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Exploring the Impact of Aryl Hydrocarbon Receptor (*AhR*) Modulation on the Blood-Testis Barrier Integrity via Tight Junction Protein-1 function

Running title: **Effects of AhR ligands on BTB via *AhR*/*Tjp1*-axis**

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Abstract

Background: Male infertility is a global concern and it tends to be increased due to miscellaneous factors such as environmental toxins, genetic, and lifestyle choices. The Aryl Hydrocarbon Receptor (AhR) has recently amassed attention due to its involvement in male infertility mechanisms and its impact on sperm production and function. AhR, a versatile receptor expressed in various tissues, including the testes, regulates genes implicated in spermatogenesis. AhR activation has been linked to cell cycle regulation and chromatin condensation during spermatogenesis.

Objectives: This study aimed to investigate the influence of AhR activation on blood-testes barrier (BTB) integrity, focusing on the role of Tight Junction Protein 1 (Tjp1) and to explore the effects of AhR modulation on spermatogenesis outcomes in adult male rats.

Methods: Forty adult male rats were divided into four groups according to their treatment regimen. Briefly, Control group kept without any treatment; RES group received i.p. injection of 100mg/kg every 72 hours; AhR antagonist (AhR^{-}) group received i.p. injection of CH223191 at a dose of 10mg/kg every 72 hours. Finally, DMSO group received dimethyl sulfoxide which was used as a solvent for preparation of resveratrol and CH223191. The study lasted for sixty days to cover entire spermatogenesis cycle. At the endpoint of the study, sperm chromatin maturity and condensation were evaluated in addition to *Tjp1* gene expression in testicular tissue supported by BTB integrity assessment.

Results: The abnormal sperm chromatin maturity and condensation were significantly ($P<0.05$) higher in AhR^{-} in comparison to all other groups. *Tjp1* gene expression was significantly upregulated in the RES group in comparison to control and AhR^{-} group which in turn tightened the BTB and maintained the testicular homeostasis for normal spermatogenesis.

Conclusions: Resveratrol treatment positively influenced sperm chromatin maturity, *Tjp1*expression, and BTB integrity, suggesting its potential as a protective agent for male reproductive health. Conversely, AhR antagonism led to compromised sperm chromatin integrity and BTB function, highlighting the critical role of AhR in spermatogenesis and BTB maintenance. These findings underscore the importance of AhR modulation in male fertility and provide insights into potential therapeutic interventions.

Keywords: Aryl Hydrocarbon Receptor (AhR), CH223191, Resveratrol, Spermatogenesis, Tight junction protein 1 (Tjp1).

Introduction

One of the significant concerns worldwide is infertility problems, these challenges arise from environmental toxins, genetic predispositions, and lifestyle choices. In recent years, research have increasingly focused on Aryl Hydrocarbon Receptor (Ahr) to underlying its role in mechanisms of male infertility and shedding light on the intricate processes involved in sperm production, maturation, and function (Bala et al., 2021; Skakkebæk et al., 2022). The AhR is a versatile and dynamic cytosolic receptor that found in a wide range of tissues throughout both developmental stages and adulthood (Gonzalez & Fernandez-Salguero, 1998; Neamah et al., 2020). Many researchers found that AhR activation has been linked to regulatory genes involved in spermatogenesis. For which, the AhR expression in the seminiferous tubule was explored in the rat, revealing a specific localization to the primary pachytene spermatocytes during stages VII–XI and round spermatids during the 2nd through 14th stage of the spermatogenic cycle. However, AhR was found to be expressed in all spermatogenic cycle stages of the human testes (Hansen et al., 2014a; Pohjanvirta, 2012; Schultz et al., 2003). The effects of AhR on the cell cycle may vary based on the presence or absence of exogenous ligands and the specific cellular context. Moreover, the AhR action depends on the absence of an exogenous ligand which may promote S-phase progression. When AhR binds to its ligand such as 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) it will lead to inhibition of DNA replication in various cell lines (Bock, 2018; Huss et al., 2019; Ribeiro et al., 2018). Furthermore, the researcher showed that the treated animals with TCDD showed lower number of cells in S phase and caused cells to be arrested in

