genes can result in abnormal production of sperms. All these mutations have been categorized as quantitative (that affect the number of sperm) and qualitative (which causes abnormalities within the sperm) spermatogenesis disorders (Figure 1). Research has indicated that there is a 15% prevalence of genetic abnormalities in males experiencing azoospermia (no sperm ejaculation) and severe oligozoospermia (low sperm count in semen). The primary genetic contributors include microdeletion on the Y chromosome, abnormalities in karyotype and mutation sin the cystic fibrosis transmembrane conductance regulator (CFTR) [11]. Apart from various Y chromosome microdeletions, abnormalities in X-chromosome can also lead to male infertility [12].

The Y chromosome was partially sequenced about two decades ago which includes 53.8% sequencing of the GRCh38 Y chromosome. The Y chromosome comprises of euchromatin and heterochromatin regions. The Euchromatin region (around 25 Mb) comprises of two pseudoautosomal regions, PAR1 and PAR2, that actively recombine with the X chromosome. The remaining chromatin (around 22 Mb) is categorized into X-degenerate regions (8.6 Mb), X-transposed regions (3.4 Mb), and ampliconic regions (9.9 Mb) with distinct

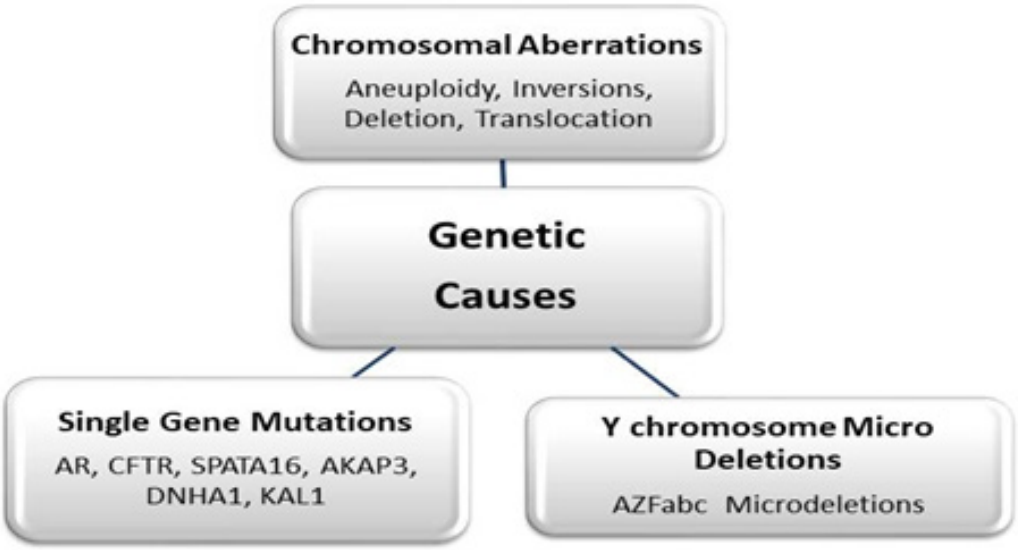

Fig. 1. Summary of various genetic causes in male infertility.

evolutionary origins [13] as shown in Figure 2. The last three segments: X-degenerate regions, X-transposed and ampliconic, are collectively known as the male specific regions of the Y-chromosome (MSY). Since these regions lack homologous sequence, they have a single copy that is directly inherited from the father[14]. These regions have highly replicative genes due to the presence of long palindromic duplicates (high identity of 99.9%) that are involved in spermatogenesis [15]. Due to these repetitive sequences this region is highly prone to intra and inter chromatid-reciprocal recombination which can lead to severe phenotypes such azoospermia and oligozoospermia. The second most common genetic aberration that can occur and leads to male infertility is the microdeletion of Yq (long arm of Y chromosome). It is observed in 1% to 7% of males experiencing severe oligozoospermia and in 13% males with azoospermia [16, 17]. Similarly,

