Assessment of Alterations in Reproductive Hormones due to Dengue Virus Infection in Patients: A Case-Control Study

Humera Naveed*, Kaleem Maqsood*, Husna Ahmad*, Waheed Haider*,

Muhammad Amir Iqbal*, Nabila Roohi*

* Institute of Zoology, University of the Punjab, Lahore

***Abstract*- Background:** Dengue fever, spread by the Aedes aegypti mosquito, is an arboviral infectious disease endemic to arid and semiarid regions of the globe. Several body organs, i.e. heart, kidney, liver, pancreas, eyes, and central nervous system, are affected by dengue fever in various ways. **Aim/Objective:** The current study is aimed to assess reproductive dysfunction in dengue patients. **Methodology:** Blood samples of 40 male patients with DF (dengue fever) and SDF (Severe dengue fever) and 30 healthy males of the same age group as the controls were collected. Blood was drawn and processed so that the serum could be separated to assess the levels of the reproductive hormones. The hormonal analysis was done through ELISA, and obtained data were analyzed statistically using One-Way ANOVA through IBM-SPSS software. **Results:** Results showed that FSH level increased significantly (P=0.039) in dengue patients' groups compared to controls. Similarly, the level of the LH prominently (P≤0.001) elevated in the patients' groups compared to controls. However, there was a significant (P≤0.001) decrease in serum testosterone levels (hypogonadism) in dengue patients' groups compared to controls. **Conclusion:** It was concluded, from the above results, that the infected patients have a disruption in reproductive hormone production which can lead to hypogonadism, ultimately.

***Index Terms*-** FSH; LH; Hypogonadism; dengue fever

I. INTRODUCTION

Regarding morbidity and mortality, dengue fever is the second-most dangerous vector-borne infectious disease globally, after only malaria [1]. So far, 47 DENV strains have been identified [2], with four dengue virus serotypes (DENV 1, 2, 3, and 4) posing a potential threat to humans [3]. An infected female Aedes aegypti mosquito spreads this virus [4]. Another possible route of dengue virus transmission is transfusion or organ transplantation with a recipient exposed to the virus [5].

It is endemic in more than 100 countries in Oceania, America, Asia, and Africa [6]. According to an estimate, around 390 million cases are recorded globally each year, 96 million with clinical symptoms [7]. More than 48,000 cases were recorded in Pakistan in 2021, with the highest number of patients being found in Punjab province [8].

The World Health Organization (WHO) defines DF as having fever episodes lasting 2 to 7 days with a temperature of 40°C, with rash, nausea, vomiting, and headache [9]. The most prominent clinical manifestations of this infection are capillaries leakage, hemorrhagic tendencies, leukopenia, thrombocytopenia, and its severe form includes abdominal discomfort, persistent vomiting, mucosal bleeding, lethargy, enlarged liver, and an increase in hematocrit. A diagnosis of DF (dengue fever) or SDF (severe dengue fever) may be made depending on the presence or absence of the characteristic clinical symptoms [10].

SDF has clinical symptoms ranging from minor to life-threatening [11]. SDF patients had 100-to-1000-fold higher viral titers than DF patients in the early stages of infection [12]. According to the results, monocytes seem to be the primary target. Infected monocytes generate interferon-a (IFN-a) and interferon-b (IFN-b). Interferon-alpha prevents viral replication, whereas interferon-beta lowers inflammation [13].

In mild cases of dengue fever, laboratory tests often show an increase in hepatic aminotransferase activity and a notable increase in leukocyte quantity which indicates disease may progress to a more severe form of SDF if treatment is delayed. The pancreas, liver, cardiovascular system, kidneys, eyes, and central nervous system are some of the organs that might be at risk of damage by the dengue virus [14]. Plasma leakage or fluid buildup may lead to severe organ damage [10].

