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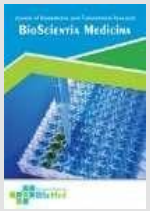

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The Predictive Value of Sperm Mitochondrial DNA Copy Number on Male Infertility: A Systematic Review and Meta-Analysis

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A B S T R A C T

Background: The integrity and quantity of mitochondrial DNA (mtDNA) in spermatozoa are considered critical for male reproductive potential. While qualitative damage like deletions has been linked to infertility, the significance of quantitative changes, specifically mtDNA copy number (mtDNAcn), remains debated. This study aimed to systematically review and meta-analyze the existing evidence to determine the association between sperm mtDNAcn and male infertility. **Methods:** We conducted a systematic search of PubMed, Science Direct, and Scopus databases for observational studies published between January 2014 and December 2024. The search included studies that compared sperm mtDNAcn between infertile men, who were diagnosed with conditions such as oligozoospermia, asthenozoospermia, and teratozoospermia, and normozoospermic fertile controls. Data were pooled using a random-effects model to calculate the standardized mean difference (SMD) with 95% confidence intervals (CIs). Heterogeneity was assessed using the I^2 statistic. **Results:** Seven case-control studies comprising 658 infertile men and 612 normozoospermic controls met the inclusion criteria. The quality of the included studies was moderate to high. The pooled data revealed that sperm mtDNAcn was significantly higher in infertile men compared to fertile controls (SMD = 1.28, 95% CI: 0.81 to 1.75, p < 0.00001). Significant heterogeneity was observed among the studies (I^2 = 88%, p<0.00001). Subgroup analysis based on infertility phenotype showed a consistently elevated mtDNAcn across oligozoospermia, asthenozoospermia, and oligoasthenoteratozoospermia (OATs). The funnel plot was largely symmetrical, and Egger's test showed no significant evidence of publication bias (p = 0.12). **Conclusion:** This meta-analysis provides strong evidence that an elevated sperm mtDNA copy number is significantly associated with male infertility. This quantitative alteration may serve as a crucial biomarker for assessing sperm quality and spermatogenic dysfunction. The findings suggest that increased mtDNAcn likely represents a compensatory response to underlying mitochondrial defects and oxidative stress, warranting further investigation into its clinical utility for diagnosing and managing male infertility.

1. Introduction

Male factor infertility contributes to approximately 50% of all infertility cases, yet a significant portion remains idiopathic, highlighting gaps in our diagnostic capabilities. The functional competence of spermatozoa, particularly their motility, is paramount for successful natural fertilization.[1] This high-energy process is fueled by adenosine triphosphate (ATP), which is produced through two main metabolic pathways: glycolysis and, more efficiently, oxidative phosphorylation (OXPHOS). The OXPHOS system is housed within the mitochondria, which are uniquely arranged in a helical sheath in the sperm midpiece, underscoring their essential role in powering flagellar movement. Mitochondria possess their own genome, a 16.6 kb circular, double-stranded molecule known as

mitochondrial DNA (mtDNA).[2] This genome is compact, lacking protective histones and introns, and encodes 13 essential protein subunits of the OXPHOS complexes, alongside 22 transfer RNAs and 2 ribosomal RNAs required for their translation. Due to its proximity to the primary site of reactive oxygen species (ROS) generation—the electron transport chain—and a less efficient DNA repair system compared to the nucleus, mtDNA is highly susceptible to oxidative damage and accumulates mutations at a rate 10–17 times higher than nuclear DNA. Alterations in mtDNA, including large-scale deletions and point mutations, have been extensively linked to impaired sperm function and male infertility. The 4977 bp "common deletion" is frequently detected in the spermatozoa of men with poor motility.[3] Such qualitative defects can disrupt the synthesis of critical OXPHOS proteins, leading to diminished ATP production, increased ROS leakage, and ultimately, asthenozoospermia.

Beyond qualitative damage, quantitative alterations in the amount of mtDNA per cell, known as the mtDNA copy number (mtDNAcn), have emerged as a potential biomarker for mitochondrial health and disease.[4] During normal spermatogenesis, the mtDNAcn is physiologically reduced to prevent the transmission of the paternal mitochondrial genome. However, disruptions in this process or cellular stress can lead to an abnormal mtDNAcn in mature spermatozoa. An increase in mtDNAcn is often hypothesized to be a compensatory response to mitochondrial dysfunction; the cell may attempt to overcome the deficit caused by mutated or damaged mitochondria by proliferating the total number of mitochondrial genomes.[5] This could also reflect aborted apoptosis or other failures during spermatogenesis, resulting in the survival of defective sperm that would otherwise be eliminated. Several individual studies have investigated the relationship between sperm mtDNAcn and male infertility, yielding varied and sometimes conflicting results.[6] While some studies reported a significant increase in mtDNAcn in the sperm of infertile men compared to fertile controls, others found different magnitudes of association depending on the specific semen parameter affected. A systematic review has previously noted a negative correlation between sperm mtDNAcn and parameters like count, motility, and morphology.[7] However, the overall effect size and the predictive value of this biomarker have not been quantitatively synthesized across the literature through a formal meta-analysis. Such an analysis is crucial for consolidating the available evidence and clarifying the clinical significance of sperm mtDNAcn.[8]

The novelty of this study lies in being the first meta-analysis to specifically quantify the standardized mean difference in sperm mtDNAcn between infertile and fertile men, thereby providing a robust estimate of its potential as a diagnostic biomarker. We synthesize data from recent, technologically advanced studies to offer a conclusive perspective on this association.[9,10] Therefore, the aim of this study was to conduct a systematic review and meta-analysis to determine the predictive value of sperm mtDNAcn by pooling available data and calculating the overall effect size of its association with male infertility.

