

Protective Role of Whole Wheat Against Stress-Induced Hormonal and Sperm Abnormalities in Male Rats

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ABSTRACT

Objective: To assess the effectiveness of whole wheat in reversing hormonal disturbances and sperm abnormalities induced by stress in male rats.

Study Design: An experimental study.

Place and Duration of the Study: Department of Physiology, Islamic International Medical College, Rawalpindi, in collaboration with the National Institute of Health, Islamabad, Pakistan, from April 2024 to April 2025.

Methodology: Thirty-six male Sprague-Dawley rats were divided into three groups: Normal Control (NC), receiving no stress; Positive Control (PC), subjected to immobilisation and a standard diet for 10 weeks; and Whole Wheat (WW), exposed to stress for 10 weeks with 4 weeks on a standard diet followed by 6 weeks on whole wheat. Serum cortisol, follicle-stimulating hormones (FSH), and testosterone levels, along with sperm count, morphology, motility, and agglutination, were assessed at weeks 0, 4, and 10. Data were analysed using Pearson's correlation, ANOVA with post-hoc Tukey, and the Chi-square test, as appropriate.

Results: Compared to NC, PC rats showed significantly elevated cortisol and reduced FSH and testosterone levels ($p < 0.001$). In contrast, WW rats exhibited decreased cortisol and increased FSH and testosterone compared to PC ($p < 0.001$). Semen analysis revealed that PC had higher sperm agglutination ($p = 0.02$) and lower sperm count, motility, and morphology (all $p < 0.001$) compared to NC. Conversely, WW showed significant improvement in all semen parameters relative to PC ($p < 0.05$). Cortisol levels correlated positively with sperm agglutination but negatively with FSH, testosterone, sperm count, motility, and morphology.

Conclusion: Whole wheat reduced stress-related hormonal imbalance and improved semen quality, suggesting protection against male reproductive damage.

Key Words: Whole wheat, Cortisol, Spermatogenesis, Stress, Sperm agglutination.

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INTRODUCTION

Spermatogenesis is a complex process of cell differentiation, during which spermatogonia progress through various stages to become mature sperms, serving as a key component of male fertility.¹ This intricate process occurs inside the seminiferous tubules of the testis, where Sertoli cells play an essential role in supporting the development of germ cells. Spermatogenesis relies on the hypothalamic-pituitary gonadal axis that affects Sertoli cells, which can proliferate and organise in response to follicle-stimulating hormones (FSH) and testosterone. Moreover, Sertoli cells maintain an immunologically privileged microenvironment by forming the blood-testis barrier (BTB) through tight junctions that protect germ cells from autoimmune reactions.²

Apart from endocrine control, spermatogenesis is also regulated by many genetic, epigenetic, endocrine, environmental, nutritional factors, and lifestyle choices.¹ Among these factors, chronic stress has emerged as a significant disrupter. It affects spermatogenesis by disturbing the secretion of neural and endocrine hormones.³ Stress influences the hypothalamus-pituitary-adrenal (HPA) axis, resulting in elevated serum cortisol levels, increased oxidative stress, and reduced testosterone levels by reducing the expression of luteinising hormone (LH) and FSH.⁴ The slow genomic actions of cortisol indicate that its suppressive effects on reproductive hormones are more likely to become significant with prolonged or repeated exposure to stress, rather than following short-term stress. Moreover, chronic stress can impair the integrity of BTB by disrupting its key structural proteins.⁵ Compromise of the BTB allows germ cell antigens to leak from the seminiferous tubules, which can trigger the production of anti-sperm antibodies. This immune response leads to impaired spermatogenesis, decreased sperm motility, and sperm agglutination.⁶

Additionally, the consumption of processed foods has been shown to negatively affect spermatogenesis.⁷ In contrast, the intake of compounds with antioxidant and anti-inflammatory properties may help counteract the detrimental effects of stress.⁴ Various

