

Diet-induced obesity disrupts hypothalamic-testicular NF- κ B signaling axis and impairs spermatogenesis: an integrated transcriptomic analysis and meta-analysis

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Background: Obesity is a recognized risk factor for male infertility, yet the involvement of the hypothalamic-testicular axis and nuclear factor kappa-B (NF- κ B)-mediated inflammation remains incompletely defined. This study aimed to investigate whether diet-induced obesity is associated with alterations in the hypothalamic-testicular NF- κ B signaling axis and spermatogenesis through integrated transcriptomic analysis and clinical meta-analysis.

Methods: Testicular single-cell RNA-seq and hypothalamic transcriptomic data from diet-induced obese mice were integrated to identify cell-type-specific transcriptional changes. The analyses included differential expression, pathway enrichment, and NF- κ B activity scoring based on target gene expression. Mechanistic findings related to steroidogenesis and germ-cell maturation were elucidated via integrated transcriptomic analysis, and a systematic clinical meta-analysis was further performed to validate these mechanistic insights and assess the association between obesity, sperm quality, and NF- κ B-related inflammation in humans.

Results: Integrated analysis revealed marked NF- κ B transcriptional activity in testicular germ cells (predominantly round

spermatids, adjusted $p = 0.00134$), whose median score was 1.82-fold higher than that of the control group. This activity inversely correlated with genes essential for steroidogenesis (*Hsd3b1*: $r = -0.47$ to -0.62), blood-testis barrier (BTB) integrity (*Cldn11*: $r \approx -0.55$), and germ-cell maturation (*Tnp1*: $r = -0.42$ to -0.58 ; all adjusted $p < 0.05$). Concurrently, a high-fat diet was associated with hypothalamic NF- κ B signaling ($\log_2FC = 1.32$, adjusted $p = 0.012$) and upregulated Protein Phosphatase 2 Catalytic Subunit Alpha (*Ppp2ca*; $\log_2FC = 1.57$, adjusted $p = 0.008$). Meta-analysis of 7 clinical studies confirmed obesity-associated reduced sperm concentration (standardized mean difference = -0.24 , 95% CI: -0.37 to -0.12 , $p < 0.001$) and 3.24-fold higher azoospermia risk (95% CI: 1.18–8.88, $p = 0.023$).

Conclusion: Collectively, these findings suggest that NF- κ B-mediated inflammation along the hypothalamus-testis axis may contribute to obesity-related male infertility and highlight NF- κ B as a potential therapeutic target. However, further interventional studies using NF- κ B inhibitors or germ cell-specific genetic models are required to establish causality and validate NF- κ B as a therapeutic target.

Key Words: diet-induced obesity, male infertility, nuclear factor kappa-B (NF- κ B) signaling, hypothalamic-testicular axis, transcriptomic integration, meta-analysis

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Introduction

Obesity has escalated into a global health challenge of critical concern. Beyond its well-documented links to metabolic syndrome, cardiovascular disease, and chronic inflammation, mounting evidence indicates

that obesity also severely compromises male fertility.¹ Epidemiological studies consistently demonstrate an inverse relationship between elevated body mass index (BMI) and key markers of semen quality, including sperm concentration, motility, and morphology, as well as reduced testosterone levels.^{2,3} Mechanistically, these adverse effects on male fertility are attributed to a confluence of endocrine disruption, systemic inflammation, oxidative stress, and metabolic dysfunction, all of which directly or indirectly impair spermatogenesis and testicular homeostasis. Despite these insights, the precise molecular pathways through which obesity disrupts spermatogenesis and undermines testicular homeostasis remain incompletely elucidated.⁴

Although obesity impairs male fertility through multiple interrelated mechanisms—including endocrine disruption, oxidative stress, and metabolic dysfunction—the specific role of NF- κ B-mediated inflammation along the hypothalamus-testis axis remains poorly defined. This study focuses on this pathway to dissect its tissue-specific contribution within a multifactorial context. Central to reproductive regulation is the hypothalamic-pituitary-gonadal (HPG) axis, which coordinates hormonal signals necessary for spermatogenesis.⁵ Obesity has been shown to alter hypothalamic inflammatory status and disrupt gonadotropin release, thereby indirectly impairing testicular function.⁶ Simultaneously, obesity exerts direct effects on the testes, influencing germ cell development, Sertoli and Leydig cell function, steroidogenesis, and the integrity of the BTB.⁷ However, how central inflammatory perturbations and local testicular dysfunction converge to disrupt spermatogenesis remains insufficiently understood.

The nuclear factor κ B (NF- κ B) pathway is a key transcriptional regulator of innate immunity and cellular stress responses.⁸ Chronic low-grade inflammation, a hallmark of obesity, potently activates NF- κ B in metabolic tissues, including the hypothalamus, liver, and adipose tissue.⁹ Acting as a pivotal transcriptional regulator, NF- κ B serves as a critical link between metabolic inflammation and reproductive dysfunction.¹⁰ In the hypothalamic arcuate nucleus, NF- κ B activation upregulates protein phosphatase 2 catalytic subunit alpha (Ppp2ca) and suppresses *Kiss1*, thereby impairing pulsatile luteinizing hormone (LH) secretion essential for Leydig cell steroidogenesis.¹¹ At the testicular level, obesity-induced inflammation can activate NF- κ B in Sertoli cells, likely downstream of pathways such as NLRP3 inflammasome signaling. Such Sertoli cell-specific NF- κ B activation contributes to BTB

disruption by upregulating proteases like MMP-8, disturbs Sertoli-germ cell adhesion, and may further impair steroidogenesis.¹² Nevertheless, how obesity activates NF- κ B in specific testicular cell types and how this relates to impaired spermatogenesis *in vivo* remains unclear.

Addressing these cell-type-specific questions requires high-resolution approaches capable of distinguishing subtle transcriptional changes across germ-cell lineages. scRNA-seq offers a powerful approach to dissect these effects at the cellular level. Spermatogenesis involves coordinated differentiation from spermatogonia through spermatocytes to mature sperm, supported by somatic cells such as Sertoli and Leydig cells.¹³ Disruption at any stage can severely compromise sperm quality. Although recent scRNA-seq studies have documented impaired testosterone synthesis and BTB dysfunction in obese mice, the role of NF- κ B signaling in these pathological processes and its potential coordination with hypothalamic regulatory mechanisms remain incompletely elucidated.

To clarify this, we integrated testicular scRNA-seq and hypothalamic transcriptomic data to examine: (1) obesity-induced changes in cellular and transcriptional profiles of the testis and hypothalamus; (2) cell-type-specific NF- κ B transcriptional activity along the hypothalamic-testicular axis; and (3) how central and peripheral NF- κ B activation jointly disrupts testicular function. By integrating testicular scRNA-seq with hypothalamic transcriptomic data, this study provides a unique integrative view of concurrent NF- κ B activation in both central and peripheral reproductive tissues under obese conditions, revealing previously unrecognized tissue crosstalk in obesity-related male infertility.

Materials and Methods

Public data acquisition

To investigate molecular mechanisms of hypothalamic-testicular axis interactions in diet-induced obesity-related male reproductive dysfunction, we integrated two public transcriptomic datasets from the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). GSE239391 is a testicular scRNA-seq dataset generated by the 10x Genomics Chromium platform, derived from male ICR mice fed a conventional diet or a high-fat diet for 16 weeks.¹⁴ GSE284408 is a hypothalamic arcuate nucleus bulk RNA-seq dataset from mice fed normal chow or high-fat diet (HFD) for

16 weeks, with ARC tissue isolated by laser capture microdissection.¹¹ Processed expression matrices were downloaded directly from GEO for downstream analyses. The integration of hypothalamic bulk RNA-seq and testicular scRNA-seq was designed to capture both population-level neuroendocrine changes and cell-type-specific germ cell responses, enabling a systems-level understanding of the hypothalamus-testis inflammatory axis.

Data processing

All bioinformatic analyses were performed using R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). For the testicular scRNA-seq dataset, 'Seurat' (version 4.3.0) was used for data processing and cell annotation. Quality control filtering excluded cells with fewer than 200 detected genes, more than 6000 detected genes, or mitochondrial content exceeding 10%. The remaining cells were normalized using SCTransform, subjected to dimensionality reduction via Principal Component Analysis with optimal principal component selection (PC1–PC20) determined by an elbow plot, and visualized through Uniform Manifold Approximation and Projection. Cell clustering was performed using a shared nearest-neighbor graph (resolution = 0.6), followed by manual curation based on canonical markers. Clusters were annotated using canonical germ cell markers for each cell type (Table A1). For the hypothalamic ARC bulk RNA-seq dataset, 'DESeq2' (version 1.42.0) was used for differential expression analysis. Raw counts were normalized by a variance-stabilizing transformation. Differentially expressed genes (DEGs) were defined as those with an absolute $\log_2$ -fold-change ($|\log_2FC|$) > 1 and a false discovery rate (FDR) < 0.05. The FDR represents the adjusted p value that controls the expected proportion of false positives among all declared DEGs.