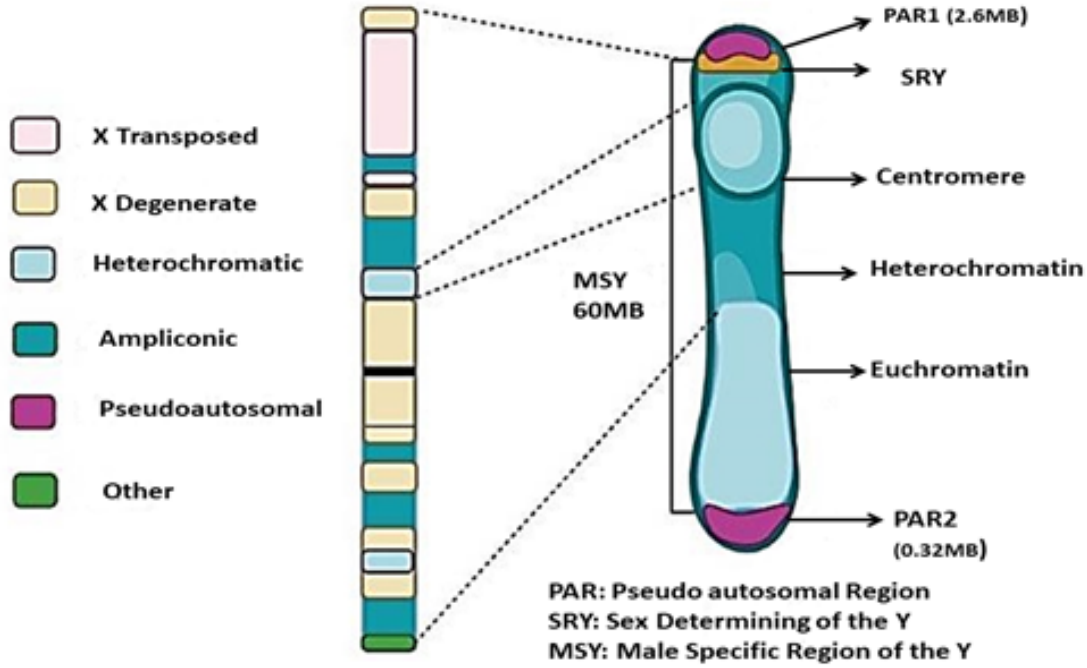

Fig. 2. Structure of Y chromosome.

the Y-chromosome has an azoospermia factor region on its long arm which has three domains: AZFa, AZFb and AZFc. Studies have revealed that complete deletion of AZFa region leads to loss of DEAD box protein 3, Y chromosomal and ubiquitin specific peptidase 9 Y-linked genes due to which there is total loss of Sertoli cells only hence absence of sperms. If the AZFb region is completely deleted affects the Y-chromosome RNA recognition motif 1 (RBMY1 and PTPN13 like Y-linked (PRY) clusters, due to which the spermatocytes are stuck in mitotic arrest during its primary stage and never fully develops. AZFc mutations are more heterogeneous as compared to AZFa and AZFb region deletion [18].

In addition to Y chromosome deletions, mutations in the X chromosome have also been reported to cause infertility in males. The primary function of sex genes is in sexual differentiation [19]. The X chromosome is heterozygous expressed in men but it does encode for multiple testicular proteins and also has been found to play a role in spermatogenesis [20]. Mutation sin the X-linked testis expressed 11 (TEX11) gene have been linked to male infertility, particularly azoospermia [21, 22]. Similarly, mutations in the human reproductive homeobox (RHOX) gene cluster have been associated with male infertility, with aberrant RHOX promoter methylation strongly linked to abnormal sperm parameters [23, 24]. Protein encoded by TEX11 plays a significant role in pathogenesis of azoospermia while RHOX gene is expressed in germ cells. Mutation in RHOX such as RHOXF1 and RHOXF2/2B causes severe oligozoospermia [21, 25]. Structural defects leading to male infertility are rare, accounting for only 1-2% of all cases [26]. These defects are most commonly caused by a birth injury to the penis or testes. Trauma to the testes can also occur as a result of accidents or sports injuries [27]. Deletion or addition of a whole chromosome during meiosis can lead to abnormal number of chromosomes due to paternal or maternal nondisjunction. The most common structural aberrations found in men suffering from infertility are reciprocal, inversions, and Robertsonian translocation. Oligozoospermic men, who have a reduced sperm count, are more likely to have autosomal structural rearrangements, with a 10% chance of such abnormalities [28]. These rearrangements can lead to defects in spermatogenesis and abnormal segregation at meiosis, contributing to the reproductive phenotype of these men [29].