G1 phase (Marlowe et al., 2004). Based on previous studies, it appears that the AhR plays an important role in the regulating of the cell cycle. Furthermore, the AhR activity, influenced by the presence or absence of its ligands and the metabolic activity of P4501A1 enzyme which plays a role in regulating cell cycle progression, particularly during the G1-to-S phase transition. The modulation of AhR activity and its downstream effects on cell cycle regulators contribute to the fine-tuning of cell cycle progression. The AhR plays a crucial role in regulating chromatin condensation during prophase, at 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" (O. A. Abdulla et al., 2021; H. Alghetaa et al., 2023; Zhang et al., 2021). Thus, the AhR activation promotes chromatin condensation by enhancing the expression of histone deacetylase (HDACs) enzymes. These enzymes remove acetyl group from histones that will lead to formation of tighten nucleosomes and inactive DNA (Garrison et al., 2000; Tsai et al., 2020; Wang et al., 2017). A substantial body of research suggests that the absence of AhR ligand binding or AhR activation may contribute to inflammation (Meng et al., 2018), apoptosis (Hansen et al., 2014a), and oxidative stress in sperm leading to DNA damage (Mostafa et al., 2017). Examining AhR expression in the rat seminiferous tubule revealed its affinity to primary pachytene spermatocytes during stages VII–XI and round spermatids through II–XIV phase of the spermatogenic cycle (Barhoover et al., 2010).

Resveratrol (3,5,4′-trihydroxystilbene; RES) is a non-flavonoid polyphenol organic compound which found naturally in various foods such as grapes, peanuts, and blueberries (Amin et al., 2023; Jawad & Sahib, 2022). RES is widely known for its renowned beneficial biological

effects which target multiple molecular pathways including oxidative stress and the AhR signal pathway (Alghetaa et al., 2023; Khayoon & Al-Rekabi, 2020). RES serves as natural antagonist to AhR, but also has partial agonistic effects on AhR when it is used in relatively high doses (Singh et al., 2007; Chitrala et al., 2018). Additionally, RES inhibits the activation of CYP1A1 and CYP1B1 via AhR/ARNT complex, this inhibition has also been correlated with a reduction in ROS production (Alghetaa et al., 2023; R. Dawood., et al., 2023; Nsaif & Al-Mualm, 2021). The protective impact of RES on ROS production holds significant importance, given that peroxidation of polyunsaturated fatty acids can lead to a loss of membrane fluidity and decreased activity of membrane enzymes and ion channels, potentially compromising sperm motility (Bustani and Baiee, 2021; Chikhaoui et al., 2024). Other researchers have elucidated the mechanism of RES on AhR, highlighting its ability to restore the reduction in ERK and p38 MAPK phosphorylation (Abdulla & Al-Okaily, 2022; Alamo et al., 2019; Liu et al., 2020). RES protects cells from DNA damage and apoptosis by modulating the anti- and pro-apoptotic mediators as well as increasing the antioxidant status. More studies showed that RES can inhibit the enzymatic activity of various cytochrome P450s and blocks their transcription through antagonism of the AhR, suggesting that RES could decrease the exposure of cells to carcinogens (Alharris et al., 2017; Mongioì et al., 2021). The tight junction protein-1 (TJP1) plays a crucial role in the formation of tight junctions in cells. It acts as a scaffold protein, cross-linking and anchoring tight junction strand proteins which are fibril-like structures within the lipid bilayer (Yan *et al.*, 2024). In the context of spermatogenesis, TJP1 protein is expressed in Sertoli cells and plays a crucial role by providing physical and nutritional support to male germ cells. It is also instrumental in maintaining the integrity of Sertoli cell tight junctions, a major component

of the blood-testes barrier (BTB) formation and the progression of spermatogenesis (Ghafouri-Fard *et al.*, 2021).

This study aims to explore the impact of activation or inhibition of AhR signaling on the sperm genetic integrity such as chromatin maturation and condensation as well as the effect of AhR signaling dysregulation on the blood-testes barrier integrity focusing on the role of *Tjp1* gene expression.

Material and methods

Experimental animals

Forty adult male rats weighed 225 – 275 grams were utilized in this study. The experimental procedures started following a two-week acclimatization period. The animals randomly divided into different cages, with each cage containing six animals at the Animal House of the College of Veterinary Medicine, University of Baghdad. Throughout the experimental period, all animals had free access to food and water.