Analytical workflow

Diet-induced transcriptional changes in testicular cell types were analyzed by FindMarkers ('Seurat') between the control diet (CD) and HFD groups. Differential expression between CD and HFD for each cell type was assessed using the Wilcoxon rank-sum test, with differentially expressed genes defined as $|\log_2FC| > 0.25$ and false discovery rate < 0.05. Functional annotation was performed via 'clusterProfiler' (version 4.10.0) for Gene Ontology Biological Process and Kyoto Encyclopedia of Genes and Genomes pathway enrichment. To quantify NF- κ B transcriptional activity, we computed module scores using the HALLMARK_TNFA_SIGNALING_VIA_NFKB gene set from the Molecular Signatures Database (MSigDB;

<https://www.gsea-msigdb.org/gsea/msigdb>). This gene set, while representing TNF- α signaling, contains well-curated NF- κ B target genes and is widely used as a surrogate for NF- κ B activity in transcriptomic analyses. The limitations of this approach are addressed in the Discussion. Group comparisons were performed by the Wilcoxon rank-sum test with Benjamini-Hochberg false discovery rate correction. Spearman correlation was used to analyze associations between NF- κ B activity scores and key testicular functional genes in CD and HFD clusters. Results were visualized as heatmaps using 'pheatmap' (version 1.0.12) with a false discovery rate < 0.05 as significant. In the correlation heatmap, the numerical values represent Spearman's correlation coefficients (ρ), with asterisks indicating statistical significance (FDR < 0.05). Sample-level average gene expression was calculated by AverageExpression ('Seurat'). Integrated heatmaps of hypothalamic-testicular axis genes were plotted with 'pheatmap' (row scaling, blue-white-red gradient). Hierarchical clustering was applied to genes and samples, with values exported as comma-separated values files.

Statistical analysis

Statistical analyses were performed using R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). Statistical methods were tailored to data type: the Wilcoxon rank-sum test for single-cell comparisons, DESeq2's Wald test for bulk RNA-seq, and Spearman correlation for continuous variable associations. The Benjamini-Hochberg method was used to calculate the false discovery rate for multiple testing. A false discovery rate < 0.05 was considered significant. For violin plots, median values are indicated by horizontal lines within each violin, and statistical comparisons between CD and HFD groups were performed using the Wilcoxon rank-sum test with Benjamini-Hochberg correction. Visualizations were generated with 'ggplot2' (version 3.4.4), 'ggpubr' (version 0.6.0), and 'pheatmap' in R.

Meta-analytical validation

To evaluate the clinical relevance of our mechanistic findings regarding the NF- κ B-mediated hypothalamic-testicular inflammatory axis, we conducted a systematic meta-analysis following the PRISMA guidelines (Supplementary Table S1, Figure A1). The primary objective was to quantify the associations between obesity, impaired sperm quality and the involvement of underlying inflammatory pathways, with a specific focus on NF- κ B signaling.

TABLE 1. Clinical studies included in the meta-analysis to evaluate the association between obesity, sperm quality, and NF- κ B related mechanisms

No.	Authors (Year)	Title
1	Abbasihormozi et al. (2023)	Nuclear factor Kappa-B protein levels in sperm of obese men with and without diabetes; Cellular approach in male infertility ¹⁵
2	Yang et al. (2022)	Diet-induced obesity impairs spermatogenesis: the critical role of NLRP3 in Sertoli cells ¹²
3	Ma et al. (2018)	Association between BMI and semen quality: an observational study of 3966 sperm donors ¹⁶
4	Fan et al. (2018)	Obesity or overweight, a chronic inflammatory status in the male reproductive system, leads to mice and human subfertility ¹⁷
5	Bieniek et al. (2016)	Influence of increasing body mass index on semen and reproductive hormonal parameters in a multi-institutional cohort of subfertile men ¹⁸
6	Belloc et al. (2014)	High body mass index has a deleterious effect on semen parameters except morphology: results from a large cohort study ¹⁹
7	Jensen et al. (2004)	Body mass index in relation to semen quality and reproductive hormones among 1558 Danish men ²⁰

A systematic search was conducted in PubMed, Web of Science, and Embase using terms for obesity, male reproductive outcomes, and NF- κ B-related inflammation. The search used Boolean terms for obesity, male reproduction, and NF- κ B inflammation. PubMed: ("obesity" [MeSH] OR "obese" [tiab] OR "overweight" [tiab] OR "high-fat diet" [tiab] OR "body mass index" [tiab]) AND ("sperm" [tiab] OR "semen" [tiab] OR "spermatozoa" [MeSH] OR "sperm concentration" [tiab] OR "azoospermia" [tiab] OR "infertility, male" [MeSH]) AND ("NF-kappa B" [MeSH] OR "NF- κ B" [tiab] OR "nuclear factor kappa B" [tiab] OR "inflammation" [MeSH] OR "inflammatory" [tiab]), adapted to Web of Science and Embase with database-specific syntax (e.g., Emtree). Limited to human studies; no language or date restrictions. Searched 1 January 2004–1 January 2025. Studies were included if they were observational in humans, compared obese/overweight versus normal-weight men, reported sperm concentration or azoospermia incidence, and provided evidence linking these outcomes to NF- κ B or obesity-related inflammation. Studies were considered to provide evidence of NF- κ B involvement if they reported direct measurement of NF- κ B components, upstream regulators, or inflammatory markers linked to NF- κ B activation in reproductive tissues. Animal studies, reviews, case reports, and studies with incomplete data were excluded. No language or date restrictions were applied. After screening, seven studies met the eligibility criteria (Table 1).

Study selection and data extraction were performed independently by two reviewers, with disagreements resolved by consensus or a third reviewer; no automation tools were used. Extracted data included study characteristics, participant details, sperm concentration, and azoospermia incidence. Numerical values were taken directly from texts or figures; standard errors were derived from confidence intervals when needed, and no assumptions were made for missing or unclear data. For sperm concentration (continuous) and azoospermia (dichotomous), the most comprehensive and fully adjusted estimate was selected when multiple time points or analyses were available. Risk of bias was assessed independently using the Newcastle-Ottawa Scale (NOS), with disagreements resolved by consensus or a third reviewer. Effect sizes were standardized mean differences (SMD) for sperm concentration and odds ratios (OR) for azoospermia. Study eligibility for each synthesis was determined by tabulating characteristics against predefined groups. Data preparation included converting medians and interquartile ranges to means and standard deviations when required. Results of individual studies and syntheses were planned to be tabulated and visually displayed using forest plots and funnel plots. A random-effects model (DerSimonian-Laird) was used; heterogeneity was assessed with I^2 statistics (>50% considered substantial). All analyses used R 4.3.1 with the 'metafor' package. Heterogeneity was

