Advances in Reproductive Sciences, 2025, 13(1), 49-62
https://www.scirp.org/journal/arsci
ISSN Online: 2330-0752
ISSN Print: 2330-0744

Mechanism of Hypoxia-Inducible Factor 1 Alpha Regulation of Apoptosis in Human Spermatozoa

Xinge Liu[1], Linfeng Mo[2], Yinjia Zhang[1], Liangzhao Liu[1], Yan Zhang[3], Yonghua He[1*], Mingxue Zhang[2], Lishan Tang[2]

[1]School of Public Health, Guilin Medical University, Guilin, China
[2]Medicine and Health Science College, Guangzhou Huashang Vocational College, Guangzhou, China
[3]Health Monitoring Section, Center for Disease Control and Prevention of Zhenhai District, Ningbo, China
Email: *hyhup@shmu.edu.cn

How to cite this paper: Liu, X.G., Mo, L.F., Zhang, Y.J., Liu, L.Z., Zhang, Y., He, Y.H., Zhang, M.X. and Tang, L.S. (2025) Mechanism of Hypoxia-Inducible Factor 1 Alpha Regulation of Apoptosis in Human Spermatozoa. *Advances in Reproductive Sciences*, **13**, 49-62.
https://doi.org/10.4236/arsci.2025.131005

Received: January 12, 2025
Accepted: February 16, 2025
Published: February 19, 2025

Abstract

Objective: To investigate the specific mechanism of hypoxia-inducible factor 1 alpha (HIF-1α) in the regulation of human sperm apoptosis, and to provide a new theoretical reference and scientific basis for the diagnosis and treatment of asthenospermia and other related conditions. Methods: Semen samples were categorized into the normal group and asthenospermia group based on sperm motility criteria. HIF-1α interfering agent cobalt chloride ($CoCl_2$) and guanylate cyclase activator (Lificiguat, YC-1) were added respectively, with a control group established accordingly. Sperm motility (using anterior viability rate as an index), apoptosis level, ATP level, mitochondrial membrane potential, and reactive oxygen species (ROS) level were measured. The expression levels of HIF-1α, p-PI3K, and Bcl-2 in the samples were analyzed using Western blotting. Results: Following $CoCl_2$ treatment, there was a significant increase in sperm apoptosis compared to the normal control group (12.51% ± 2.50% VS 11.15% ± 2.42%); additionally, sperm motility (45.34% ± 3.37% VS 51.36% ± 11.68%), ATP production (11.51 ± 2.87 nM/μL VS 14.99 ± 2.83 nM/μL), ROS levels, and mitochondrial membrane potential all decreased significantly (all $P < 0.05$). Furthermore, the expression levels of HIF-1α and p-PI3K increased significantly while Bcl-2 expression decreased (all $P < 0.05$). Conversely, following YC-1 treatment there was a significant decrease in sperm apoptosis compared to the asthenospermia control group (8.59% ± 2.86% VS 9.37% ± 3.07%); along with this change came significant increases in sperm motility (38.51% ± 5.56% VS 21.86% ± 16.43%), ATP production (13.13 ± 4.01 nM/μL VS 11.05 ± 3.67 nM/μL) and ROS levels, decrease in mitochondrial membrane potential levels (all $P < 0.05$). Moreover, compared with the control group, the expression levels of p-PI3K and HIF-1α in the YC-

1 treatment group were decreased, and the expression level of Bcl-2 was increased (all $P < 0.05$). Conclusion: HIF-1α can influence human sperm apoptosis and motility through the PI3K signaling pathway.

Keywords

Hypoxia Inducible Factor-1α, Apoptosis, Sperm Motility, Asthenospermia

1. Introduction

In recent years, the prevalence of infertility among couples has escalated globally, attributable to a myriad of factors—including environmental pollution, psychosocial stress, and suboptimal dietary patterns [1]. Current estimates indicate that over 10% of couples worldwide are impacted by infertility, with roughly half of these cases being associated with male factors [2]. Among the various etiologies of male infertility, asthenospermia—characterized by a progressive motility of spermatozoa below 32%—is one of the most prevalent [3].

Hypoxia-inducible factors (HIF) are pivotal oxygen-sensitive transcriptional regulators involved in cellular responses to hypoxic conditions [4]. Under normoxic conditions and in the absence of other metabolic disturbances, HIF-1α levels are exceedingly low [5]. However, hypoxic conditions can trigger its activation, which may lead to apoptotic pathways and subsequent cell death [6]. HIFs are instrumental in numerous physiological and pathological processes, particularly during spermatogenesis, where HIF-1α has been shown to expedite apoptotic mechanisms under low oxygen conditions [7]. It has been found that hypoxia may have adverse consequences on male reproductive health, including spermatogenic dysfunction, decreased semen quality, and reproductive hormone disorders, and that possible mechanisms include oxidative stress damage, inflammation, sperm mitochondrial dysfunction, sperm cell apoptosis, and aberrant proteins and signal transduction pathways [8]-[10].

