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RESEARCH ARTICLE

# AhR-CatSper2 Crosstalk Attenuates HFD-Induced Testicular Dysfunction

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**ABSTRACT**

High-fat diet (HFD)-induced obesity is an important cause of male reproductive dysfunction. Aryl hydrocarbon receptor (AhR) and sperm-specific CatSper2 calcium channel are critical for reproductive function. However, information on the interaction between these two pathways under metabolic stress conditions and its impact on obesity-induced male infertility remains unexplored. This study aimed to investigate functional significance of AhR-CatSper2 crosstalk in attenuating HFD-induced testicular dysfunction. Forty adult male albino rats were randomly allocated into four equal groups: control group was fed standard chow, HFD group was fed diet rich in fat, HFD-RES was fed HFD plus resveratrol 100mg/kg, and HFD-AhR$^-$ group was fed HFD with CH223191 10mg/kg intra-peritoneally every 72h). The experimental period lasted for 8 weeks. Testicular expression of *AhR* and *CatSper2* genes, sperm progressive motility, viability, acrosomal integrity, DNA fragmentation, serum testosterone levels, and testicular histopathology were assessed in experimental rats. Results revealed that HFD elevated *AhR* expression, and suppressed *CatSper2* expression compared to controls ($P<0.05$). Resveratrol upregulated AhR, with improvements in sperm progressive motility, viability, acrosomal integrity and DNA integrity compared to HFD group. In contrast, AhR antagonist reduced *CatSper2* expression and led to severe impairments across all parameters, including the highest rates of sperm DNA fragmentation, sperm with abnormal acrosomes, and the lowest testosterone levels. Histopathological examination confirmed extensive germ cell loss and Sertoli cell damage in the HFD-AhR$^-$ group, while resveratrol preserved testicular architecture close to normal. In conclusion, AhR signaling protects against metabolic stress-induced reproductive damage, while resveratrol offers partial therapeutic benefit. Conversely, AhR blockade appears detrimental for testicular function and sperm quality. Knowledge about this pathway may open new spectrums for managing obesity-related male infertility.

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## INTRODUCTION

An increasingly recognized modifiable factor related to infertility in male reproductive function is obesity, along with diet rich in fat. Studies in rodents and humans reveal that exposure to a high-fat diet leads to reduced testicular weight, impaired sperm development, concentration and motility, increased sperm DNA damage, and disruption of steroidogenesis due to oxidative stress, low-grade inflammation, endocrine disruption, damage to the blood–testis barrier (BTB), and metabolic changes in the testis (Elmorsy *et al.,* 2024).

Two proteins have been identified as particularly important for HFD-induced decrease in fertility in males, including the Aryl hydrocarbon receptor (AhR) and Cation Channel Sperm-Associated 2 (CatSper2). Aryl hydrocarbon receptor is a ligand-activated transcription factor that monitors xenobiotics, and endogenous

metabolites, coordinates metabolic, inflammatory and detoxification programs; while CatSper2 is a pore-forming subunit of the sperm-specific CatSper calcium channel, which is required for calcium influx, hyperactivated sperm motility and fertilization competence. Fertility can be compromised with even a modest dysregulation of either of these two proteins: dysregulation of AhR leads to oxidative stress, hormonal changes and break-down of the BTB; while loss or dysfunction of CatSper2 hinders the calcium signaling required to drive the hyperactivated state and fertilizing competence of sperm (Bustani *et al.,* 2025).

Aryl hydrocarbon Receptor is situated at the crossroads of diet- and environment-responsive signaling because it is activated by endogenous lipid metabolites and various environmental ligands. Once activated, it modifies expression of genes related to redox balance, steroid biosynthesis, tight junction integrity, and inflammation; all of which are dysregulated in obesity and HFD models (Zhang *et al.,* 2026). A number of recent studies and reviews demonstrate influence of AhR on steroidogenic enzymes and testicular antioxidant defenses, and it has been demonstrated to be present within BTB components, indicating that modulation of AhR can both exacerbate or reduce testicular injury based on the ligand/context (Faiad *et al.,* 2022; Bustani *et al.,* 2024; Bustani and Alghetaa, 2025).

CatSper channels, particularly CatSper2, transduce intracellular signaling into the $Ca^{2+}$ dynamics in the flagellum that causes hyperactivated sperm motility. CatSper expression and activity are affected by the testicular and epididymal environment: oxidative stress, changes in membrane lipid composition, and changes in local environmental pH and signaling lipids can all affect CatSper2 channel activity. As such, oxidative and inflammatory changes in the microenvironment of testes associated with HFD could reduce *CatSper2* expression or impair its functional coupling to sperm motility, resulting in reduced sperm motility despite preserved sperm counts. Recent mechanistic work has further established CatSper as a key player in sperm motility and fertilization, moreover, loss of *CatSper2* (the gene for CatSper2) can result in fertilization failure (Swain *et al.,* 2025).

From a mechanistic standpoint, AhR may affect CatSper2 and sperm function through various mechanisms that are not mutually exclusive. AhR activation would alter redox homeostasis and the inflammatory tone in the testis. This, in turn, may elevate reactive oxygen species (ROS) and cytokine signaling that could lead to lipid peroxidation of sperm membranes or change protein function in ways that directly affect CatSper gating or localization. In addition, AhR-driven transcriptional programs could interfere with steroidogenesis, which would indirectly affect spermatogenic maturation and the assembly of sperm ion channels. Moreover, AhR modulation of BTB integrity and/or Sertoli cell support could compromise the post-meiotic maturation environment required for optimal expression and incorporation of CatSper into sperm membranes, Finally, AhR-regulated metabolites (e.g., tryptophan-derived ligands) and downstream enzymes, could also affect the lipid microenvironment of the sperm flagellum, thereby changing CatSper channel function (Bustani *et al.,* 2025). While direct transcriptional regulation of CatSper2 by AhR has not been investigated yet, taken together, these converging pathways provide a reasonable crosstalk network linking environmental/metabolic sensing to sperm $Ca^{+2}$ handling (Wang *et al.,* 2024).