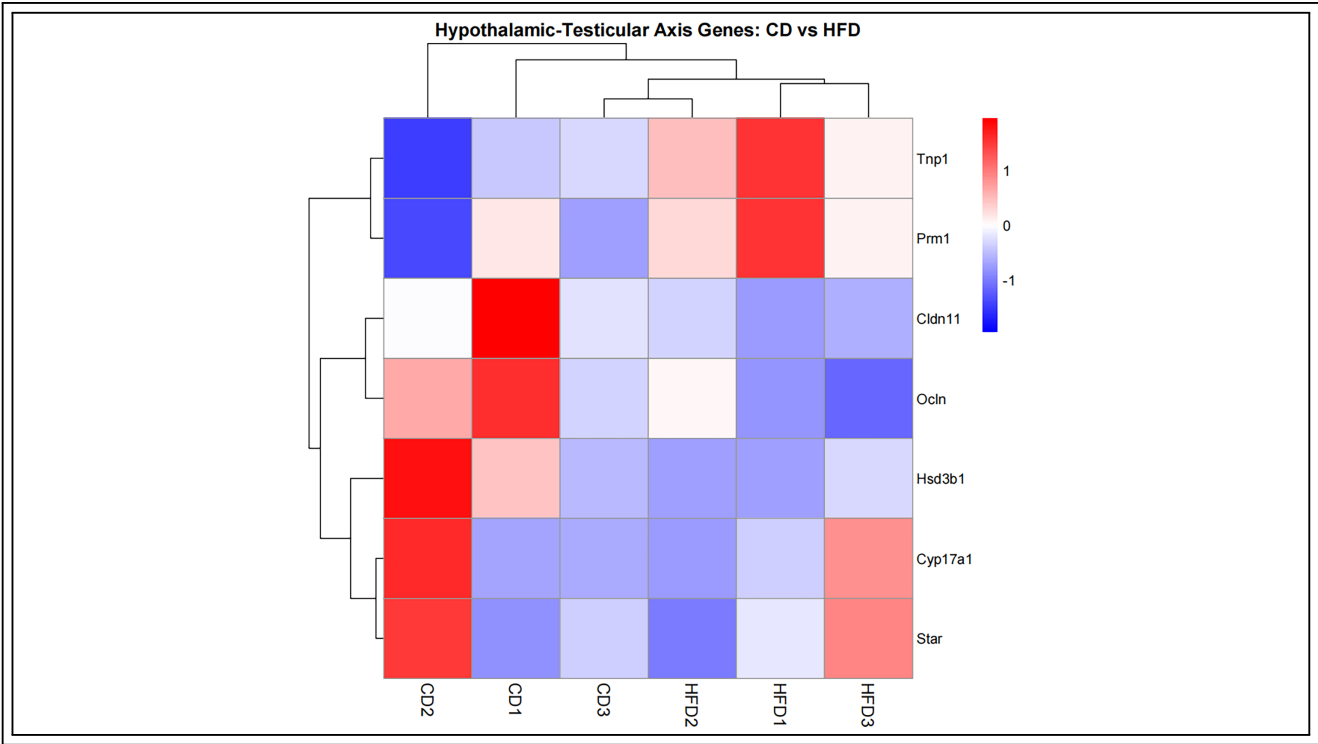


FIGURE 1. Heatmap of hypothalamic-testicular axis genes in CD and HFD mice. Row-scaled expression values (z-scores) are shown for selected genes involved in steroidogenesis (Hsd3b1, Cyp17a1, Star), BTB integrity (Cldn11, Ocln), and spermatid maturation (Tnp1, Prm1). Each column represents an individual mouse (CD1–CD3, HFD1–HFD3). Color scale: blue = lower expression, red = higher expression. CD: control diet; HFD: high-fat diet; BTB: blood–testis barrier

explored via a human-only subgroup analysis; meta-regression was not performed due to the limited number of studies. Sensitivity analyses included leave-one-out and comparisons across study designs. Publication bias was assessed using funnel plots and Egger’s test. Certainty of evidence was evaluated independently by two reviewers using the GRADE framework. This systematic review and meta-analysis were not registered, and no prior protocol was prepared.

Results

Integrated transcriptional profiling reveals diet-specific dysregulation of the hypothalamic-testicular axis

Integrated transcriptional profiling revealed distinct diet-specific reprogramming of the hypothalamic-testicular axis, with coordinated downregulation of steroidogenic and BTB genes and altered expression of spermatid maturation markers.

To evaluate how diet-induced metabolic stress reshapes reproductive signaling networks, we conducted a comprehensive cross-tissue integrated analysis of transcriptional profiles derived from the hypothalamus and testes of mice subjected to either a control diet (CD, also referred to as normal chow diet, NCD for hypothalamic samples) or an HFD. A transcriptomic heatmap (Figure 1) was generated to visualize global gene expression patterns, which revealed distinct and reproducible clustering between CD/NCD and HFD groups across all biological replicates. This clear separation reflected widespread transcriptional reprogramming of the hypothalamic-testicular axis, a key regulatory pathway that coordinates systemic metabolic homeostasis with male reproductive function. Within this axis, genes governing critical testicular endocrine activity and tissue homeostasis exhibited consistent diet-specific dysregulation patterns. Specifically, the steroidogenic gene Hsd3b1 and core BTB components Cldn11 and Ocln were significantly downregulated in HFD-fed mice compared to CD/NCD controls. In contrast, spermatid-specific markers (Tnp1 and

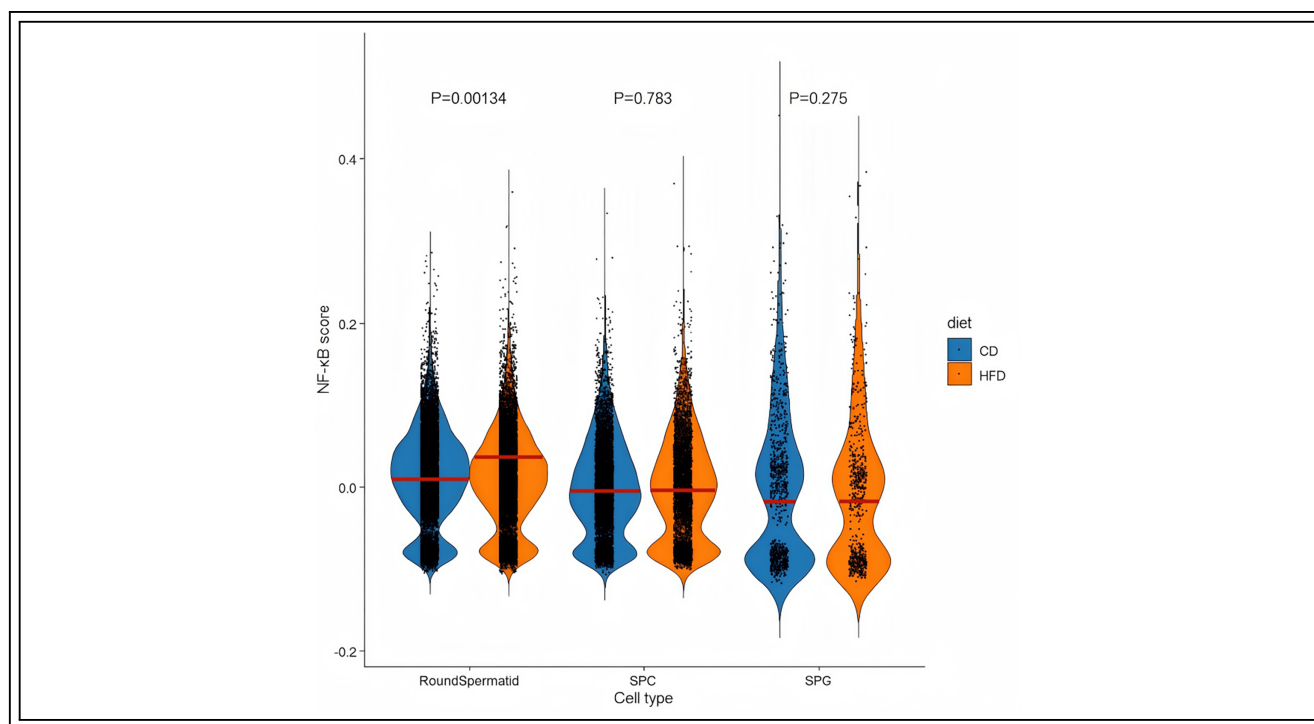


FIGURE 2. Violin plots of NF- κ B activity in key testicular germ cell populations under CD and HFD conditions. NF- κ B scores are increased most significantly in round spermatids. Horizontal lines within each violin indicate median values. Spermatocytes (SPC) show a moderate increase, while spermatogonia (SPG) show a non-significant upward trend. Statistical comparisons were performed using the Wilcoxon rank-sum test with Benjamini-Hochberg correction. These data indicate that obesity-associated transcriptional changes preferentially target late-stage germ cells, particularly round spermatids

P μ 1) were elevated in HFD samples, which likely reflects an accumulation of round spermatids (a shift in cellular composition) rather than enhanced spermatogenic maturation, and this finding is consistent with disrupted spermiogenic progression. Notably, two other steroidogenic genes, Cyp17a1 and Star, showed no significant differential expression between the two dietary groups. The coordinated shifts in steroidogenic, BTB, and spermatid maturation-related genes collectively indicate that HFD exposure disrupts the delicate balance of testicular endocrine output, structural integrity, and late-stage spermatid maturation processes.

Single-cell analysis identifies round spermatids as a key cell type exhibiting elevated NF- κ B transcriptional activity under obesity

Single-cell analysis identified round spermatids as the germ cell population showing the most pronounced and statistically significant increase in NF- κ B transcriptional activity under obese conditions.