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essential nutrients, including vitamins, minerals, amino acids, and trace elements, play vital roles in supporting male reproductive function. For example, vitamin A promotes spermatogenesis by regulating gene transcription in Sertoli cells and maintaining the integrity of the BTB. Folic acid enhances germ cell resistance to oxidative stress. Vitamin E and selenium support spermatogenesis through their antioxidant properties. Calcium and magnesium contribute to improved sperm motility and facilitate signal transduction in germ cells, while zinc is crucial for the maturation of sperm and maintaining the integrity of the germinal epithelium.⁸ Foods rich in such bioactive compounds are classified as functional foods,⁹ with whole wheat (WW) products being a prime example.

This study aimed to assess the therapeutic potential of WW supplementation in reversing stress-induced hormonal disturbances and sperm abnormalities in male rats. Specifically, the focus would be on unravelling the mechanistic pathways through which WW modulated oxidative stress, hypothalamic-pituitary-gonadal (HPG) axis regulation, and testicular function. By integrating biochemical, hormonal, and semen analyses, the research sought to provide mechanistic insights into how WW attenuated the deleterious effects of chronic stress.

METHODOLOGY

This randomised controlled trial was conducted at the Department of Physiology, Islamic International Medical College, Rawalpindi, in collaboration with the National Institute of Health, Islamabad, Pakistan, from April 2024 to April 2025, after obtaining ethical approval from both institutions (IIMC Letter No. Riphah/IRC/24/1051; NIH Letter No. F.1-5/ERC/2023). The sample size was calculated using the resource equation approach.¹⁰ Thirty-six healthy, 5-week-old Sprague-Dawley rats weighing 200-300 grams were selected by simple random sampling. Female rats and rats with visible deformities, or weighing <200 grams or >300 grams, were excluded from the study. One week before the experiment, the rats were acclimatised to an environment with a humidity level of 50-70% and a room temperature of $24 \pm 2^\circ\text{C}$, with a 12-hour light/dark cycle. A standard diet was prepared in pellet form according to the guiding principles given by the Universities Federation for Animal Welfare, and it contained wheat bran 28.5%, wheat flour 28.5%, dried skimmed milk powder 20%, fish meal 15%, common salt 0.5%, vitamins, minerals, amino acids 1%, and Soybean oil 5%.¹¹

Following acclimatisation, the rats were randomly assigned to two groups: Normal Control (NC; $n = 13$) and Interventional Group ($n = 23$). The Interventional group was exposed to immobilisation stress for 6 hours daily at 9:00 am, using wooden restraint cages with individual ventilated compartments ($6 \times 7 \times 18$ cm) designated for each rat, including an opening for tail accommodation.⁴

After four weeks of stress, the Interventional group was split into Positive Control (PC) and WW subgroups, both continuing stress for six weeks more. Groups NC and PC remained on the standard diet, while the WW group was switched to a WW-based diet after

week four, replacing wheat flour and bran with WW flour in the standard diet.

Biological sampling was performed at three time points: pre-experimental (day 0) with tail vein blood and semen from NC rats; after 4 weeks with blood and semen from interventional rats; and at 10 weeks with cardiac blood and epididymal semen from all rats. All sample collections and subsequent laboratory analyses were performed under blinded conditions to minimise observer bias.

Serum levels of cortisol (ng/mL), testosterone (ng/mL), and FSH (ng/mL) were measured using ELISA kits (Elabscience Biotechnology Co., Ltd., Japan). Sperm motility on a glass slide under $40\times$ light microscopy (Olympus CX 23, Japan),¹² mobile agglutinated sperms under $40\times$,¹³ sperm count *via* haemocytometer (Marienfeld Superior, Germany) after 200-fold dilution,¹⁴ and morphology from ethanol-fixed, carbol fuchsin-stained smears under $40\times$ magnification were observed.¹²

Data were analysed using SPSS version 26. Normality was assessed by the Shapiro-Wilk test. Repeated-measures ANOVA was used to assess changes over time, the post-hoc Tukey's test was applied for pairwise comparisons of group means (mean $\pm$ SD), and the Chi-square test was used to compare groups' qualitative data. Pearson's correlation examined cortisol and all other biological parameters' correlation, with $p \leq 0.05$ considered as significant.