From a therapeutic perspective, targeting the AhR-CatSper2 axis presents two complementary strategies to ameliorate testicular dysfunction due to HFD exposure. On the AhR side, small molecule antagonists and naturally occurring modulators (e.g., resveratrol and other polyphenols) show promising effect for decreasing AhR-mediated oxidative stress and restoring testicular function in preclinical models. On the CatSper side, interventions that re-establish testicular oxidative balance, membrane lipid composition, and/or intracellular signaling (e.g., antioxidant supplementation, metabolic modulators, or lifestyle approaches such as exercise/intermittent fasting) can indirectly support the preservation of *CatSper2* expression and functional activity, thus improving sperm motility even in the context of metabolic challenge. A recent study in rodents has indicated that correcting metabolic and inflammatory derangements in HFD animals can lead to restoration of seminal measures of spermatogenesis and sperm functional measures, which extends the translational potential of modulating this axis (Hafez *et al.,* 2025).

Thus, there is a pressing need to explore whether a functional link between AhR and CatSper2 operates within the testis and serves as a key pathway through which obesity-related dietary stress impairs male reproductive capacity. The present study was designed to investigate the existence and biological relevance of AhR–CatSper2 interaction in testicular damage provoked by a high-fat regimen. Attempts were also made to assess how altering AhR activity, either by resveratrol or by pharmacological blockade, affects *CatSper2* expression, seminal characteristics, circulating testosterone, and tissue architecture of the testes. It was hypothesized that AhR functions as a protective modulator that safeguards *CatSper2* expression and preserves sperm health under metabolic challenge, whereas disrupting AhR signaling would aggravate reproductive disturbances.

## MATERIALS AND METHODS

**Experimental animals:** Forty clinically healthy, male albino rats, aged 8-10 weeks and weighing160-200g, were used in this study, which lasted from November 2025 to January 2026. Experimental rats were kept under managed conditions with 12/12 hours of light and dark cycle. All animals were housed in plastic cages in a well-ventilated room (22-25°C), at the Facility of Animal Housing, College of Veterinary Medicine, University of Baghdad, Iraq. Rats had free access to a regular chow diet and clean water.

**Preparation of the High Fat Diet**: Composition of High Fat Diet used in the study is shown in Table 1. It was prepared weekly and stored in a clean, sealed, and opaque plastic containers, at 4°C until used as pellets (Rayshan *et al.,* 2023).

**Preparation of treatments:** The 100mg/kg body weight Resveratrol solution was prepared, as described previously

(Bordbar *et al.,* 2023). A solution sufficient for 10 animals containing 200mg of RES was dissolved in a solution of 2mL DMSO and 8mL distilled water. Vortex mixer was used for meticulously mixing the prepared mixture to ensure homogeneity. The solution of CH-223191 at a dose of 10mg/kg body weight was prepared according to the methodology outlined earlier (Cao *et al.,* 2022). This process entailed dissolving 20mg of CH-223191, adequate for 10 animals, in a 2mL of DMSO mixture. The solution was mixed properly for homogeneity by a vortex mixer.

**Study design:** The rats were assigned randomly into four equal groups ($n$=10 per group): (a) control, (b) HFD, (c) HFD-RES and (d) HFD-AhR$^-$. Rats of the control group were fed with a regular chow diet, and given Dimethyl sulfoxide (DMSO) intraperitoneally at 1µL/g BW, an organic solvent used for CH223191 preparation; HFD group was fed with HFD (Table 1); HFD-RES group was given HFD, with RES was orally administrated at a dose of 100mg/kg BW by gavage needle daily for 8 weeks for induction of obesity. Finally, HFD-AhR$^-$ group was fed with HFD, and CH223191, an AhR antagonist, was intraperitoneally administrated simultaneously at a dose of 10mg/kg every 72h. The experimental period was 8 weeks (Bustani *et al.,* 2024).

**Table 1:** Composition of High Fat Diet (HFD) fed to experimental rats of relevant group

| Ingredients | Quantity ((g/kg diet) |
|---|---|
| Starch | 150 |
| Casein | 200 |
| Tallow fat | 400 |
| Sucrose | 40 |
| Cellulose | 50 |
| Vitamin mix | 10 |
| Methionine | 3 |

**Epididymal sperm preparation:** At the end of study, rats were anesthetized by intramuscular administration of Ketamine (60mg/kg) and Xylazine (40mg/kg), the cauda epididymis of each rat was dissected and placed in 1.0mL of pre-warmed Hams F12 culture medium (5% $CO_2$, 37°C). Then, gentle tearing of the tissue was performed to drive the spermatozoa move out into the culture medium, and dishes with culture medium were incubated for 15min at 37°C. In order to assess sperm progressive motility (%), a light microscope (400x) was used to examine 10µL sperm suspension mounted onto a pre-warmed dry slide (Al-Amery *et al.,* 2022). Sperm acrosome integrity and dead sperms was examined through sperm smears stained with Eosin-Nigrosine stain, followed by evaluation under a phase contrast microscope using 1000x magnification (Kaka *et al.,* 2015). A meticulous analysis was conducted on at least 200 spermatozoa to calculate the dead sperm percentage and those with either detached or intact acrosomes.