To quantify inflammation-linked transcriptional signatures in the testis and its potential role in mediating HFD-induced reproductive dysfunction, we computed NF- κ B module scores across three major germ cell populations: round spermatids, spermatocytes (SPC), and spermatogonia (SPG). These cell populations were selected due to their critical roles in different stages of spermatogenesis, from early germ cell proliferation to late-stage sperm maturation. Although KEGG and GO analyses did not reveal global enrichment of immune-related pathways, NF- κ B activity scores—based on a predefined gene set—can detect cell-type-specific inflammatory signatures not captured by conventional pathway enrichment, highlighting the value of scoring approaches in identifying subtle transcriptional shifts. As shown in [Figure 2](#), HFD exposure induced a consistent elevation of NF- κ B transcriptional activity in all three germ cell populations, with the most striking and statistically significant induction observed in round spermatids (adjusted $p = 0.00134$, Wilcoxon rank-sum test with

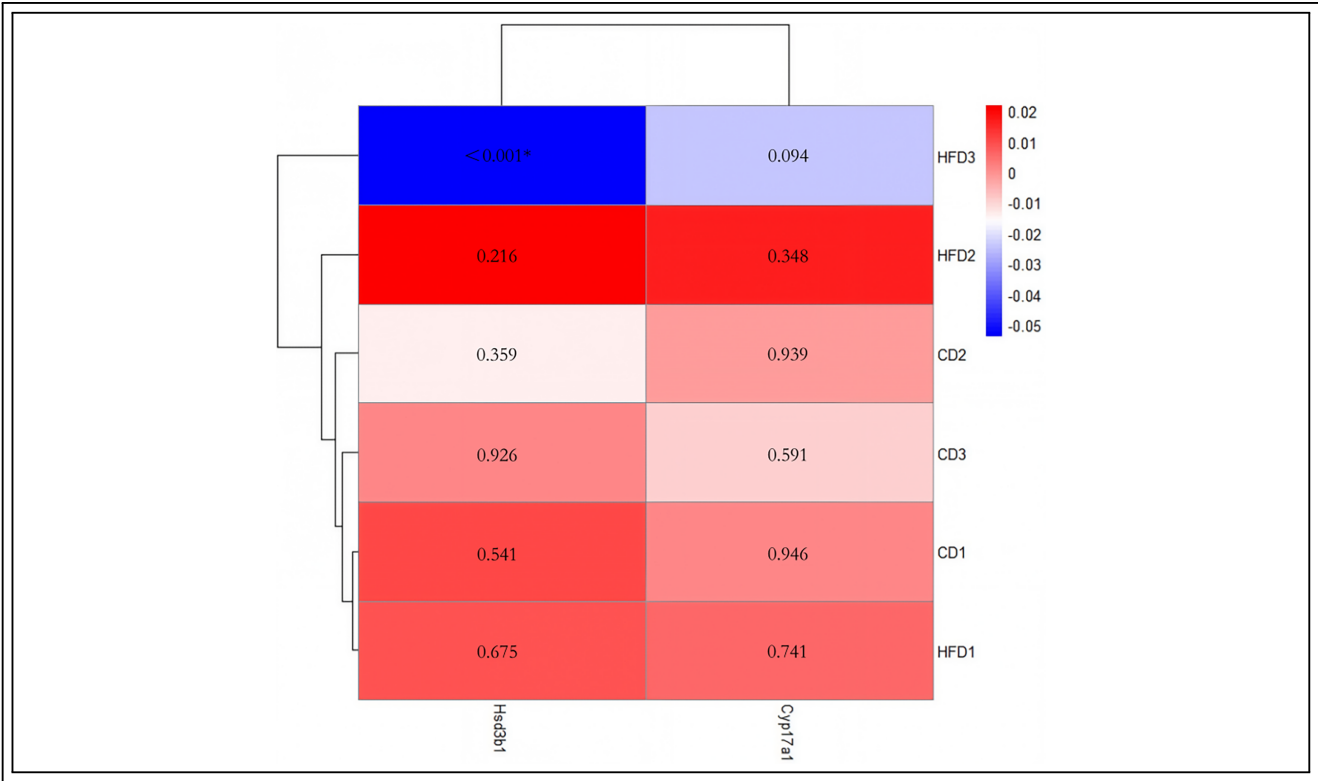


FIGURE 3. Correlation heatmap between NF-κB activity score and testicular key functional genes across cell subtypes. Each cell represents Spearman’s correlation coefficient (ρ) for a given gene-cell type cluster under CD or HFD, calculated using pooled cells from three mice per group. Asterisks indicate FDR < 0.05 (Benjamini-Hochberg corrected). Color scale: blue = negative correlation; red = positive correlation. CD: control diet; HFD: high-fat diet

Benjamini-Hochberg correction). Further analysis of the distribution of NF-κB scores revealed a robust shift in the median value of round spermatids from HFD mice, indicating a widespread transcriptional response in this cell type. Spermatocytes exhibited a moderate but statistically significant increase in NF-κB activity compared to CD/NCD controls (adjusted $p < 0.05$). In contrast, spermatogonia showed a non-significant upward trend in NF-κB activity, accompanied by greater cell-to-cell heterogeneity in HFD groups (adjusted $p > 0.05$). These results extend previous reports of somatic-cell inflammation, showing that obesity-associated transcriptional changes also affect post-meiotic germ cells, which are cell types essential for generating functional sperm.

NF-κB activation inversely correlates with key functional genes in germ cells

NF-κB transcriptional activity in HFD-derived germ cells showed strong and consistent negative correlations with genes essential for steroidogenesis, BTB

integrity, and chromatin packaging, whereas no such correlations were observed in controls.

To explore whether elevated NF-κB transcriptional activity is associated with impaired testicular function under HFD conditions, we performed a systematic correlation analysis between NF-κB activity scores and a panel of essential spermatogenic genes (Figure 3). This analysis was conducted across CD- and HFD-derived germ cell clusters (SPG: spermatogonia; SPC: spermatocytes; RS: round spermatids) to dissect diet-specific regulatory relationships. In HFD-derived germ cell clusters, NF-κB activity showed strong and consistent negative correlations with several key functional genes: Hsd3b1 (correlation coefficients $r = -0.47$ to -0.62), Cldn11 ($r \approx -0.55$), chromatin packaging genes Tnp1, Tbp1, and Prm1 ($r = -0.42$ to -0.58), and the spermatogonial maintenance regulator Dmrt1 ($r \approx -0.40$). In Figure 3, each cell represents Spearman’s correlation coefficient (ρ), and asterisks indicate statistical significance

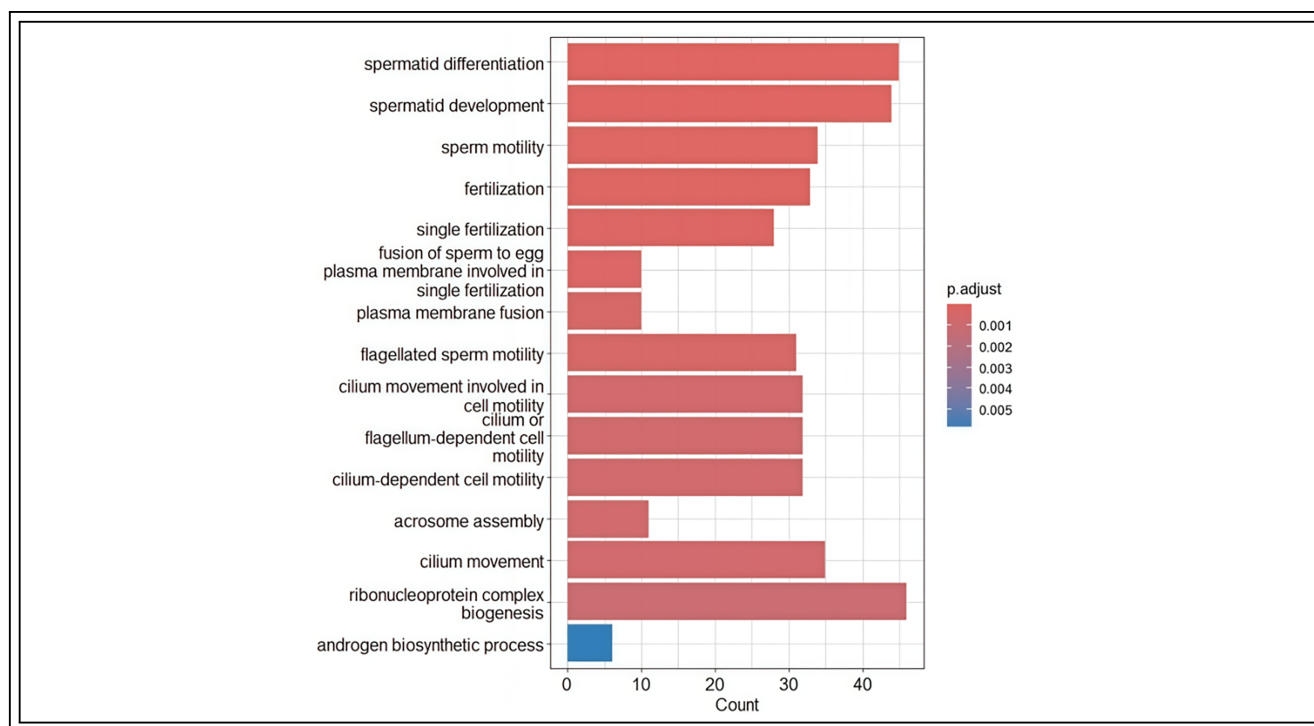


FIGURE 4. GO Biological Process (BP) enrichment analysis of differentially expressed genes (DEGs) in HFD vs. CD testes. The bar plot displays the top enriched GO BP terms for DEGs. Bar length represents gene count; color intensity indicates $\log_{10}$ (adjusted p value) (Benjamini-Hochberg correction). Key terms include spermatid differentiation, spermatogenesis, sperm motility regulation, flagellum assembly, and fertilization, indicating disruption of core spermatogenic pathways under obesity