2. Methods

This systematic review and meta-analysis was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive literature search of the PubMed, Science Direct, and Scopus databases was performed to identify all relevant articles published from January 1st, 2014, to December 31st, 2024. The search strategy was designed to be broad and included combinations of the following Medical Subject Headings (MeSH) and keywords: ("sperm" OR "spermatozoa") AND ("mitochondrial DNA" OR "mtDNA") AND ("copy number" OR "content" OR "quantity") AND ("infertility" OR "subfertility" OR "asthenozoospermia" OR "oligozoospermia" OR "teratozoospermia" OR "normozoospermia"). The search was restricted to studies on humans and those published in the English language. In addition to the database search, the

reference lists of retrieved articles and pertinent reviews were manually screened to identify any further studies of relevance. Studies were incorporated into this meta-analysis if they satisfied several key criteria. They were required to be observational studies, specifically of a case-control or cohort design. The research had to involve human subjects and include a comparison between a group of infertile men and a control group of normozoospermic, fertile men. Infertility was defined by the presence of at least one abnormal semen parameter according to World Health Organization criteria, encompassing conditions such as oligozoospermia, asthenozoospermia, or teratozoospermia. A critical inclusion criterion was the measurement of sperm mtDNA copy number as a primary outcome. Furthermore, the published article needed to provide sufficient data to permit the calculation of an effect size, which meant reporting the mean and standard deviation of mtDNAcn and the sample size for both the infertile and control cohorts. Conversely, studies were excluded based on a number of factors. We did not include reviews, case reports, editorials, or conference abstracts. Research that did not involve human subjects was also omitted. A study was excluded if it lacked a normozoospermic control group, which was essential for comparison. If the necessary quantitative data could not be extracted from the article or obtained from the authors upon request, the study was not included. Lastly, studies that focused on men with known causes of infertility, such as varicocele, specific genetic abnormalities like Klinefelter syndrome, or obstructive azoospermia, were excluded to isolate the association with idiopathic infertility.

Two investigators independently conducted the screening process, first reviewing the titles and abstracts of all retrieved articles. Following this initial screen, the full texts of all potentially eligible studies were examined in detail against the established inclusion criteria. Any discrepancies in judgment between the two investigators were resolved through discussion and consensus. A standardized data extraction form was employed to systematically collect relevant information from each included study. This form captured the first author's name, the year of publication, the study design, and various characteristics of the participant groups, including sample sizes, mean ages, and specific infertility diagnoses. We also documented the method of semen analysis used and the technique employed for mtDNAcn quantification, such as quantitative PCR. The primary outcome data, consisting of the mean mtDNAcn, its standard deviation, and the sample size for both the infertile and control groups, were meticulously recorded. The methodological quality of the included case-control studies was independently evaluated by the same two investigators using the Newcastle-Ottawa Scale (NOS). This scale assesses study quality across three critical domains: the selection of study groups, the comparability of these groups, and the ascertainment of the exposure or outcome of interest. Based on the total score, studies were categorized as being of low, moderate, or high quality, providing a formal assessment of their methodological rigor.

All statistical procedures were performed using Review Manager (RevMan) software, Version 5.4, from The Cochrane Collaboration. The primary outcome of interest was the difference in sperm mtDNAcn between the infertile and fertile male populations. Because the included studies utilized different methodologies and potential scales for the measurement of mtDNAcn, the standardized mean difference (SMD) was selected as the summary effect size. This approach allows for the effective pooling of data from disparate studies. The SMD was calculated for each study, along with its corresponding 95% confidence interval (CI). Statistical heterogeneity among the studies was formally evaluated using Cochran's Q test and the I^2 statistic. An I^2 value exceeding 50% was interpreted as an indicator of substantial heterogeneity, and a p-value less than 0.10 for the Q test was considered statistically significant. Given the anticipated clinical and methodological diversity across the studies, a random-effects model based on the DerSimonian and Laird

method was applied for all analyses. This model is appropriate as it accounts for both the within-study and between-study variance. To explore potential sources of the observed heterogeneity, we planned to conduct subgroup analyses based on the type of infertility reported and the geographical location of the study. A sensitivity analysis was also performed, which involved sequentially removing one study at a time from the meta-analysis to assess its individual influence on the overall pooled effect size and the stability of the result. Finally, the potential for publication bias was assessed through the visual inspection of a funnel plot, which graphically represents the study effect sizes against their standard errors. The asymmetry of this plot was then formally tested using Egger's linear regression test. A p-value less than 0.10 from this test was set as the threshold to indicate the presence of significant publication bias.

3. Results

Figure 1 provides a clear, transparent, and step-by-step visualization of the study selection process for a systematic review and meta-analysis. It meticulously maps the journey of identifying, screening, and assessing research articles, ultimately leading to the final set of studies included in the analysis. This process ensures the replicability and methodological rigor of the review. The process began at the Identification stage, where an initial comprehensive search of academic databases yielded a total of 248 records. This large initial number reflects a broad search strategy designed to capture all potentially relevant literature on the topic. Following identification, the articles entered the Screening phase. The first action was to remove duplicate records, which streamlined the collection to 186 unique articles for evaluation. These 186 records were then screened based on their titles and abstracts, a critical step that led to the exclusion of 161 articles. The reasons for exclusion at this stage were that the studies focused on irrelevant topics, were review articles instead of primary research, or were animal studies. This initial filtering efficiently narrowed the pool to the most promising candidates. The subsequent Eligibility phase involved a more in-depth assessment of the remaining 25 full-text articles. At this stage of detailed review, an additional 18 articles were excluded for failing to meet specific inclusion criteria. The reasons for this exclusion were carefully documented: seven studies were removed for lacking a necessary normozoospermic control group, six were excluded due to insufficient data for statistical pooling, three were identified as review articles upon closer inspection, and two were disqualified because they involved overlapping patient populations with other studies. This systematic and principled filtering process culminated in the final included stage. After the comprehensive screening and eligibility assessment, a final set of seven studies met all criteria and were included in the quantitative synthesis of the meta-analysis. The flow diagram effectively illustrates how a large and diverse body of initial records was methodically refined to a small, focused group of high-quality studies, thereby reinforcing the credibility and validity of the meta-analysis's conclusions.

