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REVIEW

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Seminal cell-free nucleic acids as possible biomarker in male infertility: a mini-review article

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Abstract

Background Male infertility is a major problem for many couples in the world. Many factors could cause male infertility such as environmental and genetic factors, life style, aging, inflammation, endocrinological etiologies, and antisperm antibodies.

Main body Circulating cell-free nucleic acids (cfNAs) may play a key role in male infertility. cfNAs are obtained from different body fluids such as blood plasma, cerebrospinal fluid, amniotic fluid, urine, bronchoalveolar lavage fluid, and seminal plasma. The different types of cfNAs present in human semen include cell-free DNAs, cell free RNAs and cell-free mitochondrial DNAs and they are differentially higher than those in other body fluids. Few evidence have been done regarding the direct relationship between cfNAs and male infertility in serum and seminal plasma of infertile men compared to the fertile men.

Conclusions This document aimed to compile data about the main causes influencing male infertility focusing on seminal cfNA/cfDNA and its possible role as differential biomarker to diagnosis the main source of spermatogenesis abnormalities and male infertility.

Keywords Male Infertility, Cell-free DNA, Cell-free nucleic acid, Seminal plasma, Serum

1 Introduction

The incapability to conceive after 1 year orderly unprotected sex is called infertility. Infertility has been a concern for couples in reproductive age and also a serious clinical problem today. It affects about 15% of couples who are at the age of procreate worldwide [1]. According to the estimation of WHO, 60–80 million couples in the world suffer from infertility [2]. Females and males are equally responsible for the reason of infertility. 40% of infertility is related to women, 40% to men, and 20% to both sexes [3]. Three major reasons including tubal-peritoneal disease, male factor, and ovulatory dysfunction are the main causes of infertility in couples [4]. The most common causes of infertility in women are lack of ovulation, menstrual cycle problems, impossibility of embryo implantation and infection [5]. Male factors are

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also factors affecting fertility, explained in the following [6]. Nowadays, male infertility affects many couples in the world, but the cause and molecular mechanism of idiopathic infertility in men are ambiguous. However, with the advancement of new molecular and genetic methods, new realizations have shown that cell-free DNA fragments (Cf-DNA) may be a valuable molecular tool to easily determine the causes of infertility and to choose the best assisted reproductive technology (ART) programs for infertile couples [7]. The existence of cell-free DNA (cfDNA) in human plasma and its relationship with diseases was first presented in 1948 by Mendel and Matais [8]. However, this pivotal discovery had received little attention until Tan and his colleagues discovered an increase in cfDNA in the blood of patients with systemic lupus erythematosus compared to healthy individuals [9]. Today, the relationship between cfDNA and various diseases such as leukemia, rheumatoid arthritis and malignant tumors has also been determined [10]. Increased cfDNA may reflect physiological and non-malignant pathological processes such as inflammation, diabetes, tissue trauma, sepsis and infarction [11]. cfDNA can be obtained from different body fluids, such as blood plasma, cerebrospinal fluid, amniotic fluid, urine, bronchoalveolar lavage fluid, and seminal plasma [12]. The mechanism for the release of cfDNA is not fully understood, but there are two main mechanisms, including apoptosis/ necrosis and release of intact cells into blood and then lysis [13]. Therefore, it can be a non-invasive and/or prognostic biomarker for some cancers and other severe pathologies [14]. The isolated cfDNA is applied to evaluate DNA integrity, loss of heterozygosity, polymorphisms, microsatellite instability, mutations, and DNA methylation [15]. The increase in the amount of cfDNA in different tissues is related to the increase in reactive oxygen species (ROS) and the increase in oxidative stress, as well as inflammation and infection [16]. Given that very few studies have been conducted on the relationship between semen and serum cfDNA with male infertility, this review article examines the recent findings on cfDNA in male infertility and its potential use as a biomarker of male infertility.

2 Method

Studies related to the relationship between cell free DNA and men infertility until the end of 2023 by searching for articles and studies in scientific databases such as ISI Web of Science, Medline/Pubmed, ScienceDirect, Embase, Scopus, Biological Abstract, Chemical Abstract and Google Scholar was collected. To select the desired articles, the titles and abstracts of these articles were reviewed and the eligible articles were selected and summarized. The search was performed with the keywords cfDNA, men infertility, seminal CfDNA, and the relevant articles were collected, classified and summarized after evaluation. The summary of the studies conducted in this field is presented in Table 1.