(FDR < 0.05). We observed some variability in correlation strength when examining individual samples, consistent with biological heterogeneity inherent to complex *in vivo* systems. However, the overall pattern across pooled clusters revealed statistically significant negative correlations (FDR < 0.05), indicating a consistent directional trend. CD-derived clusters showed near-zero correlations, confirming absent NF- κ B activation under physiological conditions. In HFD-derived clusters, despite sample heterogeneity, consistent negative correlations across key functional genes reinforce the link between NF- κ B activation and suppressed spermatogenesis. Notably, a set of inflammatory and stress-responsive transcripts showed mild positive correlations with NF- κ B scores, specifically in HFD groups. These data establish a direct and functional link between obesity-driven NF- κ B transcriptional activity and the suppression of genes essential for steroidogenesis, BTB integrity, and spermatid maturation, providing evidence that inflammation directly compromises core testicular functions.

Functional enrichment analyses confirm disruption of spermatogenic and metabolic pathways

Functional enrichment analyses confirmed that HFD exposure disrupts core spermatogenic processes, including spermatid differentiation, sperm motility, and flagellum assembly, as well as metabolic pathways such as ribosome biogenesis and amino acid metabolism.

To further characterize the scope of HFD-induced transcriptional dysregulation in the testis, we performed differential gene expression analysis between CD and HFD testes, which revealed that HFD strongly downregulates a coherent set of pathways central to male fertility (Figure 4). Gene Ontology (GO) Biological Process enrichment analysis was conducted to annotate the functional relevance of these differentially expressed genes. The analysis highlighted highly significant (adjusted p < 0.001) enrichment of terms spanning nearly every major stage of germ cell development and sperm maturation. Key enriched terms included spermatid differentiation, spermatogenesis, regulation of sperm

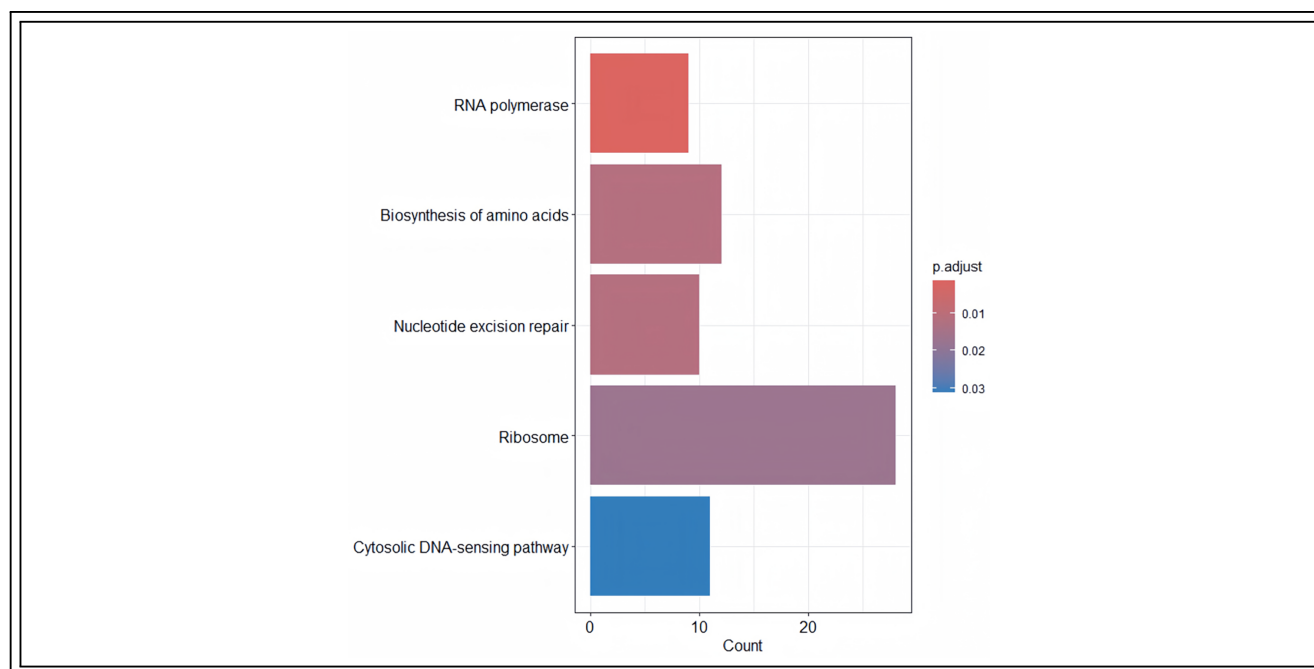


FIGURE 5. KEGG pathway enrichment analysis of DEGs in HFD vs. CD testes. The bar plot shows the top enriched KEGG pathways for testicular DEGs. Bar length represents gene count; color intensity indicates $-\log_{10}$ (adjusted p value) (Benjamini–Hochberg correction). Significantly enriched pathways (adjusted $p < 0.05$) include ribosome biogenesis, amino acid biosynthesis, RNA polymerase, and the cytosolic DNA-sensing pathway, revealing that a high-fat diet disrupts both core metabolic and immune signaling in the testis

motility, flagellum assembly, and fertilization-related processes. These terms collectively cover the full spectrum of germ cell maturation, from meiosis and post-meiotic differentiation to the acquisition of sperm motility and fertilization capacity. The enrichments observed in the GO analysis align precisely with the previously documented dysregulation of *Tnp1*, *Prm1*, *Tbp1*, and *Cldn11* in HFD groups. This alignment reinforces a model where obesity-induced inflammation impairs both the structural maturation, including chromatin packaging, flagellum formation, and functional capacity, such as motility and fertilization potential of developing sperm.

Consistent with the GO enrichment results, KEGG pathway enrichment analysis (Figure 5) identified significant HFD-associated alterations in pathways that are critical for germ cell viability, protein metabolism, and immune response. The most prominent enriched pathways included ribosome biogenesis, amino acid biosynthesis, RNA polymerase, and the cytosolic DNA-sensing pathway. Notably, enrichment of ribosome-biogenesis pathways suggests impaired translational capacity, a hallmark of defective spermatid elongation. Amino acid biosynthesis and RNA polymerase pathways

are essential for maintaining germ cell growth and transcriptional activity, respectively, and their dysregulation further supports a disruption of core metabolic processes in the testis under HFD conditions. Additionally, enrichment of the cytosolic DNA-sensing pathway, a key mediator of sterile inflammation that activates the NF- κ B signaling cascade, further validates the role of inflammation in driving testicular dysfunction. Collectively, these findings demonstrate that HFD exposure disrupts both the core metabolic machinery required for normal germ cell development and the immune homeostasis of the testis. This dual disruption provides a comprehensive mechanistic framework for explaining the reduced sperm production and impaired male fertility that are consistently associated with obesity.

