



Aryl Hydrocarbon Receptor Modulation and Resveratrol Influence on *Dnah1* Expression: Implications for Male Reproductive Health

Ghadeer S Bustani^{*1,2} , Nabil Al-Humadi³ , Hasan FK Alghetaa¹

¹Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, ²The Islamic University in Najaf, Najaf, Iraq, ³Office of Vaccine and Review, CBER, Food and Drug Administration, 10904 New Hampshire Avenue, Silver Spring, MD 20903 USA

A B S T R A C T

Male reproductive health is intricately regulated by molecular and physiological processes, with the aryl hydrocarbon receptor (AhR) playing a crucial role by being activated by various ligands and influencing the onset and progression of diseases. The present study aimed to evaluate the role of AhR on spermatogenesis in adult male rats that were affected by resveratrol (RES) as agonist and CH223191, as an AhR antagonist. The study included forty rats which were randomly divided into four equal groups: The control group, DMSO group (positive control), RES group, and AhR⁻ group. The rats were received respective treatments intraperitoneally twice weekly for 60 days. Parameters related to male reproductive health were evaluated. The results showed that activation by the RES treatment enhanced the sperm morphology, sperm acrosomal integrity, and luteinizing hormone. The testicular tissue integrity was maintained by RES. Moreover, the *Dnah1* gene expression were upregulated and played a major role in sperm morphology, it was upregulated and testosterone levels were elevated by RES. On the other hand, sperm morphology, sperm acrosomal integrity, testosterone hormone levels, and *Dnah1* expression were reduced in AhR⁻ rats group. The testicular tissue integrity was maintained by RES. These findings highlight the role of AhR in male reproductive health and suggest RES as a potential therapeutic agent.

Keywords: Aryl hydrocarbon receptor, Resveratrol, CH223191, Spermatogenesis, Sperm Morphology, Acrosomal Integrity, Testosterone, *Dnah1* gene expression

*Correspondence:

kashifalkitaa@covm.uobaghdad.edu.iq

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INTRODUCTION

Male reproductive health is influenced by intricate molecular and physiological processes (1). The aryl hydrocarbon receptor (AhR) as a transcription factor activated by ligand-binding chemical compounds is linked to the onset and progression of several diseases, including autoimmune diseases, cancers, metabolic syndromes, and allergies. In general, AhR responds to and interacts with environmental toxins/ligands, dietary ligands, and allergens to regulate toxic, biological, and cellular responses. Furthermore, more recently, scientists have discovered that the AhR may also play a vital role in

regulating reproduction and immunity (2). Human AhR cDNA was identified in 1993. It was expressed at its highest levels in the placenta, lungs, and heart. Studies illustrated that the AhR Knockout Mouse showed developmental abnormalities that highlight the roles of the receptor in female fertility (3), perinatal growth, and increased susceptibility to colitis (4,5). Based on previous studies, it appears that the AhR plays a role in regulating the S-phase of the cell cycle. Furthermore, the G1-to-S phase transition and the presence or absence of its ligands, as well as the metabolic activity of cytochrome P4501A1 (CYP1A1), are affected by AhR activity, which in turn controls cell cycle progression. The adjustment of cell cycle progression is

aided by the manipulation of AhR activity and its subsequent effects on cell cycle regulators. Additionally, the G1-to-S phase transition and the presence or absence of its ligands, as well as the metabolic activity of CYP1A1, affect AhR activity, which in turn controls cell cycle progression. The cell cycle progression is fine-tuned by AhR activity modulation and its downstream effects on cell cycle regulators (6). The AhR is shown to regulate the xenobiotic-metabolizing enzymes through cytochrome P450 and other enzymes (6–8). Moreover, the major role of AhR in the different phases of mitosis is not studied and the information available is limited. However, its specific involvement in different phases of mitosis remains understudied. In prophase, the chromosomes condense and become visible, the nuclear envelope breaks down, and the mitotic spindle begins to form. The AhR plays a crucial role in regulating chromatin condensation during prophase, at the first stage of mitosis “Chromatin condensation involves the compaction of DNA into a more organized and compact structure, enabling proper chromosome alignment and segregation in subsequent stages” (9). Thus, the AhR activation promotes chromatin condensation by enhancing the expression of histone deacetylases. Histone deacetylases enzymes (which remove acetyl groups from histones) facilitate chromatin compaction and allowing the histones to wrap the DNA more tightly (10–12). Resveratrol (3,5,4'-trihydroxystilbene; RES) is a non-flavonoid polyphenol organic compound, it is found naturally in various foods such as grapes, peanuts, and blueberries (13). The RES is widely known for its renowned beneficial biological effects which involve multiple molecular targets including oxidative stress and the AhR signal pathway (14,15). Moreover, studies found that the antagonist of AhR and one of the RES mechanisms inhibit AhR expression. The RES also inhibits the activation of CYP1A1 and CYP1B1, which have been shown to be associated with reactive oxygen species (ROS) production (16). The protective effects of RES on the generation of ROS are especially significant because polyunsaturated fatty acid peroxidation results in a decrease in the fluidity of the membrane and in the functioning of ion channels and membrane enzymes, which impair sperm motility (1,17).

This study aimed to evaluate the effect of resveratrol (RES) as agonist and CH223191 as antagonist on AhR and AhR in adult male rats by assessing sperm integrity, hormone levels, and *Dnah1* gene expression related to male reproductive health following treatment with RES and CH223191. Additionally, the study has examined the histopathological features of testicular tissue to evaluate the effects of these interventions on testicular parenchyma.

MATERIALS AND METHODS

Study Design

The study has aimed to investigate how RES and CH223191 (Hebei Guanlang Biotechnol, China) impact AhR

and spermatogenesis in adult male rats. To this end, four groups from forty rats were randomly assigned: Naive group (n=10) served as control; RES group (n=10) received 100 mg/kg of resveratrol intraperitoneally twice weekly [18]; AhR⁻ group (n=10) received 10mg/kg of CH223191 intraperitoneally twice weekly [19] (n=10), received twice weekly of i.p injection of dimethyl sulfoxide which was used to dissolve and in preparation of RES as well as CH223191 treatments. All experimental animals were euthanized 60 days post treatment, the endpoint of the experiments, and their testes, blood, and epididymis were taken for further downstream analysis.

Treatment Preparation

Resveratrol working solution was freshly prepared at 100 mg/kg body weight, following the protocol described by Bordbar et al. [18]. This involved dissolving 400 mg of Resveratrol, suitable for 20 animals, in a mixture of 2 ml dimethyl sulfoxide (DMSO) and additional 2 ml dH₂O were added. The solution was carefully mixed for homogeneity using a vortex, and each rat received a dose based on its weight (1 µL/1g BW) of the resultant solution. Similarly, a CH-223191 solution was prepared at 10 mg/kg body weight, as per the method detailed by Cao et al. [19]. This involved dissolving 40 mg of CH-223191, sufficient for 20 animals, in 2 ml of DMSO. The solution was thoroughly mixed using a vortex, and each rat received a dose based on its weight (0.5 µL/1 g BW) of the working solution.