However, certain karyotype abnormalities (variations in the number or structure of chromosomes) are not detrimental but are silent mutations hence resulting in normozoospermic (normal sperm) men, the prevalence for such cases are mere 0.75 to 1.0%. Therefore, the number for deleterious karyotype mutations is approximately high i.e., 15% for nonobstructive azoospermia and 4% for severe oligozoospermia [30].

Some very obvious indicators for a karyotype abnormality are low sperm count (less than 5 million per mL), recurrent miscarriages, fetal malformation or cognitive developmental disability. Chromosomal aberrations are not only numerical but structural too and they not only necessarily result on Y chromosome but can also involve X chromosome and autosomes as well. Klinefelter syndrome (KS; 47, XXY) is a chromosomal abnormality that results in aneuploidy, leading to nonobstructive azoospermia in men [31]. The prevalence for this disorder is 14.8% men with azoospermia, 5.4% men with severe oligozoospermia, and 4% of all overall male infertility [32] Whilst, there are other variants present too, about 20% of affected men have higher levels of structural defect due to 47XXY/46, XY [33]. Studies have reported that sperm obtained through microsurgical testicular sperm extraction method (micro-TESE) offers higher sperm count as compared to conventional testicular sperm extraction (TESE), especially with men that have nonobstructive azoospermia this also includes Klinefelter syndrome patients [34, 35]. In non-mosaic Klinefelter patients, the sperm aneuploidy rate is 2-25% while in mosaic Klinefelter patients it is 1.5%-7%. This increases the chances of affected individuals to have offspring [36].

Another condition, 46, XX Male Syndrome also known as de la Chapelle syndrome, is a rare genetic condition in which an individual has two X chromosomes (typically found in females) but exhibits male characteristics. This occurs due to the presence of male-determining genes, usually transferred from the Y chromosome to one of the X chromosomes through a process called translocation. At the time of birth such patients have normal external male genitalia; the condition is only

noticeable upon puberty because of infertility and hypogonadism. The testes of these patients contain hyalinized seminiferous tubules lined with Sertoli cells only (SCO). It has been observed that about 90% of the affected individuals have an SRY gene translocated on the terminal end of the short arm of X chromosome or a rare autosomal chromosome [37]. Generally, no treatment is available for such genetic disorders except symptomatic management. Fortunately, genetic screening and genetic counseling could be an opted as a possible solution. Along with this, patients with aneuploidy opting for in vitro fertilization should also consider preimplantation genetic testing to ensure a healthy offspring.

3.2. Hormonal Imbalance

Hormones play a key role in the reproductive process, and imbalances can lead to fertility problems. Hormonal defects account for about 15% of all cases of male infertility [38] . They are most commonly caused by a problem with the hypothalamus or the testes, but they may also be due to a problem with other endocrine male infertility. It is important to note that hormonal problems usually affect sperm quality, not the total number of sperm [39]. A hormonal imbalance caused by too much prolactin may lead to low sperm production as well as undescended testes or other problems with sexual development [40]. Too little testosterone or an abnormal ratio of testosterone to estrogen may result in fatigue, loss of sex drive, erectile dysfunction, and reduced sperm production [41]. There are many different types of hormone imbalances that can cause infertility, including: Hypothyroidism – an underactive thyroid gland [42]. The symptoms include fatigue, weight gain, depression, and slowed heart rate and muscle weakness. Patients with hypothyroidism often have abnormal levels of prolactin in their blood [43]. Hyperprolactinemia – abnormally high levels of prolactin in the blood due to a problem with the hypothalamus or pituitary gland that causes low testosterone production. Low testosterone can lead to infertility [44]. Hyperthyroidism – an overactive thyroid gland that causes the body to produce too much of the hormone thyroxine. Patients with Diabetes have a higher risk of developing fertility problems due to changes in hormones and sperm quality. There is also evidence that diabetes may damage testicles and reduce testosterone production, thus causing infertility [45]. Hormonal medications can interfere with sperm production and lead to the development of female sex characteristics such as breast enlargement. Exposure to certain chemicals or radiation may cause a hormonal imbalance in some men that leads to fertility problems [46]. According to cancer research, exposure to high levels of heat or certain chemicals may cause a man's testicles to shrink. Shrunken testicles are unable to produce sperm, which leads to infertility. Heavy exposure to chemicals such as lead or cadmium can damage sperm and make them less capable of fertilizing the egg [47]. Therefore, the high mutation rates resulting in extensive structural polymorphism among human Y chromosomes and considering the multifactorial nature of male infertility, developing new diagnostic panels is essential for transforming the current landscape of prevention, diagnosis, and management [48, 49].