RESULTS

Significant changes in all hormone levels were noted in the PC and WW groups over time, as shown in Table I.

A significant increase in serum cortisol levels was noted in the PC group at both week 4 and 10 compared to the NC group ($p < 0.001$). WW intake depicted a significant reduction in cortisol levels at week 10, compared to the PC group, with $p < 0.001$ (Figure 1A).

At week 4 and 10, the PC group showed a notable decline in serum FSH levels as compared to the NC group, with a statistically highly significant p -value ($p < 0.001$, Figure 1B). In contrast, the WW group demonstrated a significant increase in serum FSH levels relative to the PC group at week 10 ($p < 0.001$), as illustrated in Figure 1B. A similar trend was observed in serum testosterone levels, which significantly declined in the PC group at both week 4 and 10 ($p < 0.001$). However, the WW group showed a significant elevation in testosterone levels relative to the PC group at week 10 ($p < 0.001$, Figure 1C).

As shown in Table II, by week 4, stress had a significantly negative impact on sperm parameters. Compared to week 0, there was a notable decrease in sperm count, rapid progressive motility, and normal morphology. Additionally, stress led to an increase in the percentage of immotile sperms, abnormal morphology, and headpiece defects. By week 10, the WW group showed significant improvement in these parameters compared to the PC group, with increased sperm count, enhanced motility, and reduced immotile sperms and headpiece defects.

Table I: Changes in serum hormone levels in the three groups over time.

Parameters	Time of sampling	NC Mean ± SD	PC Mean ± SD	WW Mean ± SD
Serum cortisol levels (ng/mL)	Week 0	46.83 ± 7.15	42.88 ± 5.82	46.24 ± 6.88
	Week 4	47.05 ± 4.04	96.91 ± 5.55	97.27 ± 5.47
	Week 10	45.91 ± 6.63	99.37 ± 6.23	69.77 ± 5.49
	p-values	0.784	<0.001	<0.001
Serum FSH levels (ng/mL)	Week 0	8.26 ± 0.37	8.02 ± 0.54	8.04 ± 0.45
	Week 4	8.18 ± 0.38	3.60 ± 0.46	3.60 ± 0.42
	Week 10	8.04 ± 0.45	3.41 ± 0.19	7.02 ± 0.67
	p-values	0.638	<0.001	<0.001
Serum testosterone levels (ng/mL)	Week 0	3.91 ± 0.64	3.45 ± 0.69	3.65 ± 0.78
	Week 4	3.67 ± 0.69	0.73 ± 0.40	0.95 ± 0.45
	Week 10	3.45 ± 0.63	0.84 ± 0.54	3.78 ± 0.62
	p-values	0.368	<0.001	<0.001

Comparison of mean ± SD of serum cortisol, FSH, and testosterone by repeated measure ANOVA.

Table II: Comparison of sperm parameters among the groups (NC, PC, and WW).