Clinical meta-analysis validates the association between obesity and impaired spermatogenesis in humans

Meta-analysis of seven clinical studies confirmed that obesity is significantly associated with reduced sperm concentration and a threefold increased risk of

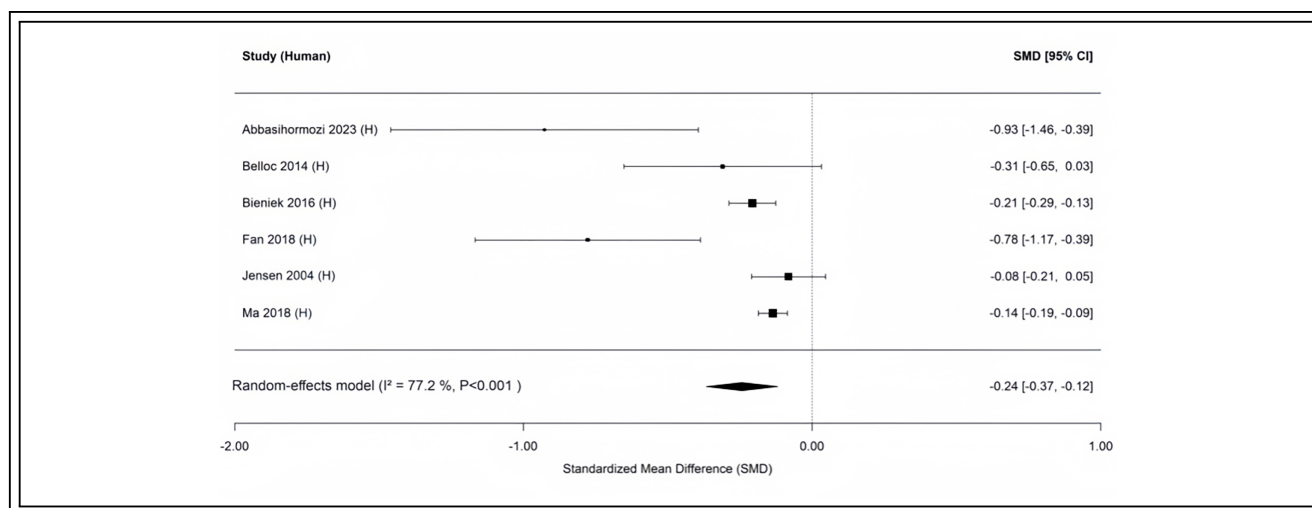


FIGURE 6. Forest plot of the association between obesity and sperm concentration in humans. Squares indicate study-specific standardized mean differences (SMDs) weighted by inverse variance, with horizontal lines showing 95% confidence intervals (CIs); the diamond represents the pooled random-effects estimate. Obesity was associated with reduced sperm concentration (SMD = -0.24 , 95% CI: -0.37 to -0.12 , $p < 0.001$), with moderate-to-substantial heterogeneity ($I^2 = 77.2\%$). Heterogeneity likely reflects population, methodological, and geographic differences; however, the consistent effect direction supports the robustness of this finding. References for the included studies: Abbasihormozi et al.,¹⁵ Belloc et al.,¹⁹ Bieniek et al.,¹⁸ Fan et al.,¹⁷ Jensen et al.,²⁰ and Ma et al.¹⁶

azoospermia in humans, providing population-level validation of our mechanistic findings.

To determine whether the inflammatory hypothalamic-testicular pathway identified in HFD mice is also supported by clinical evidence, we conducted a systematic meta-analysis of seven eligible human studies examining the association between obesity and male reproductive outcomes. Human-only pooled estimates showed a consistent negative impact of obesity on spermatogenesis across independent cohorts. Risk of bias was assessed using the Newcastle-Ottawa Scale, with all studies scoring ≥ 7 out of 9, indicating moderate to high methodological quality. In six studies reporting quantitative sperm concentration, obese men exhibited significantly lower sperm production compared with normal-weight controls (Figure 6).

Similarly, analysis of four studies providing dichotomous data demonstrated that obesity markedly increased the risk of azoospermia. The pooled random-effects odds ratio was 3.24, indicating that obese men had more than threefold greater odds of producing no detectable sperm (Figure 7).

Based on GRADE assessment, the certainty of evidence was moderate for sperm concentration and low for azoospermia, primarily due to heterogeneity and the observational design of included studies.

Although only one included study directly measured NF- κ B protein levels, the overall inflammatory context of obesity supports the relevance of NF- κ B signaling in human reproductive impairment. Visual inspection of the funnel plot showed no evident asymmetry, and Egger's regression test did not detect significant publication bias (Figure 8).

Building on our findings, we propose a unified model (Figure 9) in which diet-induced obesity co-activates NF- κ B in both the hypothalamus and testes. This model focuses on the inflammatory axis elucidated in this study and is not intended to exclude other well-established mechanisms—including endocrine disruption, oxidative stress, and metabolic dysfunction—that operate in parallel to collectively impair male fertility. This central-peripheral inflammatory synergy disrupts the HPG axis, impairing LH pulsatility, testosterone production, and spermatogenesis, ultimately leading to decreased sperm quality.

Discussion

In this study, we employed an integrated transcriptomic approach, combining single-cell transcriptomic profiling of the testis with bulk RNA sequencing of the hypothalamic ARC, to

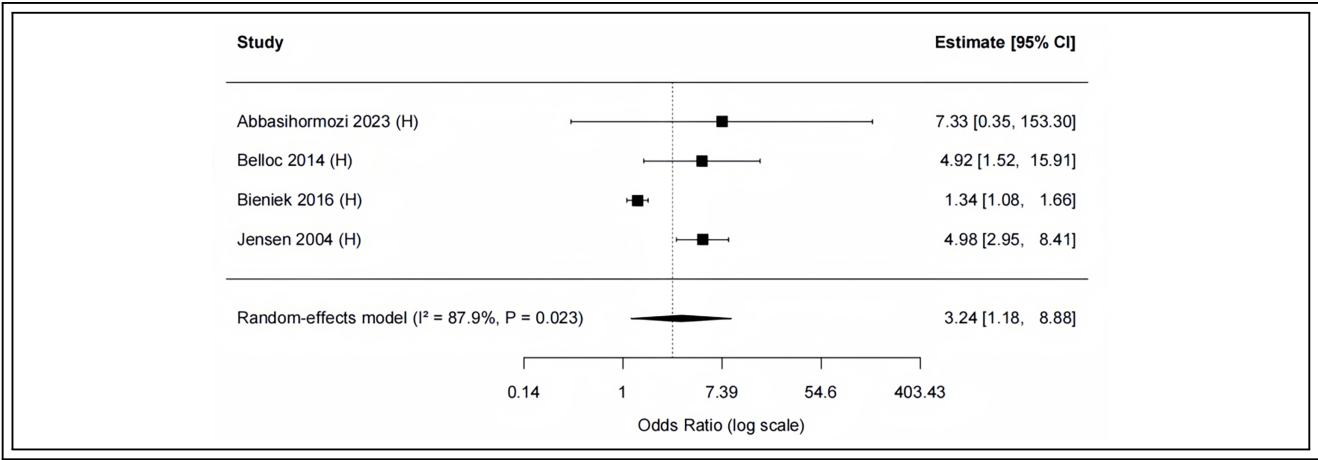


FIGURE 7. Meta-analysis of human clinical evidence linking obesity to impaired spermatogenesis. Squares indicate study-specific odds ratios (ORs) weighted by inverse variance, with horizontal lines showing 95% confidence intervals (CIs); the diamond represents the pooled random-effects estimate. Obesity was associated with a significantly increased risk of azoospermia compared with normal-weight controls (OR = 3.24, 95% CI:1.18–8.88, $p = 0.023$), with substantial between-study heterogeneity ($I^2 = 87.9\%$). Heterogeneity likely reflects population, methodological, and geographic differences; however, the consistent effect direction supports the robustness of this finding. References for the included studies: Abbasihormozi et al.,¹⁵ Belloc et al.,¹⁹ Bieniek et al.,¹⁸ and Jensen et al.²⁰

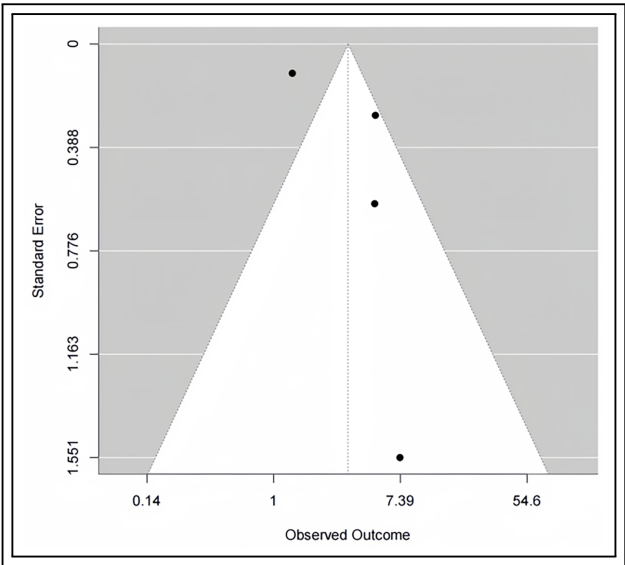


FIGURE 8. Funnel plot assessing publication bias in the azoospermia meta-analysis. Points represent individual studies vs. standard error. No evident asymmetry on visual inspection; Egger’s regression test showed no significant publication bias

reproductive function. The integrated dataset bridges central neuroendocrine regulation with peripheral testicular physiology, providing compelling evidence that HFD exposure triggers coordinated and sustained inflammatory transcriptional responses across both tissues. These cross-tissue inflammatory signatures drive key pathological events, including impaired testosterone synthesis, loss of BTB integrity, and defective spermatogenesis.^{21,22} Through comprehensive mapping of NF-κB-associated transcriptional signatures, our findings establish inflammation as a central molecular node linking metabolic stress to reproductive dysfunction in obesity.