3.3. Epigenetics

The term epigenetics can be defined as changes in phenotype caused by methods and mechanisms other than changes in DNA sequences, these can be DNA modifications, histone modifications, and RNA interference [50].

3.3.1. DNA methylation in male infertility

DNA methylation predominantly occurs in CpG islands and is controlled by DNA methyltransferases (DNMT) and can influence gene expression by suppressing transcription [51]. Correct DNA methylation is essential for various cellular processes crucial for male fertility, including spermatogenesis and chromatin stability [51, 52]. Recent evidence depicts the connection between proper DNA methylation and male fertility. Abnormal DNA methylation patterns are observed in spermatozoa of infertile men, impact sperm quality, motility, and DNA integrity [53–55]. Hypermethylation of promoters of specific genes such as MTHFR, IGF2, H19, PLAG1 and SNRPN, is associated with poor sperm quality and an increased risk of infertility [56]. Spermatogenesis a critical process for male fertility is influenced by DNA methylation abnormalities in genes H19, MEST, and RHOX clusters, leading to conditions such oligozoospermia [57, 58]. Additionally,

hypomethylation of H19 has been observed in men with teratozoospermia [59, 60].

3.3.2. Histone modification in male infertility

The intricate landscape of nucleosome, the fundamental units of chromatin, is a key player in male fertility. Consisting of DNA wrapped around histone octamer, including histones, H2A, H2B, H3 and H4, this structural organization lends rigidity to chromatin. Crucial to this dynamic is the realm of histone modifications, a cascade of covalent post-translational changes primarily occurring on the lysine-rick tail of histone proteins, notably H3 and H4 [61]. Histone acetylation is a reversible process catalyzed by acetyltransferases and deacetylases, acting as a significant regulator. This modification neutralizes positive charge of the amino acid on histone tail therefore reducing DNA affinity and facilitating an open chromatin structure for transcription. It has been found that H4 hyperacetylation (Hypac-H4) is a crucial modification during spermatogenesis, observed spermatogonia, spermatocytes and spermatids. Histone modifications such as H4K8ac, H4K5ac, H4K20me2, and H4me exhibit distinct patterns during various stages of spermatogenesis. Studies have been conducted that elaborate the intricate relationship between Hypac-H4 and impaired spermatogenesis [62]. Histone methylation, another facet of epigenetic regulation, dictates the activation or repression of chromatin states. Diverse patterns of histone methylation have been unveiled in human spermatozoa, and abnormal methylation is associated with severe damage to the spermatogenesis [63]. Additionally, histone phosphorylation has also been found to be involved in transcriptional activation and chromatin rearrangement during spermatogenesis. Defects in histone phosphorylation are involved in sperm dysfunction and male infertility issues. It has also been found that histone phosphorylation can regulate several biological events including mitotic/meiotic chromosome condensation, activation and inactivation of genes transcription, chromatin remodeling and double-strand DNA break repairs (DSB) which are all essential processes in sperm development [64]. In addition to this the process of histone-to protamine exchange during spermatogenesis marks as a crucial juncture. The somatic histones are replaced by protamine, the core of the spermatids condense and the process ensure the safety of sperm genome against the rigors of fertilization [65]. Recent studies have indicated that epigenetic modifications of histone play a pivotal role in orchestrating histone-to-protamine exchange in human spermatozoa [64].