Rats were randomly assigned to four equal groups includes: Control group receiving no treatment, DMSO group was given i.p. injection of dimethyl sulfoxide (50% solution), RES group received i.p. injection of resveratrol at a dose of 100mg/kg every 72 hours (Bordbar et al., 2022) and lastly, AhR^{-} group was i.p. injection of CH223191, an AhR antagonist, at a dose of 10 mg/kg every 72 hours (Cao et al., 2022). The duration of all experiments was 60 days to cover whole entire spermatogenesis cycle in rats (Clouthier et al., 1996; Sadighara et al., 2024). At the end of experiments, 5 animals from each group were sacrificed to collect testes and the tail of the

epididymis for further assessments. While the other 5 animals were taken to assess testes barrier integrity.

Preparation of drug solutions:

Resveratrol purchased from Hebei Guanlang Biotechnologies and its solution was prepared to achieve protocol dosage at 100mg/kg B.W as following. Briefly, 400 mg of Resveratrol was dissolved in 2 ml of dimethyl sulfoxide (DMSO) before being further diluted in another 2 ml of distil water. The resulting solution was accurately mixed for homogeneity using a vortex mixer, then each rat received 1µL/g B.W. of this solution (Bordbar et al., 2022). Similarly, CH223191 solution, was prepared by dissolving 40 mg of CH223191 (Hebei Guanlang Biotechnolies) in 2 ml DMSO then once the homogenous solutions is obtained, another 2ml of distil water were added. The solution was thoroughly mixed for homogeneity using a vortex mixer, then each rat received 1µL/g B.W. of the resulting solution (Alharris et al., 2017; Cao et al., 2022). Two ml of DMSO (Hebei Guanlang Biotechnolies) were mixed with 2ml of distil water to be used in DMSO group where each rat received 1µL/g B.W.

Sample collection:

At the end of the experiment on the 60th day, the animals were anaesthetized by using ketamine (90 mg/kg body weight) and xylazine (40 mg/kg body weight). Post anesthesia, the bilateral testes, epididymis, and blood samples were collected for downstream analysis. The left tail of the epididymis was then rinsed and incubated in 2 ml of normal saline at 37°C. It was cut

using micro-scissors to extract the spermatozoa for further evaluation (Ngaha Njila et al., 2019; Al-Mousaw et al., 2022).

Fixation process for cytogenetic examination:

The fixation process involved placing a 10µl aliquot of the sperm sample on a clean microscope slide and smearing, allowing it to briefly air dry, and subsequently immersing the slide in a 3:1 solution of methanol and glacial acetic acid for 5 minutes. Following fixation, the slide was left to air dry completely. This meticulous procedure ensures the preservation of sperm morphology on the slide(Tejada et al., 1984).

Abnormal Sperm Chromatin Maturity test (ASCM)

Sperm smears were delicately soaked with 0.1N HCl at 5ºC for 5 minutes, then rinsed with distilled water. The smears were subjected to staining with 5% Toluidine Blue (TB) dye in a 50% citrate-phosphate buffer (pH=3.5). The evaluation process involved the enumeration of two hundred spermatozoa on each slide, using a light microscope with a magnification of ×1000. Sperms with unstained or lightly blue nuclei were categorized as normal sperm (TB−), while those exhibiting dark blue nuclei were deemed as abnormal (TB+). This meticulous methodology ensures a comprehensive assessment of sperm chromatin maturity, (Abbasi et al., 2011; Pourmasumi et al., 2019) Figure 1.

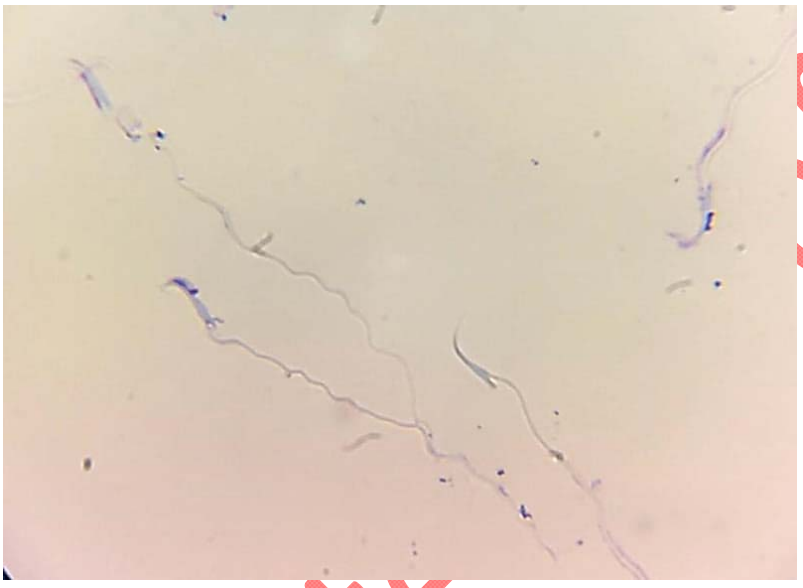

Figure 1: Assessment of Sperm Nuclear Chromatin Condensation using Aniline Blue Staining - A Comparative Analysis of Immature and Mature Sperm (400×).