Animals and Sample Preparation

At the endpoint of the experiment, 60 days post treatments, the animals were euthanized by overdose of intramuscular injections of combination of Xylazine (Micopite, USA, 40 mg/kg) and Ketamine (Alpha Than, Holand, 90 mg/kg). Approximately five milliliters of blood was collected via heart puncture. Scrotal area was gently cleaned with sterile normal saline (NS), then bilateral testicles and epididymal tissues were collected. The epididymal tail was incubated at 37 °C in 2 mL of normal saline. Following the study's methodology, it was then divided into segments and spermatozoa were extracted using anatomical micro-scissors for sperm characteristics evaluation (20).

Sperm Morphology and Acrosomes Integrity Evaluation

For evaluation of rat sperm morphology and Acrosomes, the Eosin-Nigrosin (Amichem Research Lab LLP, India) stain solution was prepared by creating a 1% Eosin Y solution In combination with 10% Nigrosin solution, the eosin stain to evaluate the sperm membrane integrity, while nigrosine was only for giving contrast (21). Ten to twenty microliters of epididymal sperm suspension was mixed with the stain, smeared on a warm slide, and allowed to dry. The slide was then examined under a light

microscope, with sperm heads partially or completely stained pink were recorded as dead sperms (1). The dead and morphology tests were examined simultaneously under 40× magnification power. While acrosome integrity was assessed by using 100× magnification power (22).

Circulatory Luteinizing and Testosterone Hormones

Rat blood samples were used to evaluate the levels of luteinizing hormone (LH) and testosterone (T). The study employed the SL1061Ra and SL1093Ra assay kits from SunLong Biotech Co., Ltd., China to assess the levels of testosterone and LH levels, respectively.

Gene Expression of Testicular *Dnah1*

Testicular tissue was excised, stored at -80°C in a deep freezer (Arctoko, Denmark) in Triazol reagent (Trans® Chinese; Cat No: ET101-01). The extracted RNA was then used for cDNA synthesis, where reverse transcription into cDNA was performed using a One-Step RT-PCR Premix Kit (Promega's, USA). The resulting cDNA was subsequently used for gene expression analysis via quantitative PCR (qPCR). Primers were designed by using an available tool on NCBI database. Then the sequence of targeted genes were manufactured through Integrated DNA Technologies, Inc (IDT) in USA. The used primer details are listed in Table 1.

For qPCR, SYBR Green master mix was used with conditions, employing the 7500 real-time PCR system (Applied Biosystems™ 4351106). The procedure included a holding stage at 37°C for 15 minutes followed by another holding stage at 95°C for 10 minutes. PCR cycling consisted of denaturation at 95°C for 15 seconds, annealing at a temperature specific to the primer used for 1 minute, and 40 cycles of amplification. The extension was carried out at 72°C for 30 seconds. The melt curve stage involved three steps: 95°C for 15 seconds, 60°C for 1 minute, and 95°C for 30 seconds. Real-time monitoring was performed using an instrument equipped with fluorescence detection capabilities, measuring the fluorescence signal emitted by the SYBR Green DNA-binding dye throughout each amplification cycle to monitor the amplification process continuously.

Evaluation of analgesic Formalin Induced Acute Pain

The study involved mice given a subcutaneous injection of 10 µL of 4% formalin (Central Drug House, India) to assess acute pain, on the right side of the hind paw of mice. The formalin model reveals the biphasic nociceptive response which means of frequency of licking of the injected paw or flinching, a pain response characterized by spontaneous, fast, transient shaking or raising of the paw. Data were recorded the average frequency of licking and flinching during the initial acute phase (phase I) within the first five minutes and the lasting phase (phase II) between 20 and 60 minutes after the injection (25).

Assessment of Serum Prostaglandin E2 (PGE2) Level After Induced Pain

Upon the end of the experiment, five mice from each group were euthanized using chloroform, and blood samples were collected from the heart using a sterile 1 mL syringe that was fitted with a 25-gauge needle. The blood samples were collected in gel tube for serum isolation. The serum was then pipetted and transferred to sterile centrifuge tubes for ELIZA analysis. The serum levels of prostaglandin E2 (PGE2) were assessed using a mouse ELISA kit catalog number: MBS729260 (MyBioSource, USA). The kit contains a polyclonal anti-PGE2 antibody and a PGE2-HRP conjugate. The PGE2-HRP conjugate was incubated with the assay sample and buffer for one hour. After decantation and washing, the wells were used for the incubation of the horseradish peroxidase (HRP) enzyme substrate. A final solution was introduced, resulting in a noticeable yellow coloration. The intensity of the color was measured at 450 nm using a spectrophotometer (HUMA READER HS, 65205 Wiesbaden, Germany), in a microplate reader. The intensity of the color is inversely related to the concentration of PGE2, as there is competition between PGE2 in the samples and the PGE2 HRP conjugate for binding to the antiPGE2 antibody site.

Statistical Analysis

The collected data was analyzed by using the GraphPad Prism 9 program. The statistical approach used the One-Way Analysis of Variance (ANOVA) method for determining significant differences among multiple groups, especially under various experimental conditions and treatments. The analysis also involved the Holm-Sidak correction for multiple test comparisons, with a significance threshold set at $p < 0.05$. Significance levels were denoted as * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$. The t-test statistical analysis method was used for the analysis of gene expression (* for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$).

RESULTS AND DISCUSSION

This study was aimed to investigate the interactions between RES and CH223191 on the AhR and shed light on their potential implications for the intricate processes governing male reproductive health. With a meticulous approach, the study includes a range of assessments, including sperm Integrity (Morphology, Acrosomal, Hormonal Evaluation, and *Dnah1* gene expression analysis) as well as histopathological examination.

Sperm Morphology Integrity

In the evaluation of sperm morphology, as depicted in (Figure 1), distinct trends emerge among the experimental groups since the control group exhibited a high level of normal sperm morphology, with a mean percentage of (97.60 ± 0.40) %, while the DMSO group demonstrated a comparable morphology with a mean of (97.95 ± 0.36) %.