**Sperm DNA-fragmentation test:** The process of sperm fixation consisted of mounting a small drop of the sperm sample on a clean slide. Following air-dried, slides were immersed for 5min in a 3:1 solution of methanol and glacial acetic acid. Following fixation, slides were left to dry completely. Subsequently, slides were placed in a trough containing 0.01% solution of acridine orange stain. After staining for 2min, slides were washed with distilled water, air-dried and examined under a fluorescent microscope (Tejada *et al.,* 1984; Da Costa *et al.,* 2021). Green fluorescence indicated intact sperm DNA. However, red fluorescence was indicative of damaged DNA. Therefore, different colorations were considered as qualitative marker for integrity of condensation and chromatin condition (Bustani and Alghetaa, 2025).

**Testosterone hormone concentration:** The rat serum testosterone hormone (T) was measured in rat blood samples collected from the heart at the end of trail after anesthetizing the rats by intramuscular injection of Ketamine 60mg/kg and Xylazine 40mg/kg. Following collection, blood samples were processed to obtain serum, and then analysis was conducted using testosterone Enzyme-Linked Immunosorbent Assay ELISA kit (Cat No: ER1462; Fine test®/ Wuhan fine Biotech. China). The assay was conducted according to the guidelines given with the kit (Koohpeyma *et al.,* 2022), and had sensitivity of 0.188ng/mL, intra-assay CV of <8%, and inter-assay CV of <10%).

**Testicular *AhR* and *CatSper2* gene expression:** Testicular tissues were collected from rats at the termination of study after intramuscular injection of Ketamine 60mg/kg and Xylazine 40mg/kg. After excision of testes, specimens were placed in Trizol to estimate gene expression analysis. Other tissues of testes were fixed in formalin 10% for histopathological study.

Following the excision, testicular tissue was preserved in a deep freezer at -80°C, which facilitated the RNA extraction process utilizing the Triazole reagent (Trans® Chinese; Cat No: ER501-01). Then the complementary DNA (cDNA) synthesis was executed utilizing the extracted RNA as a template. The RNA underwent reverse transcription into cDNA through the application of Premix Kit of a one-step RT-PCR (Promega's/USA) during the reverse transcription reaction. The resultant cDNA was subsequently employed for the analysis of quantitative PCR (qPCR) gene expression.

The analysis of gene expression was performed using custom-designed primers sourced from the GeneBank database and produced by Integrated DNA Technologies, Inc. The subsequent primers (for *Rattus norvegicus*) were employed for the amplification of designated target and housekeeping genes, as delineated in Table 2. SaCycler-96 Real Time PCR SYSTEM and the SYBR Green master mix were employed to perform Rt-qPCR using the parameters listed in Table 3.

**Table 2:** Sequence of primers used for gene expression analysis of testicular *AhR*, CatSper2 and *GAPDH*

| Gene ID | Forward sequence 5"-->3" | Reverse sequence 5"-->3" | NCBI reference sequence | product size |
|---|---|---|---|---|
| *AhR* | GTCAGCCATGGTCAGTCCTCAG | GCTCGGACTCTGAAACTTGCTT | NM_013149.3 | 154 |
| *CatSper2* | GAAGTCAGGGCTGAGTGGG | TGGACAAAGCAACAGCCTGA | NM_001012220.1 | 173 |
| *GAPDH* | AGAGACAGCCGCATCTTCTT | ATGAAGGGGTCGTTGATGGC | NM_017008.4 | 157 |

**Table 3:** Rt-qPCR setting for cDNA synthesis and amplification of targeted genes, *AhR*, CatSper2 and housekeeping gene *GAPDH*

| | Phase | Condition | Time | Cycler |
|---|---|---|---|---|
| Holding (cDNA synthesis) | Holding stage 1 | 37C° | 15min | 1 |
| | Holding stage 2 | 95C° | 10min | 1 |
| PCR cycle | Denaturation | 95C° | 15s | |
| | Annealing | According to the primer | 1min | |
| | Extension | 72C° | 30s | 40 |
| Melt curve stage | 1st | 95C° | 15s | |
| | 2nd | 60C° | 1min | |
| | 3rd | 95C° | 30s | |

**Evaluation of testicular histopathology:** After excision of testes, specimens of testes were fixed in 10% formalin for histopathological studies. After 3 days of fixation, testes dehydration was performed by alcohol, and clearing by xylene. Tissues were then immersed in paraffin and cut into a thickness of 5 microns. Following hematoxylin and eosin staining, the sections were examined under 400× magnification using phase contrast microscope (Olympus) for histopathological examination (Mohammadi *et al.,* 2018).

**Statistical analysis:** Obtained data were subjected to one-way ANOVA using Graph Pad Prism software version 9.1.0 (San Diego, CA, United States). When ANOVA showed significant effects, the data were subjected to Tukey's test *of post hoc* testing. Data are presented as mean ± standard error (SE), and number of animals expressed as (n). P value less than 0.05 (P<0.05) was regarded as statistically significant.

## RESULTS

***AhR* and *CatSper2* genes:** The results in Figure 1A show that the values of fold change represented the normalized fold-change in AhR receptor gene expression among different study groups fed a high fat diet (HFD, RES, or AhR⁻) compared to the normal diet group. *AhR* gene expression in all HFD groups showed significant increase (P<0.05) compared to normal diet animals. However, administration of CH223191 resulted in significant (P<0.05) downregulation of expression of *AhR* gene, compared to RES group.

The analysis of fold change gene expression of *CatSper2* (Fig. 2B) revealed that diet rich in fat caused significant (P<0.05) reduction in *CatSper2* gene expression in all obese study groups compared to lean group despite modulation of AhR pathway. In HFD-RES group, a significant decrease (P<0.05) was noted with a fold change (0.49±0.09) compared to control group (1.00±0.01). Moreover, HFD-AhR⁻ animals exhibited *CatSper2* expression significantly decreased, with an approximately 0.26±0.12 fold compared to control group.