Testosterone is a primary male sex hormone as it is well recognized for its function in spermatogenesis and developing secondary sexual characteristics in males [15]. It is primarily produced by interstitial cells in the testes in a complex process mainly controlled by luteinizing hormone (LH). Abnormalities in LH production and function can disrupt spermatogenesis, resulting in infertility [16]. Follicle-stimulating hormone (FSH) is another crucial hormone by the pituitary gland that targets male gonadal cells necessary for spermatogenesis to begin. Still, it seems to be essential for sperm viability and motility also [17].

The medical name for low libido caused by a lack of sexual hormones is hypogonadism [18]. It is a syndrome caused by any disruption in the hypothalamic-pituitary-testicular axis. Ultimately, the testes fail to produce normal physiological testosterone levels and an average number of spermatozoa [19]. The acute decline in testosterone levels and rise in LH and FSH levels are recognized as a primary disorder of testes or primary hypogonadism, while Secondary hypogonadism results from hypothalamic-pituitary axis disorder, which is associated with low

testosterone levels, impaired spermatogenesis, and low or inappropriate normal LH and FSH levels [20].

To our knowledge, serum levels of reproductive hormones in male dengue patients have not been documented previously. So, in the current investigation, attention was paid to assessing dengue fever's effects on the pituitary-testicular axis.

II. MATERIALS AND METHODS

Forty patients, including 26 with dengue fever (DF) and 14 with severe dengue fever (SDF) from Mayo Hospital, Lahore, were recruited for this investigation. Thirty healthy males of the same age group were also recruited as study controls from University of the Punjab, Lahore. The Ethical Review Board of University of the Punjab, Lahore approved the research plan. Participants' socio-demographic characters and clinical features were recorded on a pre-designed proforma.

For phlebotomy, a registered technician was recruited. After that, collected blood samples were poured into clot activator tubes to separate the serum. The blood samples were immediately carried to the Physiology Lab of the Institute of Zoology, University of the Punjab, Lahore and left for 30-40 minutes to allow coagulation. Following that, centrifugation was done for 15 minutes at 3000 rpm. Finally, the serum was transferred to labelled Eppendorf vials and stored at -80°C till hormonal analysis.

Analysis of serum levels of reproductive hormones (Testosterone, LH, and FSH) by commercially available ELISA kits of PerkinElmer (USA) at the Physiology/Endocrinology Laboratory, Institute of Zoology, University of the Punjab, Lahore.

Statistical analysis of obtained data from the hormonal analysis was performed through One-Way ANOVA to find the variations between the study groups. At the same time, Chi-Square Fishers Exact Test analyzed the association between the severity of dengue fever and clinical symptoms at a significance level of $P \leq 0.05$. IBM-SPSS and GraphPad Prism software were used for this purpose. Results were presented as mean ± SEM in tabular and graphical form.

III. RESULTS

For this investigation, 40 dengue patients and 30 controls were enrolled. The age range was 10-50 years, and all the subjects were males (Table 1). Among the patients, 26 subjects were with dengue fever (DF), while 14 were with severe dengue fever (SDF) (Table 3).

Table 1: Frequency (%) of socio-demographic features of dengue patients and controls.

Parameters	Category	Control (N=30)		Cases (N=40)	
		N	%	N	%
Age	10-20	1	3.33	7	17.50
	20-30	21	70.00	17	42.50
	30-40	5	16.67	9	22.50
	40+	3	10.00	7	17.50
BMI	Underweight	1	3.33	0	0.00
	Normal weight	16	53.33	18	45.00
	Overweight	12	40.00	14	35.00
	Obese	1	3.33	8	20.00
Education	Primary	0	0.00	7	17.50
	Intermediate	2	6.67	3	7.50
	Higher	28	93.33	4	10.00
	Illiterate	0	0.00	7	17.50
Residence	Rural	12	40.00	12	30.00
	Urban	18	60.00	28	70.00
Marital Status	Unmarried	18	60.00	12	30.00
	Married	12	40.00	28	70.00
Hospitalization	0-2	N/A	N/A	21	52.50
	3-4	N/A	N/A	12	30.00
	5-7	N/A	N/A	7	17.50

Among clinical features, nausea, rashes, aches/pain, and tourniquet tests were the most prevalent clinical symptoms among all the patients. Tenderness, vomiting, lethargy, HCT increase, and medical intervention were more commonplace among the patients with severe dengue fever, while shock, rapid breathing, severe bleeding, and impaired consciousness were only linked to severity (Table; 2, Fig; 1-2).