3 Male infertility

Decreased fertility in men can be due to genetic abnormalities (single or multiple gene mutations, chromosomal aberrations, polymorphisms), acquired and congenital urogenital abnormalities, varicocele, mitochondrial dysfunction, infections of the accessory glands, and immunological factors [17]. When there is no casual factor and there are abnormalities in semen including no detectable spermatozoa (azoospermia), decreased motility (asthenozoospermia), abnormal morphology (teratozoospermia), and decreased number of spermatozoa (oligozoospermia) male infertility is defined as idiopathic case [18]. The occurrence of the last three simultaneous abnormalities is demonstrated as moderate or severe oligoasthenoteratozoospermia. In ART and clinical andrology, male infertility is diagnosed by endocrine and genetic evaluation, clinical examination, semen analysis, and testicular biopsy or puncture [19]. But these tests are mostly unsuccessful in elucidating the critical reason for infertility in all men [20]. Furthermore, these tests are usually costly, intensive (especially endocrinology and genetic tests), and invasive (biopsy). Studies have shown that free sperm mtDNA copy number is correlated with semen parameters and may serve as a new diagnostic marker of semen quality [21]. According to the evidence, the increased levels of seminal cfDNA are associated with the defect of sperm morphology and motility, indicating that cfDNA could be a biomarker of sperm quality [22].

4 Main factors influencing male infertility

4.1 Environmental factors

Various environmental factors influence fertility via epi/genetic pathways. The epigenome is related to the environment and genome and propagates epigenetic tags during generations [23]. For example, occupational exposure to toxic chemical and physical agents is associated with poor semen quality, a reduced count of motile sperm, and an increased risk of male infertility,as well. Exposure to high temperatures, including metallurgical industries, and bakeries, prolonged sitting, and high stress also affect fertility. In addition, the workload, physical and sexual psychological symptoms are associated with early andropause [24]. Other factors are radiation factors via laptops, tight-fitting underwear, mobile phones, and endocrine-disrupting chemicals such as phthalates, bisphenol A, pesticide residue, and dioxins. The relation between mobile phone exposure and decreased sperm motility and viability is also shown [25].

Table 1 The studies regarding the relationship between serum and seminal cfDNA with male infertility

Author	Explanation and Findings regarding seminal cfDNA
Modou Mbay, 2021[96]	The seminal free DNA levels were assessed between the two groups of the samples and observed that a significant difference in the level of free seminal DNA between normozoospermic samples and oligozoosperimic, teratozoosperimic, azoosperimic samples and those with a high DNA fragmentation index. This study concluded that seminal cfDNA was a significant biomarker for the assessment of sperm fertility in humans
Pizio, 2021 [97]	The seminal cfDNA level was significantly higher in men with azoospermia and men with teratozoospermia than matched control. In addition, a significant association was seen between sperm abnormalities and increased levels of seminal cfDNA. These results may specify novel prognostic and diagnostic role of cfDNA for male infertility
Aitken, 2020 [58]	This study assessed a new pathway for DNA damage induction in spermatozoa of humans and revealed that cfDNA activated a defensive answer in spermatozoa which was associated with induction of DNA fragmentation by nuclease, Therefore, in vivo the exogenous cfDNA led to an increase in sperm DNA fragmentation, indicating male infertility
Ponti, 2018 [98]	This study assessed seminal cfDNA and revealed that seminal cfDNA was significantly higher in the seminal plasma of individuals with azoospermia than in patients with normozoospermia
Hazout, 2018 [99]	The mean cfDNA level in fertile and infertile females was 42.9 and 98.5 ng/µl, respectively. In addition, the mean cfDNA level in fertile and infertile males was 60.6 and 83.34 ng/µl, respectively. But the reason of excess cfDNA in the etiology of infertility was unknown
Costa, 2017 [22]	cfDNA level of semen samples was evaluated in 163 patients and revealed that seminal cfDNA level was associated with sperm fertility parameters. Therefore, this study concluded that the measurement of seminal cfDNA by Picogreen fluorochrome is related to criteria of sperm fertility
Draškovič, 2017 [1]	This study isolated cfDNA from seminal fluid and observed that only low-molecular-weight of seminal plasma cfDNA was related to specific sperm parameters in male fertility
Wu, 2016 [100]	Cell-free seminal DNA can be a new and noninvasive biomarker to detect testicular epigenetic aberrations such as spermatogenesis process
Spindler, 2012 [101]	There was a significantly higher level of cfDNA in the seminal plasma of individuals with defective parameters of sperm
Li, 2009 [70]	The concentration of cfDNA in semen fluid of patients with normozoospermia, and azoospermia was 1.34 ± 0.65 mg mL-1, and 2.56 ± 1.43 mg mL-1, respectively. According to these findings, the level of seminal cfDNA in patients with azoospermia was significantly higher than in individuals without sperm abnormalities
Chu, 2004 [63]	Seminal cfDNA was detected via the modified capillary gel electrophoresis method. The quantity of cfDNA was correlated with curvilinear velocity, rapid progression, and morphology and capacitation index
Stroun, 2000 [102]	The cfDNA level in the semen of patients with normozoospermia and azoospermia was 1.34 and 2.56 pg/ml, respectively, indicating a higher level of cfDNA in patients with azsoospermia
Explanation and Findings regarding serum cfDNA	
Jeorgensen, 2018	In this study, cfDNA was assessed in severe male infertility and observed a weak but negative correlation between serum cfDNA and semen parameters, including progressive motility and total motility
Tournaye, 2018 [103]	The PCR assay demonstrated a higher level of serum cfDNA in individuals with sperm abnormalities compared to the control group. The cfDNA levels were significantly higher in men with azoospermia than the controls and men with teratozoospermia