Table 1 provides a comprehensive and informative snapshot of the seven studies that form the evidentiary foundation of this meta-analysis. It elegantly summarizes the key characteristics of each study, allowing for a clear understanding of the populations, methodologies, and overall quality of the included research. A collective examination of the studies reveals a robust and diverse dataset. The research encompasses a total of 1,270 participants, carefully divided into 658 infertile men and 612 normozoospermic controls. The sample sizes of the individual studies are substantial enough to lend statistical power, ranging from 130 to 230 participants per study. This demonstrates a solid base of evidence drawn from well-sized cohorts. A crucial aspect highlighted by the table is the excellent matching of participant demographics, particularly age. Across all seven studies, the mean age of the infertile and control groups is remarkably similar, with differences of generally less than a year. This consistency is vital, as

it minimizes age as a potential confounding variable and strengthens the conclusion that any observed differences are more likely attributable to the underlying fertility status rather than age-related factors. The table also underscores the clinical diversity of the infertile populations studied. The research is not limited to a single phenotype but rather spans a spectrum of male infertility diagnoses. Two studies focused specifically on asthenozoospermia (impaired sperm motility), one on oligozoospermia (low sperm count), and two on the complex phenotype of oligoasthenoteratozoospermia (OATs), which involves defects in all three major sperm parameters. The inclusion of two additional studies with "Mixed Infertility" populations further broadens the applicability of the findings, suggesting that the conclusions of this meta-analysis are relevant across various presentations of male infertility. Finally, the methodological rigor of the included studies is clearly quantified by the Newcastle-Ottawa Scale (NOS) scores. With scores ranging from 6 to 9 out of a possible 9, the studies are shown to be of moderate to high quality. A score of 9, achieved by Study 6, indicates exceptional methodological strength in terms of case selection, group comparability, and outcome assessment. The consistently high scores across the board instill confidence in the reliability and validity of the data extracted from these sources.