A core novel finding is the cell-type-specific elevation of NF-κB transcriptional activity in germ cells of HFD-fed mice, most prominently in round spermatids. Unlike prior studies focusing on testicular somatic cells,^{23–25} our single-cell analysis reveals that post-meiotic germ cells—particularly round spermatids—are direct targets of obesity-induced inflammatory reprogramming, complementing previous reports of somatic dysfunction. This is biologically significant, as round spermatids undergo critical chromatin remodeling and structural assembly essential for functional sperm formation;^{26,27} perturbations at this stage can irreversibly impair sperm quality.^{28–30}

systematically dissect the molecular mechanisms through which diet-induced obesity (DIO) disrupts the hypothalamus-testis axis and impairs male

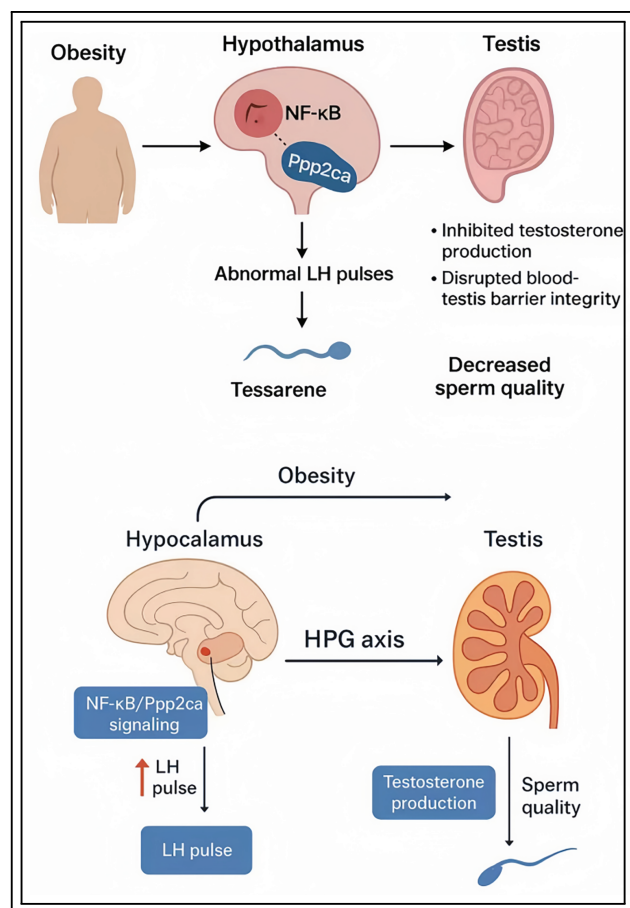


FIGURE 9. The mechanism of obesity on the male reproductive endocrine system. Obesity activates NF-κB signaling in the hypothalamus, leading to upregulation of Ppp2ca and abnormal luteinizing hormone (LH) pulses. Concurrently, obesity-induced NF-κB activation in the testes disrupts testosterone production and BTB integrity, ultimately impairing sperm quality. The red upward arrow indicates an increase in LH pulse frequency that is driven by the activation of hypothalamic NF-κB/Ppp2ca signaling in the context of obesity. This figure was created using Adobe Illustrator (version 27.0, Adobe Inc., San Jose, CA, USA)

The targeted NF-κB gene set enabled detection of cell-type-specific inflammatory signatures masked by bulk analysis, underscoring the sensitivity of module scoring approaches. However, it is important to acknowledge that the gene set used (HALLMARK_TNFA_SIGNALING_VIA_NFKB) represents TNF-α signaling and contains NF-κB target genes, but does not directly measure NF-κB activity itself. This is a limitation of transcriptomic-based scoring approaches, and future studies should

incorporate direct measurements of NF-κB activation, such as phosphorylated p65 protein levels or nuclear translocation assays, to validate these findings. Strong negative correlations between NF-κB activity and spermatogenic genes (*Tnp1*, *Prm1*, *Tbp1*, *Dmrt1*) support that inflammation disrupts germ cell maturation.^{31–33} Despite sample-level heterogeneity, significant cluster-level correlations (FDR < 0.05) and consistent trends across functionally related genes indicate that NF-κB activation is associated with suppressed spermatogenic gene expression. Mechanistically, NF-κB may interfere with chromatin remodeling in round spermatids via competition for coactivators or epigenetic regulators. Additionally, reduced BTB-related gene expression (*Cldn11*, *Ocln*) suggests compromised barrier integrity, potentially facilitating inflammatory propagation and disrupting germ-somatic communication critical for spermatogenesis.^{34,35}

Our findings extend previous knowledge by establishing a direct link between diet-induced hypothalamic neuroinflammation and testicular dysfunction. The hypothalamic ARC RNA-seq dataset used in this study demonstrated pronounced activation of NF-κB signaling and overexpression of the phosphatase Ppp2ca in HFD-fed mice. Previous studies have shown that Ppp2ca inhibits the AKT and CREB signaling pathways, two key regulators of *Kiss1* neuron function in the ARC.¹¹ *Kiss1* neurons play a pivotal role in triggering the pulsatile release of GnRH, which is essential for the subsequent secretion of LH from the pituitary gland.³⁶ This mechanistic axis provides a plausible and well-supported explanation for the previously observed reduction in LH pulse release in DIO males, a phenomenon known to be critical for driving normal Leydig cell steroidogenesis and testosterone production.³⁷ Consistent with this central regulatory defect, integrated heatmap analysis in the present study demonstrated that key steroidogenic genes in the testes, including *Hsd3b1* and *Cyp17a1*, were significantly downregulated in mice fed an HFD. Whether NF-κB activation originates centrally and propagates to the testis, or represents parallel peripheral responses to systemic stress, remains unclear. Circulating factors and neuronal pathways may mediate crosstalk, but tissue-specific manipulation is needed to establish causality. Moreover, the observed NF-κB activation may also reflect systemic metabolic disturbances associated with obesity—such as insulin resistance, lipotoxicity, and elevated cytokines—which could independently or synergistically impair testicular function. Thus, distinguishing local from

systemic inflammation will require models that isolate tissue-specific NF- κ B activation while controlling for systemic metabolic parameters.

The pathway enrichment results from our functional annotation analyses further reinforce the multifactorial and systemic nature of obesity-induced infertility. GO Biological Process enrichment analysis overwhelmingly pointed to defects in core spermatogenesis-related processes, including spermatid differentiation, spermatogenesis, regulation of sperm motility, and fertilization capacity. These enriched terms reflect the coordinated downregulation of a coherent set of genes that govern every major stage of sperm differentiation and maturation, from meiosis to the acquisition of functional competence. Complementing these GO results, KEGG pathway analysis revealed significant disruption of ribosome biogenesis, amino acid metabolism, and RNA polymerase activity in HFD testes. These pathways are essential for supporting the intense translational bursts required during spermatid elongation and flagellum assembly, which are highly energy-dependent and particularly vulnerable to metabolic stress.³⁸ Concurrently, the enrichment of cytosolic DNA-sensing pathways and other immune-related terms in HFD groups indicates activation of innate immune sensors within the testis, possibly triggered by lipid accumulation, increased oxidative stress, or mitochondrial DNA leakage, and all of which are well-documented consequences of obesity.^{39,40} Together, these pathway enrichment findings provide additional mechanistic insight into how testicular inflammation may be initiated and maintained in the context of diet-induced obesity, further validating the central role of NF- κ B-mediated inflammation in driving reproductive dysfunction.¹⁷