Abnormal Sperm Chromatin Condensation test (ASCC)

Sperm nuclear chromatin condensation was evaluated by using aniline blue staining (Wong et al., 2008). In brief, after the fixation process, the slides were immersed in a 5% aniline blue (AB) solution in 4% acetic acid (pH 3.5) for 5 minutes. Subsequently, the slides were rinsed in distilled water, followed by staining in 0.5% eosin for 1 minute, and allowed to air-dry. The stained slides were then examined at 1000X magnification using a light microscope. Immature sperm, characterized by nuclear histone proteins, appeared dark blue, while mature sperm with

protamine exhibited a red-pink stain. A minimum of 100 spermatozoa per slide were counted to analyze the percentage of red-pink spermatozoa (Figure 2).

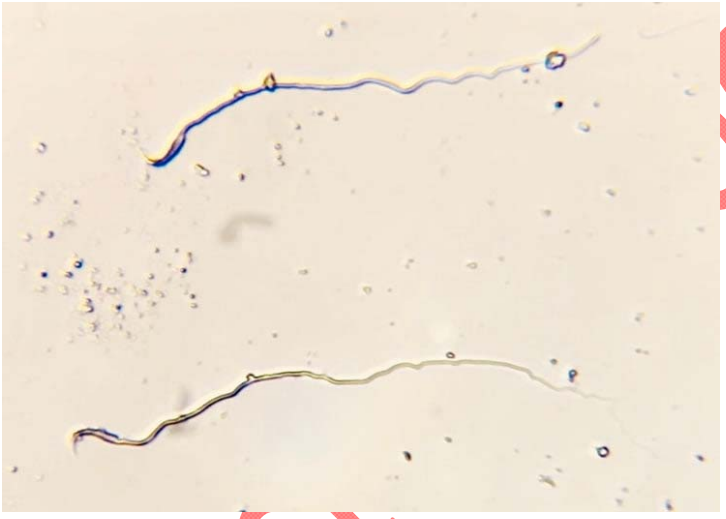

Figure 2: Assessment of Sperm Chromatin Maturity using Toluidine Blue Staining - A Comparative Analysis of TB- and TB+ Sperm (400X)

qRt-PCR assessment of Tjp1 gene expression

Gene expression analysis was conducted by first extracting RNA from testicular tissue stored at -80°C using triazole reagent. The extracted RNA was then reverse transcribed into cDNA using a One-Step RT-PCR Premix Kit. Custom-designed primers obtained from the Gene bank database were used for amplification of target genes. The qPCR was performed using SYBR Green master mix under specific thermocycler conditions mentioned in table 1 and 2. Real-time monitoring of the amplification process was achieved using a 7500 real-time PCR system, which

measured the fluorescence signal emitted by the SYBR Green dye throughout each amplification cycle.

Table 1: *Tjp1* and housekeeping gene *Gapdh* primers used in this study.

#	ID Gene	Reverse	Forward	NCBI Reference Sequence
1	*Tjp1*	5'- TCA CAG TGT GGC AAG CGT AG -3'	5'- TTC CAC AAG GAG CCA TTC CT -3'	NM_001106266.1
2	*Gapdh*	5'- ATG AAG GGG TCG TTG ATG GC -3'	5'- AGA GAC AGC CGC ATC TTC TT -3'	NM_017008.4

Table 2: Thermocycler running conditions.