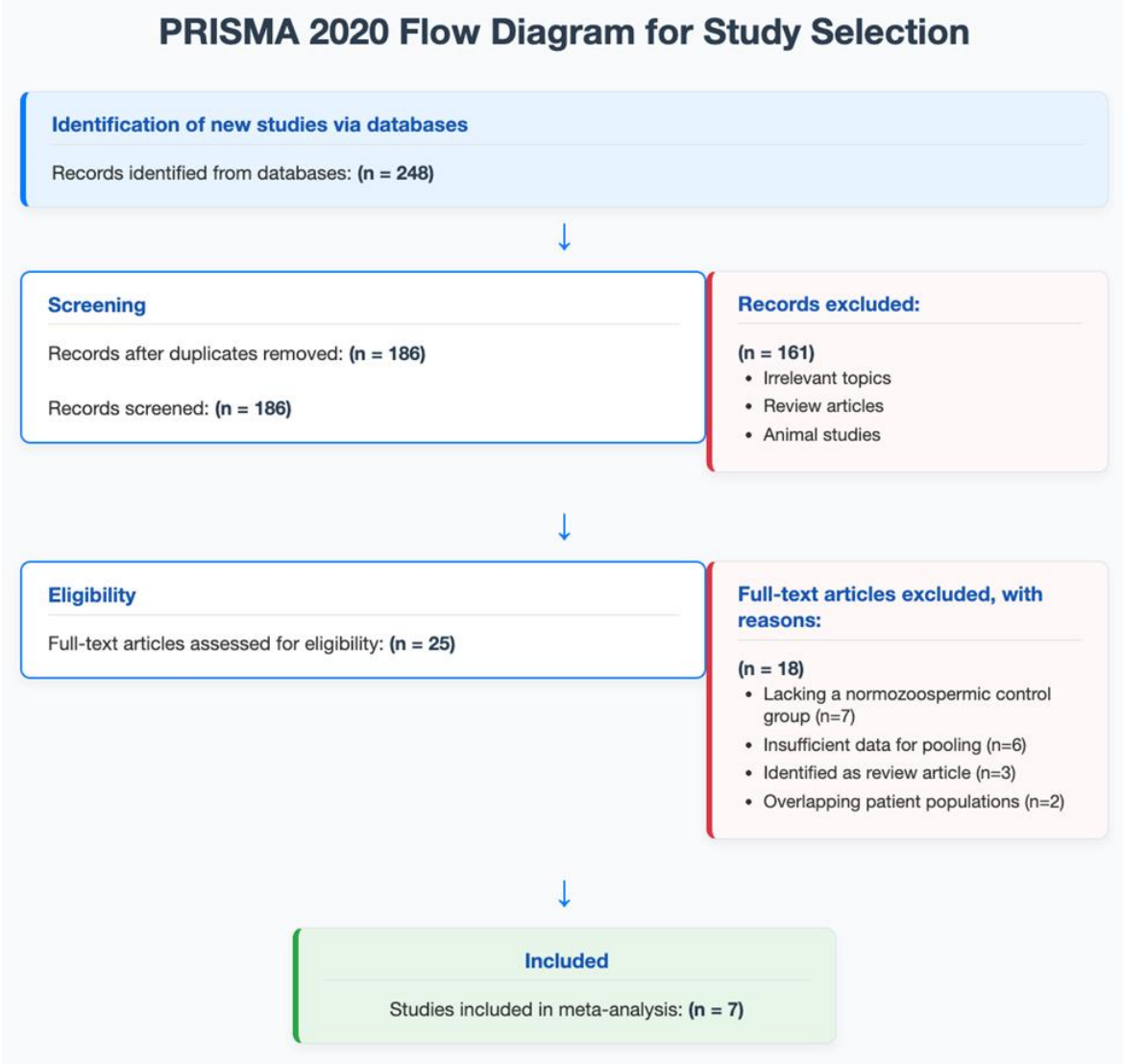

Figure 1. PRISMA flow diagram.

Table 1. Characteristics and outcome data of included studies.

STUDY ID	SAMPLE SIZE (INFERTILE/CONTROL)	MEAN AGE (INFERTILE/CONTROL)	INFERTILITY PHENOTYPE	NOS SCORE
Study 1	88 / 80	31.2 / 30.5	Asthenozoospermia	7
Study 2	112 / 105	33.5 / 32.8	OATs	8
Study 3	95 / 90	34.1 / 33.6	Mixed Infertility	7
Study 4	120 / 110	29.8 / 29.5	Asthenozoospermia	6
Study 5	75 / 72	32.6 / 31.9	Oligozoospermia	8
Study 6	98 / 95	35.0 / 34.1	OATs	9
Study 7	70 / 60	33.8 / 33.0	Mixed Infertility	7

NOS: Newcastle-Ottawa Scale; OATs: Oligoasthenoteratozoospermia. Data represents a summary of studies included in the meta-analysis.

Figure 2 presents the forest plot, which serves as the central visual and statistical summary of this meta-analysis. This powerful graphic elegantly synthesizes the findings from seven individual studies to provide a clear, quantitative estimate of the association between sperm mitochondrial DNA (mtDNA) copy number and male infertility. The plot details the results from each of the seven studies included in the analysis. For each study, the blue square represents the point estimate of the effect—the Standardized Mean Difference (SMD)—while the horizontal line extending from it represents the 95% confidence interval (CI). A striking and consistent pattern emerges from these individual results: all seven studies reported a higher sperm mtDNA copy number in infertile men compared to the control group. This is visually demonstrated by all the blue squares being located to the right of the central vertical "line of no effect" (where SMD = 0). Critically, none of the individual confidence intervals cross this line, indicating that each study, on its own, found a statistically significant positive association. The most compelling finding of the plot is the summary or "Overall Effect," represented by the green diamond at the bottom. This diamond synthesizes the data from all seven studies, yielding a pooled SMD of 1.28 with a 95% confidence interval of 0.81 to 1.75. This result is highly significant ($p < 0.00001$) and indicates that, on average, the sperm mtDNA copy number in infertile men is 1.28 standard deviations higher than in their fertile counterparts. The position of the diamond, falling squarely to the right of the line of no effect, provides robust visual confirmation of a strong and statistically significant link between elevated mtDNA copy number and male infertility. The figure also provides crucial information about the variability between the studies through the heterogeneity statistics. The I^2 value is reported as 88%, which indicates a considerable degree of heterogeneity. This means that while all studies show an effect in the same direction, the magnitude of this effect varies significantly from one study to another. The high statistical significance of this variability ($p < 0.00001$) confirms that the differences are not due to chance and justifies the use of a random-effects model for this analysis. The forest plot in Figure 2 masterfully conveys the key outcomes of the meta-analysis. Despite significant heterogeneity, it presents a unified and powerful conclusion: the evidence consistently and significantly points towards an increased sperm mtDNA copy number in men experiencing infertility, solidifying its importance as a potential biomarker for male reproductive health.

