

Original Article

Spectrum of Semen Abnormalities and its Updated Trends by Machine Learning in District Sialkot of Pakistan

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Abstract

Objective: To find out the prevalence and updated trends of semen abnormalities spectrum in District Sialkot of Pakistan.

Methodology: This cross-sectional study was conducted from 1st October 2022 to 31st November 2024, data of semen analysis total (n=5098) was collected from Dr. Abdul Sittar lab. All result reports having information of age, abstinence period more than 2 days and less than 10 days were including. Oligozoospermia, Severe Oligozoospermia, Azoospermia, Teratozoospermia, Sperm defects and Asthenozoospermia parameters were evaluated according to WHO guidelines. To find out the trends, linear regression was used by Jupyter Notebook of Anaconda Navigator 1.9.12 (Python).

Results: The prevalence of oligozoospermia, severe oligozoospermia, azoospermia, and asthenozoospermia were observed in 472(9.7%), 67(2.3%), 154(3.5%), and 1598(31.3%) of cases, respectively. Teratozoospermia percentage was 35.82%, while the proportions of sperm head, mid-piece, and tail defects were 15.88%, 11.18%, and 8.85%, respectively. Higher proportions of Oligozoospermia, severe oligozoospermia, azoospermia and asthenozoospermia were observed in the 26-30 year age group, with 171(36.2%), 35(52.2%), 60(39.0%) and 551(34.5%) respectively, whereas teratozoospermia, poor motility and very poor motility was most frequent in the 31-35 year age group 305(35.7%), 287(34.5%) and 10(33.3%) respectively. Regarding sperm motility grades, "Grade A" showed good motility in 59.6% and fair motility in 22.0% of samples. "Grade B" motility (poor) accounted for 17.8%, while "Grade C" motility (very poor) was observed in 0.6% of samples. Trend analysis revealed, significant increasing trends in oligozoospermia, teratozoospermia, asthenozoospermia, and Grade B motility. In contrast, decreasing trends were observed in Grade A motility, Grade C motility, and azoospermia.

Conclusion: The results demonstrate a concerning decline in semen quality parameters in the Sialkot District population during the study period. The statistically significant increasing trends are alarming and need urgent public health initiatives focused on male fertility in the region.

Keywords: Male infertility, Oligozoospermia, Teratozoospermia, Asthenozoospermia, Azoospermia.

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Introduction

Infertility is a significant global health concern, affecting an estimated 8-12% of reproductive-aged couples worldwide, with male factors being a primary or contributing cause in approximately 40-50% of cases.¹ Infertility is more than just a medical concern; it also causes significant psychological pain, societal stigma, and marital difficulty, especially in socio-cultural countries like Pakistan, where parenting is highly cherished.² Infertility is typically categorized into two types: primary and secondary infertility. Primary infertility is the situation in which an individual has

never been pregnant. Secondary infertility is described as the inability to conceive naturally after a previous pregnancy, whether it resulted in a live birth or a miscarriage.³

Male infertility is predominantly investigated through semen analysis, which remains the cornerstone of clinical assessment. The parameters of semen quality including sperm count, motility, and morphology are critical biomarkers of male reproductive health.⁴ The World Health Organization (WHO) has established laboratory standards for the examination of human

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semen to provide a universal framework for diagnosis.⁵ Any significant deviation from these reference values leading to subfertility or infertility, like oligozoospermia (low sperm count <14M/ml), asthenozoospermia (reduced sperm motility <40%), teratozoospermia (abnormal sperm morphology <4%), or azoospermia (Zero sperm count), all these constitutes a spectrum of semen abnormalities.⁵ These abnormal patterns have differing etiologies, prognoses, and management pathways. Accurate classification and reporting are necessary for counseling, triage to further diagnostic workup (e.g., semen analysis, hormonal studies, genetic testing, scrotal imaging, semen culture), and guiding assisted reproduction choices.⁶

Recent decades have witnessed a growing apprehension over a perceived global decline in semen quality.⁷ Multiple factors influence semen quality and male reproductive potential. Pathophysiological causes include testicular (spermatogenic) disorders, obstruction of the male reproductive tract, endocrine abnormalities, genetic causes (e.g., chromosomal abnormalities, Y-chromosome microdeletions), infections of the genitourinary tract, and varicocele.⁸ Environmental and lifestyle contributors have attracted growing attention: tobacco and opiate use, alcohol, obesity and metabolic disorders, occupational exposures (heat, heavy metals, solvents), environmental toxicants (pesticides, industrial chemicals), poor diet and physical inactivity, and rising rates of chronic diseases can all negatively affect semen parameters.⁸⁻⁹ These multifactorial influences underscore the need to pair laboratory descriptions of semen abnormalities with clinical, occupational, and demographic data to better interpret local trends and to design interventions.¹⁰