Sperm quality parameters: Obese group (HFD) showed a slight but non-significant decrease in sperm progressive motility compared to the lean (control) group (Fig. 2A). Notably, resveratrol group (HFD-RES) showed a significantly increased (P<0.05) sperm progressive motility in comparison to AhR⁻ and HFD groups. Conversely, AhR antagonist group (HFD-AhR⁻) exhibited the lowest sperm progressive motility compared to control, HFD and HFD-RES groups (P<0.05; Fig. 2A).

The high-fat diet group showed a slightly higher but non-significant percentage of dead sperm compared to control group (Fig. 2B). Notably, the HFD-RES group showed an improvement in live sperm. Although the percentage of dead sperm in HRD-RES group differed non-significantly from the control rats, it was significantly lower (P<0.05) than the HFD and HFD-AhR⁻ animals. Notably, the HFD-AhR⁻ group exhibited a significant (P<0.05) reduction of sperm viability in comparison to other groups (Fig. 2B).

The results regarding the abnormal sperm acrosome (Fig. 2C) showed that the HFD group had a slightly but non-significantly increase in abnormal sperm acrosomes in comparison with the control group. However, the HFD-RES group revealed a slight but non-significant reduction in abnormal sperm acrosomes when it was compared with the control rats. In contrast, the HFD-AhR⁻ group showed a significant (P<0.05) increase in abnormal sperm acrosomes in comparison to the other three groups. Photographical results regarding sperm DNA fragmentation in rats of four experimental groups are shown in Figure 3A. Statistical analysis of data revealed that groups fed diet rich in fat exhibited a significant (P<0.05) increase in sperm DNA fragmentation than the control rats (Fig. 3B). Although, the HFD-RES group showed significantly (P<0.05) higher sperm DNA fragmentation than the control group, however, it was significantly (P<0.05) decreased in comparison to HFD and HFD-AhR⁻ groups. Conversely, the HFD-AhR⁻ rats showed significant (P<0.05) increase of fragmentation in sperm DNA compared to other study animals (Fig. 3B).

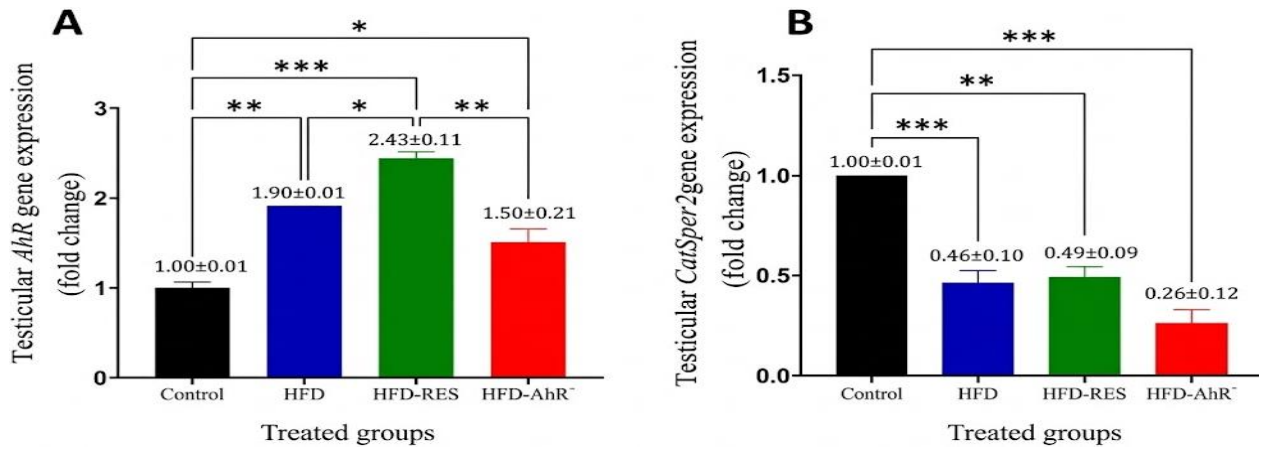



**Fig. 1:** Effect of HFD and Resveratrol on testicular gene expression; A): *AhR* gene expression. B): *CatSper2* gene expression. Stars denote significant difference (P<0.05). The values are presented as mean ± SEM (n=10).

**Serum testosterone concentrations:** Results of serum testosterone concentrations showed that the control group had the highest average testosterone concentration of 3.20±0.25ng/ml (Fig. 4), which served as baseline for comparison. The HFD and HFD-AhR⁻ groups, on the other hand, exhibited significant (P<0.05) reduction in the concentration of serum testosterone in comparison to control rats. The HFD-RES treated group also showed significantly decreased serum testosterone concentration when it was compared with the testosterone concentration of the control animals as well. However, HFD-AhR⁻ group showed the lowest (P<0.05) concentration of serum testosterone compared to other three groups.

**Histopathological examination of the testes:** Histopathologic analysis of the testicular tissue exhibited remarkable differences between experimental groups, with the possible ameliorative effects of resveratrol (RES) on spermatogenesis. The sections obtained from the control subjects revealed normal histology and architecture of seminiferous tubules and intact germinal cells at all development stages (Fig. 5A). The Sertoli cells were evident in association with the germ cells development, and Leydig cells were present in the interstitial spaces (Fig. 5A).

Conversely, the HFD group showed remarkable testicular impairment. Seminiferous tubules exhibited severe damage of the germinal cell population, including necrotic cell remnant (Fig. 5B). The densification of germ cells density was severely diminished along with damage to tubular wall. Of note, Sertoli cells were not readily apparent within the affected tubules, suggesting impaired support of germ cell development (Fig. 5B).