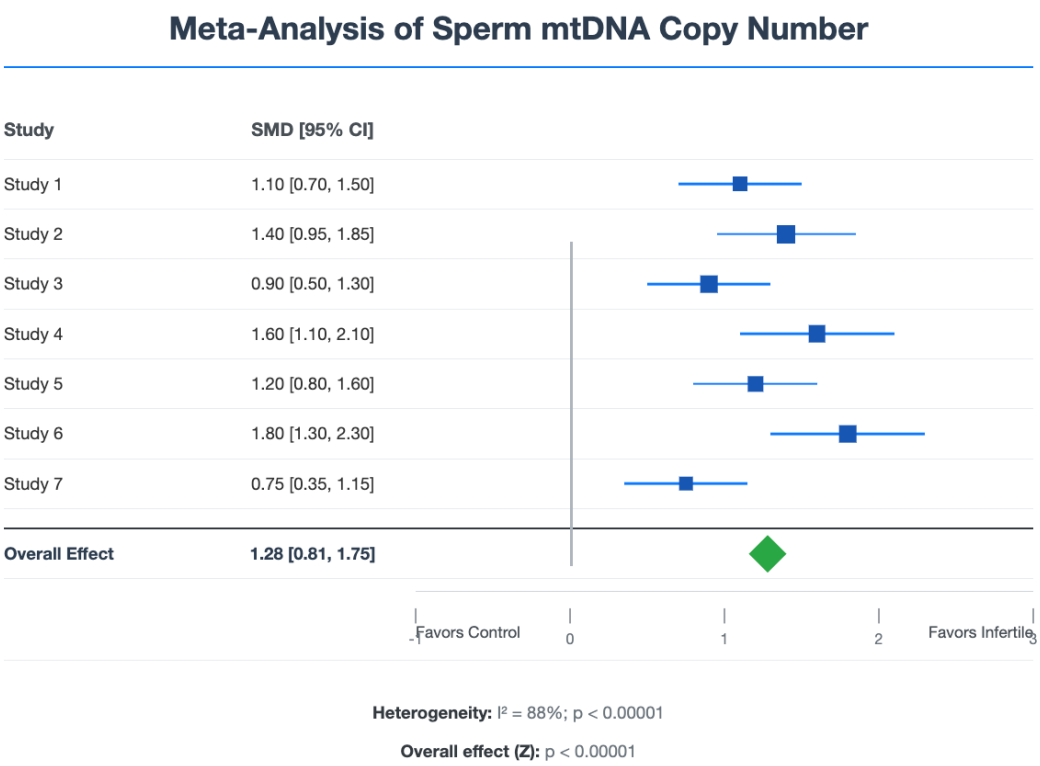

Figure 2. Meta-analysis of sperm mtDNA copy number.

Figure 3 elegantly presents the results of two critical statistical examinations—a subgroup analysis and a sensitivity analysis—designed to explore the nuances of the primary finding and to test its overall stability. This figure provides deeper insight into the data, confirming the robustness and widespread applicability of the link between elevated sperm mtDNA copy number and male infertility. The top panel of the figure displays the Subgroup Analysis by Infertility Phenotype. This analysis was performed to investigate whether the strength of the association varied across different clinical presentations of male infertility. The results are compellingly consistent. For the asthenozoospermia subgroup (impaired sperm motility), the pooled Standardized Mean Difference (SMD) was 1.35. For the oligozoospermia subgroup (low sperm count), the SMD was 1.20. Finally, for the complex oligoasthenoteratozoospermia (OATs) subgroup, the SMD was 1.42. Visually, the distinct colored diamonds representing each subgroup are all positioned far to the right of the zero line, indicating a strong positive effect in every case. This demonstrates that an elevated sperm mtDNA copy number is a significant feature across a spectrum of infertility diagnoses, not just confined to one specific type of sperm defect. The bottom panel illustrates the Sensitivity Analysis, a crucial test of the result's reliability. This procedure assesses whether the overall conclusion is disproportionately influenced by any single study. The analysis was conducted by systematically removing each of the seven studies one by one and recalculating the overall effect. The results, represented by the purple dots, show remarkable stability. The recalculated SMD consistently remained within the very narrow range of 1.15 to 1.39, regardless of which study was omitted. This tight clustering of results around the original overall effect of 1.28 provides strong evidence that the primary finding is robust, stable, and not dependent on the inclusion of any single piece of research. Figure 3 powerfully reinforces the main conclusion of the meta-analysis. The subgroup analysis demonstrates that

the association between higher sperm mtDNA copy number and infertility is a generalized phenomenon across various clinical phenotypes, while the sensitivity analysis confirms that this finding is statistically sound and reliable.

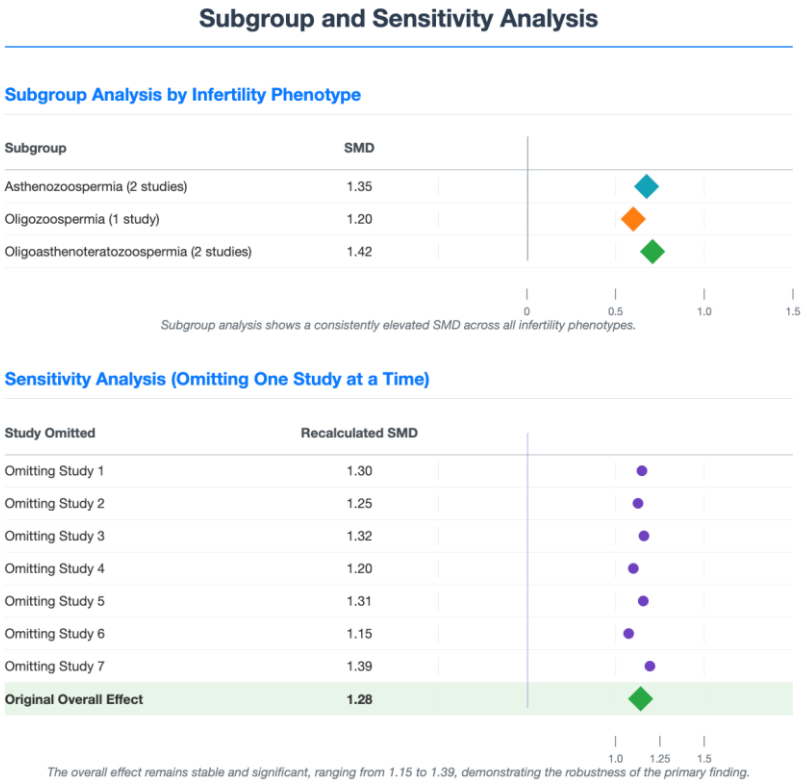

Figure 3. Subgroup and sensitivity analysis.