#	Phase	Condition	Time	cycle
Holding	Holding stage 1	37°C	15 minutes	1
	Holding stage 2	95°C	10 minutes	1

PCR cycle	Denaturation	95°C	15 seconds	40
	Annealing	60°C	1 minute	
	Extension	72°C	30 seconds	
Melt curve stage	1st	95°C	15 seconds	1
	2nd	60°C	1 minute	1
	3rd	95°C	30 seconds	1

Blood-Testis Barrier test

To assess testes-barrier integrity, at the end of the 60-day experimental period, 5 animals from each groups were intravenously injected with 0.2 ml of 1% Evans blue in saline via the tail vein. Evans blue upon intravascular injection potently binds to albumin, facilitating the study of vascular permeability and albumin leakage in various organs, including the testes (Alves-Lopes et al., 2018; Belvitch and Dudek, 2018; Domínguez-Salazar et al., 2020). Following a 30-minute interval, the animals were euthanized, and the testes were extracted and incubated in formamide solution for 24 hours at 37°C in water bath. Estimation of extravasated Evans blue levels was done by using a spectrophotometer absorbance at 620 nm and plotted the results on slope equation after measuring standards prepared for this purpose (Alves-Lopes et al., 2018).

Statistical analysis

GraphPad Prism 9.1 version for Windows (GraphPad Software, Boston, Massachusetts USA, www.graphpad.com) was employed to analyze the collected data, utilizing the One-Way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test and corrected by Holm-Sidak correction for multiple test comparison. $P<0.05$ considered as a significant threshold.

Results

Abnormal Sperm Chromatin Maturity (ASCM)

Analyzed data of spermatozoa in this study revealed that the percentage of mature chromatin in spermatozoa of AhR^{-} group has been significantly ($P<0.05$) increased in comparison with all other study groups. However, administration of resveratrol to RES group has driven the percentage of mature chromatin to be at lowest level but insignificant in comparison with Control and DMSO groups (Figure 3).

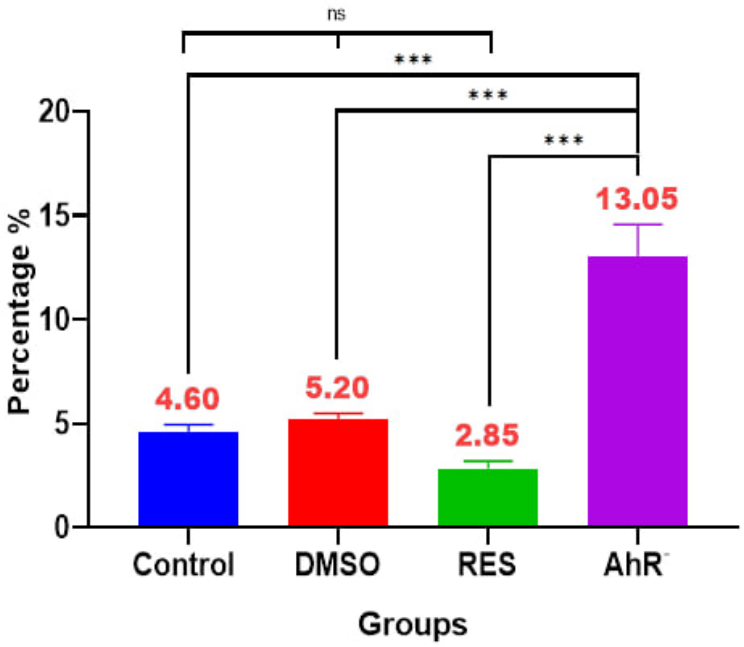

Figure (3): Sperm Chromatin Maturity
- Values are expressed as the means, SEM.
- DMSO: Administered dimethyl sulfoxide intraperitoneally twice weekly for 60 days.
- RES: Receiving 100mg/kg resveratrol intraperitoneally twice weekly for 60 days.
- AhR⁻: Provided with CH223191, intraperitoneally at 10 mg/kg twice weekly for 60 days.
- *: Denotes differences between groups, * for $p<0.05$, ** for $p<0.01$, and *** for $p<0.001$.
- ns: Denotes note significant different between groups

Abnormal Sperm Chromatin Condensation (ASCC)

Abnormal sperm chromatin condensation (ASCC) is one of the important quality tests associated with the ability of sperm in fertility capacity. Aniline blue dye is a traditional method to estimate ASCC. In this study, the collected data revealed that abolishing AhR pathway by using CH223191 led to significant ($P<0.05$) increase of percentage of ASCC in AhR⁻ group in

comparison with other groups. In contrast, resveratrol treatment demonstrated lowest value of ASCC in RES among all study groups (Figure 4).