The RES group also showed evidence of normal testicular structure, similar to the control group, with normal and complete developmental germ cell populations within the seminiferous tubules and maintained Sertoli cells (Fig. 5C). Interestingly, the HFD-AhR- group exhibited extensive disruption throughout the seminiferous tubules similar to the HFD group. Seminiferous tubules from the HFD-AhR⁻ group showed a remarkable decline in germinal cell populations, and evidence of apoptosis was also clear with clear basophilic apoptotic bodies, especially in spermatogonia and spermatocytes. Sertoli cells were absent in the seminiferous tubule that exhibited such phenotype (Fig. 5D).

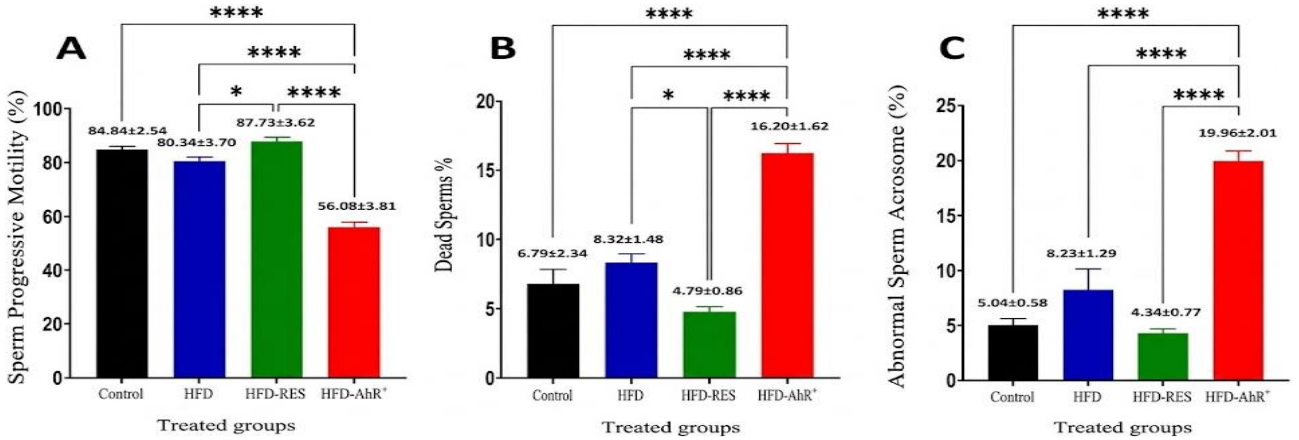



**Fig. 2:** Effect of HFD and Resveratrol on sperm parameters; A): Sperm progressive motility. B): Dead sperm. C): Abnormal sperm acrosome. Stars denote significant difference (P<0.05). The values are presented as mean ± SEM (n=10).

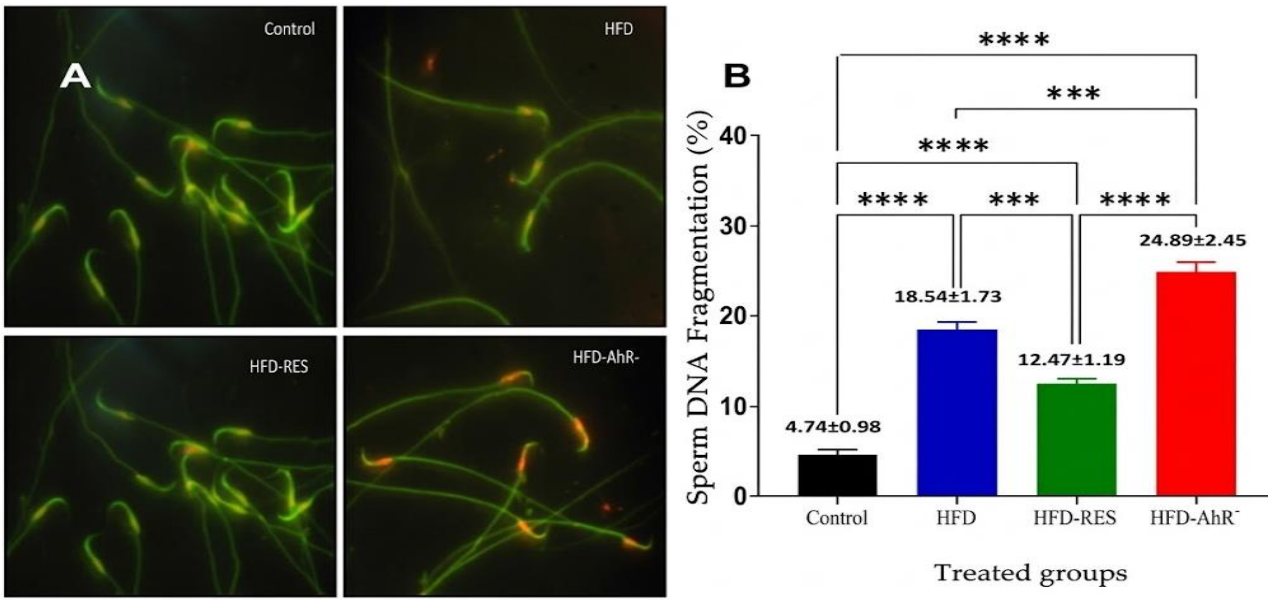



**Fig. 3:** Effect of HFD and Resveratrol on spermatozoa DNA integrity (%); A): Acridine orange-stained picture for each group. B): Sperm DNA fragmentation. Stars denote significant difference (P<0.05). The values are presented as means ± SEM (n=10).