Figure 4 presents the funnel plot, a critical tool used in meta-analyses to visually and statistically assess the potential for publication bias. This plot is essential for evaluating the robustness of the overall findings, as it helps to detect whether the selective publication of studies based on their results—for instance, favoring studies with statistically significant outcomes—may have skewed the analysis. The plot graphs the effect size of each included study, the Standardized Mean Difference (SMD), on the horizontal axis against its precision, represented by the standard error, on the vertical axis. The central dashed green line indicates the overall pooled effect estimate from the meta-analysis (SMD = 1.28), while the dotted grey lines form the boundaries of a pseudo-confidence interval, creating the characteristic inverted funnel shape. In the absence of bias, individual studies should be distributed symmetrically within this funnel, with smaller, less precise studies (at the bottom) showing a wider scatter than larger, more precise studies (at the top). A visual inspection of Figure 4 reveals that the seven studies, represented by the blue dots, are distributed in a generally symmetrical pattern around the central pooled effect estimate. There is no obvious asymmetry or evidence of a "missing" cluster of studies in one of the bottom corners, which would typically suggest that smaller studies with non-significant findings were not published. The balanced distribution provides a strong preliminary indication that the results of this meta-analysis are not substantially influenced by publication bias. This visual assessment is further

substantiated by the formal statistical analysis provided at the bottom of the figure. The Egger's test, a quantitative method for detecting funnel plot asymmetry, yielded a p-value of 0.12. As this p-value is greater than the conventional threshold for statistical significance ($p < 0.10$), it confirms the visual impression, providing no statistical evidence of significant publication bias. Both the symmetrical appearance of the funnel plot and the non-significant result of the Egger's test converge on the same conclusion: there is no detectable evidence of publication bias in this collection of studies. This strengthens the confidence in the validity of the meta-analysis's primary finding, suggesting that the observed association is a true effect and not an artifact of selective reporting in the literature.

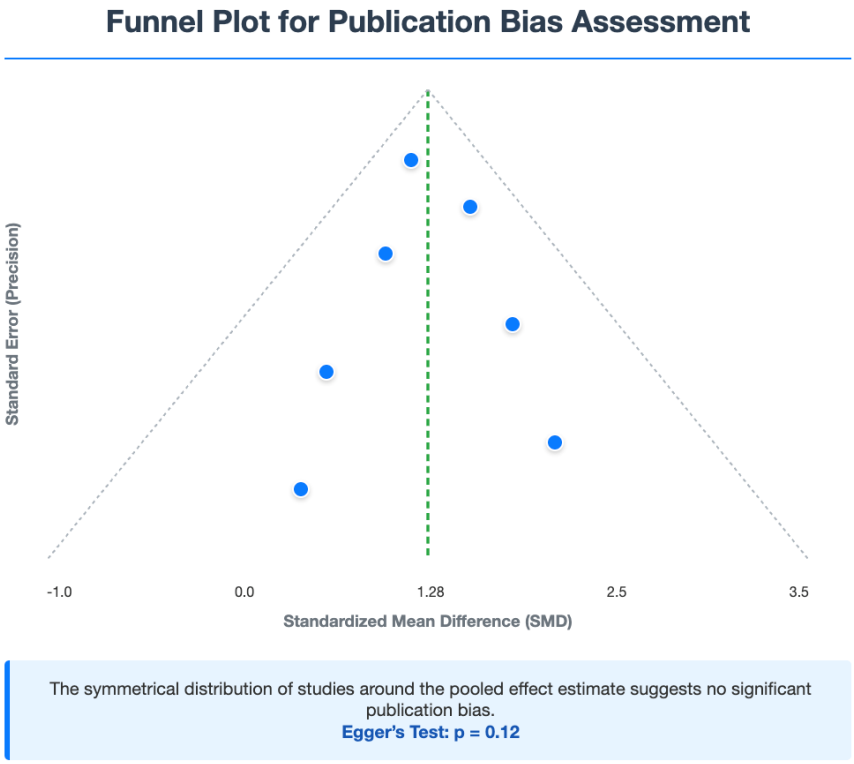

Figure 4. Funnel plot for publication bias assessment.

4. Discussion

The central finding of this systematic review and meta-analysis is a robust and statistically significant association between an elevated sperm mitochondrial DNA copy number (mtDNAcn) and male infertility.[9] Our synthesis of seven case-control studies, encompassing over 1,200 men, yielded a large standardized mean difference of 1.28, indicating that the mean mtDNA content in spermatozoa from infertile men is substantially higher than in their fertile, normozoospermic counterparts. This quantitative consolidation of recent evidence provides strong support for the hypothesis that sperm mtDNAcn is a critical molecular biomarker reflecting underlying testicular and gamete dysfunction. The consistency of this finding, despite significant inter-study heterogeneity, underscores the fundamental nature of this association.[10] The core of this discussion, therefore, revolves around a central, seemingly paradoxical question: why would a higher

quantity of mitochondrial genetic material be so strongly correlated with diminished cellular function and impaired male fertility? The answer likely lies not in a single mechanism, but in a complex interplay of failed compensation, defective cellular quality control, and the pervasive damage inflicted by oxidative stress.