3.3.3. Chromatin remodeling in male infertility

Chromatin remodeling is a dynamic process which consists of protein complexes like SWI/SNF, ISW1 and MI2 which alter the nucleosome location and structure through ATP-dependent process [66]. This process has been found to be crucial in condensing chromatin in spermatozoa to transmit epigenetic information to the embryo [67]. Previous studies revealed that correct DNA packaging during spermatogenesis, where 85% histones are replaced by protamine's is vital [68, 69]. Aberration in this process, such as mutation in Calcium/calmodulin-dependent protein kinase 4 (CaMK4), which participates in phosphorylation of protamine 2 has been linked with impaired spermatogenesis and male infertility [70]. In addition to this, PRM1 md PRM2 which are key proteins in sperm function and fertilization are crucial and their haploinsufficiency can lead to reduced protein levels that can result in abnormal chromatin structure and a damaged DNA in sperm [58]. The PRM1 and PRM2 ratio needs to be tightly regulate, any deviation has the potentially to impact the sperm quality, DNA integrity and male fertility [71]. Studies have also associated abnormal histone H4 acetylation to impaired spermatogenesis, since H4 hyperacetylation is crucial for histone to protamine transition [60, 72]. It has been observed especially in conditions like Sertoli cell-only syndrome (SCOS) [73].

3.3.4. Genetic imprinting in male infertility

Genetic imprinting is an epigenetic process which dictates allele expression in a parent-of-origin-specific manner through CpG island methylation changes. It has been observed that men suffering from male infertility have aberrations in imprinted genes like GTL2 and H19 indicating the significance of genetic imprinting during spermatogenesis [58, 74, 75]. It has been observed that fertile males have high IGF2/H19 imprinting control region 1 (ICR1) methylation levels while reduced MEST methylation is linked to low sperm counts, this

serves as a strong indicator of sperm quality in infertile males [76]. Assisted reproductive techniques (ART), including ICS1, IVF and ROSI are linked with an increase prevalence of imprinting defects, potentially impacting embryonic development and elevating the risk of infertility and congenital abnormalities in offspring [77]. Optimizing ART techniques and ensuring long-term follow-up for ART offspring are crucial for comprehensive clinical understanding [78].

3.3.5. miRNAs in male infertility

Epigenetic modifications including non-coding RNAs like microRNAs (miRNA), play a crucial role in male infertility. miRNA make a substantial portion of cellular RNAs and regulate post-transcriptional gene expression. Dysregulation of miRNA expression in sperm cells has been associated with severe abnormalities in these cells and can impact subsequent generation [79]. Experimental studies have highlighted transgenerational inheritance of altered DNA methylation and non-coding RNA in sperm, emphasizing the importance of understanding miRNA involvement in male infertility [80]. Eating a healthy diet, exercising regularly, maintaining a healthy weight, and avoiding toxins and excessive alcohol intake are all good steps toward improving fertility. Reducing stress levels is also important, and may be accomplished through exercise, relaxation techniques, or simply spending time with friends and family. Medications such as antidepressants and high blood pressure medicine can negatively affect fertility by reducing sex drive or causing erectile dysfunction that makes intercourse difficult if not impossible. Men who smoke are more likely to have low sperm count than non-smokers [81]. Obesity also increases the risk for diabetes which can cause testicular damage that impairs sperm production or causes sexual dysfunction that makes intercourse impossible [82]. A healthy diet with plenty of fruits, vegetables, lean meats, whole grains, and other unprocessed foods will go a long way toward preventing obesity [83]. Excessive alcohol intake has been associated with reduced sperm counts as well as reduced fertility in general by increasing estrogen levels in men which may lead to fertility problems [84]. Environmental toxins such as heavy metals and pesticides can also cause epigenetic changes that lead to male infertility [85]. Treatments for male infertility depend on the underlying cause and may include lifestyle changes such as quitting smoking, losing weight, and eating a healthy diet to reduce obesity-related testosterone reduction and improve sperm count; supplements such as vitamins or antioxidants to treat hormone imbalances or vitamin B12 shots to increase sperm production [86]. For men with more serious fertility problems, assisted reproductive technologies such as in-vitro fertilization (IVF) may be necessary [87].