Sperm parameters	Week 0	Week 4	Week 10		
	Mean ± SD	Mean ± SD	NC Mean ± SD	PC Mean ± SD	WW Mean ± SD
Sperm count (million/mL)	5.10 ± 0.65	2.46 ± 0.95	5.15 ± 0.57	3.37 ± 0.67	4.61 ± 1.10
	p ^a <0.001	p ^b <0.05		p ^c <0.001	p ^d <0.05
Rapid progressive motility (%)	73.0 ± 3.0	14.00 ± 5.29	68.0 ± 4.21	32.13 ± 22.87	63.10 ± 14.07
	p ^a <0.001	p ^b <0.001		p ^c <0.001	p ^d <0.001
Non-rapid progressive motility (%)	19.67 ± 3.51	45.00 ± 5.0	23.60 ± 3.95	41.88 ± 17.91	26.00 ± 16.5
	p ^a = 0.076	p ^b = 0.110		p ^c <0.05	p ^d <0.05
Immotile (%)	7.33 ± 3.51	41.00 ± 6.55	8.30 ± 2.11	26.0 ± 16.54	10.50 ± 2.87
	p ^a <0.05	p ^b <0.001		p ^c <0.001	p ^d <0.001
Normal morphology (%)	75.0 ± 5.0	49.33 ± 1.15	72.60 ± 6.11	59.63 ± 7.63	64.60 ± 8.79
	p ^a <0.001	p ^b <0.05		p ^c <0.001	p ^d = 0.365
Abnormal morphology (%)	25.0 ± 5.0	50.67 ± 1.15	27.40 ± 6.11	40.38 ± 7.63	34.30 ± 8.12
	p ^a <0.001	p ^b <0.05		p ^c <0.001	p ^d = 0.207
Headpiece defect (%)	8.67 ± 1.15	32.67 ± 2.51	12.90 ± 4.01	28.50 ± 9.75	19.00 ± 8.02
	p ^a <0.001	p ^b <0.05		p ^c <0.001	p ^d <0.05

a = Comparison between week 0 and week 4. b = Comparison between week 4 and week 10 (WW Group). c = Comparison between NC and PC groups at week 10. d = Comparison between PC and WW groups at week 10. Through one-way ANOVA and post-hoc Tukey test.

Table III: Comparison of sperm agglutination among the groups.

Time point / group	Sperm Agglutination		p-values
	Present (%)	Absent (%)	
Week 0	0	100	<0.05 ^a
Week 4	100	0	<0.05 ^b
Week 10	NC	10	<0.001 ^c
	PC	75	<0.05 ^d
	WW	30	<0.05 ^e

a = Comparison of sperm agglutination between week 0 and week 4. b = Comparison of sperm agglutination between week 4 and week 10. c = Comparison of sperm agglutination between the NC and PC groups at week 10. d = Comparison of sperm agglutination between week 0 and PC at week 10. e = Comparison of sperm agglutination between the PC and WW groups at week 10. Through the Chi-square test.

As shown in Table III, the frequency of sperm agglutination was significantly higher in the PC group compared to the NC group. However, WW intake markedly reduced the sperm agglutination to 30%, compared to the 75% observed in the PC group.

A negative correlation was found between serum cortisol levels and both serum FSH and testosterone levels, as well as key sperm parameters such as sperm count, rapid progressive motility, and normal morphology. In contrast, serum cortisol levels showed a positive correlation with non-rapid progressive motility, immotile sperms, headpiece defects, and sperm agglutination, as illustrated in Figure 2.