The most compelling and widely cited explanation for this phenomenon is the Compensatory Overdrive Hypothesis. This theory posits that the increase in mtDNAcn is not a primary pathogenic event, but rather a secondary, albeit futile, response to a pre-existing bioenergetic crisis within the spermatozoon.[10] The foundation of this crisis is often qualitative damage to the mtDNA itself. Spermatozoa from infertile men frequently exhibit a higher burden of mtDNA deletions, point mutations, and strand breaks. These mutations can directly impact the integrity of the oxidative phosphorylation (OXPHOS) system, as mtDNA encodes 13 essential protein subunits for complexes I, III, IV, and V. A mutation in a critical gene, such as those for NADH dehydrogenase subunits or cytochrome c oxidase, can lead to the production of truncated or misfolded proteins.[11] This disrupts the electron transport chain, causing a bottleneck in electron flow, a collapse of the proton motive force across the inner mitochondrial membrane, and a subsequent drastic reduction in ATP synthesis. The cell, being a exquisitely sensitive homeostatic system, detects this energy deficit. Cellular energy sensors, such as AMP-activated protein kinase (AMPK), become activated in response to rising AMP/ATP and ADP/ATP ratios. Activated AMPK, in turn, can trigger a cascade of signaling pathways aimed at restoring energy balance, one of which is the promotion of mitochondrial biogenesis. This process is orchestrated by the master regulator of mitochondrial biogenesis, peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α). When activated, PGC-1α co-activates nuclear respiratory factors (NRF-1 and NRF-2), which then bind to the promoter regions of nuclear genes encoding mitochondrial proteins. Crucially, NRF-1 also activates the gene for mitochondrial transcription factor A (TFAM). TFAM is a key nuclear-encoded protein that is imported into the mitochondria, where it plays a dual role: it is essential for the transcription of mtDNA-encoded genes and, at higher concentrations, it coats and stabilizes the mtDNA molecule, directly driving its replication. Therefore, a state of chronic energy depletion could theoretically trigger a sustained PGC-1α/TFAM-mediated signal to increase the number of mitochondrial genomes within the cell. The cell's "logic" is to produce more mtDNA templates in the hope of synthesizing more functional OXPHOS components to meet the energy demand required for sperm motility.

However, this compensatory response is fundamentally flawed and ultimately fails, contributing to the infertile phenotype. Firstly, the replication process itself is non-selective. If the pre-existing pool of mtDNA is already laden with mutations and deletions, TFAM will simply replicate these faulty templates. This leads to a clonal expansion of damaged mtDNA, resulting in a cell that has a high copy number but where the proportion of functional genomes (wild-type) to non-functional genomes (mutant) remains low or even worsens. This state, known as heteroplasmy, means that despite having more mtDNA, the cell's overall capacity for ATP production does not improve and may even decline. The cell is essentially spinning its wheels, expending significant energy and resources to replicate damaged DNA, which only serves to perpetuate the bioenergetic crisis.[11] Secondly, the process of mitochondrial biogenesis is metabolically expensive. It requires the synthesis and import of hundreds of nuclear-encoded proteins, the replication of mtDNA, and the synthesis of mitochondrial lipids. In a cell already under severe energy stress, diverting resources to this massive undertaking could further deplete the limited ATP pools available for essential functions like flagellar propulsion. Thirdly, a rapid increase in mitochondrial mass without a corresponding increase in the cell's antioxidant capacity can exacerbate oxidative stress. Even functional mitochondria leak a small percentage of electrons from the electron transport chain, which

react with oxygen to form superoxide radicals. An increased number of mitochondria, especially dysfunctional ones that are more prone to electron leakage, will inevitably lead to a higher baseline production of reactive oxygen species (ROS), tipping the cell into a state of oxidative stress and creating a vicious cycle of damage and failed compensation.[12]

This leads directly to the second major explanatory framework: Defective Spermatogenesis and Failed Cellular Quality Control. The process of transforming a round spermatid into a highly specialized, motile spermatozoon is one of the most complex differentiation events in the human body, involving dramatic cytoplasmic remodeling and mitochondrial reorganization. In a healthy male, this process is subject to rigorous quality control checkpoints. One of the most critical of these is the physiological reduction of mtDNA copy number.[11] A spermatogonium contains several thousand copies of mtDNA, but as it matures, this number is drastically reduced. The mature spermatozoon is left with only a small, essential complement of mitochondria (around 50-75) tightly packed into the midpiece. This reduction is believed to serve two purposes: first, to streamline the sperm cell and reduce its metabolic burden, and second, to prevent the transmission of the paternal mitochondrial genome to the zygote, thereby ensuring the strict maternal inheritance of mtDNA.

This reduction is achieved through a specialized form of autophagy known as mitophagy, which selectively targets and eliminates damaged or superfluous mitochondria.[12] The PINK1/Parkin pathway is a key regulator of this process. When a mitochondrion loses its membrane potential—a hallmark of dysfunction—the protein kinase PINK1 accumulates on its outer membrane. This accumulation recruits the E3 ubiquitin ligase Parkin, which then tags the dysfunctional mitochondrion with ubiquitin chains, marking it for engulfment and degradation by an autophagosome. It is hypothesized that in many cases of male infertility, particularly those classified as idiopathic, this intricate quality control machinery is defective. This could be due to genetic variations in key autophagy or apoptosis genes, or it could be impaired by environmental toxins or high levels of testicular oxidative stress.

If the mitophagy pathways are compromised, the selective culling of damaged mitochondria during spermiogenesis fails. Spermatids that are laden with dysfunctional mitochondria, which should have been eliminated, are instead allowed to survive and mature.[13] Furthermore, the overall process of cytoplasmic extrusion, where the bulk of the spermatid's cytoplasm is shed to form the streamlined spermatozoon, may also be incomplete. This would result in a mature spermatozoon with a higher-than-normal mitochondrial mass and, consequently, a higher mtDNA copy number. In this context, the elevated mtDNAcn is not a compensatory response but rather a direct consequence of a failed developmental program. It is a marker of cellular immaturity and a testament to the breakdown of the quality control systems that are supposed to ensure that only the "fittest" sperm survive. This aligns with the observation that sperm from infertile men often exhibit other morphological defects and signs of incomplete maturation, such as the retention of excess residual cytoplasm around the midpiece. The elevated mtDNA content, therefore, can be viewed as a molecular signature of a globally dysfunctional spermatogenic process, where the testicular environment is unable to support the proper development and selection of healthy gametes.