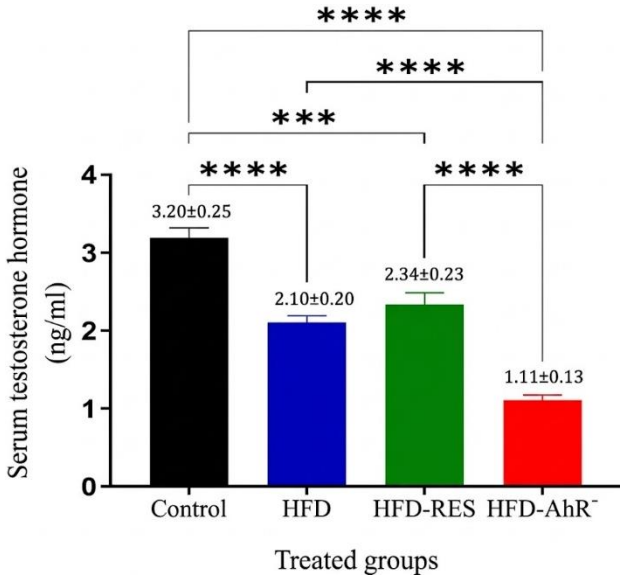



**Fig. 4:** Effect of HFD and Resveratrol on serum testosterone concentrations. Stars denote significant difference (P<0.05). The values are presented as means ± SEM (n=10).

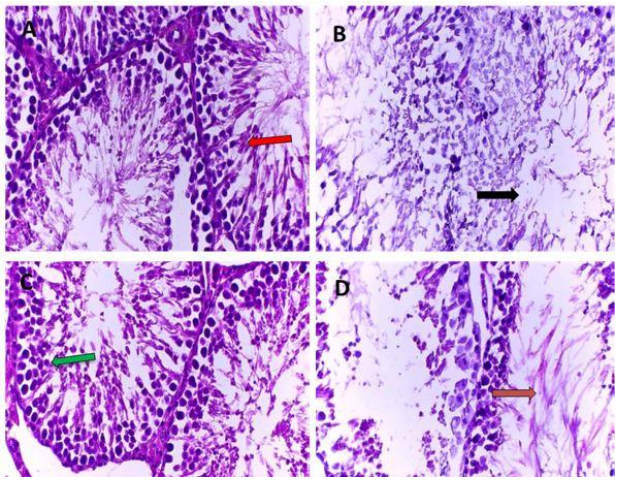



**Fig. 5:** Histopathological examination of testicular tissue. A): Control; red arrow shows intact germinal cells at all development stages. B): HFD group; black arrow shows severe damage of the germinal cell population. C): HFD+RES group; green arrow shows intact germinal cells. D): HFD-AhR- group; orange arrow shows severe damage of the germinal cell population (Hematoxylin & Eosin stain, 400X).

## DISCUSSION

High fat diet resulted in a significance increase in *AhR* mRNA, which may suggest that high fat dietary patterns stimulate AhR-dependent signaling pathways in the testis (Moyer *et al.,* 2016). Previous assessments of obesity in animal studies have shown similar observations of HFD feeding, which included induction of *AhR* expression in the liver and testis, and stimulating inflammatory signaling pathways (Danfeng *et al.,* 2023). Aryl hydrocarbon receptor induction is likely stimulated through elevated levels of endogenic AhR ligands formed through lipid peroxidation and metabolism by gut microbiota (Kang *et al.,* 2022).

Interestingly, resveratrol supplementation (HFD-RES) resulted in a higher expression level of AhR compared to the control and HFD groups. Resveratrol has been shown to make a complex with AhR as a selective modulator of AhR depending on dose, concentration and tissue (Szaefer *et al.,* 2024). The antioxidant (Zhang *et al.,* 2014) and anti-inflammatory properties could potentially promote AhR transcription indirectly through a compensatory feedback mechanism to reinstate redox balance in the cellular system (Lee *et al.,* 2024). These results show that resveratrol positively regulates the anti-inflammatory activity linked to *AhR* expression, while potentially downregulating its downstream inflammatory activity; this finding is in line with suggested function of resveratrol as a modulator rather than solely as an AhR antagonist (Busbee *et al.,* 2013). In contrast, the HFD-AhR- cohort revealed a statistically significant decrease in *AhR* expression, consistent with pharmacologic inhibition of AhR. However, the reduction of AhR did not reach the level of expression noted in the control rats.

On the other hand, HFD group demonstrated ~54% lower *CatSper2* expression than control, which agrees with the previous findings indicating that HFD and obesity negatively affect male reproductive parameters including testis size, sperm motility and fertility rates (Choudhuri *et al.,* 2023). A meta-analysis, for example, provides strong evidence from animal models that HFD reduces sperm quality, mating success, and fertilization success (Crean and Senior, 2019). From a mechanistic standpoint, HFD is linked with the inflammation and oxidative stress in the testis, perturbed hormone milieu (e.g., decreased testosterone) and epigenetic changes in the germ cell population (Vega *et al.,* 2012). Within this context, downregulation of *CatSper2* may indicate either direct transcriptional repression, damage to the germ cell population that normally expresses it (i.e., mature spermatids/spermatozoa), or disruption of cellular milieu that support its expression (Shi *et al.,* 2025).

In the present study, *CatSper2* expression was lower in the HFD-RES group than control, while it was slightly higher than the HFD group, suggesting resveratrol supplementation may have partially reduced HFD-mediated suppression of *CatSper2*. Resveratrol has been shown to protect spermatogenesis, sperm motility, and testicular architecture after stress. According to Bustani and Alghetaa (2025), resveratrol improves sperm viability, survival, and motility while decreasing fragmentation of DNA, preserving architecture of testicles, and enhancing expression of the *AhR* gene in a rat model. Even though it does not indicate a full recovery to control levels, the improvement suggests that resveratrol could support the preservation of *CatSper2* expression level during metabolic stress conditions, likely through its ability to inhibit oxidative damage, enhance the Sertoli/germ cell environment, or influence epigenetic or transcriptional regulation (Maroto *et al.,* 2025).