4. DIAGNOSTIC TECHNIQUES

Next-generation sequencing, biomarkers, enzymatic tools, and miRNA technology are widely used for diagnosis of male infertility [88]. Moreover, epigenetic studies support an in-depth understanding of this disease other than the genetic contribution; this allows the researchers to diagnose male infertility at an early stage [89]. There are multiple conventional diagnostic approaches for male infertility that help the physician to select appropriate treatment methods for patient [90]. The scrotal ultrasound test is used in which high-frequency sound waves produce images of the body. This test provides information regarding the varicocele-related problems in testes [91]. Transrectal ultrasound provides the sample for the prostate, hormone testing is performed to diagnose the abnormalities in organ systems that contribute to male infertility. Post-ejaculation analysis is performed to determine the sperm in urine and indicates that the sperm travels back into the bladder rather than into the penis during ejaculation [92]. A blood test (Y-chromosome microdeletion test) is performed to reveal the subtle changes in the Y chromosome. This test shows sign of genetic abnormality. Testicular Biopsy involves the sample removal from the testicle with the use of a needle. These test results are particular helpful in cases of obstructive azoospermia, since they help to identify whether the absence of sperm in the ejaculate is due to a blockage in the reproductive tract. A positive report of the biopsy means that presence of sperms has been identified in testicular tissue, which indicates that the absence of sperm in ejaculate is likely due a blockage in the reproductive tract. [93–96]. Specialized sperm function tests are the accumulation of the series of tests that investigate the survival of sperm after ejaculation, also how well sperm penetrates an egg.

4.1. Next-Generation Sequencing

Genetic investigation has made exceptional advances owing to the availability of NGS platforms. Contrary to single gene mutation identification through exon-by-exon amplification and Sanger sequencing, NGS enables the interrogation of large panels of genes in a single experiment and at a reasonable cost. Also, the cost of whole exome sequencing and whole genome sequencing has dropped in the last decades, therefore, novel genes causing male infertility can be rapidly identified [97].

4.2. Sperm Analysis

Sperm analysis is done by different methods. In which sperm morphology, motility, and size of sperm are analyzed. Microfluidic techniques were combined with high-speed imaging that was given the full 3F swimming patterns of the sperm that was present in the bulk fluid mixture. This technique shows an in-depth study of the chemo-taxis and rheotaxis of the sperm (34). Genetic imbalances are determined by using fluorescence in situ hybridization (FISH) which identifies the abnormal number of chromosomes that are analyzed for this disease. FISH uses fluorescent tag to particular DNA elements to determine the aneuploidies which are because of the mitotic errors [39, 98–100]. While FISH is primarily used to identify chromosomal abnormalities, it can also be employed to estimate the number of sperm with specific genetic traits or abnormalities Other emerging techniques are super-resolution microscopy, digital holography, and next-generation sequencing [101]. Computer-aided sperm analysis (CASA) can be utilized to measure sperm motility. Sperm RNA-based Analysis was done in which different types of RNAs including non-coding RNA (siRNA, piRNA, miRNA, IncRNA, and Tisense RNA), and coding RNA. It was seen that these RNA affects the fertility of the men, and the RNA amount in the somatic cells was much higher than in spermatozoa, therefore somatic cells were considered to be removed from the transcripts of sperms. RNA sequencing and Microarray profiling were used to identify the transcription levels of spermatozoa [102].

4.3. Assays

Genetic and epigenetic markers are used to diagnose male infertility. Presently available tests include the DNA fragmentation index, anti-sperm antibody assays, sperm fluorescence in situ hybridization, and other sperm functional tests. The basic challenge in finding the predictive biomarkers is the poor diagnostic capability of all existing assays. Novel male infertility biomarkers are based on emerging research areas such as proteomics, epigenetics, genomics, metabolomics, and transcriptomics. As one of the leading biomarkers for male infertility Reactive Oxygen Species (ROS) has gained interest, because of its wide application in diagnosis of male infertility. For the metabolomics approach, ROS in semen is most widely used [103]. An anti-sperm antibody (ASA) assay is used for evaluating male infertility. It confirms the presence of the immunoglobulins that bind with the patient's sperm. These immunoglobulins clumped with the male sperm and reduced the motility and function of the sperm. This assay is reported to not provide efficient, and reliable results. Another assay that is used is the DNA fragmentation index (DFI) assay; this assay measures the integrity of the sperm DNA. As exposure to certain kinds of stress issues has a bad impact on fertilization, and embryo development [104]. Other assays that were used in different studies were protein-based assays ECM1, ACCRV1, and TEX101. All of these assays are reported to be useful in the diagnosis of sperm concentrations, and different aspects of sperm survival such as motility, morphology, size, and others. But there is a need to develop an efficient assay, and that does not require stepwise testing [105]. Therefore, these diagnostic methods can be utilized to decide the treatment options for affected individuals.