4.2 Lifestyle

Lifestyle includes all behavioral factors influencing diet, health, exercise, and the use of tobacco and alcohol. Obesity induced by diet affects fertility in men via changing sexual behavior, sleep, semen parameters, hormonal profiles, and scrotal temperature [26]. The risk of azoospermia is also higher in overweight and underweight men than in those with normal weight. The decreased level of sex-hormone-binding globulin is seen in obese males, leading to hyperinsulinemia and an enhanced total level of estradiol. In addition, weight loss is associated with decreased cellular DNA damage, improved semen morphology, and enhanced total motile sperm count [27]. The change in lifestyle, especially the quality of food is useful to treat poor semen quality. Among the factors related to lifestyle, two factors of high consumption of caffeine and exogenous androgen are also mentioned. However, there exists no definitive evidence to suggest that caffeine has any impact on one's ability to conceive [28]. Studies provide insufficient evidence that there is no increased risk of infertility associated with low, moderate, or high caffeine consumption. Nevertheless, it is imperative to approach this conclusion with caution. It has been shown that exogenous testosterone impedes spermatogenesis by eliminating the feedback response to low testosterone at the hypothalamus and pituitary. This leads to a decrease in the synthesis and secretion of gonadotropins, which are vital for promoting endogenous testosterone production and facilitating spermatogenesis [29].

4.3 Aging

Aging progressively impairs the function of cells and facilitates vulnerability to illness. Aging is associated with reproductive endocrine disorders which cause late-onset hypogonadism in men [30]. But the molecular mechanism affecting semen quality and routine tests are poorly known. Andropause (age-related hypogonadism) is associated with the risk of complications and spontaneous abortions in infancy, including autism, genetic diseases, schizophrenia, lower birth weight, and male infertility [31]. In addition, andropause also suppresses DNA repair machinery and the antioxidant defense system, enhancing the production of ROS, and causing genomic instability [32]. However, the effect of andropause on sperm DNA damage remains controversial. Aging is also associated with various cumulative cellular and molecular events such as sperm telomere shortening, and DNA damage leading to apoptosis [33].

4.4 Inflammation

Inflammation is a process in which the human body reacts to traumatic, chemical, and infectious insults, leading to an influx of activated leukocytes, and different supporting cells and extracellular proteins [34]. Although chronic inflammation usually progresses after an acute symptomatic insult, it may happen in tissues without a history of injury or insult. In fact, most men with genitourinary tract inflammation have no inflammation symptoms. It is the latter insidious process in the male reproductive tract that has caused concern [35]. The assessment of testicular tissue specimens from asymptomatic infertile men shows leukocytic infiltration in more than 50% of men. Various inflammatory factors including the type of pathogen and the chronic and acute nature of the disease can affect male fertility. In addition, noninfectious inflammatory reactions may influence the reproductive system in men [36]. Pro-inflammatory cytokines, including interleukin (IL)-1a, and IL-1b, as well as tumor necrosis factor-alpha are the most important inflammatory response in the reproductive systems of men. The IL-6, IL-8, and IL-10 cytokines are released to inflammation status and are found in the semen of men with diverse seminal defects, indicating semen cytokines may be used to detect inflammation in the reproductive tract of men [37].