The most impressive finding, and perhaps mechanistically informative, is the one group treated with HFD and an AhR antagonist showed the lowest expression of *CatSper2,* which is a far greater downward shift than HFD alone. This would indicate that an AhR antagonist severely inhibits *CatSper2* when treated under HFD. A previous study has shown that AhR is increasingly thought of as a key player in male reproductive physiology (Bustani and Alghetaa, 2025). Both endogenous (dietary or microbiome-derived) and exogenous ligands stimulate AhR in the testes. However, knocking out or excessive

AhR activation disrupts spermatogenesis, alters gene expression of germ cells, and affects fertility (Bustani *et al.,* 2025). In particular, AhR antagonism (or knockout) leads to deteriorated sperm parameters and testicular histology in animal models (Bustani and Alghetaa, 2025).

Sperm progressive motility is an important indicator of male fertility since it determines the ability of spermatozoa to penetrate and fertilize the oocyte. As shown in the present study, the control group had a very high sperm progressive motility characterized by viable specimens of spermatozoa that exhibited normal physiological activity in healthy rats. The group fed HFD showed a slight but non-significant reduction in sperm progressive motility. This slight but non-significant reduction (probably due to sample size) in sperm motility could be a result of metabolic malfunctions related to HFD-induced obesity, including increased levels of oxidative stress, lipid peroxidation, and reversible mitochondrial dysfunction in sperm cells, as has been reported earlier (Han *et al.,* 2023). Excess lipid deposition in the testes can affect sperm energy metabolism and axonemal structure, in addition to hindering the flagellar propulsion due to reactive oxygen species (ROS)-induced damage (Moreira *et al.,* 2025).

Notably, resveratrol administration in HFD-RES group significantly enhanced sperm motility compared to the HFD and $AhR^-$ animals. Resveratrol likely improved sperm motility by virtue of its anti-oxidative, anti-inflammatory, and mitochondrial protective properties, improving sperm bioenergetics and motility (Elewa *et al.,* 2025). Resveratrol activates the SIRT1/PGC-1α pathway to regulate anti-oxidant enzymes, like superoxide dismutase and catalase, to restore ATP synthesis in sperm mitochondria (Liu *et al.,* 2021). Additionally, lipid-regulating properties of resveratrol could ameliorate testicular oxidative damage that had negative influences on sperm function induced by HFD metabolic dysfunction.

However, there was a significant reduction in sperm progressive motility in the AhR antagonist–treated group compared to other three groups. This interrelation suggests that AhR signaling is fundamentally important for sustaining sperm motility and energy homeostasis. Aryl hydrocarbon receptor is responsible for regulating redox-sensitive genes and steroidogenic enzymes, as well as detoxification pathways that are required for functional testicular and sperm health (Esser and Rannug, 2015). Aryl hydrocarbon receptor inhibition likely alters expression of calcium and CatSper2 channel proteins that are essential for sperm hyperactivation and motility (Zhang *et al.,* 2020).

The percentage of dead spermatozoa is an effective indicator of viability of spermatozoa and general semen quality, as it takes into consideration both structural integrities, as well as combined metabolic and oxidative stress-related injury (de Oliveira *et al.,* 2019). In the current study, the dead sperm percentage in control rats was within normal range under basal conditions. The HFD animals revealed a slight increase in the percentage of dead spermatozoa; however, this difference was not statistically significant compared to the control rats. However, previous studies have indicated that an obesogenic diet promotes the production of reactive oxygen species and peroxidation of lipids in the testes, which decreases sperm viability (de Oliveira *et al.,* 2019).

Importantly, the HFD-RES group exhibited an improved sperm viability. Although this did not reach statistical significance in comparison to the control group, it was significantly better compared to the HFD and specifically the HFD-$AhR^-$ groups. These data support the idea that resveratrol exhibits cytoprotective properties on sperm viability in an adverse metabolic stressor environment. According to de Oliveira *et al.,* (2019), resveratrol (30mg/kg) improved sperm viability and motility in animals fed a high-fat diet for two months. Furthermore, acknowledged antioxidant effects of resveratrol also reduce testicular oxidative stress which helps improve sperm cell viability (de Oliveira *et al.,* 2019).

On the other hand, the HFD-$AhR^-$ group had the highest percentage of dead sperm. This supports emerging findings identifying AhR signaling as an integral part of male reproductive health, including sperm viability. Recently, Bustani and Alghetaa (2025) reported a substantial decrease in survival and viability of sperm in rats, with remarkable increases in oxidative stress when AhR was inhibited via CH223191 (Bustani and Alghetaa, 2025). This suggests that disrupting AhR function under obesity-prone conditions may increase sperm cell death, potentially through redox balance and apoptosis related signaling pathways.

The integrity and physiological functional activity of the sperm acrosome are necessary for an effective fertilization process, as the acrosome contains many hydrolytic enzymes. In the present study, control rats had an overall low rate of sperm with abnormal acrosomes, suggesting a stabilizing effect of control diet on the acrosomal membrane and sperm function. In the rats fed HFD, there was a slight but non-significant increase of sperm with abnormal acrosomes compared to control and HFD-RES groups, suggesting that the dysregulation of lipids and the resultant oxidative stress associated with high fat intake could lead to loss of acrosomal membrane stability. According to Chen *et al.* (2025a), obesity and consumption of HFD are also associated with lipid peroxidation and reactive oxygen species levels increase, which can disrupt sperm membrane stability and impair the acrosome.

Remarkably, the administration of resveratrol improved these indices, demonstrated by a decrease in the percentage of abnormal acrosomes, although it was statistically non-significant, however, the reduction was towards control levels. Previous studies have shown that resveratrol improves redox homeostasis in the testes via the upregulation of anti-oxidant enzymes including SOD and GPx, and by mitigating peroxidative damage to sperm membrane lipids and acrosome vesicles (Zhang *et al.,* 2024a; Elewa *et al.,* 2025). In addition, resveratrol activates the SIRT1 and PGC-1α signaling pathways responsible for both mitochondrial biogenesis and cellular energy balance to enhance the process of spermatogenesis and improve sperm quality during metabolic stress (Liu *et al.,* 2021).