In contrast, the AhR⁻ group exhibited a significant reduction in sperm morphology compared with others, with a mean of (79.60 ± 1.4) %. At the cellular level, an AhR establishes functional interactions with signaling pathways governing cell morphology (23). In addition, the activation of AhR is associated with a reorganization of the cytoskeleton mainly due to a redistribution of actin and vinculin and to the activation of the focal adhesion and non-receptor tyrosine kinases (24), furthermore, these cellular effects are

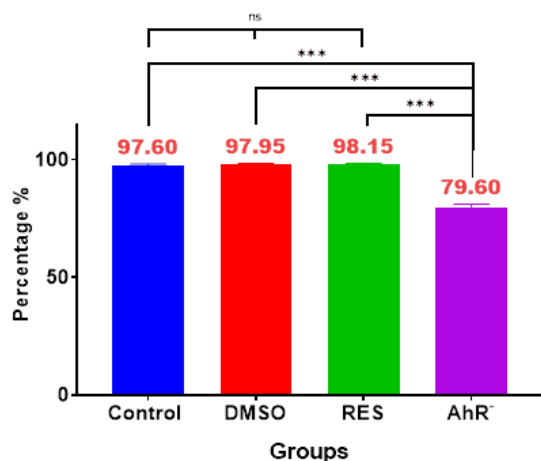


Figure 1. The Sperm Morphology Integrity Test evaluated the percentage of normal sperm morphology among different treatment groups over a 60-day period. Rats were administered dimethyl sulfoxide (DMSO), resveratrol (RES), or CH223191 (AhR antagonist) intraperitoneally twice weekly. Results, presented as means with SEM, indicated significant differences between groups - *** for $p < 0.001$.

Sperm Acrosomal Integrity

Regarding acrosomal integrity in (Figure 2), the control group demonstrated a high percentage of intact acrosomes, with a mean of (98.60 ± 0.17) %. The DMSO group displayed comparable acrosomal integrity with a mean of (98.20 ± 0.20) %. Similarly, the RES group exhibited a slightly enhanced acrosomal integrity, recording a mean of (98.55 ± 0.25) %, although the difference was not statistically significant compared to the control group. Conversely, the AhR⁻ group displayed a significant decrease in acrosomal integrity, with a mean of (96.70 ± 0.29) % compared with the control group. Generally, the sperm acrosomal plays a major role in the fertilization process which contains enzymes that are important in the penetration of the zona pellucida of the egg (29). Intact acrosomes mean that the sperm can successfully bind to and penetrate the egg (30). The AhR localized in the sperm acrosome and its activation modulates the expression of acrosomal enzymes (31). The study showed knock-down for AhR in mice leads to increased oxidative stress and morphologic abnormalities in sperm head and acrosomal (31–34). On the other hand, the acrosomal was formed during spermatogenesis since AhR has a critical role in the spermatogenesis and

accompanied by changes in the activation of c-Jun N-terminal kinases where it regulates morphology through the phosphorylation of cytoskeletal regulatory proteins (25). Building on this foundation, in the current study, a significant decrease in the integrity of sperm morphology was observed in the AhR⁻ group, as shown in (Figure 1). On the other hand, the improvement in sperm morphology due to RES can be explained by the activation of AhR leading to an increase in morphological integrity (26–28).

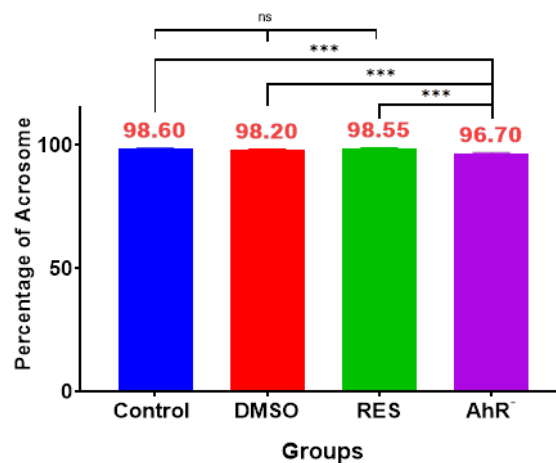


Figure 2. The Sperm Acrosomal Integrity Test assessed the percentage of intact acrosomes in different treatment groups over a 60-day period. Rats were treated with dimethyl sulfoxide (DMSO), resveratrol (RES), or CH223191 (AhR antagonist) intraperitoneally twice weekly. Results, presented as means with SEM, indicated significant differences between groups *** for $p < 0.05$.

spermiation process. The current data showed the significant role of RES as an antioxidant and as a promoting effect on AhR compared to the AhR⁻ group, which showed the adverse effect of stopping the AhR activation.

LH and Testosterone Hormone Evaluation

The circulatory hormonal evaluation conducted in this study serves as a critical component for understanding the intricate dynamics of male reproduction since assessing hormonal levels provides valuable insights into the endocrine regulation that governs various stages of spermatogenesis. Testosterone plays a pivotal role in supporting spermatogenesis, and alterations in its levels impact directly sperm production. Luteinizing hormones, produced by the pituitary gland, are essential for regulating the function of the testes which stimulates the production of testosterone by Leydig cells.

Testosterone Hormone.

In Figure 3, the outcomes of the Testosterone Hormone Test have delineated that the Control group exhibited a mean testosterone concentration of (0.14 ± 0.01) ng/ml, serving as a baseline for comparison, while, the DMSO group displayed a slightly reduced but non-significant

different to control testosterone level, with a mean of (0.13 ± 0.008) ng/ml as showed in previous study which reduces reproductive potential which may lead to subfertility or infertility in dogs [35]. Notably, the group treated with RES demonstrated an increased testosterone level, reaching a mean of (0.17 ± 0.010) ng/mL, although the difference was not significant compared to the Control. In contrast, the AhR⁻ group exhibited a significantly $p < 0.05$ diminished testosterone concentration of (0.09 ± 0.007) ng/mL compared to all other groups. In Figure 3, the hormonal profile reveals notable changes in testosterone which findings indicate a potential impact on the hypothalamic-pituitary gonadal (HPG) axis. Animal studies illustrated that RES has been shown to increase blood testosterone levels significantly [36] and instance, a study found that when

mice were given a large dose of RES, their testosterone levels went up by 51.6% [37,38]. In addition, studies showed that RES decreases the conversion of testosterone to estrogen (aromatization) and potentially leads to a higher overall level of testosterone in the body [39]. In addition to the previous role of AhR, studies showed its involvement in various molecular pathways, including those related to glucose homeostasis and insulin secretion as illustrated studies on AhR^{-/-} mice and found that reduced serum testosterone levels which AhR regulating Notch signaling since lead to germ cell apoptosis and reduction of expression of Notch1 gene and Notch3 gene in Sertoli cells resulting the decreased testosterone level [40] Thus loss of AhR lead to decrease in testosterone in male as exhibited in AhR⁻ group of the study result.

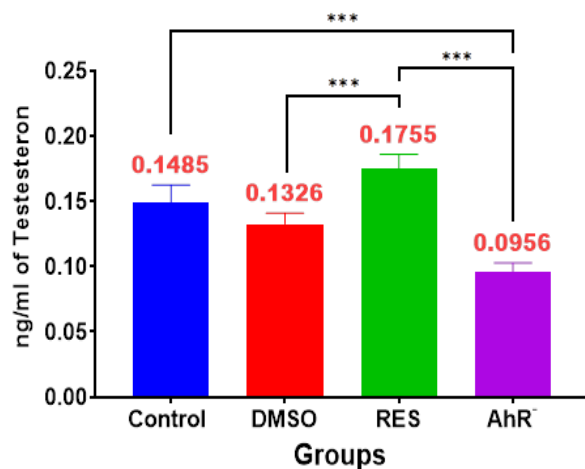


Figure 3. The Testosterone Hormone Test measured testosterone levels in different treatment groups over a 60-day period. Rats were treated with dimethyl sulfoxide (DMSO), resveratrol (RES), or CH223191 (AhR antagonist) intraperitoneally twice weekly. Results, presented as means with SEM, indicated significant differences between groups *** for $p < 0.05$.