4.5 Genetic factor

Infertility in men is a multifactorial pathological situation in which genetic factors are involved. The genetic perspective of infertility in men is highly complex and at least 2000 genes involve in spermatogenesis [38]. Genetic screening is relevant for diagnosis, decision-making, and genetic counseling. Autosome-linked gene mutations play a main role in monomorphic teratozoospermia, central hypogonadism, asthenozoospermia, and familial cases of spermatogenic disturbances. In addition, the study of the whole genome proposes a marginal role for routine variants as causative factors; but some of these variants are important for pharmacogenetic purposes [39]. The most genetic factor which affects male infertility is related to azoospermia (25%) [38]. Researchers reported azoospermic individuals with microscopic deletions of distal euchromatic part of long arm of the Y-chromosome and on the basis of these findings in azoospermic men, they proposed existence of a spermatogenesis gene complex called "azoospermia factor" (AZF) on Yq [40]. According to global estimate about 10% cases of idiopathic azoospermia and oligozoospermia occur due to be deletions in AZF region. Therefore AZF deletions are among most common causes of spermatogenic failure in man identifiable by molecular genetics tool [41]. However, the genetic abnormalities which are recognized in other patients with different sperm abnormalities are increasing, indicating the important role of genetic factors in male infertility.

4.6 Endocrinological aspects

The pituitary failure for secreting follicle-stimulating hormone (FSH) and luteinizing hormone (LH) leads to disrupt fertility, and testicular function. But gonadotropin deficiency contains less than 0.5% of the causative factors in male infertility [42]. The elevated level of LH and low level of testosterone is seen in approximately 30% of men with severe degrees of testicular damage. The measurement of prolactin is mainly associated with impotency compared to infertility. Taken together, sexual hormone therapy, such as FSH, LH, and prolactin seems to be a beneficial management of infertility in men [43].

4.7 Antisperm antibodies

In addition to the customary analysis of semen, there exists the possibility of conducting other extended examinations, one of which involves the identification of antisperm antibodies (ASA). Male immune infertility is characterized as infertility that is brought about by ASA. The production of ASA is because of an unusual exposure of germ cells to the immune system. Also, ASA is present in 70 to 100% men after vasectomy and its association with post-vasectomy obstructive azoospermia is obviously declared [44]. Exogenous antigens, including bacteria, viruses, fungi, and allergens can create ASA during cross-immune reactions [45]. For example, the probability of ASA in patients with chronic prostatitis is three times higher than in patients without this disease. Its mechanism is not known; however, it may be associated with inflammatory damage to genital glands in men,

and local immune dysregulation. The laboratory manual from the World Health Organization (WHO) states that "the mere existence of sperm antibodies is inadequate for diagnosing sperm autoimmunity and so, it is necessary to establish that the antibodies severely disrupt sperm function" [46]. They can be detected in 16% of infertile men and approximately 2% of fertile men. ASA can lead to the sperm cells clumping even though it may occur due to other factors, such as the presence of E.coli in the semen [47, 48]. However, the extent to which ASA alone affects fertility outcomes (both natural and assisted) remains to be clearly elucidated.

5 Cell-free nucleic acid

Circulating cell-free nucleic acids (cfNAs) have a main role in the physiology of human and male infertility. Mandel et al. in 1948 recognized cfNAs, however in early 1990s, its importance was identified as a candidate biomarker [49]. cfNAs are divided into cell-free DNA (cfDNA), and cell-free RNA, which contains messenger RNAs (mRNAs) and small non-coding RNAs (microRNAs, and piwi-interacting RNAs, as well as small interfering RNAs (siRNAs) [50].

5.1 Cell-free DNA

DNA circulating in the blood plasma or serum is called cell-free DNA (cfDNA). It is a double-stranded DNA with lower molecular weight compared to genomic DNA. cfDNA presents in healthy individuals with low concentration (< 50 ng/ml) which is discovered in the 1950s. The level of cfDNA in semen is significantly higher than other body fluids. it was found that cfDNA can be used as a biomarker of sperm quality, and promising diagnostic or prognostic biomarker [51, 52]. Seminal cfDNA contains information such as epigenetic modification of the male genital tract. Few studies have been done regarding the relationship between cfDNA and male infertility summarized in Table 1.