According to the results of the Chi-square and Fisher's exact test, tenderness, HCT increase, medical intervention, and impaired consciousness were strongly associated ($P \leq 0.0001$) with the severity of dengue fever (Table; 2).

Table 2: Frequency (%) of clinical symptoms among dengue patients with DF and SDF.

Symptoms	DF (N=26)		SDF (N=14)		Total (N=40)		*P*-value
	N	%	N	%	N	%	
Nausea/vomiting	17	65.38	11	78.57	28	70.00	0.385[a]
Rashes	18	69.23	11	78.57	29	72.50	0.528[a]
Aches/pains	19	73.08	13	92.86	32	80.00	0.136[a]
Torniquet test	16	61.54	13	92.86	29	72.50	0.034[a]
Tenderness	4	15.38	10	71.43	14	35.00	0.001[b]
Vomiting	6	23.08	10	71.43	16	40.00	0.006[b]
Lethargy	7	26.92	9	64.29	16	40.00	0.041[b]
HCT increase	6	23.08	13	92.86	19	47.50	0.000[b]
Medical intervention	3	11.54	10	71.43	13	32.50	0.000[b]
Shock	1	3.85	6	42.86	7	17.50	0.004[b]
Rapid Breathing	0	0.00	5	35.71	5	12.50	0.003[b]
Severe bleeding	0	0.00	8	57.14	8	20.00	0.003[b]
Impaired consciousness	0	0.00	8	57.14	8	20.00	0.000[b]

[a]Chi-square test; [b]Fisher's exact test. DF; Dengue Fever, SDF; Severe Dengue Fever

Figure 1: Represents the frequency of clinical symptoms associated with dengue fever in patients having dengue fever (DF) and severe dengue fever (SDF).

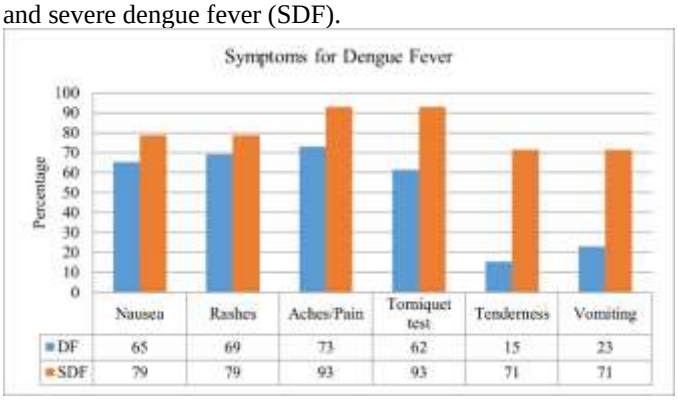
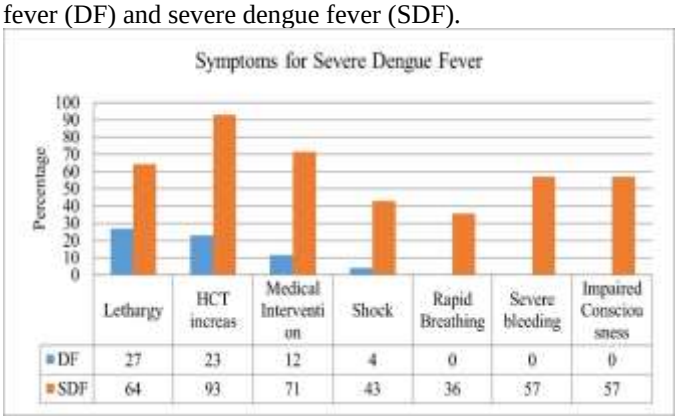
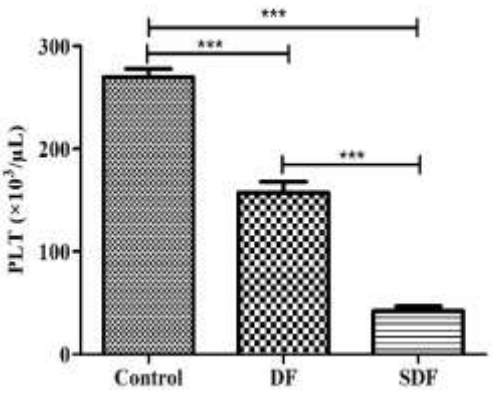