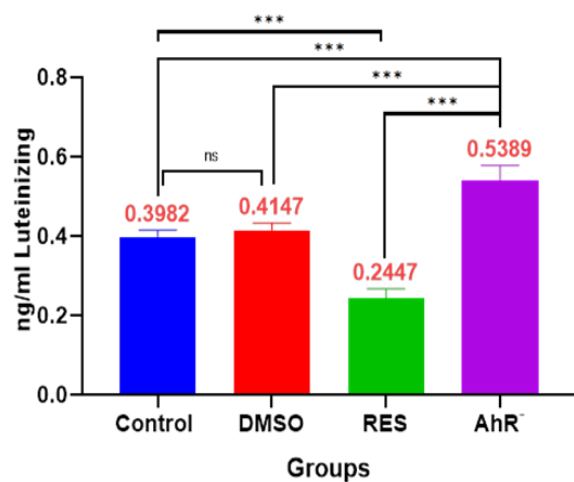


Figure 4. The Luteinizing Hormone Test measured luteinizing hormone levels in different treatment groups over a 60-day period. Rats were treated with dimethyl sulfoxide (DMSO), resveratrol (RES), or CH223191 (AhR antagonist) intraperitoneally twice weekly. Results, presented as means with SEM, indicated significant differences between groups (***) for $p < 0.05$.

Luteinizing Hormone

In (Figure 4), the results of the Luteinizing Hormone test are illustrated, since the control group displayed a baseline mean luteinizing hormone concentration of (0.39 ± 0.01) ng/ml while the DMSO group exhibited a slightly elevated but statistically not significantly comparable level, with a mean of (0.41 ± 0.01) ng/ml., the RES group demonstrated a significantly reduced luteinizing hormone concentration, recording a mean of (0.24 ± 0.02) ng/ml compared to the control and DMSO groups. In contrast, the AhR⁻ group exhibited a significantly increased luteinizing hormone concentration of (0.53 ± 0.03) ng/ml, indicating a distinct hormonal response compared with all other groups. The (Figure 4) illustrates the outcomes of the luteinizing

hormone (LH) test across the experimental groups, highlighting the pivotal role of LH in orchestrating testosterone production and subsequent spermatogenesis. Specifically, the group treated with resveratrol (RES) exhibited a reduction in LH concentration, whereas the AhR⁻ group displayed a significant increase in LH concentration. The observed reduction in LH concentration in the RES-treated group can be attributed to the modulatory effects of polyphenolic compounds on the HPG axis. Resveratrol has been shown to influence the HPG axis by enhancing the sensitivity of Leydig cells to LH, thereby promoting testosterone production even at lower LH levels. Additionally, resveratrol may directly upregulate steroidogenic enzymes involved in testosterone biosynthesis, leading to increased testosterone levels

without a corresponding increase in LH. In contrast, the AhR⁻ group's significant increase in LH concentration, despite decreased testosterone levels, suggests a disruption in the normal feedback mechanisms of the HPG axis. The absence of the aryl hydrocarbon receptor (AhR) can impair the negative feedback loop that regulates LH secretion. As a result, the hypothalamus and pituitary gland may respond to the lower testosterone levels by increasing LH production in an attempt to stimulate testosterone synthesis, despite the impaired response. Previous studies have explored these complex interactions, demonstrating that polyphenolic compounds like resveratrol can exert both stimulatory and inhibitory effects on the HPG axis, depending on the context and experimental conditions [41,42]. These studies provide insights into the dual nature of resveratrol's action, which can either enhance or suppress hormonal activity based on various factors such as dosage, duration of treatment, and the physiological state of the organism. In summary, Figure 4 highlights the distinct hormonal responses of the experimental groups, with resveratrol reducing LH levels through enhanced Leydig cell sensitivity and direct enzymatic effects, while AhR deficiency leads to increased LH levels due to disrupted feedback regulation. On the same side, some studies suggest that RES influences LH secretion through its antioxidant and estrogenic properties, which bind to estrogen receptors and mimic the actions of estrogen that provide negative feedback to the hypothalamus and pituitary gland which this feedback mechanism helps regulate the production of GnRH and LH (43,44). Based on the present results information in histological and gene expression proved the atrophy and distraction of the Sertoli cell lead reduction in its function and hormonal change one of the most significant roles of its function sequence leads to the increased LH levels as negative feedback as illustrated in AhR⁻ group

Gene Expression

Figure 5 illustrates the fold change analysis of *Dnah1* gene expression which the control group, the baseline expression is normalized to 1. In the RES group, there is a significant increase in *Dnah1* gene expression, as reflected by a fold change of approximately 2.676. Conversely, in the AhR⁻ group, there is a significant decrease in *Dnah1* expression, with a fold change of approximately 0.011. This substantial down regulation indicates a potent inhibitory effect of the AhR antagonist on *Dnah1* gene expression. Exploring *Dnah1* gene expression, as illustrated in (Figure 5), further elucidates the impact of AhR modulation. The *Dnah1* gene plays a vital role in spermatozoa, specifically in the formation of the inner dynein arms, which are microtubule-associated motor protein complexes composed of several heavy, light, and intermediate chains. It is crucial for the biogenesis of the axoneme, with its expression spanning the entire length of the sperm

flagellum. Previous studies have shown that in the absence or presence of mutations in *DNAH1*, the inner dynein arms are largely missing, resulting in the disorganization of these structures and the mislocalization of microtubule doublets, ultimately leading to decreased sperm motility (45–48) which agrees with the result which showed the RES group significant increase in *Dnah1* gene expression, conversely, in the AhR⁻ group, there is a significant decrease in *Dnah1* expression, which substantial downregulation indicates a potent inhibitory effect of the AhR antagonist on *Dnah1* gene expression.