In recent years, some progress has been made in the etiology, diagnosis and treatment of oligozoospermia but the exact pathogenesis needs to be studied in depth. Few previous studies have been conducted on the use of experimental subjects for interventions to explore the mechanisms of sperm apoptosis in oligozoospermia. The present study focuses on human spermatozoa to elucidate the role of HIF-1α in modulating sperm motility and apoptosis. By administering HIF-interfering agents within the sperm culture medium, the researchers aim to investigate alterations in sperm motility and apoptotic incidence, thereby uncovering the mechanisms governing HIF-1α-mediated apoptosis in human sperm. This study aspires to contribute novel insights that may enhance the diagnostic approach to asthenospermia.

2. Materials and Methods

2.1. Study Population

A total of 265 male subjects who underwent physical examinations at the

Reproductive Medicine Center of the Affiliated Hospital of Guilin Medical University between November 2022 and May 2023 were included in this study. This project was approved by the Medical Ethics Committee of the Affiliated Hospital of Guilin Medical University (approval number: QTLL202129). Informed written consent was obtained from all participants.

Inclusion Criteria: Participants aged between 18 and 45 years were eligible if they presented with a semen volume of at least 1.5 mL, alongside the absence of severe manifestations of azoospermia or necrospermia, and no indications of cryptozoospermia.

Exclusion Criteria: Individuals were excluded from the study if they had a history of infectious diseases, including but not limited to hepatitis, tuberculosis, or syphilis; a documented history of urogenital tract infections; significant comorbidities affecting major organ systems, such as the cardiovascular or renal systems; or if they demonstrated an inability to comply with the study protocols.

2.2. Methods and Observational Indicators

2.2.1. Sample Collection and Processing

Following a 3 - 7 day period of sexual abstinence, study participants provided semen samples in non-toxic containers at the Reproductive Medicine Center. The samples were subsequently incubated in a 37˚C thermostatic water bath for 30 minutes to ensure complete liquefaction. After liquefaction, 1 mL of each sample was transferred to an EP tube for further analysis.

Participants with total sperm motility (PR + NP) ≥ 40% and progressive motility (PR) ≥ 32% were categorized into the normal motility group. Conversely, those with PR < 32% were classified as the weak spermatozoa group.

For the experimental setup, 200 μL of semen from the normal motility group was treated with 100 μM cobalt chloride ($CoCl_2$, Mackin, China) in an EP tube for 40 minutes, while another 200 μL of semen from the same group served as the control. Similarly, 200 μL of semen from the weak spermatozoa group was treated with 5000 μM YC-1 (Selleck, USA) in an EP tube for 60 minutes, with another 200 μL serving as the control. Regarding the selection of specific concentrations of $CoCl_2$ and YC-1, we explored them in a pre-experiment and took the doses with appropriate differences in relevant parameters between groups.

2.2.2. Sperm Motility Evaluation

Sperm motility was assessed utilizing a computer-assisted sperm analysis system (CASA, ZJ-3000E, Xuzhou, China), permitting the evaluation of sperm motility parameters through PR.

2.2.3. Sperm Apoptosis Detection

The level of sperm apoptosis was determined using an Annexin V-FITC apoptosis detection kit (Beyotime, China). Firstly, a 200-microliter aliquot of the sample was processed by washing it three times with phosphate-buffered saline (PBS). Following centrifugation at 1000 g for 5 minutes, the supernatant was discarded.

Subsequently, 195 microliters of Annexin V-FITC conjugate was added to the post-wash suspension, and after resuspending the cells, 5 microliters of Annexin V-FITC was introduced and thoroughly mixed. Additionally, 10 microliters of propidium iodide (PI) staining solution was added and mixed well. After incubating the mixture for 10 minutes at room temperature in the dark, apoptotic cells were enumerated using a multifunctional enzyme marker (Thermo Fisher, USA), maintaining the samples in darkness throughout the process.

2.2.4. Sperm ATP Level Assay

To initiate the procedure, 800 μL of pre-warmed human fallopian tube fluid (HTF, MCE, USA) was added to an EP tube. Subsequently, 200 μL of the sample was aspirated, tilted at a 45-degree angle, and incubated at room temperature for 1 hour. Following incubation, the supernatant was carefully aspirated from the tube and subjected to centrifugation at 2000 rpm for 5 minutes to eliminate excess liquid. The resulting cells were collected and washed with an appropriate volume of PBS that had been frozen. The cell pellet was then resuspended in 100 μL of ATP assay lysate (Beyotime, China). Using a cryo-centrifuge, the suspension was centrifuged at 4˚C at 12,000 g for 5 minutes. The supernatant was subsequently collected and transferred to a new tube, maintained on ice, and evaluated for ATP levels using a multifunctional enzyme marker within 30 minutes of preparation.

2.2.5. Measurement of the Mitochondrial Membrane Potential (MMP) in Spermatozoa

The mitochondrial membrane potential (MMP) of sperm was determined using the MMP detection kit (Beyotime, China) with the JC-1 method. 200ul of semen sample was resuspended in 0.5mL of cell culture medium, followed by adding 0.5 mL of JC-1 staining working solution and mixing, and then incubated in a cell culture incubator at 37˚C for 20min. An appropriate amount of JC-1 staining buffer (1×) was prepared according to the ratio of adding 4 mL of distilled water for every 1 mL of JC-1 staining buffer (5×) and placed in an ice bath. When the incubation was finished, centrifuge at 600g 4˚C for 3-4min, discard the supernatant, then wash with JC-1 staining buffer (1×) twice, remove the supernatant, and re-suspend the appropriate amount of JC-1 staining buffer (1×) and analyze it by using the enzyme marker.