5. TREATMENT STRATAGIES

There are several treatments methods available to treat male infertility which increase sperm production and improves fertility rate.

5.1. Surgical Treatments

In cases of infertility that can be attributed to epigenetic factors, surgical procedures such as testicular sperm extraction (TESE) may be used to recover sperm from unreceptive areas. However, this approach is currently only feasible for a few types of cancer patients and is not readily available [106].

Sperms retrieval for Males who have Obstructive Azoospermia and varicocele can be treated by using surgical treatments. Microdissection testicular sperm extraction method is also a surgical method and after this surgery 70-90% of men returns to the normal ejaculation of sperms [107].

5.1.1. Vasovasostomy

Vasovasostomy (VV) is a surgery that involves the reconnection of the vas deferens to restore sperm flow. This surgery is often used as part of infertility treatment procedures for men with obstructive azoospermia, an otherwise irreversible condition caused by occlusion of the vasa deferentia. Vasovasostomy can be planned based on the patient's age, comprehensive medical history, family medical history, overall health status, and psychological condition. Vasovasostomy process is done under mild anesthesia, the part under examination is thoroughly shaved then the patient is laid down in comfortable position both vasa are carefully examined in most of normal cases only one side is needed to be repaired [108, 109].

5.1.2. Vasoepididymostomy

Vasoepididymostomy (VE) is a sperm retrieval technique used to bypass an obstruction in the vas deferens and epididymis, restoring fertility to men who suffer from non-obstructive azoospermia [97]. The procedure involves the reattachment of the epididymal tubule and spermatic cord to create a longer path for sperm flow, thus increasing chances of successful fertilization. Vasoepididymostomy is a complex surgery as compared to VV; it is only performed if there are no sperms that are visualized after the analysis of fluid taken from vas deferens. The selection of procedure depends upon the quality of sperms [110].

5.1.3. Sperm retrieval techniques in OA (Obstructive Azoospermia)

Sperm retrieval techniques are another option for men with non-obstructive azoospermia who wish to conceive children through IVF. The sperm retrieval method is commonly used for patients who do not undergo reconstruction; the main aim of this technique is hankering of sperms from testis and epididymis percutaneous or opens [111]. Percutaneous Epididymal Sperm Aspiration (PESA) is a straightforward procedure that does not require an operating room, anesthesia, or specialized microsurgical staff. In this process, a small needle is used to cannulate the epididymis and extract some epididymal fluid, which is then analyzed for sperm. If necessary, the procedure is followed by Testicular Sperm Extraction (TESE) [112]. The TESE procedure may also be employed to locate and extract sperm from the testes of patients with obstructive azoospermia. Sperm from these procedures can be used for ICSI to fertilize eggs from female partners. In TESE after making a small incision in the tubules for the extraction of sperms. TESE success rate in men with NOA is 20-45% [106]. Microsurgical testicular sperm extraction (MicroTESE) is another technique in which a microscope is used and more keen extraction is done under microscopic conditions in sperm retrieval the success rate of MicroTESE is up to 60% [113]. MESA Microsurgical epididymal sperm aspiration is usually performed under local anesthesia, where the sperm is aspirated from the different ducts or epididymis under an operating microscope [114].

5.2. Nonsurgical Treatments

5.2.1. Sclerotherapy

Sclerotherapy means sclera (Hardness) and therapy (treatment) Sclerotherapy is a non-surgical treatment in which blockage in the vessels is addressed. It is an effective treatment for obstructive azoospermia where spermatic cord occlusion is caused by blood vessel malfunction. The procedure involves injecting a substance known as a sclerosant in case of NOA the injection of ethanol into these blood vessels to dissolve them. This method can restore fertility in patients with unilateral or bilateral non-obstructive azoospermia, allowing them to conceive children through IVF [115].