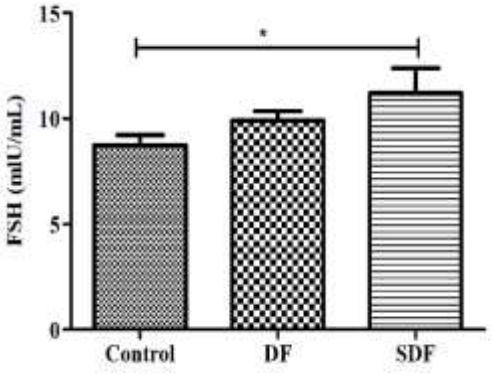
Figure 2: Represents the frequency of clinical symptoms associated with severe dengue fever in patients having dengue fever (DF) and severe dengue fever (SDF).



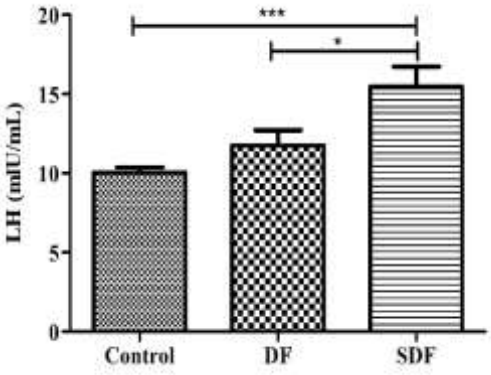
Intergroup comparison of mean±SEM values of serum hormones level between DF, SDF, and controls was made through One-Way ANOVA. Platelets in DF and SDF were significantly ($P \leq 0.001$) lower than the control. Since their level was also significantly ($P \leq 0.001$) low in SDF when compared to DF (Fig; A). Among the hormones, the FSH level was raised in DF nonsignificantly while significantly ($P \leq 0.05$) in the SDF group when compared to the controls (Fig; B). Similarly, levels of LH were also prominently ($P \leq 0.001$) raised in patients with SDF and nonsignificantly in DF patients in comparison to controls, while in SDF, the level was significantly ($P \leq 0.05$) higher than in DF (Fig; C). However, the serum testosterone level declined significantly in patients with DF and SDF ($P \leq 0.01$ and 0.001, respectively) than in controls. Since in SDF, it was too less ($P \leq 0.05$) as compared to DF (Fig; D).



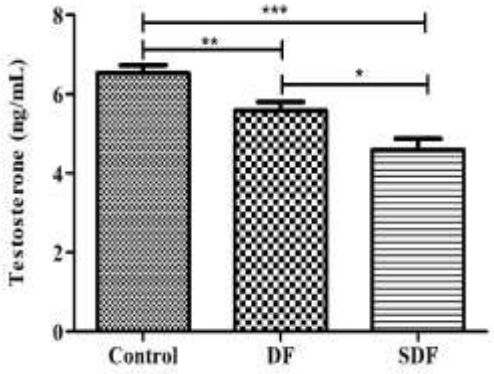
(Fig: A)



(Fig: B)



(Fig: C)



(Fig: D)

Figure (A-C): Intergroup comparison of Mean ± SEM of Platelets and serum reproductive hormones in controls and patients with dengue fever (DF) and Severe Dengue Fever (SDF). *, **, *** represents the significance value at $P \leq 0.05$, 0.01, and 0.001, respectively.