2.2.6. Measurement of the Reactive Oxygen Species (ROS)

A fluorescent probe, DCFH-DA, from the ROS detection kit (Beyotime, China), was employed to measure ROS levels in sperm. Sample sperm concentration was adjusted to 10^6~2×10^7/mL and centrifuged at 1500 rpm for 3 min. Subsequently, DCFH-DA was diluted to 10 uM/L with PBS, and the cells were suspended in diluted DCFH-DA and incubated in an incubator at 37˚C for 20 min. After washing with PBS three times, the cell precipitates were collected and resuspended in PBS. The fluorescence intensity was measured at an excitation wavelength of 488 nM and an emission wavelength of 525 nM using an enzyme marker.

2.2.7. Western Blot (WB) to Detect the Expression Level of Relevant Proteins

Total protein was extracted from the cells, and the concentration was quantified using the BCA method. Subsequently, SDS-PAGE was utilized to separate the extracted proteins. Following electrophoresis, the proteins were transferred to a membrane and subjected to blocking to prevent nonspecific binding. The primary antibody was diluted in blocking buffer, achieving a final concentration of 1:1000 for the internal reference antibody, and was incubated either for 1.5 hours at room temperature or overnight at 4˚C. After thorough washing of the membrane, the secondary antibody was diluted in blocking buffer to a concentration of 1:1000 and incubated for 1 hour. Eventually, the membrane was developed, exposed, and the resultant protein density values were analyzed.

2.3. Statistical Analyses

Statistical analysis of the data was performed using SPSS16.0 (SPSS Inc., Chicago, IL, USA). Categorical data were expressed as rate or percentage (%), and the chi-squared test was used to test the difference between groups; the measurement data conforming to normal distribution were expressed as mean ± standard deviation. The paired-sample t-test was used for comparison between matched groups, and the independent-sample t-test was used for pairwise comparison between unmatched groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Baseline Data of Participants

The mean age of the study participants was 34.19 ± 5.55 years, with an average abstinence duration of 4.23 ± 1.32 days. Notably, the mean sperm concentration, total sperm motility, and PR were found to be significantly lower in the weak spermatozoa group compared to the normal group ($P < 0.05$). Additionally, no significant differences were observed between the two groups concerning body mass index (BMI), smoking prevalence, or alcohol consumption rates ($P > 0.05$) (Table 1).

Table 1. General information about the subjects.

Project	Normal group (n = 157)	Asthenospermia group (n = 108)	t/χ^2	P
Average age (years)	33.12 ± 5.50	35.15 ± 5.55	−1.923	0.061
Average days of abstinence (days)	4.20 ± 1.44	4.15 ± 1.23	0.921	0.357
Average sperm concentration ($\times 10^6$/mL)	114.37 ± 98.54	83.45 ± 70.20	3.213	<0.05
Average total sperm motility (%)	73.52 ± 11.49	36.24 ± 12.29	28.156	<0.05
Average progressive motility rate (%)	55.76 ± 13.00	19.33 ± 8.01	28.571	<0.05
Body mass index (kg/m^2)	23.54 ± 1.40	23.71 ± 1.16	−0.435	0.630
Smoke (%)	38.49	35.96	0.121	0.728
Drink (%)	45.35	44.95	0.001	0.981

3.2. Effect of HIF Interfering Agents on Sperm Motility

Sperm motility was determined after treatment with HIF inhibitors. The results showed that the $CoCl_2$ group had significantly lower sperm motility than the normal control group ($P < 0.05$), and the YC-1 group had significantly higher sperm motility than the asthenospermia control group ($P < 0.05$) (Table 2).

Table 2. Sperm motility in the experimental and control groups.

Group	Cases	Sperm motility (%)	t	P
Normal sperm motility control group	37	51.36 ± 11.68	2.188	0.033
Normal sperm motility + $CoCl_2$ treated group	19	45.34 ± 3.37		
Asthenospermia control group	15	21.86 ± 16.43	−4.504	<0.001
Asthenospermia group + YC-1 treated group	34	38.51 ± 5.56		

3.3. Effect of HIF Interfering Agents on Sperm Apoptosis

The apoptosis level of the samples was determined after the experimental group was treated with the HIF inhibitor. The results showed that the $CoCl_2$ group had a significantly higher level of sperm apoptosis than the control group ($P < 0.05$), and the YC-1 group had a significantly lower level of sperm apoptosis than the control group ($P < 0.05$) (Table 3).

Table 3. Sperm apoptosis rate in experimental and control groups.