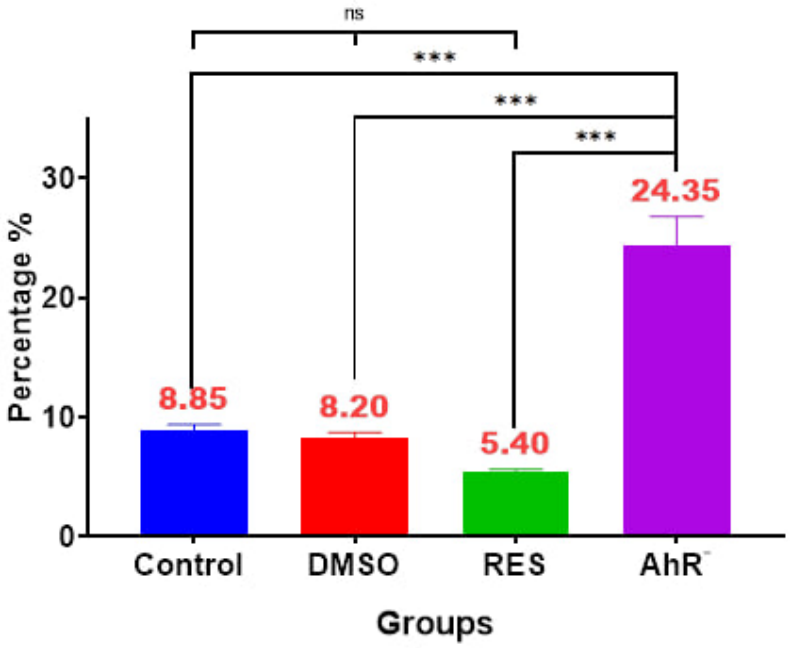

Figure (4): Sperm Nuclear Chromatin Condensation
- Values are expressed as the means, SEM.
- DMSO: Administered dimethyl sulfoxide intraperitoneally twice weekly for 60 days.
- RES: Receiving 100mg/kg resveratrol intraperitoneally twice weekly for 60 days.
- AhR⁻: Provided with CH223191, intraperitoneally at 10 mg/kg twice weekly for 60 days.
- *: Denotes differences between groups, * for $p<0.05$, ** for $p<0.01$, and *** for $p<0.001$.
- ns: Denotes note significant different between groups

Tight junction protein-1 (Tjp1) gene expression in testes:

Taking testicular tissue for studying this gene expression revealed that inhibiting AhR pathway led to significant (P<0.05) reduction in the mRNA expression of this protein in AhR⁻ group (Figure 5). In contrast, activation of AhR by using resveratrol has significantly (P<0.05) boosted the gene expression in RES group in comparison to control group as well as AhR⁻ group (Figure 5).

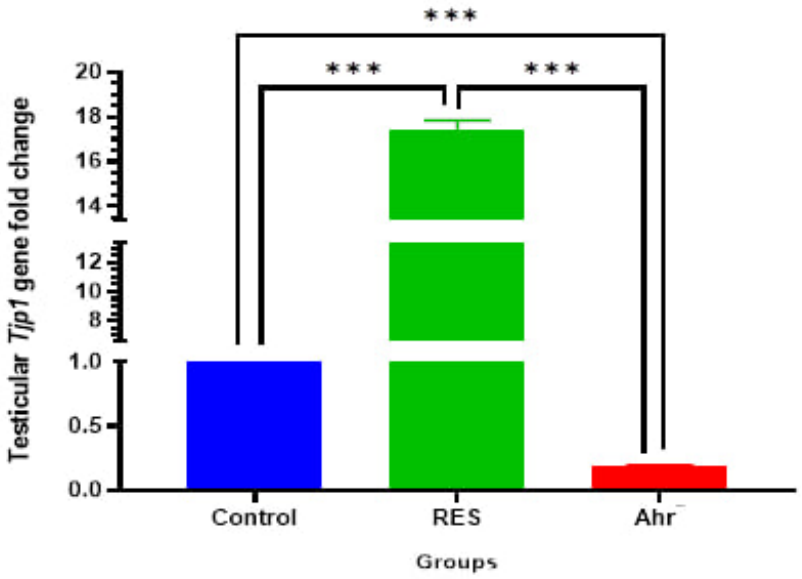

Figure (5): Fold Change Analysis of Tjp1 Gene Expression in Response to Resveratrol and Ahr Antagonist Treatments

- Values are expressed as the means, SEM.
- RES: Receiving 100mg/kg resveratrol intraperitoneally twice weekly for 60 days.
- AhR⁻: Provided with CH223191, intraperitoneally at 10 mg/kg twice weekly for 60 days.
- *: Denotes differences between groups, * for p<0.05, ** for p<0.01, and *** for p<0.001.
- ns: Denotes note significant different between groups

Blood-Testis Barrier

In the assessment of the Blood-Testis Barrier (BTB) using the Evans blue test (Figure 6), distinct variations were observed among the experimental groups. Blocking AhR signals in AhR^{-} group has significantly ($P<0.05$) elevated the extravasated Evans blue dye to the testicular tissue in comparison with control and RES groups (Figure 6). Remarkably, DMSO administration led to significant ($P<0.05$) increase in amount of leaked Evans blue dye in DMSO group in comparison with the control and RES groups (Figure 6).