Conversely, the $AhR^-$ animals exhibited a significant increase in sperm with abnormal acrosomes compared to other three groups, suggesting that disrupting the Aryl hydrocarbon receptor pathway has a dramatic negative effect on acrosome integrity. The AhR has an intrinsic regulatory role in spermatogenesis and sperm maturation by modulating oxidative stress responses, steroidogenesis, and detoxification pathways (Esser and Rannug, 2015).

The increase in damaged acrosomes in the AhR$^-$ group may indicate a deletion of AhR-mediated antioxidant defenses and modification of sperm membrane protein expression (Bustani and Alghetaa, 2025). Recent studies indicate that depletion of AhR has been associated with increased oxidative load in the testis and disrupted Sertoli-germ cell interactions, which resulted in premature breakdown of acrosomal vesicles and defects in sperm function (Zhang *et al.,* 2024b).

Results of the sperm DNA fragmentation assay indicated that HFD, resveratrol supplementation, and AhR deficiency affected sperm genomic integrity. The control group showed the lowest level of DNA fragmentation compared to other three groups, which is consistent with previous findings in healthy male rodents (Merino *et al.,* 2018), who stated that sperm DNA fragmentation increased in rats fed HFD but not with control diet. However, HFD group showed a significant increase in DNA fragmentation compared to control. These findings are consistent with prior data demonstrating HFD exposure induces oxidative stress, lipid peroxidation, and apoptosis of germ cells that results in sperm DNA integrity impairment (Ahmad and Haque, 2022).

Resveratrol supplementation in the HFD-RES group interestingly reduced DNA fragmentation compared to HFD group, suggesting a degree of protection possibly related to antioxidant function and suppressed apoptosis (Rivera *et al.,* 2019; Kumar *et al.,* 2024), as indicated by the improvement in sperm quality and DNA fragmentation in a model of oxidative stress (Malak *et al.,* 2026). The DNA fragmentation levels in the HFD-RES group were still higher than the control group, however, it demonstrated a reduction when compared to group fed HFD without resveratrol, indicating some therapeutic potential of resveratrol to decrease reproductive toxicity induced by diet rich in fat.

Conversely, there was a significant increase in sperm DNA fragmentation following HFD containing treatments and the highest level of DNA damage was found in the HFD-AhR$^-$ group, indicating that lack of AhR is deleterious to sperm DNA damage caused in response to HFD. The AhR receptor has a crucial effect in modulating oxidative stress response and maintenance of testicular homeostasis (Bustani and Alghetaa, 2025). The significant increase in sperm DNA damage in the absence of the AhR signaling suggests that AhR is protective against HFD-induced genotoxic stress in sperm cells, thereby providing evidence for the importance of AhR signaling in mediating detrimental effects on male fertility in response to HFD (Safe *et al.,* 2020).

In the current study, HFD group had significantly lower serum testosterone levels than control rats. Previous literature supports a correlation between high fat diets and Leydig cell dysfunction, leading to impaired steroidogenesis (Chen *et al.,* 2025b). This decrease in serum testosterone in HFD group could be due to increased oxidative stress and inflammation from HFD exposure which negatively impacts the hypothalamic–pituitary–gonadal axis (Ahmad and Haque, 2022).

Interestingly, resveratrol treatment in the HFD-RES group had a partial effect on testosterone restoration, although this was still significantly lower than that of control, which is consistent with the literature showing that resveratrol can increase testosterone levels by mitigating oxidative stress impact on Leydig cells and manipulating steroidogenic enzymes. The small amount of testosterone increase compared to HFD group suggests that while resveratrol has a protective effect, it was not able to fully restore testosterone levels compromised due to the HFD (Rivera *et al.,* 2019).

On the other hand, the HFD-AhR$^-$ group had the lowest serum testosterone concentration compared to control, HFD and HFD-RES groups, reflecting that reduced AhR leads to further suppression of testosterone caused by HFD (Wada *et al.,* 2016). Aryl hydrocarbon receptor signaling has been suggested important for maintaining testicular function and regulating steroidogenesis, and its deficiency may impair Leydig cell function and increase susceptibility to metabolic and oxidative stress. Thus, our findings suggest that both environmental dietary exposure and AhR signaling may be important for testosterone homeostasis (Yasunaga *et al.,* 2013).

**Conclusions:** The results of the present study revealed that AhR-CatSper2 crosstalk offers critical protection against HFD-induced testicular dysfunction. Resveratrol supplement can ameliorate adverse effects of HFD on reproductive parameters through AhR upregulation and CatSper2 restoration, while AhR antagonism severely compromised sperm quality and testicular integrity. The bidirectional modulation of this axis reveals AhR as a protective rheostat rather than merely a xenobiotic sensor, with *CatSper2* expression strongly linked to AhR signaling integrity. These findings establish AhR-CatSper2 signaling as a promising therapeutic goal for managing obesity-related infertility in the male.

**Authors contribution:** MAK and AAA conceived the research idea, and both individuals made equal contributions to develop the concept and monitored the proposal developments. YMA and DRA performed the experiment, processed and analyzed the sera and tissue samples. All authors interpreted the data, reviewed the manuscript for important intellectual contents and approved the final version.

**Ethics statement:** The experimental procedures conducted in this study were reviewed and approved by the Committee of Animal Care and Use at the University of Baghdad, College of Veterinary Medicine, Baghdad, Iraq (code ethics: P.G. 887, 2-5-2023).

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**Data availability:** All data related to this study is reported and archived in the Laboratory of Physiology, Biochemistry and Pharmacology in the College of Veterinary Medicine, University of Baghdad, Iraq.

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