Group	Cases	Apoptosis rate (%)	t	P
Normal sperm motility control group	26	11.15 ± 2.42	10.199	<0.001
Normal sperm motility + $CoCl_2$ treated group	26	12.51 ± 2.50		
Asthenospermia control group	22	9.37 ± 3.07	10.159	<0.001
Asthenospermia group + YC-1 treated group	22	8.59 ± 2.86		

3.4. Effect of HIF Inhibitors on Sperm

Following the treatment of the experimental group with the HIF inhibitor, the ATP levels in the samples were measured. The results indicated that the ATP level in the $CoCl_2$ group was significantly lower than that in the control group, while the ATP level in the YC-1 group was significantly higher than that in the control group ($P < 0.05$) (Table 4).

3.5. Effect of HIF Interfering Agents on Sperm Membrane Potentials

Following the treatment of the experimental group with a HIF interfering agent, the sperm membrane potential was measured. The results indicated that the sperm membrane potential in the $CoCl_2$ group was significantly lower than that in the control group, and similarly, the YC-1 group exhibited a significantly lower

sperm membrane potential compared to the control group (P < 0.05) (Table 5).

Table 4. Sperm ATP levels in experimental and control groups.

Group	Cases	ATP level (nM/μL)	t	P
Normal sperm motility control group	29	14.99 ± 2.83	11.797	<0.001
Normal sperm motility + $CoCl_2$ treated group	29	11.51 ± 2.87		
Asthenospermia control group	24	11.05 ± 3.67	11.221	<0.001
Asthenospermia group + YC-1 treated group	24	13.13 ± 4.01		

Table 5. Sperm mitochondrial membrane potential levels in experimental and control groups.

Group	Cases	Fluorescence intensity	t	P
Normal sperm motility control group	27	4.29 ± 0.85	17.007	<0.001
Normal sperm motility + $CoCl_2$ treated group	27	3.26 ± 0.72		
Asthenospermia control group	11	4.14 ± 0.97	2.934	0.016
Asthenospermia group + YC-1 treated group	11	3.72 ± 0.94		

3.6. Effect of HIF Interfering Agents on Sperm ROS

Following the treatment of the experimental group with the HIF inhibitor, the ROS levels were assessed in the samples. The ROS levels in the $CoCl_2$ group were significantly lower compared to the control group, whereas the ROS levels in the YC-1 group were markedly higher than those in the control group. These differences were statistically significant (P < 0.05) (Table 6).

Table 6. Sperm ROS levels in experimental and control groups.

Group	Cases	Fluorescence intensity	t	P
Normal sperm motility control group	38	5.51 ± 4.45	2.885	0.004
Normal sperm motility + $CoCl_2$ treated group	38	5.06 ± 4.16		
Asthenospermia control group	17	5.92 ± 5.86	2.249	0.025
Asthenospermia group + YC-1 treated group	17	6.13 ± 5.97		

3.7. Expression of Hif-1α, p-PI3K and Bcl-2 in Sperm Treated with HIF Interfering Agents

Following the treatment with the HIF inhibitor, the expression levels of HIF-1α, p-PI3K, and Bcl-2 proteins in the PI3K pathway were quantified in spermatozoa (Figure 1(a), Figure 1(b)). The results demonstrated that, compared to the control group with normal motility, the $CoCl_2$-treated group exhibited significantly increased expression levels of both p-PI3K and HIF-1α, while the expression level of Bcl-2 was significantly decreased (P < 0.05) (Figure 2(a)). In contrast, the YC-1-

treated group showed significantly decreased expression levels of both p-PI3K and HIF-1α, whereas the expression level of Bcl-2 was significantly increased compared to the control group with impaired spermatogenesis ($P < 0.05$) (Figure 2(b)).

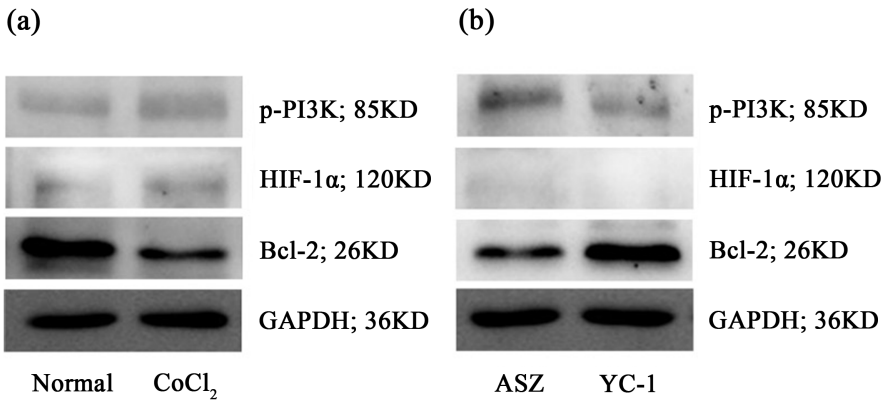

Figure 1. The expression levels of p-PI3K, HIF-1α and Bcl-2 ((a) $CoCl_2$ experimental group and control group; (b) YC-1 experimental group and control group; GAPDH protein expression was used as a control).