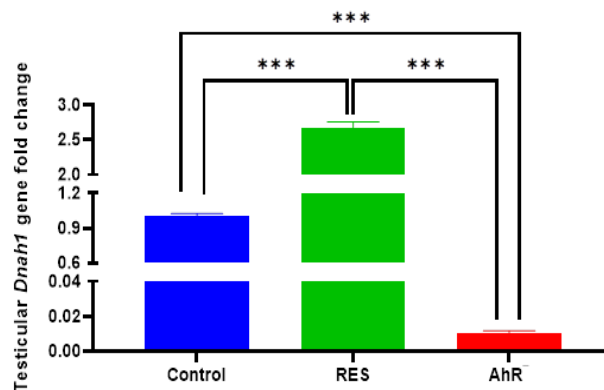


Figure 5. The Fold Change Analysis of *Dnah1* Gene Expression evaluated the expression of the *Dnah1* gene in response to resveratrol (RES) and the AhR antagonist CH223191 treatments. Rats were treated with RES or CH223191 intraperitoneally twice weekly for 60 days. Results, presented as means with SEM, indicated significant differences between groups (***) for $p < 0.001$.

Histopathological Examination

The histopathological found a clear divergence among the experimental groups, each presenting a distinctive narrative of testicular differentiation that examination begins with the control group, serving as the benchmark for normalcy (Figure 6A). The histological section unveils the expected, characteristic architecture of testicular tissue seminiferous tubules enveloped by a thin basement membrane, exhibiting a typical arrangement with sparse interstitial blood vessels, extends to the presence of mature spermatozoa within the tubular lumens, maintaining the structural integrity of the epithelial layer with Sertoli cells and germ cells representing various stages of spermatogenesis (49). Intriguingly, the RES group (Figure 6C). Mirrors the normalcy observed in the control, indicating that 100 mg/kg RES intraperitoneally twice weekly for 60 days does not induce significant histopathological alterations in testicular tissue that suggests a degree of histological preservation of RES on testicular tissue due to its health benefits due to its anti-inflammatory and antioxidative properties (50). Generally, the RES has shown promising results in protecting rat testicular tissue against various forms of damage (36, 51,

52). Furthermore, RES can directly scavenge free radicals, which are unstable atoms that cause damage to cells, contributing to aging and disease consequently neutralizing these free radicals and preventing cellular damage and reducing oxidative stress [53,54]. The AhR⁻ group exhibited significant abnormalities in the histopathological examination, as shown in (Figure 6D), indicating structural and functional irregularities in testicular tissue. The examination revealed deformed seminiferous tubules characterized by tubular atrophy (reduced size) and tubular separation, with notable separations between tubules. Some tubules showed abnormal attachment of epithelial cells, leading to disorganization and destruction of the germinal epithelium, accompanied by a reduction in germ cells. The lumen of these deformed tubules contained immature germ cells and an accumulation of cell fragments. Additionally, interstitial fibrosis and expansion were observed between seminiferous tubules, indicating structural alterations in the testicular tissue of the AhR⁻ group. Furthermore, AhR signaling plays a crucial role in regulating germ cell survival and preventing excessive apoptosis, which is essential for

maintaining the requisite pool of germ cells necessary for spermatogenesis. Previous studies on AhR knockout (AhR^{-/-}) mice have demonstrated that the absence of AhR correlates with a reduction in the expression of genes involved in the defense against reactive oxygen species. The AhR signaling pathway is implicated as a stress rheostat, influencing spermatogenesis and histopathological features of the seminiferous epithelium since the evaluation of AhR^{-/-} males revealed Sertoli cell degeneration, defects in mature sperm, and architectural distortions at stages IV–XII of the seminiferous epithelium [31, 55, 56]. In parallel, our findings align with previous research indicating RES's interaction with AhR, supporting the notion that RES exhibits agonism for AhR and binding and modulating the effects of AhR activation, offering a prospect for improvement in the AhR function (57). Finally, the result in (Figure 6B) illustrates the DMSO group showed normal but minor alterations including tubular shrinkage in seminiferous tubules, some interstitial expansion between seminiferous tubules furthermore, and some Basement membrane thickening of tubules.

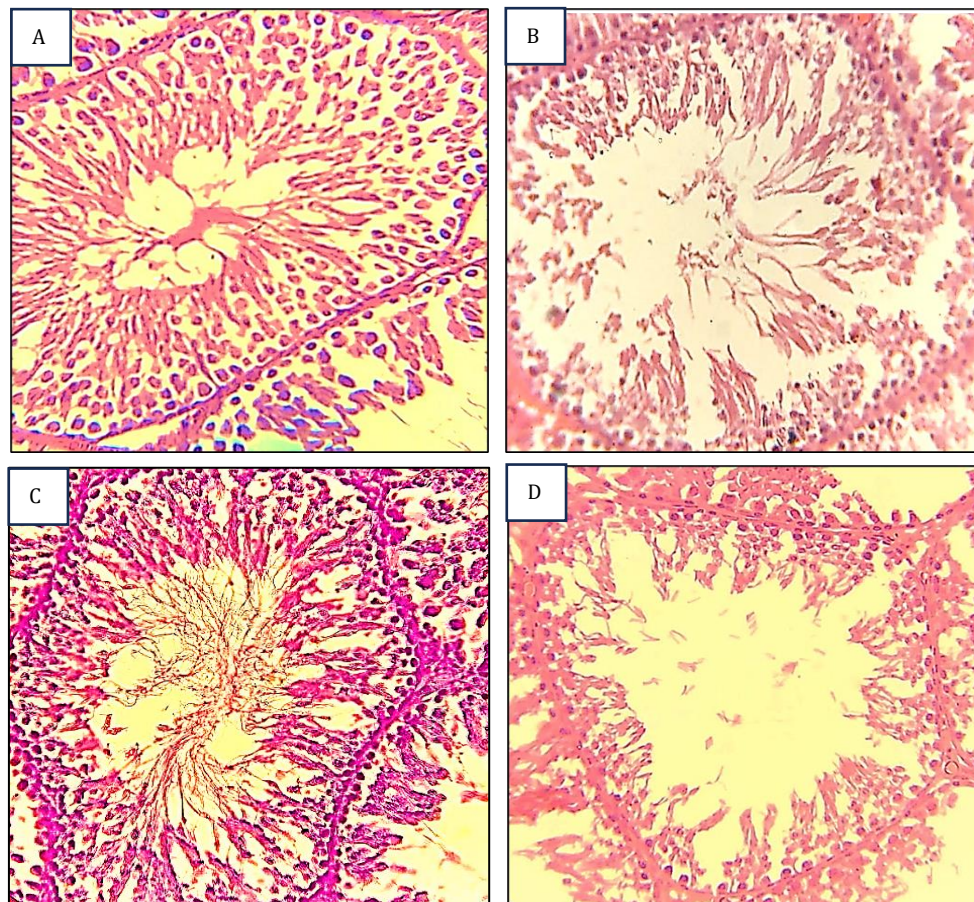


Figure 6. Histological Section of seminiferous tubules under 40×. **(A)** Control Group Testicular Tissue (Normal histological architecture of testicular tissue). **(B)** DMSO Group Testicular Tissue (Minor alterations in histological architecture of testicular tissue). **(C)** RES Group Testicular Tissue (Normal histological architecture of testicular tissue). **(D)** AhR⁻ Group Testicular Tissue (Abnormal histological architecture of testicular tissue)

In conclusion, this work elucidates the complex interactions between AhR, RES, and spermatogenesis in adult male rats. The significant abnormalities observed in the AhR⁻ group, including reduced sperm morphology, compromised acrosomal integrity, decreased testosterone levels, and altered gene expression, particularly *Dnah1*, underscore the critical role of AhR in maintaining male reproductive health. Conversely, RES treatment resulted in enhanced *Dnah1* gene expression, higher testosterone levels, and improved sperm morphology and acrosomal integrity, indicating potential therapeutic benefits. While some parameters did not reach statistical significance, the overall trends observed in the data suggest that AhR activation is crucial for preserving healthy testicular architecture and spermatogenesis. Additionally, the beneficial effects of RES-mediated modulation of AhR highlight its potential as a therapeutic approach for conditions affecting male reproduction. Future studies should further investigate these mechanisms to confirm and expand upon these findings.