IV. DISCUSSION

Although the prevalence of both male infertility and sexually transmitted infections (STIs) continues to increase annually, research in these areas remains predominantly centered on women. Consequently, there is still a lack of understanding regarding the contribution of viral infections and resulting immunopathology to male factor infertility. The primary objective of this discussion is to shed light on the critical testicular cell types involved in spermatogenesis, as well as to examine ho

Table 3: Overall statistical comparison of serum level of reproductive hormones in patients having dengue fever (DF) and severe dengue fever (SDF) with controls.

Parameters	Mean ± SEM			ANOVA P-Value
	Control (N=45)	DF (N=26)	SDF (N=14)	
Platelets ($x10^3/\mu L$)	269.80±8.21	157.40±10.47	42.00±4.73	≤0.001
FSH (mIU/mL)	8.73±0.48	9.90±0.44	11.19±1.19	0.039
LH (mIU/mL)	10.04±0.33	11.76±0.96	15.46±1.28	≤0.001
Testosterone (ng/dL)	6.53±0.20	5.58±0.22	4.59±0.28	≤0.001

inflammation caused by viral infections can impact these cells' functions. Additionally, this discussion will suggest a potential mechanism for the inflammation and damage observed during dengue fever [21].

Viruses are among the most common pathogens in humans and can contribute to normal and abnormal hormonal functioning. Viruses may affect the endocrine system directly and indirectly, leading to abnormal hormone production or suppression levels. The endocrine glands are vital to human health, and any temporary or permanent disruption will devastate the human body. Specific viral proteins can be synthesized by viral encoding, which may be similar to human hormones' structure and function [22]. The male reproductive system may serve as a safe refuge from diseases since viruses live in semen for far longer than in blood, urine, or saliva [23].

Studies examining the hormonal changes that occur in dengue patients are pretty rare. This research aims to investigate how DENV infection affects the endocrine system and to determine whether these alterations correlate with disease severity. In men, testosterone deficiency is the primary cause of infertility [24].

Dengue patients in our investigation had low testosterone in their serum than healthy controls, with a statistically significant difference. These findings show that dengue has a devastating impact on testosterone biosynthesis and its level.

Based on the available data, we aim to suggest a potential mechanism that could account for the pathogenesis of Dengue Hemorrhagic Fever (DHF). Dengue virus (DENV) is known to replicate in macrophages and trigger CD4+ T cells to produce a distinct cytokine that, in turn, prompts macrophages to generate free radicals, nitrite, reactive oxygen, and peroxynitrite. In addition to inducing apoptosis in the target cells, these free radicals also stimulate the production of proinflammatory cytokines such as IL-1α, TNF-α, IL-6, IL-8, and hydrogen peroxide within macrophages [25]. Testicular macrophages (Tmφ) generate reactive oxygen species, which can negatively affect the testosterone production and 3β-hydroxysteroid dehydrogenase activity of Leydig cells. This implies that the presence of persistent inflammation resulting from an infection may hinder steroidogenesis by altering Leydig cell function. This, in turn, can directly impact spermatogenesis and male fertility [26]. Under normal circumstances, the production of IL6 by Sertoli cells serves to suppress meiotic DNA synthesis in spermatocytes. Therefore, it is plausible that an elevation in IL6 levels during dengue fever could result in decreased sperm production, potentially leading to male infertility [27].

Testicular health is crucial to the success of the male reproductive system. Testosterone production and spermatogenesis are two of their critical functions in men. This primary circulating androgen is produced by Leydig cells in the testes. Hypogonadism is characterized by inadequate quantities of testosterone [26].

Follicle-stimulating hormone (FSH) regulates development from infancy to maturity and sexual organ maturation. Male reproductive organs like the testes and the prostate gland, as well as enhanced masculinity, bone density, and secondary sexual traits, rely on testosterone, which FSH produces in conjunction with the Luteinizing hormone (LH) [28].