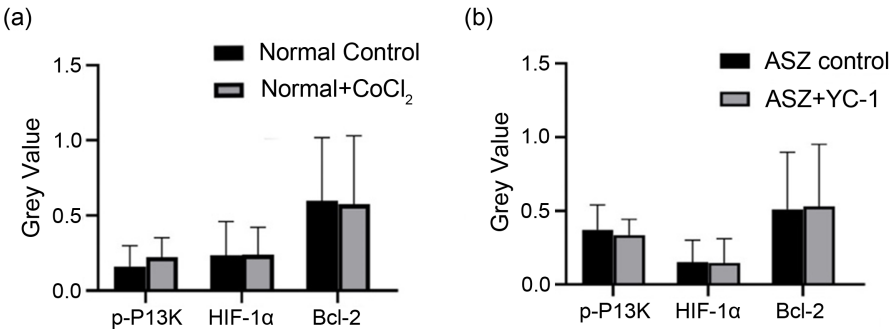

Figure 2. Changes in p-PI3K, HIF-1α and Bcl-2 protein levels in sperm ((a) Comparison between the group with normal sperm motility and the $CoCl_2$-treated group; (b) Comparison of asthenospermia group and YC-1 treated group).

4. Discussion

Research indicates that hypoxia can lead to a significant decline in sperm count, a phenomenon that may be associated with increased apoptosis of germ cells [10]. HIF-1, a critical transcription factor involved in cellular adaptation to hypoxic conditions, was initially identified in 1992 during genetic studies of erythropoietin (EPO) [11]. HIF-1α is the key functional subunit of HIF-1, which exhibited elevated expression levels in hypoxic tissues, a finding that has been corroborated by preceding studies [12]. Under hypoxic conditions, HIF-1 plays a crucial role in modulating cellular metabolism by facilitating the shift from aerobic to anaerobic pathways [13]. This metabolic transition not only decreases mitochondrial oxygen consumption but also sustains cellular energy metabolism under low-oxygen conditions [11] [14].

In this study, two experimental strategies were adopted to thoroughly investigate the effect of HIF-1α on sperm motility under hypoxic conditions. Firstly,

$CoCl_2$ was added to the normal sperm motility group to simulate the hypoxic environment, and then the changes in sperm motility and apoptosis were observed when HIF-1*α* expression was up-regulated. Secondly, the HIF inhibitor YC-1 was added to the weak spermatozoa group to inhibit the expression of HIF-1*α*, and the specific effect of HIF-1*α* on sperm motility and its potential mechanism was further analyzed by comparing with the control group. The results showed that the over-activation of HIF-1*α* was significantly associated with decreased sperm motility and increased sperm apoptosis.

4.1. HIF-1*α* Levels Affect Apoptosis and Motility of Human Spermatocytes

As the core of cellular metabolism, mitochondria not only provide cells with critical energy and material support by synthesising ATP but also consume oxygen in a significant way [15]. Furthermore, mitochondria are pivotal in the regulation of apoptosis [16]. Disruption of mitochondrial function may result in diminished ATP synthesis, consequently engendering insufficient cellular energy and ultimately promoting cell death. In mature spermatocytes, ATP is chiefly produced by mitochondria, and its abnormal production may directly induce mitochondrial apoptosis [17]. Thus, HIF-1*α* appears to play a critical role in hypoxia-induced mitochondrial apoptosis. Our study showed that cobalt chloride treatment resulted in a significant reduction in ATP levels in spermatozoa compared to the control group. Conversely, treatment with YC-1 was associated with an upward trend in ATP levels within the spermatozoa. These findings suggest that excessive activation of HIF-1*α* may hinder sperm energy production, thereby contributing to an adverse effect on sperm cell apoptosis.

MMP has been identified as a critical mechanism that contributes to the maintenance of normal mitochondrial physiological function. Its stability exerts a substantial influence on mitochondrial function and the process of apoptosis [18]. Research shows that HIF-1*α* could affect mitochondrial function and apoptotic process by regulating MMP [19]. It is widely accepted that alterations in the permeability of the inner and outer mitochondrial membranes act as a catalyst for a series of reactions, ultimately resulting in the process of apoptosis. In conditions of hypoxia, there is an increased expression level of HIF-1*α*, which has been shown to lead to an increase in the concentration of sodium ions and a decrease in the concentration of potassium ions. This, in turn, causes a change in the membrane potential, thus disrupting the ionic balance between the interior and exterior of the cell and consequently triggering apoptosis [20]. Mitochondria are also a major source of intracellular ROS in hypoxic environments. Such conditions are frequently characterized by an overproduction of ROS, which subsequently induces oxidative stress [21]. However, studies have shown that in hypoxia, the increased stability of the HIF-1*α* subunit promotes its translocation to the nucleus, where it facilitates the formation of heterodimers and activates downstream gene expression pathways. This process consequently reduces oxygen consumption, minimises

ROS production, and restores oxygen delivery [22]. Besides, the primary role of HIF-1α in mediating mitochondrial adaptation is to downregulate the activity of these organelles, thereby preventing excessive ROS generation [19].