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N/A

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Bustani GS, Baiee FH. Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. *Vet World*. 2021;14(5):1220. 10.14202/vetworld.2021.1220-1233
- Esakky P, Hansen DA, Drury AM, Moley KH. Cigarette smoke condensate induces aryl hydrocarbon receptor-dependent changes in gene expression in spermatocytes. *Reprod. Toxicol*. 2012;34(4):665-76. 10.1016/j.reprotox.2012.10.005.
- Abbott BD, Schmid JE, Pitt JA, Buckalew AR, Wood CR, Held GA, Diliberto JJ. Adverse reproductive outcomes in the transgenic Ah receptor-deficient mouse. *Toxicol Appl Pharmacol*. 1999;155(1):62-70. 10.1006/taap.1998.8601.
- Wang SY, Cheng YY, Liu SC, Xu YX, Gao Y, Wang CL, Wang ZG, Feng TQ, Lu GH, Song J. A synonymous mutation in IGF-1 impacts the transcription and translation process of gene expression. *Mol. Ther Nucleic Acids*. 2021;26:1446-65. 10.1016/j.omtn.2021.08.007
- Alghetaa H, Mohammed A, Sultan M, Busbee P, Murphy A, Chatterjee S, et al. Resveratrol protects mice against SEB-induced acute lung injury and mortality by miR-193a modulation that targets TGF- β signalling. *J Cell Mol Med*. 2018;22(5):2644-55. 10.1111/jcmm.13542.
- Levine-Fridman A, Chen L, Elferink CJ. Cytochrome P4501A1 promotes G1 phase cell cycle progression by controlling aryl hydrocarbon receptor activity. *Mol Pharmacol*. 2004;65(2):461-9. 10.1124/mol.65.2.461.
- Wincent E, Bengtsson J, Bardbori AM, Alsberg T, Luecke S, Rannug U, et al. Inhibition of cytochrome P4501-dependent clearance of the endogenous agonist FICZ as a mechanism for activation of the aryl hydrocarbon receptor *Proc Natl Acad Sci*. 2012;109(12):4479-84. 10.1073/pnas.1118467109.
- Pinne M, Raucy JL. Cytochrome P450 Gene Regulation: Reporter Assays to Assess Aryl Hydrocarbon Receptor (HLHE76, AhR) Activation and Antagonism. In: *Cytochrome P450*. Springer; 2021. p. 157-74. 10.1007/978-1-0716-1542-3_10
- Zhang J, Gao X, Yu L. Roles of histone deacetylases in acute myeloid leukemia with fusion proteins. *Front Oncol*. 2021;11:741746. 10.3389/fonc.2021.741746
- Wang LT, Chiou SS, Chai CY, Hsi E, Wang SN, Huang SK, et al. Aryl hydrocarbon receptor regulates histone deacetylase 8 expression to repress tumor suppressive activity in hepatocellular carcinoma. *Oncotarget*. 2017;8(5):7489. 10.18632/oncotarget.984
- Garrison PM, Rogers JM, Brackney WR, Denison MS. Effects of histone deacetylase inhibitors on the Ah receptor gene promoter. *Arch Biochem Biophys*. 2000;374(2):161-71. 10.1006/abbi.1999.1620.
- Tsai CH, Li CH, Liao PL, Chang YW, Cheng YW, Kang JJ, et al. Aza-PBHA, a potent histone deacetylase inhibitor, inhibits human gastric-cancer cell migration via PKC α -mediated AHR-HDAC interactions. *Biochim Biophys Acta Mol Cell Res*. 2020;1867(2):118564. 10.1016/j.bbamcr.2019.118564
- Abdulla JM, Al-Okaily BN. Histomorphometric and histopathological alterations of rat testis following exposure to hydrogen peroxide: Protective role of resveratrol supplement. *Iraqi J. Vet. Med*. 2022;46(1):17-23. 10.30539/ijvm.v46i1.1313
- Longobardi V, Zullo G, Salzano A, De Canditiis C, Cammarano A, De Luise L, Puzio MV, Neglia G, Gasparriani B. Resveratrol prevents capacitation-like changes and improves in vitro fertilizing capability of buffalo frozen-thawed sperm. *Theriogenology*. 2017;88:1-8. 10.1016/j.theriogenology.2016.09.046
- Aghetaa HFK, Dawood RA, Aladhami AK. Resveratrol Administration Ameliorates Hepatotoxicity in Mercuric Chloride- Induced Liver Injury in Rats. *Iraqi J. Vet. Med*. 2023;47(2):1-8. Doi: 10.30539/ijvm.v47i2.1482.
- Huang Y, Zhang J, Tao Y, Ji C, Aniaqu S, Jiang Y, Chen T. AHR/ROS-mediated mitochondria apoptosis contributes to benzo [a] pyrene-induced heart defects and the protective effects of resveratrol. *Toxicology*. 2021;462:152965. 10.1016/j.tox.2021.
- Abdulla OA, Neamah W, Sultan M, Alghetaa HK, Singh N, Busbee PB, et al . The ability of AhR ligands to attenuate delayed type hypersensitivity reaction is associated with alterations in the gut microbiota. *Front Immunol*. 2021;12:684727. 10.3389/fimmu.2021.684727.
- Bordbar H, Yahyavi SS, Noorafshan A, Aliabadi E, Naseh M. Resveratrol ameliorates bisphenol A-induced testicular toxicity in adult male rats: a stereological and functional study. *Basic Clin Androl*. 2023;33(1):1. 10.1186/s12610-022-00174-8.19
- Cao W, Lu J, Li L, Qiu C, Qin X, Wang T, et al. Activation of the Aryl Hydrocarbon Receptor Ameliorates Acute Rejection of Rat Liver Transplantation by Regulating Treg Proliferation and PD-1 Expression. *Transplantation*. 2022;106(11):2172-81. 10.1097/TP.0000000000004205.
- Ngaha Njila MI, Massoma Lembè D, Koloko BL, Yong Meng G, Ebrahimi M, Awad EA, et al. Sperm parameters quality and reproductive effects of methanolic extract of *Alchornea cordifolia* leaves on senescent male rats. *Andrologia*. 2019;51(9):e13359. 10.1111/and.13359.
- Al-Ghetaa HFK. Effect of Environmental High Temperature on the Reproductive activity of Awassi Ram Lambs. *Iraqi J. Vet. Med*. 2012; 36(2), 244-253. 10.30539/iraqijvm.v36i2.502.
- Kaka A, Wahid H, Rosnina Y, Yimer N, Khumran AM, Sarsaifi K, et al. α -Linolenic acid supplementation in BioXcell® extender can improve the quality of post-cooling and frozen-thawed bovine sperm. *Anim Reprod Sci*. 2015;153:1-7. 10.1016/j.anireprosci.2014.12.001.
- Mulero-Navarro S, Fernandez-Salguero PM. New trends in aryl hydrocarbon receptor biology. *Front Cell Dev Biol*. 2016;4:45. Doi: 10.3389/fcell.2016.00045.
- Larigot L, Juricek L, Dairou J, Coumoul X. AhR signaling pathways and regulatory functions. *Biochim Open*. 2018;7:1-9. 10.1016/j.biopen.2018.05.001.
- Komulainen E, Varidaki A, Kuleskaya N, Mohammad H, Sourander C, Rauvala H, et al. Impact of JNK and its substrates on dendritic spine morphology. *Cells*. 2020;9(2):440. 10.3390/cells9020440
- Shaito A, Posadino AM, Younes N, Hasan H, Halabi S, Alhababi D, et al. Potential adverse effects of resveratrol: A literature review. *Int J Mol Sci*. 2020;21(6). 10.3390/ijms21062084.