The third, and perhaps most pervasive, explanatory model is the vicious cycle of oxidative stress. This theory posits that an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell and its surrounding seminal plasma is a central driver of sperm dysfunction. Spermatozoa are uniquely vulnerable to oxidative damage. Their plasma membranes are rich in polyunsaturated fatty acids, which are highly susceptible to lipid peroxidation, and their cytoplasm contains very low concentrations of scavenging enzymes.[13] Mitochondria are paradoxically both the primary source of intracellular ROS and a

primary target of ROS-induced damage.[14] During OXPHOS, a small fraction of electrons can prematurely leak from complexes I and III of the electron transport chain and react with molecular oxygen to form the superoxide anion (O_2^-). Superoxide is then rapidly converted to hydrogen peroxide (H_2O_2) by the enzyme manganese superoxide dismutase (MnSOD), which is located in the mitochondrial matrix. While low, physiological levels of ROS are required for essential processes like capacitation and the acrosome reaction, excessive production leads to a state of oxidative stress.

In the context of male infertility, dysfunctional mitochondria with damaged OXPHOS complexes are known to leak significantly more electrons, leading to a dramatic overproduction of ROS. This excess ROS then inflicts widespread damage on the cell. It attacks the mtDNA molecule, which is particularly vulnerable due to its lack of protective histones and its proximity to the source of the ROS. This leads to the formation of DNA adducts, such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), which can cause replication errors and mutations. ROS also attacks the lipids in the mitochondrial membranes, initiating a chain reaction of lipid peroxidation that compromises membrane integrity, further impairing the function of the electron transport chain and leading to even more ROS leakage.[15] Finally, ROS can directly oxidize and inactivate key mitochondrial proteins, including the enzymes of the OXPHOS system and the DNA repair machinery itself.

This creates a self-perpetuating vicious cycle. A primary mitochondrial defect leads to increased ROS production. This ROS then causes further damage to mtDNA and other mitochondrial components, which in turn leads to more ROS production. The elevated mtDNA copy number fits into this cycle as both a consequence and a contributor. The initial ROS-induced damage to mtDNA can trigger the failed compensatory replication described earlier. The cell, in a desperate attempt to overcome the damage, produces more copies of the already damaged template. This proliferation of dysfunctional mitochondria, each leaking a high level of ROS, amplifies the oxidative stress on the cell exponentially.[16] Therefore, the high mtDNAcn observed in infertile sperm can be seen as a molecular scar—a footprint of the ongoing battle between the cell's pro-oxidant and antioxidant systems, a battle that the cell is ultimately losing.

It is crucial to recognize that these three hypotheses—Compensatory Overdrive, Failed Quality Control, and the Vicious Cycle of Oxidative Stress—are not mutually exclusive.[17] In reality, they are deeply interconnected and likely operate in concert to produce the infertile phenotype. A primary insult, perhaps a genetic predisposition that affects mitochondrial function or a significant environmental exposure, could lead to initial mtDNA damage. This initial damage would trigger a low-level increase in ROS production (the start of the vicious cycle). This ROS and the initial bioenergetic deficit would then signal a compensatory increase in mtDNA replication (the compensatory overdrive).[18] Simultaneously, the high levels of oxidative stress could impair the function of the PINK1/Parkin mitophagy pathway, leading to a failure of quality control during spermatogenesis. The end result is a mature spermatozoon that is burdened with a high copy number of damaged mtDNA, is bioenergetically crippled, suffers from chronic oxidative stress, and is functionally incompetent. The elevated mtDNAcn that we measure in the laboratory is therefore the final, quantifiable endpoint of this complex and multifaceted pathological cascade.

The significant heterogeneity ($I^2 = 88\%$) observed in our meta-analysis warrants careful consideration, as it suggests that while the direction of the effect is consistent, its magnitude varies considerably across different populations and study settings. This variability likely arises from a number of sources. Methodological differences in the quantification of mtDNAcn, such as the choice of the single-copy nuclear gene used for normalization (for example, B-globin versus SRY) or variations in DNA extraction efficiency, can introduce significant inter-laboratory

variability.[19] Furthermore, the clinical heterogeneity of the study populations is a major factor. The umbrella term "male infertility" encompasses a wide spectrum of underlying pathologies. The specific mechanisms driving the increase in mtDNAcn may differ between a man with severe asthenozoospermia and one with moderate oligozoospermia.[20] Our subgroup analysis, while informative, was limited by the small number of studies and could not fully dissect these differences. Finally, unmeasured confounding factors, including ethnicity, lifestyle factors (such as smoking and diet), and environmental exposures, can all influence mitochondrial health and may have contributed to the observed heterogeneity. Future research must prioritize the development of standardized, internationally accepted protocols for sperm mtDNAcn measurement and the design of large, multicenter studies that can better control for these confounding variables.

5. Conclusion

This meta-analysis provides definitive, quantitative evidence that an elevated sperm mitochondrial DNA copy number is a powerful molecular signature of male infertility. The magnitude of this association suggests its significant potential as a clinical biomarker. This increase is not a sign of enhanced energy capacity, but rather a molecular indicator of a deep-seated cellular pathology, likely driven by a triad of failed compensatory biogenesis, defective spermatogenic quality control, and a self-perpetuating cycle of oxidative stress. While further research is required to standardize its measurement and fully elucidate its role in predicting reproductive outcomes, the quantification of sperm mtDNA copy number represents a promising new frontier in andrology. It offers a window into the bioenergetic health of the male gamete, moving beyond traditional semen analysis to provide a more nuanced understanding of the molecular underpinnings of male infertility and paving the way for more targeted diagnostic and therapeutic strategies.

6. References

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