In this study, the group treated with the HIF-1α promoter exhibited a reduction in ROS levels compared to the control group, while the YC-1 experimental group demonstrated an increase in ROS levels. These results indicate that HIF-1α may have the dual capacity to diminish ROS production and modulate mitochondrial function, facilitating cellular adaptation to hypoxic conditions and enhancing the organelle's role in the adaptive response [23]. These findings offer new insights into the mechanistic actions of HIF-1α in response to hypoxia. In this study, the sperm membrane potential level in the YC-1 group was lower than that in the control group, possibly because the sperm of asthenospermia patients may contain a higher level of ROS, which causes apoptosis through the mitochondrial pathway, and destroys the mitochondrial membrane structure, showing decreased the sperm MMP levels [24]. Therefore, more in-depth exploration and research are needed.

4.2. Influence on Apoptosis and Motility of Human Spermatocytes through the PI3K Signalling Pathway

The key components of the PI3K signaling pathway include phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt), which play crucial roles in cell survival, growth, and apoptosis [25]. Previous studies have shown that the activation of the PI3K/Akt pathway significantly influences the expression of the downstream anti-apoptotic protein Bcl-2 [26]. The Bcl-2 protein family is integral to the regulation of apoptosis, comprising both pro-apoptotic and anti-apoptotic members [27]. Bcl-2 itself, as a member of this apoptosis-inhibiting protein family, is a fundamental component of the apoptotic regulatory network, wherein elevated levels typically suppress apoptotic processes. Notably, previous studies have identified a negative correlation between the expression of HIF-1α and Bcl-2 [28] [29]. In the present study, the cobalt chloride-treated group exhibited a significant decline in Bcl-2 expression compared to the motility-normal control group. In contrast, the YC-1-treated group showed an increase in Bcl-2 expression relative to the weak spermatogenesis control group. This observation may reflect the activation of a negative feedback mechanism in response to HIF-1α inhibition, which subsequently enhances the expression of the anti-apoptotic protein Bcl-2, thereby attenuating apoptosis.

The PI3K pathway also holds potential therapeutic promise for other diseases. Inhibition of PI3K signaling may be effective in treating many types of cancer [30]. PI3K also has a regulatory role in aging-related diseases. Studies have shown that the PI3K signaling pathway is involved in a variety of biological processes associated with aging, including epigenetic changes, telomere loss, mitochondrial dysfunction, and others. These studies have opened up new ways for the diagnosis and treatment of aging-related diseases [31]. The PI3K signaling pathway has an increasingly prominent role in the fibrosis process, and this pathway not only

regulates idiopathic pulmonary fibrosis (IPF) alone, but also interacts with many signaling pathways and is involved in multiple aspects of pathogenesis, which is potentially important for the development of new anti-fibrosis strategies [32].

In conclusion, our findings indicate that excessive activation of HIF-1α may lead to decreased sperm motility and increased apoptosis of sperm cells. This observation provides a novel perspective on the impact of hypoxic conditions on sperm motility and establishes a significant theoretical foundation for future intervention strategies targeting related pathologies. Nonetheless, the study has certain limitations. Firstly, due to the limited sample size, it was not possible to set up separate promoter and inhibitor groups in different populations for in-depth study. Secondly, the study exclusively analysed the expression levels of certain HIF-1α-related proteins, which precluded comprehensive elucidation of the underlying mechanism of action. Therefore, further research is necessary to elucidate the complex relationship between HIF-1α, sperm motility, and apoptosis, utilizing larger sample sizes and multi-omics approaches.

5. Conclusion

HIF-1α may modulate mitochondrial function through the PI3K signaling pathway, leading to a reduction in ATP synthesis, a decrease in ROS levels, and a modification of mitochondrial membrane potential. These alterations subsequently initiate the apoptotic cascade response.

Authors' Contributions

Yonghua He conceived and designed the study. Xinge Liu, Linfeng Mo and Yinjia Zhang recruited the participants, and analyzed the data. Liangzhao Liu and Yan Zhang collected data on the patients. Xinge Liu and Linfeng Mo wrote the manuscript. Lishan Tang, Mingxue Zhang and Yonghua He edited the manuscript and provided response to comments. Yonghua He, Lishan Tang and Mingxue Zhang supervised the study. All authors read and approved the final manuscript.

Acknowledgements

We would like to express our special thanks to Dr. Xuming Liang for his great coordination that allowed the project to proceed smoothly.

Funding Source

This study was supported by the Natural Science Foundation of Guangxi Autonomous Region (grant/award number: 2020JJD140016), and the Postgraduate Innovation Project of Guangxi Autonomous Region (grant/award number: YCSW2021245).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Harris, E. (2023) Infertility Affects 1 in 6 People Globally. *JAMA*, **329**, Article 1443. https://doi.org/10.1001/jama.2023.6251

[2] Eisenberg, M.L., Esteves, S.C., Lamb, D.J., Hotaling, J.M., Giwercman, A., Hwang, K., *et al*. (2023) Male Infertility. *Nature Reviews Disease Primers*, **9**, Article No. 49. https://doi.org/10.1038/s41572-023-00459-w