27. Revel A, Raanani H, Younglai E, Xu J, Han R, Savouret JF, et al. Resveratrol, a natural aryl hydrocarbon receptor antagonist, protects sperm from DNA damage and apoptosis caused by benzo (a) pyrene. *Reprod Toxicol.* 2001;15(5):479–86. 10.1016/s0890-6238(01)00149-6.
28. Alghetaa H, Mohammed A, Sultan M, Busbee P, Murphy A, Chatterjee S, et al. Resveratrol protects mice against SEB-induced acute lung injury and mortality by miR-193a modulation that targets TGF- β signalling. *J Cell Mol Med.* 2018;22(5):2644–2655. 10.1111/jcmm.29.
29. Barroso G, Valdespin C, Vega E, Kershenovich R, Avila R, et al. Developmental sperm contributions: fertilization and beyond. *Fertility and sterility.* 2009; 92(3), 835–848. 10.1016/j.fertnstert.2009.06.030.
30. Bustani GS, Jabbar MK, AL-Baghdady HF, Al-Dhalimy AMB. Protective effects of curcumin on testicular and sperm parameters abnormalities induced by nicotine in male rats. In: *AIP Conference Proceedings.* 2022; 2386(1); article id.020042. 10.1063/5.0066855.
31. Hansen DA, Esakky P, Drury A, Lamb L, Moley KH. The aryl hydrocarbon receptor is important for proper seminiferous tubule architecture and sperm development in mice. *Biol Reprod.* 2014;90(1):1–8. 10.1095/biolreprod.113.108845.
32. Esakky, P., Moley, K. Preventing germ cell death by inactivating aryl hydrocarbon receptor (AHR). *Cell Death Dis.* 2016; 7, e2116.: 10.1038/cddis.2016.20.
33. Serra L, Bourdon G, Estienne A, Fréville M, Ramé C, Chevaleyre C, et al. Triazole pesticides exposure impaired steroidogenesis associated to an increase in AHR and CAR expression in testis and altered sperm parameters in chicken. *Toxicol Rep.* 2023; 10:409–27. Doi: 10.1016/j.toxrep.2023.03.005
34. Hashemi M, Azarnia M, Hajebrahimi Z, Ebrahimi SN. The effect of hydroalcoholic extract of *Cichorium intybus* leaf on aryl hydrocarbon receptor expression in the testis of Wistar rats exposed to cigarette smoke. *Avicenna J Phytomed.* 2023;13(1):58. Doi: 10.22038/AJP.2022.21307.
35. Vannucchi CI, Angrimani D de SR, Eyherabide AR, Mazzei CP, Lucio C de F, Maiorka PC, et al. Effects of intratesticular administration of zinc gluconate and dimethyl sulfoxide on clinical, endocrinological, and reproductive parameters in dogs. *Theriogenology.* 2015;84(7):1103–10. Doi: 10.1016/j.theriogenology.2015.06.005.
36. Bordbar H, Yahyavi SS, Noorafshan A, Aliabadi E, Naseh M. Resveratrol ameliorates bisphenol A-induced testicular toxicity in adult male rats: a stereological and functional study. *Basic Clin Androl.* 2023;33(1):1. Doi: 10.1186/s12610-022-00174-8.
37. Shin S, Jeon JH, Park D, Jang MJ, Choi JH, Choi BH, et al. trans-Resveratrol relaxes the corpus cavernosum ex vivo and enhances testosterone levels and sperm quality in vivo. *Arch Pharm Res.* 2008;31:83–7. 10.1007/s12272-008-1124-7.
38. Korkina, Liudmila G, Wolfgang Mayer, Chiara De Luca. Meristem plant cells as a sustainable source of redox actives for skin rejuvenation. *Biomolecules* 2017;7(2):40 10.3390/biom7020040
39. Sainz-Urruela C, Vera-López S, San Andrés MP, Díez-Pascual AM. Graphene-based sensors for the detection of bioactive compounds: A review. *Int J Mol Sci.* 2021;22(7):3316. Doi: 10.3390/ijms22073316.
40. Huang B, Butler R, Miao Y, Dai Y, Wu W, et al. Dysregulation of Notch and ER α signaling in AhR $-/-$ male mice. *PNAS.* 2016;113(42):11883–8. Doi: 10.1073/pnas.1613269113
41. Meng X, Zhou J, Zhao CN, Gan RY, Li HB. Health benefits and molecular mechanisms of resveratrol: a narrative review. *Foods.* 2020;9(3):340. Doi: 10.3390/foods9030340.
42. Wu Y, Baumgarten SC, Zhou P, Stocco C. Testosterone-dependent interaction between androgen receptor and aryl hydrocarbon receptor induces liver receptor homolog 1 expression in rat granulosa cells. *Mol Cell Biol.* 2013;33(15):2817–28. Doi: 10.1128/MCB.00011-13.
43. Morita Y, Wada-Hiraike O, Yano T, Shirane A, Hirano M, Hiraike, et al. Resveratrol promotes expression of SIRT1 and StAR in rat ovarian granulosa cells: an implicative role of SIRT1 in the ovary. *RB&E.* 2012;10(1):1–10. Doi: 10.1186/1477-7827-10-14
44. Elsayy H, Famurewa AC, Sedky A. Resveratrol Mitigates Diabetic Testicular Dysfunction, Endocrine Deficits, and Insulin Resistance via Suppression of Sperm-Endocrine Aberrations and Oxidative Inflammation in Rats. *Andrologia.* 2023;2023. Doi: 10.1155/2023/6385767
45. Wang M, Yang QY, Zhou JP, Tan HP, Hu J, Jin L, et al. Novel compound heterozygous mutations in *DNAH1* cause primary infertility in Han Chinese males with multiple morphological abnormalities of the sperm flagella. *Asian J Androl.* 2023;25(4):512–9. 10.4103/aja.202292.
46. Jiang C, Zhang X, Zhang H, Guo J, Zhang C, Li J, et al. Novel bi-allelic mutations in *DNAH1* cause multiple morphological abnormalities of the sperm flagella resulting in male infertility. *Transl Androl Urol.* 2021;10(4):1656. 10.21037/tau-20-1434.
47. Zhuang BJ, Xu SY, Dong L, Zhang PH, Zhuang BL, Huang XP, et al. Novel *DNAH1* mutation loci lead to multiple morphological abnormalities of the sperm flagella and literature review. *World J Mens Health.* 2022;40(4):551. 10.5534/wjmh.210119.
48. Ghafouri-Fard S, Shoorei H, Mohaqiq M, Raza SHA, Taheri M. The role of different compounds on the integrity of blood-testis barrier: A concise review based on in vitro and in vivo studies. *Gene.* 2021;780:145531. 10.1016/j.gene.2021.145531
49. Balci A, Ozkemahli G, Erkekoglu P, Zeybek ND, Yersal N, Kocer-Gumusel B. Histopathologic, apoptotic and autophagic, effects of prenatal bisphenol A and/or di (2-ethylhexyl) phthalate exposure on prepubertal rat testis. *Environmental Science and Pollution Research.* 2020;27:20104–16. 10.1007/s11356-020-08274-6
50. Assefa EG, Yan Q, Gezahegn SB, Salissou MTM, He S, Wu N, et al. Role of resveratrol on indoxyl sulfate-induced endothelial hyperpermeability via aryl hydrocarbon receptor (AHR)/Src-dependent pathway. *Oxid Med Cell Longev.* 2019;2019. 10.1155/2019/5847040
51. Chi KK, Zhang WH, Chen Z, Cui Y, He W, Wang SG, et al. Comparison of quercetin and resveratrol in the prevention of injury due to testicular torsion/detorsion in rats. *Asian J Androl.* 2016;18(6):908. Doi: 10.4103/1008-682X.167720
52. Sener TE, Atasoy BM, Cevik O, Cilingir Kaya OT, Cetinel S, et al. Effects of resveratrol against scattered radiation-induced testicular damage in rats. *Turkish Journal of Biochemistry.* 2020;46(4):425–33. 10.1515/tjb-2020-0320
53. Gu T, Wang N, Wu T, Ge Q, Chen L. Antioxidative stress mechanisms behind resveratrol: a multidimensional analysis. *J Food Qual.* 2021;2021:1–12. 10.1155/2021/5571733
54. Amin GM, Marouf BH, Namiq H. The Effects of Resveratrol Supplementation on the Quality of Life of Diabetic Patients with Neuropathy: Small Randomized Clinical Trial. *IJPS.* 2023;32(3):118–27.
55. Zhang W, Ou N, Liang Z, Hu R, Song Y, Yang Y, et al. Relationship between aryl hydrocarbon receptor and spermatogenic dysfunction in male patients with varicocele. *Andrologia.* 2020;52(11):e13792. 10.1111/and.13792
56. Alghetaa H, Mohammed A, Singh N, Wilson K, Cai G, Putluri N, et al. Resveratrol attenuates staphylococcal enterotoxin B-activated immune cell metabolism via upregulation of miR-100 and suppression of mTOR signaling pathway. *Front Pharmacol.* 2023;14:1106733. 10.3389/fphar.2023.1106733.
57. Lee S, Gross SE, Pfaff H, Dresen A. Waiting time, communication quality, and patient satisfaction: an analysis of moderating influences on the relationship between perceived waiting time and the satisfaction of breast cancer patients during their inpatient stay. *Patient Educ Couns.* 2020;103(4):819–25. 10.1016/j.pec.2019.11.018.