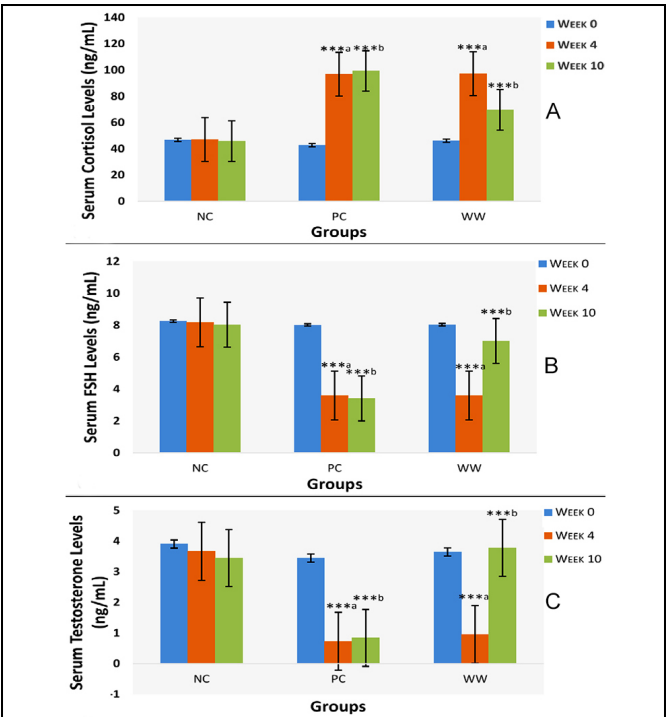


Figure 1: (A) Serum cortisol at 0, 4, and 10 weeks; (B) Serum FSH at 0, 4, and 10 weeks; (C) Serum testosterone at 0, 4, and 10 weeks. a = Comparison between the NC and PC groups at weeks 4 and 10; b = Comparison between the PC and WW groups at weeks 4 and 10 through Post-Hoc Tukey test. *** = p <0.001.

Variables	Pearson's Correlation	Cortisol (ng/mL)	FSH (ng/mL)	Testosterone (ng/mL)	SC (million/mL)	RPM (%)	NRPM (%)	Immotile (%)	NSM (%)	ASM (%)	HPD (%)	SA
Cortisol (ng/mL)	r-value	1										
	p-value											
FSH (ng/mL)	r-value	-0.925	1									
	p-value	<0.001										
Testosterone (ng/mL)	r-value	-0.728	0.807	1								
	p-value	<0.001	<0.001									
SC (millions/mL)	r-value	-0.639	0.637	0.616	1							
	p-value	<0.001	<0.001	<0.001								
RPM (%)	r-value	-0.641	0.673	0.711	0.768	1						
	p-value	<0.001	<0.001	<0.001	<0.001							
NRPM (%)	r-value	0.459	-0.459	-0.474	-0.77	-0.849	1					
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001						
Immotile (%)	r-value	0.572	-0.63	-0.682	-0.403	-0.726	0.253	1				
	p-value	<0.001	<0.001	<0.001	<0.05	<0.001	0.193					
NSM (%)	r-value	-0.565	0.501	0.368	0.629	0.699	-0.703	-0.370	1			
	p-value	<0.001	<0.001	<0.05	<0.001	<0.001	0.052	<0.001				
ASM (%)	r-value	0.587	-0.535	-0.435	-0.705	-0.75	0.751	0.402	-0.973	1		
	p-value	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001	<0.05	<0.001			
HPD (%)	r-value	0.629	-0.646	-0.556	-0.676	-0.792	0.692	0.552	-0.808	0.873	1	
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
SA	r-value	0.498	-0.49	-0.562	-0.645	-0.681	0.593	0.475	-0.58	0.632	0.584	1
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001	

Figure 2: Correlation among various study parameters [FSH, testosterone, sperm count (SC), rapid progressive motility (RPM), non-rapid progressive motility (NRPM), immotile, normal sperm morphology (NSM), abnormal sperm morphology (ASM), head piece defect (HPD), and sperm agglutination (SA)]. Through Pearson's correlation.

DISCUSSION

This study evaluated the role of WW in reversing stress-induced hormonal and reproductive disturbances in male rats. Stress markedly increased cortisol level, reduced FSH and testosterone levels, and impaired semen quality, whereas WW supplementation lowered cortisol, improved hormone levels, and restored sperm parameters compared to stressed controls.