[3] Lin, Q., Ge, X., Gao, L., Chen, Y., Su, T., Ma, M., *et al*. (2024) Betaine Alleviates Spermatogenic Cells Apoptosis of Oligoasthenozoospermia Rat Model by Up-Regulating Methyltransferases and Affecting DNA Methylation. *Phytomedicine*, **129**, Article ID: 155713. https://doi.org/10.1016/j.phymed.2024.155713

[4] Aschner, M., Skalny, A.V., Lu, R., Santamaria, A., Zhou, J., Ke, T., *et al*. (2023) The Role of Hypoxia-Inducible Factor 1 *α* (HIF-1*α*) Modulation in Heavy Metal Toxicity. *Archives of Toxicology*, **97**, 1299-1318. https://doi.org/10.1007/s00204-023-03483-7

[5] Nath, B. and Szabo, G. (2012) Hypoxia and Hypoxia Inducible Factors: Diverse Roles in Liver Diseases. *Hepatology*, **55**, 622-633. https://doi.org/10.1002/hep.25497

[6] Rana, N.K., Singh, P. and Koch, B. (2019) $CoCl_2$ Simulated Hypoxia Induce Cell Proliferation and Alter the Expression Pattern of Hypoxia Associated Genes Involved in Angiogenesis and Apoptosis. *Biological Research*, **52**, Article No. 12. https://doi.org/10.1186/s40659-019-0221-z

[7] Tang, X., Chang, C., Hao, M., Chen, M., Woodley, D.T., Schönthal, A.H., *et al*. (2021) Heat Shock Protein-90*α* (HSP90 *α*) Stabilizes Hypoxia-Inducible Factor-1*α* (HIF-1*α*) in Support of Spermatogenesis and Tumorigenesis. *Cancer Gene Therapy*, **28**, 1058-1070. https://doi.org/10.1038/s41417-021-00316-6

[8] He, T., Guo, H., Xia, L., Shen, X., Huang, Y., Wu, X., *et al*. (2022) Alterations of RNA Modification in Mouse Germ Cell-2 Spermatids under Hypoxic Stress. *Frontiers in Molecular Biosciences*, **9**, Article 871737. https://doi.org/10.3389/fmolb.2022.871737

[9] Oyedokun, P.A., Akhigbe, R.E., Ajayi, L.O. and Ajayi, A.F. (2022) Impact of Hypoxia on Male Reproductive Functions. *Molecular and Cellular Biochemistry*, **478**, 875-885. https://doi.org/10.1007/s11010-022-04559-1

[10] Li, Z., Wang, S., Gong, C., Hu, Y., Liu, J., Wang, W., *et al*. (2021) Effects of Environmental and Pathological Hypoxia on Male Fertility. *Frontiers in Cell and Developmental Biology*, **9**, Article 725933. https://doi.org/10.3389/fcell.2021.725933

[11] Huang, X., Zhao, L. and Peng, R. (2022) Hypoxia-Inducible Factor 1 and Mitochondria: An Intimate Connection. *Biomolecules*, **13**, Article 50. https://doi.org/10.3390/biom13010050

[12] Fratantonio, D., Virgili, F., Zucchi, A., Lambrechts, K., Latronico, T., Lafère, P., *et al*. (2021) Increasing Oxygen Partial Pressures Induce a Distinct Transcriptional Response in Human PBMC: A Pilot Study on the "Normobaric Oxygen Paradox". *International Journal of Molecular Sciences*, **22**, Article 458. https://doi.org/10.3390/ijms22010458

[13] Qannita, R.A., Alalami, A.I., Harb, A.A., Aleidi, S.M., Taneera, J., Abu-Gharbieh, E., *et al*. (2024) Targeting Hypoxia-Inducible Factor-1 (HIF-1) in Cancer: Emerging Therapeutic Strategies and Pathway Regulation. *Pharmaceuticals*, **17**, Article 195. https://doi.org/10.3390/ph17020195

[14] Infantino, V., Santarsiero, A., Convertini, P., Todisco, S. and Iacobazzi, V. (2021) Cancer Cell Metabolism in Hypoxia: Role of HIF-1 as Key Regulator and Therapeutic Target. *International Journal of Molecular Sciences*, **22**, Article 5703. https://doi.org/10.3390/ijms22115703

[15] Andrieux, P., Chevillard, C., Cunha-Neto, E. and Nunes, J.P.S. (2021) Mitochondria as a Cellular Hub in Infection and Inflammation. *International Journal of Molecular Sciences*, **22**, Article 11338. https://doi.org/10.3390/ijms222111338

[16] Keerthiga, R., Pei, D. and Fu, A. (2021) Mitochondrial Dysfunction, UPR^{mt} Signaling, and Targeted Therapy in Metastasis Tumor. *Cell & Bioscience*, **11**, Article No. 186. https://doi.org/10.1186/s13578-021-00696-0

[17] Park, Y. and Pang, M. (2021) Mitochondrial Functionality in Male Fertility: From Spermatogenesis to Fertilization. *Antioxidants*, **10**, Article 98. https://doi.org/10.3390/antiox10010098