The temporary changes observed in sperm could be attributed to the viral infection, fever, or a combination of both, given that all patients reported experiencing febrile episodes. While the exact cause of these alterations following DENV infection remains unclear, it is worth noting that heightened sperm DNA fragmentation has previously been observed in cases of testicular or epididymal hyperthermia [29].

In this investigation, LH and FSH levels were considerably higher in dengue patients than in the control group. Finally, a negative feedback process working to restore testosterone levels may be responsible for the increased LH and FSH.

Our prospective study had some limitations, including a small sample size and the lack of a control group consisting of non-infected men for the assessment of semen and hormonal characteristics. Additionally, the study focused on men with mild or minor symptoms of dengue infection, and thus, the effects on individuals with asymptomatic or severe dengue remain unknown.

Our understanding of the interactions between DENV and the reproductive tract is still in its early stages. The present study highlights the necessity for additional research in this area, which could have significant implications for public health policy by improving diagnostic accuracy and limiting the sexual transmission of DENV. Furthermore, our findings are pertinent to the counseling of DENV-infected patients and couples who are considering starting a family.

Conclusively, reproductive hormones in dengue patients have not been the subject of much research anywhere globally. In the present study, dengue patients' FSH and LH levels were significantly elevated. On the other hand, the testosterone levels of dengue patients dropped dramatically. These results suggested that dengue infection poses substantial risks to male reproductive capacity. Finally, a negative feedback process working to restore testosterone levels may be responsible for the increased LH and FSH.

ACKNOWLEDGMENT

The authors of the present investigations gratefully acknowledge Institute of Zoology, University of the Punjab for providing lab.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Rodenhuis-Zybert, I.A., J. Wilschut, and J.M. Smit, *Dengue virus life cycle: viral and host factors modulating infectivity.* Cellular and molecular life sciences, 2010. **67**(16): p. 2773-2786.
2. Katzelnick, L.C., et al., *Dengue viruses cluster antigenically but not as discrete serotypes.* Science, 2015. **349**(6254): p. 1338-1343.
3. Ratnam, I., et al., *Dengue fever and international travel.* Journal of travel medicine, 2013. **20**(6): p. 384-393.
4. Pozzetto, B., M. Memmi, and O. Garraud, *Is transfusion-transmitted dengue fever a potential public health threat?* World journal of virology, 2015. **4**(2): p. 113.
5. Pouliot, S.H., et al., *Maternal dengue and pregnancy outcomes: a systematic review.* Obstetrical & gynecological survey, 2010. **65**(2): p. 107-118.
6. Wu, J.-Y., et al., *Dengue fever in mainland China.* The American journal of tropical medicine and hygiene, 2010. **83**(3): p. 664.
7. WHO. *Dengue and severe dengue*. 2022 [cited 2022 18-04]; Available from: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue.
8. WHO. *Dengue fever – Pakistan*. 2021 [cited 2022 16-04-2022]; Available from: https://www.who.int/emergencies/disease-outbreak-news/item/dengue-fever-pakistan.
9. Calderón-Peláez, M.-A., et al., *Dengue virus infection of blood–brain barrier cells: Consequences of severe disease.* Frontiers in microbiology, 2019. **10**: p. 1435.
10. Beaumier, C., M.N. Garcia, and K.O. Murray, *The history of dengue in the United States and its recent emergence.* Current Tropical Medicine Reports, 2014. **1**(1): p. 32-35.
11. Chan, M. and M.A. Johansson, *The incubation periods of dengue viruses.* PloS one, 2012. **7**(11): p. e50972.
12. Vaughn, D.W., et al., *Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity.* The Journal of infectious diseases, 2000. **181**(1): p. 2-9.
13. Scott, R., *McN.* A. Nisalak, KH Eckels, M. Tingpa-lapong, VR Harrison, DJ Gould, FE Chapple, and PK Russell, 1980: p. 181-186.