Among the groups, the PC group, which was subjected to stress without any nutritional intervention, showed a marked increase in serum cortisol levels compared to the NC group. This elevation aligns with findings from Hidayatik *et al.*, who reported that chronic stress triggered the HPA axis, consequently elevating serum cortisol concentrations.¹⁵

Beyond cortisol, stress exposure also significantly affected reproductive hormones. Specifically, the PC group exhibited a pronounced decline in serum FSH and testosterone levels. These findings align with those of the previous studies by Hidayatik *et al.* and Belhan *et al.*, which documented stress-related reductions in serum testosterone and FSH levels, respectively, in animal models subjected to psychological and physical stress.^{15,16} However, the current study contrasts with those of Mohamadpour *et al.*, who reported elevated FSH levels in stressed groups. This discrepancy is likely attributable to differences in the sources and duration of the stress employed.¹⁷

Notably, rats supplemented with WW flour (WW group) demonstrated stabilised serum parameters, indicating the potential stress-buffering effects of WW. The significant reduction in serum cortisol observed in the WW group may

be attributed to the presence of quercetin, a flavonoid abundant in WW. As highlighted by Quraishi *et al.*, quercetin modulates the HPA axis, thereby aiding in cortisol regulation during stress exposure.¹⁸

Furthermore, the protective influence of WW on reproductive hormones, *i.e.*, testosterone and FSH can be linked to its rich content of vitamin E, zinc, selenium, and quercetin. Existing literature supports this mechanism: vitamin E, zinc and selenium, and quercetin (Bin-Jaliah) have all been shown to attenuate stress-induced decline in these hormones.¹⁹⁻²¹ In a related study, Adibmoradi *et al.* demonstrated increased testosterone levels following wheat sprout extract supplementation, which they attributed to its antioxidative properties counteracting oxidative stress induced by heavy metals.²²

In terms of semen quality, chronic stress in the PC group led to reduced sperm count, motility, and normal morphology, along with increased sperm agglutination. These findings align with those of Belhan *et al.*, who reported impaired sperm parameters in stress-related depressive states.¹⁶ Interestingly, the WW group exhibited significant improvement in all sperm quality markers. These improvements may be credited to the antioxidant and micronutrient components of WW. Adibmoradi *et al.* also reported similar enhancements in sperm parameters with wheat sprout supplementation, emphasising the contributions of vitamin E and phenolic compounds.²² In this study, the reduction in sperm agglutination in the WW group is likely due to the zinc content in WW. Supporting this, Al-Ani *et al.* found that zinc supplementation mitigated cadmium-induced oxidative damage, enhancing sperm count, motility and normal morphology, and reducing sperm agglutination.²³ Additionally, the protective role of quercetin against stress-induced deterioration of sperm parameters was affirmed by Bin-Jaliah, further validating the current observations.²¹

While the present findings provide valuable mechanistic insights, the study is limited by its reliance on an animal model, fixed intervention duration, and lack of direct measurement of individual bioactive compounds. To build on these findings, future studies should focus on identifying and quantifying the specific bioactive components of WW responsible for the observed protective effects, as well as exploring their synergistic interactions. Importantly, well-designed clinical trials in humans, integrating broader dietary patterns and lifestyle factors, are needed to confirm the translational relevance of WW in mitigating stress-induced hormonal and reproductive dysfunction.

CONCLUSION

This study demonstrates that WW consumption effectively mitigates stress-induced hormonal imbalances and improves sperm quality in male rats. These findings suggest its potential as a dietary intervention for supporting reproductive

health under chronic stress conditions. Further research, particularly in human subjects, is recommended to validate these results and explore their clinical applicability.

ETHICAL APPROVAL:

Ethical approval was obtained from the Institutional Review Committee of Islamic International Medical College (Approval No. Riphah/IRC/24/1051) and the Ethics Committee for Animal Care and Experimentation (IECACE) of NIH (No.F.1-5/ERC/2023).

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

RN: Contribution to data analysis, interpretation of the results, and drafting of the manuscript.

SA: Conception and design of the study, data analysis, interpretation, and drafting of the manuscript.

RA: Conception of the study, data collection, and analysis.

HFK: Drafting of the work and critical revision of the manuscript.

SF: Conception and design of the study.

GJ: Drafting of the work and review.

All authors approved the final version of the manuscript to be published.

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