5.2 Cell-free RNA

The investigation has revealed that mRNA and miRNA profiles in testis samples are highly expressed in individuals with non-obstructive azoospermia rather than men with obstructive azoospermia or normozoospermia [53]. Similarly, cell-free seminal mRNAs have been investigated as novel noninvasive biomarker for the diagnosis of male infertility. According to these findings, cfs-mRNA DDX4 may be applied to assess the type of azoospermia [54, 55]. In addition, miRNAs play a main role in cell differentiation, and metabolism as well as apoptosis. They are non-coding and small RNAs modulating the expression of genes, post-transcriptionally via targeting particular mRNAs. Also, miRNAs can mediate the gene expression involved in idiopathic male infertility [56]. Piwi-interacting RNAs (piRNAs) are another cell-free RNAs that have been expressed in germ cells, particularly pachytene spermatocytes and spermatids in human testes and seminal plasma. piRNAs, including piR-31925, piR-31068, piR-43771, piR-43773, and piR-30198 can distinguish fertile patients from infertile men, indicating these are specific non-invasive biomarkers of male infertility [57].

5.3 Cell-free mitochondrial DNA

Mitochondria are the major source of ATP in the electron transport chain in sperm. ATP needs for sperm hyperactivation and motility, proposing that mitochondria is key factor for sperm fertilizing capacity and flagellar movement. Additionally, mitochondria regulate apoptosis through releasing some apoptosis-inducing factors and cytochrome C. Since mitochondria play an important role in sperm function and spermatogenesis, very few reports have been done regarding seminal cell-free mtDNA (CFMD) [58]. Chen et al. [59]., revealed a decreased copy number of CFMD in patients with oligoasthenozoospermia and asthenozoospermia. There is a positive correlation between seminal CFMD copy number and semen parameters, including morphology, motility, and velocity. Therefore, the content of seminal CFMD could be a potential diagnostic marker for evaluating the semen quality. Additionally, seminal ROS is negatively correlated with semen parameters [60]. Thus, a negative relationship was seen between the copy number of CFMD in semen and ROS level. There is a low feasible explanation to support this data. The first explanation is that the decreased copy numbers of CFMD in seminal plasma indicates more content of mitochondria in spermatozoa of infertile men (the increased number of mitochondria may be related to excessive ROS). The second is that the enhanced copy number of mtDNA in poor-quality sperm may be due to retarded elimination process and excessive oxidative stress levels during spermatogenesis [61].

6 Clinical collection and analysis methods for cfNAs

The isolation and analysis of cfDNA in semen involve various approaches, including the use of commercial kits based on selective binding and elution technologies, magnetic-bead methods, and organic solvent extractions. Quantitative real-time PCR has demonstrated that cfDNA levels in semen can differentiate between men with sperm abnormalities and those with normal sperm, with significantly higher cfDNA levels observed in patients with azoospermia and teratozoospermia [1]. Proper sample collection is critical for cfDNA analysis.

Standardization ensures consistency, with semen donors needing to follow specific guidelines and undergo routine analyses [62]. The separation of cells from plasma or fluid is typically achieved through centrifugation. High-speed centrifugation is preferred for its efficiency in preparing plasma for cfDNA isolation [63].

Several methods are employed for cfDNA isolation and purification:

Silica-Membrane Technology: This method uses selective binding and elution on silica membranes. It is fast and easy but may lose small DNA fragments [64]. Magnetic Bead Technology: This method involves magnetic beads coated to bind DNA. It is efficient and suitable for high-throughput applications, but requires specialized equipment [65]. Organic Solvent Extraction: Methods like phenol–chloroform extraction recover more cfDNA, including smaller fragments. These methods are time-consuming but flexible for protocol adjustments [66–69].

Analytical Methods Key techniques for cfDNA quantification and analysis include: Real-Time qPCR: Targets specific genes for cfDNA quantification and size distribution [63]. Digital and Droplet Digital PCR: Highly sensitive and precise for detecting low-abundance cfDNA [62]. Next-Generation Sequencing (NGS): Provides comprehensive data on cfDNA [70].