14. Chawla, P., A. Yadav, and V. Chawla, *Clinical implications and treatment of dengue.* Asian Pacific journal of tropical medicine, 2014. **7**(3): p. 169-178.
15. Coates, J.M., M. Gurnell, and Z. Sarnyai, *From molecule to market: steroid hormones and financial risk-taking.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2010. **365**(1538): p. 331-343.
16. Ramanujam, L., et al., *association of molecular variants of luteinizing hormone with male infertility.* Human Reproduction, 2000. **15**(4): p. 925-928.
17. Casarini, L., et al., *FSH for the Treatment of Male Infertility.* International journal of molecular sciences, 2020. **21**(7): p. 2270.
18. Surampudi, V. and R.S. Swerdloff, *Hypogonadotropic and Hypergonadotropic Hypogonadism*, in *Testosterone*. 2017, Springer. p. 133-145.
19. Lunenfeld, B., et al., *Recommendations on the diagnosis, treatment and monitoring of hypogonadism in men.* The Aging Male, 2015. **18**(1): p. 5-15.
20. Bhasin, S. and S. Basaria, *Diagnosis and treatment of hypogonadism in men.* Best practice & research Clinical endocrinology & metabolism, 2011. **25**(2): p. 251-270.
21. Ndulue, C., *Std pong 2.0: an african-centric persuasive game for promoting risky sexual behaviour change.* 2019.
22. Somasundaram, N.P. and S.S. Gunatilake, *Infections in Endocrinology: Viruses.* Endotext [Internet], 2021.
23. Epelboin, S., et al., *Zika virus and reproduction: facts, questions and current management.* Human Reproduction Update, 2017. **23**(6): p. 629-645.
24. Joensen, U.N., et al., *Do perfluoroalkyl compounds impair human semen quality?* Environmental health perspectives, 2009. **117**(6): p. 923-927.
25. Chaturvedi, U., et al., *Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis.* FEMS Immunology & Medical Microbiology, 2000. **28**(3): p. 183-188.
26. Bryan, E.R., et al., *Testicular inflammation and infertility: Could chlamydial infections be contributing?* American Journal of Reproductive Immunology, 2020. **84**(3): p. e13286.
27. Hakovirta, H., et al., *function of interleukin-6 as an inhibitor of meiotic DNA synthesis in the rat seminiferous epithelium.* Molecular and cellular endocrinology, 1995. **108**(1-2): p. 193-198.
28. Meriggiola, M.C., et al., *Annual patterns of luteinizing hormone, follicle stimulating hormone, testosterone and inhibin in normal men.* Human reproduction, 1996. **11**(2): p. 248-252.
29. Sergerie, M., et al., *High risk of temporary alteration of semen parameters after recent acute febrile illness.* Fertility and sterility, 2007. **88**(4): p. 970. e1-970. e7.

AUTHORS

First Author: Humera Naveed
Institute of Zoology, University of the Punjab, Lahore -54590
https://orcid.org/0009-0005-6988-4945

Second Author: Kaleem Maqsood
Institute of Zoology, University of the Punjab, Lahore -54590
https://orcid.org/0000-0003-2330-3162

Third Author: Husna Ahmad
Institute of Zoology, University of the Punjab, Lahore -54590
https://orcid.org/0000-0003-4160-5148

Fourth Author: Waheed Haider
Institute of Zoology, University of the Punjab, Lahore -54590

Fifth Author: Muhammad Amir Iqbal
Institute of Zoology, University of the Punjab, Lahore -54590
https://orcid.org/0000-0002-5097-2065

Sixth Author: Dr. Nabila Roohi
Institute of Zoology, University of the Punjab, Lahore -54590
https://orcid.org/0000-0002-2396-5433

.
Corresponding Author: Dr. Nabila Roohi
Institute of Zoology, University of the Punjab, Lahore -54590
https://orcid.org/0000-0002-2396-5433