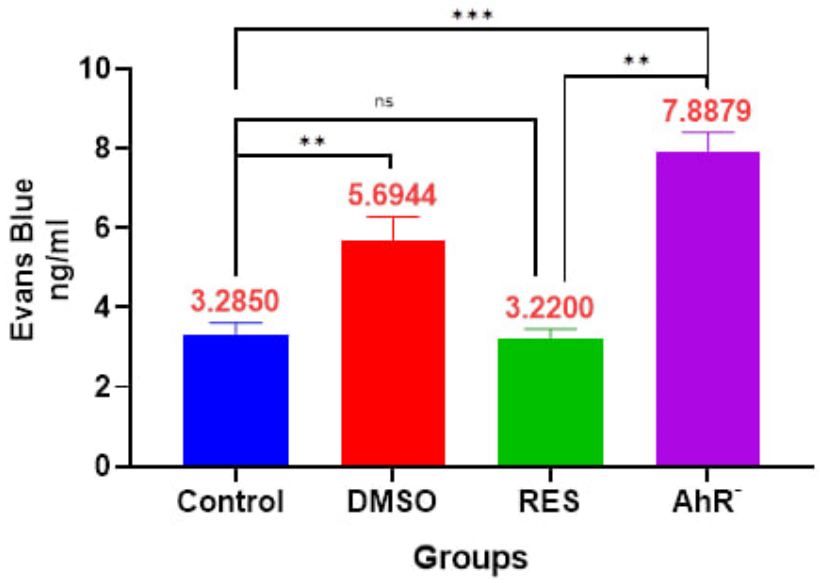

Figure 6: Blood-Testis Barrierr
- Values are expressed as the means, SEM.
- DMSO: Administered dimethyl sulfoxide intraperitoneally twice weekly for 60 days.

- RES: Receiving 100mg/kg resveratrol intraperitoneally twice weekly for 60 days.
- AhR⁻: Provided with CH223191, intraperitoneally at 10 mg/kg twice weekly for 60 days.
- *: Denotes differences between groups, * for $p<0.05$, ** for $p<0.01$, and *** for $p<0.001$.
- ns: Denotes note significant different between groups

Discussion

In this study we examined the effect of dysregulated AhR signaling on the sperm integrity through its genetical components by using specific stains namely Toluidine blue and Aniline Blue shown in figures 3 and 4, respectively. The Sperm chromatin maturity reflects the level of compaction and condensation of the genetic material within the sperm nucleus. In sperm nuclei with loosely packed chromatin or damaged DNA, the phosphate residues of the DNA are more exposed and accessible to Toluidine Blue dye, and a metachromatic alteration occurs. Generally, chromatin condensation occurs during the final stages of spermatogenesis. Histones pack DNA in the cell nucleus by protamines that results in tighter DNA packaging and increased stability of the sperm nucleus. Moreover, abnormalities in chromatin condensation led to DNA damage (Pourmasumi *et al.*, 2019; Sharma & Agarwal, 2018; Zini *et al.*, 2014). The Toluidine Blue test results presented in Figure 3 provide insights into sperm chromatin maturity in which the RES group showed improved sperm chromatin maturity percentage compared to control and DMSO groups but with insignificant differences. In contrast, the AhR⁻ group exhibited significant high values in comparison to all other groups, suggesting significant compromised sperm chromatin maturity which will impair the sperm fertility capacity (Kim et al., 2013). The results of the current study are in accordance with previous studies which examined the role of AhR on the sperm chromatin maturity (Karman *et al.*, 2012). The previous study showed that

sperm of AhR-null mice undergo apoptosis, and the cells also showed an early onset of chromatin condensation, nuclear fragmentation, and DNA laddering together with very low levels of DNA synthesis (Karman *et al.,* 2012). These findings collectively underscore the intricate role of AhR in governing not only sperm DNA fragmentation but also sperm nuclear chromatin condensation and chromatin maturity (Bustani et al., 2024). Furthermore, previous studies illustrated the expression of genes involved in sperm chromatin condensation was adversely affected by loss of AhR as shown in AhR−/− mice via different target genes such as *Prm1*, *Prm2*, and *Hsp70a* which play critical roles in maturity of germ cell and replace histones throughout the late phase of spermatogenesis and are responsible for sperm head condensation (Hansen *et al.,* 2014b).