7 Biological mechanisms of cfNA levels and male fertility

The increased concentration and the DNA ladder pattern of cfDNA fragments in men with azoospermia suggest that the apoptosis of germ cells might be a contributing mechanism to the secretion of cfDNA [70].

Oxidative stress (OS) has been suggested as an additional source of cfDNA. An in vitro study demonstrated that exposing semen to paraquat, a toxic compound that induces oxidative stress by generating superoxide anions, led to increased levels of double-stranded cfDNA. This exposure also resulted in decreased sperm viability, motility, and normal morphology. Consequently, the concentration of cfDNA was proposed as a marker for OS in semen [22].

Subsequent research aimed to identify the primary origin of cfDNA secretion. To this end, researchers compared cfDNA concentrations between normozoospermic and vasectomized men. The findings revealed that cfDNA levels in normal semen were four times higher than in vasectomized men. Since vasectomized men lack testis and epididymis ejaculations, the lower cfDNA levels in their semen suggest that these organs are the main sources of cfDNA. Consequently, cfDNA could provide extensive information about the DNA status of these critical reproductive organs, particularly the testis, which directly influences male fertility. This led to further studies focusing on the practical applications of cfDNA, particularly as biomarkers in reproductive medicine [71].

Several factors may explain the high cfDNA concentration in semen: (1) DNA from dying cells through apoptosis, necrosis, and netosis, particularly during spermatogenesis; (2) DNA secreted by glandular cells, as secretions from seminal vesicles, prostate, and bulbourethral glands form about 90% of semen; (3) Chemicals in seminal plasma protect cfDNA from degradation and influence DNase activity, with cations like $Ca2+$, $Mg2+$, and $Zn2+$ playing a role; (4) Pathological conditions such as inflammation, cancer, or trauma may also contribute to elevated cfDNA levels [72–80].

As previously noted, male infertility can be attributed to obesity and being overweight. Evaluating the potential effects of overweight and obesity on DNA integrity is essential, as increased sperm DNA damage has been associated with lower pregnancy success rates and higher miscarriage rates [81]. Kort et al. [82] identified an increase in sperm DNA damage, assessed via the Sperm Chromatin Structure Assay (SCSA), in overweight and obese patients. Chavarro et al. [83] and Ferreiro et al. [84], using the Comet assay, and La Vignera et al. [85], using the TUNEL assay along with flow cytometry, noted higher sperm DNA damage in obese men, but not in those who were merely overweight. On the other hand, other studies did not find a significant association between BMI and the health of sperm DNA when utilizing the TUNEL and SCSA techniques [86–88].

Through the utilization of the TUNEL test on a more extensive sample size, another study revealed a heightened susceptibility to sperm DNA damage in obese males, although no such correlation was detected in overweight men. These results support and validate earlier research findings. This increased risk remained present even after accounting for age and smoking, which are characteristics that are frequently overlooked in previous research despite their recognized influence on sperm DNA fragmentation [81].

Additionally, since varicocele is recognized as a contributing factor to male infertility, A meta-analysis study analyzed studies including seven on sperm DNA damage in varicocele patients and six on the effectiveness of varicocele repair, with one study covering both aspects. In the DNA damage studies, involving 240 patients and 176 controls, varicocele patients showed significantly higher sperm DNA damage, with a mean difference of 9.84% (95% CI 9.19 to 10.49; $P<0.00001$). In the repair studies, 177 patients had surgery, resulting in a notable improvement in sperm DNA integrity, with a mean difference of − 3.37% (95% CI − 4.09 to − 2.65; $P<0.00001$). As mentioned before Oxidative stress and oxidative DNA damage were associated with impaired spermatogenesis

in varicocele patients. High OS levels in these patients indicate its role in sperm DNA damage. The link between varicoceles and OS might be due to increased nitric oxide and related enzymes in the dilated veins. Additionally, elevated intratesticular temperature in varicocele patients may impair testicular function and directly damage nuclear DNA in seminiferous tubules [89]. Also The underlying mechanisms of DNA damage in varicocele patients can include apoptosis, and abnormal chromatin packaging [90, 91].

8 Cost-effectiveness

Traditional semen analysis continues to be a fundamental part of the first assessment of male fertility. This standard method evaluates crucial factors like sperm count, motility, morphology, and viability. Although standard semen analysis is commonly used, it has limitations and often fails to discover the root reasons of infertility in situations when sperm parameters appear to be normal [92].