تأثير تعديل مستقبل الأريل هيدروكربون وتأثير الريسفيراترول على تعبير جين *Dnah1* : الآثار على الصحة التناسلية الذكورية

غدير صباح بستاني^{٢،١} ، حسن فلاح كاشف الغطاء^١ ، نبيل حسين الحمادي^٣

^١ فرع الفلسفة والكيمياء الحياتية والادوية، كلية الطب البيطري، ^٢ جامعة بغداد، بغداد، العراق، الجامعة الإسلامية في النجف، النجف الاشرف، العراق، ^٣ ادارة الغذاء والدواء الامريكية، ولاية ماريلاند، الولايات المتحدة الاميركية

الخلاصة

يعد تنظيم صحة الجهاز التناسلي للذكور بشكل دقيق من خلال العمليات الجزيئية والفسبولوجية، ويلعب مستقبل الأريل هيدروكربون (AhR) دورًا حاسمًا في تنظيمها إذ ينشط AhR بواسطة مجموعة متنوعة من الرابطات ويؤثر على تقدم الأمراض. ان الهدف من هذه الدراسة تقييم دور AhR في التخصيب عند ذكور الجرذان البالغة والتي تأثرت بالريسفيراترول (RES) او CH223191 المضاد لل AhR. شملت الدراسة أربعين جرذًا قُسمت عشوائيًا في أربع مجموعات متساوية: مجموعة ضابطة، والمجموعة الضابطة الموجبة DMSO، ومجموعة RES، ومجموعة AhR⁻. تلقى الجرذان العلاجات في الخلب مرتين أسبوعيًا لمدة 60 يومًا، وقيمت مختلف معايير صحة الجهاز التناسلي للذكور. أظهرت النتائج تفعيل AhR بعلاج RES معززا شكل الحيوانات المنوية، وسلامة الحيوانات المنوية، وهرمون منشط للجسم الأصفر. علاوة على ذلك، حفز تعبير الجين *Dnah1*، الذي لعب دورًا رئيسيًا في شكل الحيوانات المنوية، وارتفاع مستويات هرمون التستوستيرون بواسطة RES. ومن ناحية أخرى، قلل تشوهات الحيوانات المنوية، وسلامة حائل الحيوانات المنوية، ومستويات هرمون التستوستيرون، وتعبير جين *Dnah1* في جردان AhR⁻. حافظ على تماسك الأنسجة الخصية بواسطة RES، وفقًا لتحليل نسيجي تشريحي. تسلط هذه النتائج الضوء على دور AhR في صحة الجهاز التناسلي للذكور وتقتصر RES كعامل علاجي محتمل

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