From other side, we have examined the effects of dysregulated AhR signaling on the soundness of seminiferous tubule barrier through its tight junction protein gene expression particularly *Tjp1* (Figure 5) supported by the ability of this barrier to avoid extravasation of Evans blue dye from the circulatory blood into testicular tissue (Figure 6). Tight junction protein-1 (TJP1) is integral to the formation and maintenance of the tight junctions in the seminiferous epithelium. A previous study reported that germ cell impairment occurs when the disruption of *TJP1's* is taken place indicating potential impact on BTB integrity which in turn will influence the overall composition of the testicular tissue (Noguchi *et al.,* 2021). In this study, the resveratrol treatment led to significant increase in *Tjp1* expression (Figure 5) which resulted in empowering the BTB of the same treated group (Figure 6). These findings in agreement with a previous study that found resveratrol sustains intestinal barrier integrity via upregulation of *Tjp1* expression, improves antioxidant capacity, and alleviates inflammation

(Yang *et al.,* 2021). Conversely, the AhR^{-} group displays a significant reduction in *Tjp1* expression indicating a disruption in the barrier function of testis. These findings emphasize the potential regulatory role of RES in enhancing the expression of genes associated with sperm development and integrity that agree with previous studies that suggest the sustained normalcy of the BTB hinges primarily on the expression levels of *Tjp1* which in the absence of these proteins, there is a perpetual augmentation of the body's autoimmune response to spermatogenesis, inevitably leading to a diminished efficiency of this crucial reproductive process (Ghafouri-Fard *et al.,* 2021; Koşal et al., 2024; Yan *et al.,* 2024). The results depicted in Figure 6, highlighting the BTB, unveil crucial insights into its role and potential implications. The BTB has a pivotal role in the intricate process of spermatogenesis, serving as a robust physical impediment that separates blood vessels from seminiferous tubules. Formed by tight junctions among Sertoli cells, the BTB meticulously governs the developmental milieu for germ cells, offering protection against toxins and upholding a distinctive chemical composition within the seminiferous tubules' lumen. Essential for spermatogenesis, any disruption to the BTB poses a potential threat to fertility (Yan *et al.,* 2024). The BTB's physical structure demarcating the seminiferous epithelium into basal and abluminal compartments, distinguishes it as one of the most impermeable tissue barriers. Beyond its anatomical function, the BTB also serves as an immunological barrier, intricately shaping the physiological environment for spermatogenesis by sequestering germ cells and lymphatic systems while concurrently implementing local immune suppression (Noguchi *et al.,* 2021; Barfourooshi et al., 2023). The result of RES group showed significant increase of *Tjp1* gene expression (Figure 5) along with reduction of leaky Evans blue dye (Figure 6) in the testicular tissue in comparison with the other study groups which aligns

with prior study that demonstrated the BTB's pivotal role in safeguarding germ cells and shaping the intricate milieu necessary for successful sperm development (Yan *et al.,* 2024). Conversely, the AhR^{-} group displays a significant reduction in BTB integrity that indicated the negative effect of decreased *Tjp1* expression by antagonist on the BTB.

Conclusion

In conclusion, our study highlights the pivotal role of Aryl Hydrocarbon Receptor (AhR) activation in male reproductive health, particularly in sperm chromatin maturity and Blood-Testis Barrier (BTB) integrity. Resveratrol treatment showed promising results in improving sperm chromatin maturity, *Tjp1*expression, and BTB integrity, suggesting its potential as a therapeutic agent for male infertility. Conversely, AhR antagonism led to compromised sperm chromatin integrity and BTB function, emphasizing the importance of AhR modulation in spermatogenesis. These findings provide valuable insights into potential therapeutic interventions for male reproductive issues.

Author Contributions

Conceptualization, GB and HA Investigation, GB and HA; Writing—original draft preparation, GB and HA; Writing—review and editing, GB and HA; Visualization, GB and AM; Supervision, HA. All authors have read and agreed to the current version of the manuscript.

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