[18] Bock, F.J. and Tait, S.W.G. (2019) Mitochondria as Multifaceted Regulators of Cell Death. *Nature Reviews Molecular Cell Biology*, **21**, 85-100. https://doi.org/10.1038/s41580-019-0173-8

[19] Li, H., Zhou, Y., Li, L., Li, S., Long, D., Chen, X., *et al*. (2019) HIF-1α Protects against Oxidative Stress by Directly Targeting Mitochondria. *Redox Biology*, **25**, Article ID: 101109. https://doi.org/10.1016/j.redox.2019.101109

[20] Yang, H., An, B., Choi, K. and Jeung, E. (2013) Change of Genes in Calcium Transport Channels Caused by Hypoxic Stress in the Placenta, Duodenum, and Kidney of Pregnant Rats. *Biology of Reproduction*, **88**, 1-12. https://doi.org/10.1095/biolreprod.112.103705

[21] Peoples, J.N., Saraf, A., Ghazal, N., Pham, T.T. and Kwong, J.Q. (2019) Mitochondrial Dysfunction and Oxidative Stress in Heart Disease. *Experimental & Molecular Medicine*, **51**, 1-13. https://doi.org/10.1038/s12276-019-0355-7

[22] Janbandhu, V., Tallapragada, V., Patrick, R., Li, Y., Abeygunawardena, D., Humphreys, D.T., *et al*. (2022) HIF-1α Suppresses Ros-Induced Proliferation of Cardiac Fibroblasts Following Myocardial Infarction. *Cell Stem Cell*, **29**, 281-297.e12. https://doi.org/10.1016/j.stem.2021.10.009

[23] Wu, X., Pan, J., Yu, J.J., Kang, J., Hou, S., Cheng, M., *et al*. (2023) DiDang Decoction Improves Mitochondrial Function and Lipid Metabolism via the HIF-1 Signaling Pathway to Treat Atherosclerosis and Hyperlipidemia. *Journal of Ethnopharmacology*, **308**, Article ID: 116289. https://doi.org/10.1016/j.jep.2023.116289

[24] Marchi, S., Giorgi, C., Suski, J.M., Agnoletto, C., Bononi, A., Bonora, M., *et al*. (2012) Mitochondria-Ros Crosstalk in the Control of Cell Death and Aging. *Journal of Signal Transduction*, **2012**, Article ID: 329635. https://doi.org/10.1155/2012/329635

[25] Xie, Y., Shi, X., Sheng, K., Han, G., Li, W., Zhao, Q., *et al*. (2018) Pi3k/Akt Signaling Transduction Pathway, Erythropoiesis and Glycolysis in Hypoxia (Review). *Molecular Medicine Reports*, **19**, 783-791. https://doi.org/10.3892/mmr.2018.9713

[26] Wei, L., Zeng, K., Gai, J., Zhou, F., Wei, Z. and Bao, Q. (2020) Effect of Acupuncture on Neurovascular Units after Cerebral Infarction in Rats through PI3K/AKT Signaling Pathway. *Clinical Hemorheology and Microcirculation*, **75**, 387-397. https://doi.org/10.3233/ch-190659

[27] Smith, V.M., Dietz, A., Henz, K., Bruecher, D., Jackson, R., Kowald, L., *et al*. (2019) Specific Interactions of BCL-2 Family Proteins Mediate Sensitivity to BH3-Mimetics in Diffuse Large B-Cell Lymphoma. *Haematologica*, **105**, 2150-2163. https://doi.org/10.3324/haematol.2019.220525

[28] Wang, X., Wei, L., Li, Q. and Lai, Y. (2022) HIF-1α Protects Osteoblasts from Ros-Induced Apoptosis. *Free Radical Research*, **56**, 143-153. https://doi.org/10.1080/10715762.2022.2037581

[29] Cho, T.J., Lee, D., Choi, B., Shinn, H.K. and Park, C. (2021) Hypoxia-Induced

Suppression of Antiapoptotic BCL-2 Expression in Human Bladder Tumor Cells Is Regulated by Caveolin-1-Dependent Adenosine Monophosphate-Activated Protein Kinase Activity. *International Neurourology Journal*, **25**, 137-149. https://doi.org/10.5213/inj.2040444.222

[30] Yang, J., Nie, J., Ma, X., Wei, Y., Peng, Y. and Wei, X. (2019) Targeting PI3K in Cancer: Mechanisms and Advances in Clinical Trials. *Molecular Cancer*, **18**, Article No. 26. https://doi.org/10.1186/s12943-019-0954-x

[31] Liu, Y., Liu, Q., Zhang, Z., Yang, Y., Zhou, Y., Yan, H., *et al*. (2023) The Regulatory Role of PI3K in Ageing-Related Diseases. *Ageing Research Reviews*, **88**, Article ID: 101963. https://doi.org/10.1016/j.arr.2023.101963

[32] Wang, J., Hu, K., Cai, X., Yang, B., He, Q., Wang, J., *et al*. (2022) Targeting PI3K/AKT Signaling for Treatment of Idiopathic Pulmonary Fibrosis. *Acta Pharmaceutica Sinica B*, **12**, 18-32. https://doi.org/10.1016/j.apsb.2021.07.023