To confirm the clinical translatability of our mechanistic findings, we integrated a systematic meta-analysis of seven eligible clinical studies. Results demonstrated that obesity is significantly associated with reduced sperm concentration and increased azoospermia risk. Despite substantial inter-study heterogeneity (I^2 ranging from 77.2% to 87.9%), all studies reported consistent directional effects, and no significant publication bias was detected. Meta-analysis heterogeneity arose from variations in study design, population traits, BMI cutoffs and sperm assessment methods; only one study directly measured NF- κ B protein levels, highlighting the lack of direct human NF- κ B evidence and the need for NF- κ B-related biomarkers in clinical fertility assessments. Future studies should quantify phosphorylated p65, target gene expression or other NF- κ B pathway components in reproductive

tissues/seminal plasma to directly validate the pathway's role in human obesity-related infertility. These population-level data provide quantitative clinical validation of our transcriptomic-derived mechanistic framework, confirming a conserved cross-species model wherein chronic metabolic inflammation compromises spermatogenesis. Importantly, our study bridges the gap between central neuroendocrine dysfunction and local testicular pathology, identifying NF- κ B as a unifying trans-tissue driver of reproductive impairment, which potentially explains the high prevalence of idiopathic infertility in obese individuals.^{41,42}

This study has notable strengths: (1) The integrated transcriptomic approach enables unprecedented resolution of central-peripheral metabolic-reproductive crosstalk; (2) Single-cell transcriptomics precisely identifies vulnerable germ cell subpopulations, informing targeted therapeutic development; (3) Convergent results from two independent public datasets enhance robustness and generalizability. Limitations include: (1) Bulk ARC RNA-seq cannot resolve cell-type-specific hypothalamic responses, requiring future single-cell/spatial transcriptomics; (2) The absence of direct interventional validation—such as NF- κ B inhibitor studies or germ cell-specific genetic knockdown models—limits our ability to establish causality, and future studies employing such approaches are needed to determine whether blocking NF- κ B signaling can rescue spermatogenesis; (3) The role of systemic metabolic factors (insulin resistance, lipotoxicity, circulating cytokines) requires further investigation; (4) Integrated proteomic/metabolomic analyses are needed to capture multi-layered molecular disruptions; (5) The meta-analysis has limitations including substantial inter-study heterogeneity ($I^2 = 77.2\%–87.9\%$), a small number of studies for azoospermia analysis ($n = 4$), the observational design of included studies precluding causal inference, and potential for unpublished negative findings despite no detected publication bias; (6) The NF- κ B activity scores were derived from a transcriptomic signature (HALL-MARK_TNFA_SIGNALING_VIA_NFKB) that reflects TNF- α signaling rather than direct NF- κ B activation, representing an analytical surrogate that requires experimental validation.

Despite these limitations, our findings provide compelling evidence that DIO disrupts male reproductive function via a coordinated hypothalamic-testicular inflammatory mechanism centered on NF- κ B. Targeting NF- κ B signaling, restoring LH pulsatility, or improving testicular

metabolic resilience may represent promising therapeutic strategies. Given the global rise in obesity prevalence, these mechanistic insights offer a timely foundation for developing targeted interventions to address metabolic disease-related reproductive disorders, while our integrated multi-tissue approach provides a template for future studies investigating metabolic-reproductive crosstalk.^{43–45}

Conclusion

In conclusion, this study suggests that diet-induced obesity impairs male fertility via an NF- κ B-mediated hypothalamus-testis inflammatory axis, leading to neuroendocrine disruption, testicular dysfunction, and defective germ cell maturation. These findings, supported by a meta-analysis confirming reduced sperm quality and an elevated risk of azoospermia in obese men, position NF- κ B as both a potential biomarker and therapeutic target for obesity-associated male infertility. Nonetheless, causal inference is limited by the observational nature of our data; future studies employing pharmacological or genetic blockade of NF- κ B are necessary to confirm whether targeting this axis can rescue spermatogenesis *in vivo*.

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Author Contributions

Guowei Du and Xuanyi Chen designed the experiments. Guowei Du, Heng Wang, Shanshan Chen, Yanting Wang, Jing Wang, Guangzhao Yang and

Xuanyi Chen performed the experiments. Guowei Du, Jianhuai Chen, Siwen Liu and Xuanyi Chen analyzed the results. Guowei Du, Jianhuai Chen, Siwen Liu and Xuanyi Chen wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials

The data of the current study were available from the corresponding author on reasonable request.

Ethics Approval

This study utilized publicly available data from the Gene Expression Omnibus (GEO) database (GSE239391 and GSE284408). The original studies were approved by their respective institutional ethics committees. The meta-analysis part of this study did not involve direct contact with human subjects or animals and thus did not require additional ethics approval.

Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Materials

The supplementary material is available online at <https://www.techscience.com/doi/10.32604/cju.2026.078745/s1>.

Appendix A

TABLE A1. **Marker genes used for testicular germ cell annotation in scRNA-seq analysis**

Cell type	Marker genes used
Spermatogonia	Gfra1, Plzf, Id4
Spermatocytes	Sycp3, Hormad1, Rec8
Round spermatids	Prm1, Tnp1, Hils1

Note. Cell type annotation was performed based on canonical marker genes described in the original dataset publication.¹⁴

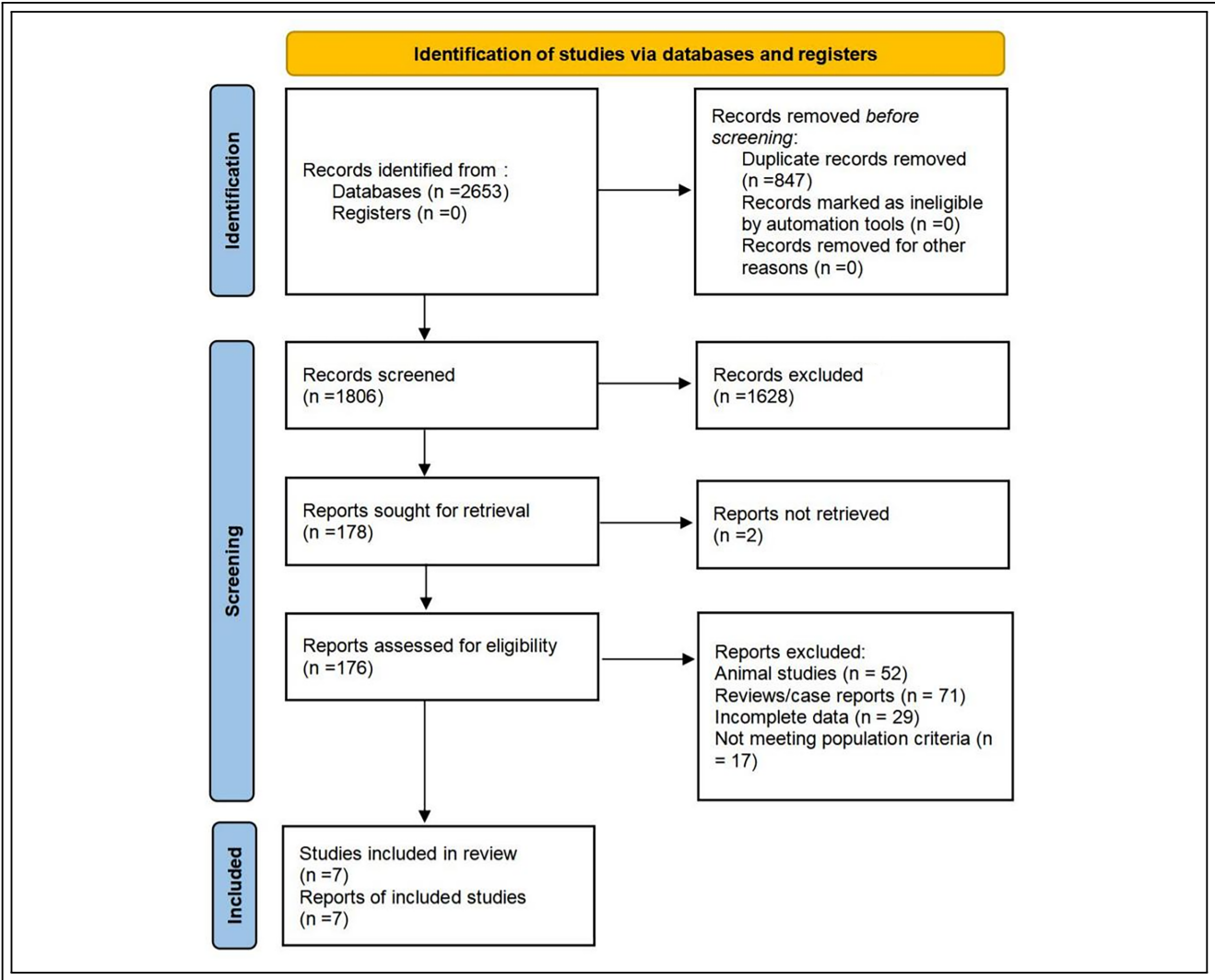


FIGURE A1. PRISMA 2020 flow diagram

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