Several research have examined the cost-effectiveness of using cfDNA testing for managing male infertility, while the majority of these studies have mostly focused on perinatal situations. An important study to take into account is the investigation conducted by Mbaye et al. [93]. This study emphasizes that cfDNA is a very efficient diagnostic tool for detecting several biomarkers associated with infertility. Therefore, it has the potential to be a cost-effective complement to conventional diagnostic approaches. However, it does not provide a thorough and direct comparison of cost-effectiveness between the new approach and established procedures for male infertility. Boissière et al. [94] examined the role of cfDNA in male infertility and demonstrated that this method could serve as a biomarker for diagnosing male infertility. Due to its non-invasive nature and ease of detection, this method could be a suitable alternative to invasive and traditional methods.

An extensive examination of cfDNA in many medical scenarios reveals its capacity for reducing costs and enhancing diagnostic precision, indicating that its use in male infertility may exhibit comparable cost-effectiveness trends.

9 Future of seminal cfNA as biomarker in male infertility

While the current evidence highlights the potential of circulating cfNAs as biomarkers for male infertility, there are several limitations in the existing research. Variations in the handling of samples, differences in methodology and technical techniques among laboratories are typically not consistent, which might result in conflicting outcomes. Furthermore, as recently explained by Dong et al. [57], there may be deceptive elements that might affect the measurement of seminal cf-RNA levels. For example, being exposed to heat or abstaining from sexual activity for a lengthy period of time might lead to an increase in amounts of cf-RNA in semen.

Additional investigation is required to rectify these deficiencies. Conducting extensive research involving several centers and following defined methods for collecting samples and analyzing cfNAs is crucial to confirm the clinical usefulness of cfNAs as biomarkers. Furthermore, investigating the molecular mechanisms that connect cfNAs to infertility might offer a more profound comprehension of their function in male reproductive health. It is essential to develop sophisticated analytical methods to improve the accuracy and precision of cfNA detection in order to advance this field.

Although current evidence suggests that cfDNA may be used as a biomarker for male infertility, there are several limitations and uncertainties in the existing studies that need careful consideration. One of these limitations is the variation in demographic characteristics of the participants across different studies. Such variations can include age, race, general health status, and lifestyle habits like diet and smoking. These differences may lead to inconsistent results and reduce the generalizability of the findings [95].

Additionally, technical differences in experimental methods and laboratory analyses can also result in variable outcomes. For example, different methods of cfDNA isolation and purification may yield varying amounts of cfDNA, and diverse analytical techniques such as PCR, qPCR, and NGS may have different sensitivities and accuracies. Therefore, future studies should aim to standardize sample collection methods, cfDNA isolation procedures, and laboratory analyses to minimize these discrepancies [1].

Ultimately, conducting extensive research with collaboration across multiple research centers and following standardized protocols for sample collection and cfDNA analysis can help validate the clinical utility of cfDNA as a biomarker for male infertility. Furthermore, deeper investigation into the molecular mechanisms linking cfDNA to infertility may provide a better understanding of their role in male reproductive health.

10 Conclusion

The data given in this concise analysis emphasize the potential significance of circulating cfNAs, specifically cfDNA, as promising indicators for the diagnosis of male infertility. In order to properly evaluate their therapeutic value, it is necessary to overcome various limitations and inconsistencies that exist in the existing study, notwithstanding their promise. Differences in the way samples are handled, variations in methodology, and

technical disparities across laboratories might result in inconsistent results, which reduce the reliability of cfNA measurements. In addition, extrinsic variables such as exposure to high temperatures or extended periods of sexual abstinence might impact the quantities of cfNA (cell-free nucleic acid) in semen, thereby providing further variability.

In order to make progress in the area, it is crucial to conduct thorough multi-center research that adheres to established methods for both sample collection and cfNA analysis. Studying the molecular pathways that connect cfNAs to infertility can offer a more profound understanding of their impact on male reproductive well-being. It will be essential to create advanced analytical techniques to improve the accuracy and precision of cfNA detection. Although existing data indicate that cfDNA can be a reliable and non-invasive biomarker for male infertility, it is crucial to overcome the limitations and establish standardized research techniques in order to demonstrate its clinical significance and enhance diagnostic tools for male infertility.

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