5.2.2. Gonadotropin-releasing hormone therapy

Gonadotropin-releasing hormone (GnRH) is a peptide hormone that regulates follicle-stimulating hormone and luteinizing hormone. Excessive levels of these hormones can cause the degeneration and death of germ cells, ultimately leading to infertility in men and women. GnRH agonists

such as leuprolide acetate can be administered to suppress the release of these hormones, increasing the survival rate of sperm in men with azoospermia. GnRH agonist therapy is also used as a treatment for chronic testicular pain [116].

5.2.3. Antiestrogens

Selective estrogen receptor modulators (SERMs) such as tamoxifen and clomiphene can be used in conjunction with gonadotropin and antiandrogen treatment to stimulate spermatogenesis in men with non-obstructive azoospermia [117].

5.2.4. Aromatase inhibitors

Aromatase inhibitors are being developed as a treatment for male-factor infertility, acting to eliminate the levels of estrogen within the body. Furthermore, inhibiting the activity of aromatase can block the conversion of testosterone into estrogen, thus increasing the amount of free testosterone in men [118].

5.2.5. Flavonoids

Flavonoids are low molecular weight polyphenols ubiquitously synthesized by green plants that may show various pharmacological attributes according to their chemical structures. So far, different flavonoids like quercetin (Q), rutin (R), naringenin (N) and epicatechin (E) have shown their potential in improving sperm motility and viability. Specifically, rutin has been reported to improve the kinematic parameters of post-thawing sperm, as well as its fertilizing characteristics. Quercetin, which is a reddish pigment found in 70 plant species and is present in many plant-based food products [119]. Flavonoids can also be found abundantly in apple skins, red wine, and red onions. Quercetin has been mostly explored for its free radical scavenging and metal chelating properties [120–122]. Furthermore, it can be easily taken from apple skins, in red wine and in red onions. Q has been mostly explored for its free radical scavenging and metal chelating properties [120, 121]. There are multiple factors like Oxidative stress, apoptosis, inflammation which may prompt male reproductive system dysfunction. Studies suggest that oxidative stress is observed in around half of all infertile men. These factors may lead to small testis in the weight and may disrupt testicular structural which can eventually lead to inhibition of spermatogenesis with compromised sperm quantity and quality [9, 10]. Similarly, quercetin lowered the endocrine and testicular abnormalities caused by the heavy metal cadmium in male rats. Apigenin, EGCG, and luteolin have been shown to elevate gene expressions of steroidogenic acute regulatory protein (StAR), cytochrome P450 11A (CYP11A), and CYP17A. These effects are beneficial for restoring Leydig cell function and testosterone secretion [123, 124]. Various in vitro and animal studies have reported therapeutic potential of rutin in male infertility but the exact mechanisms are yet to be explored. Contrary to a lot of therapeutic potentials of flavonoids, some studies have claimed little antioxidant activity due to limited bioavailability of rutin in in vivo conditions [125]. Moreover, male Infertility can be caused due to metal toxicity which eventually results in ROS induction [126]. Consequently, antioxidant therapy is an encouraging strategy for treatment of individuals with heavy metal poisoning [127]. Despite carotenoids and vitamin E, flavonoids provide protection against metal toxicity [128]. It has been suggested that three flavonoids, rutin, naringin, and kaempferol have been shown to restore motility of AlCl3 -, CdCl2 -, and PbCl4 exposed sperm cells. Whereas, other two flavonoids, catechin and quercetin, had no positive effects on motility of metal-exposed sperm; rather, they decreased sperm motility compared to untreated control samples [129]. Therefore, to translate such findings into clinical reality, more studies should be conducted to see how these flavonoids could be utilized on larger scale to deal with sperm abnormalities.

6. CONCLUSIONS

Male infertility is a very complex biological condition that can be caused by different factors. Despite, involvement of various factors; genetic, environmental, and psychological there exist many treatment options for patients, and most cases can be treated successfully. Treatment options include medication, dietary supplements like rutin, surgery, and assisted reproductive technologies (ART). In some cases, lifestyle changes such as quitting smoking or drinking may also be recommended. Society should also encourage men to get checked for infertility.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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