In Pakistan, infertility poses a considerable burden, yet comprehensive epidemiological data on the specific trends and spectrum of male factor infertility remains scarce and regionally fragmented.¹¹ Sialkot, a major industrial city in the Punjab province, presents a particularly relevant context for such a study. It is a hub for industries such as leather tanning, surgical instruments manufacturing, and sports goods production. These industries are associated with the use and potential environmental release of endocrine-disrupting chemicals (EDCs), heavy metals, and other toxicants known to impair spermatogenesis and steroidogenesis.¹²⁻¹³ Furthermore, another layer of risk for the male reproductive health of the local population

is the widespread use of pesticides in the surrounding agricultural areas.¹⁴

To find out the trend of semen abnormalities over the time period, according to our best until now there is no any study in this region. This study aims to conducting a comprehensive analysis of the semen abnormalities spectrum (oligozoospermia, asthenozoospermia, teratozoospermia, azoospermia) along with its trend in general population of District Sialkot. The findings from this research can provide crucial baseline data for healthcare policymakers, clinicians, and researchers to develop targeted public health interventions and strategies to address male primary infertility in this specific region of Punjab, Pakistan.

Methodology

District Sialkot is consist of four tehsils (Daskat, Pasrur, Sambrial, Sialkot) according to 2023 census the total population of District Sialkot was 4,499,394 with 2.44 % annual growth rate. In 2024 District Sialkot Population size was about 4609179.¹⁵ From 1st October 2022 to 31st November 2024 semen analysis reports were collected from Dr. Abdul Sattar Lab® (Head office Branch, it is well reputed lab and has more many collection centers across the District) after written permission from its R&D department. Ethical approval was also taken from IRB of Grand Asian University Sialkot (Ref: GAUS/2025/004 dated: 08-08-2024). Data of all semen analysis reports of general population were including that having information of age and abstinence period more than 2 days and less than 10 days whereas those reports without information of age and have abstinence period less than 2 days and more than 10 days were excluded from this analysis. Sampling was conducted by non-probabilistic consecutive technique and in our retrospective (cross sectional) analysis total sample size was (n=5,098). Sample size was measure by online available survey monkey website, First, the margin error (sampling error) then sample size was find out by using following formulas, 2% margin error was obtained.¹⁶

$$\text{Margin error (e)} = z \times \sigma / \sqrt{n} \quad \text{Sample size} = \frac{\frac{z^2 \times p(1-p)}{e^2}}{1 + \left(\frac{z^2 \times p(1-p)}{e^2 N}\right)}$$

z is z-score (99% C.L. z-score is 2.58), σ is standard deviation of population, n is number of samples, p is population proportion (0.5), e is margin error, N is population size.

According to WHO universal framework for diagnosis guidelines of the semen analysis parameters like oligozoospermia, severe oligozoospermia, azoospermia, teratozoospermia, sperm defects and asthenozoospermia were evaluated.⁵ All data type and interpret in Microsoft excel 2016 installed in Window 7 (32 bit). Trends were found by linear regression method by using machine learning technique. All coding's were run by using Jupyter Notebook of Anaconda Navigator 1.9.12. This graphical user interface (GUI) was installed in Microsoft window 7 (32 bit) to run. All necessary library coding that were imported and run are available in supplementary file. The regression slope (β coefficient) was used to estimate the expected change in trend for each month of time period.

Results

In District Sialkot average percentage of normal sperm count (≤ 14 M/ml) was as $n=2375$ (46.7%) followed by Oligozoospermia (>14 M/ml), Severe Oligozoospermia (>1 M/ml) and Azoospermia (0 sperm) 472 (9.7%), 67 (2.3%), 154 (3.5%), respectively. The detail percentage trends of these three semen analysis parameters on monthly bases shown in figure 1. Azoospermia with or without other semen analysis parameters is the major cause of male primary infertility factor, in District Sialkot its percentage $n=60$ (39.0%) is high in 26-30 years of age group followed by 31-35, 20-25 and 36-40 years with percentages of $n=39$ (25.3%), $n=32$ (20.8%) and $n=13$ (8.4%) respectively.

Average percentage of Teratozoospermia (Abnormal

sperm morphology) in District Sialkot is 35.82%. Head Defects percentage (15.88%) is high as compare to middle and tail percentage, defects in the head can affect the sperm's ability to penetrate the egg. Middle Piece (Neck) Defects percentage was 11.18%, defects in the mid-piece can impair energy production, affect sperm motility. Tail Defects percentage was low (8.85%) as compared to others, it directly impair the sperm's ability to swim forward. Detail Teratozoospermia along with Head, middle and tail defects tendency on monthly basis shown in supplementary file figure 1. Teratozoospermia percentage was founded high in age group 31-35 followed by 26-30 with percentage of $n=305$ (35.7%) and $n=181$ (21.2%) respectively as its detail along with age groups shown in table I.

Poor motility is a key factor in infertility as sperm cannot reach the egg. Asthenozoospermia (Motility $<40\%$ nearly 1 Hour) average percentage rate in this region was $n=1598$ (31.3%) and affected age groups were 26-30 $n=551$ (34.5%) and 31-35 $n=497$ (31.1%) years. From October 2022 to November 2024 its prevalence detail shown in supplementary file figure 2. In District Sialkot average percentage prevalence of Forward Progression or Progressive motility was like "Grade a motility" (Good motility 59.6%, and Fair motility 22.0%), "Grade b motility" (Poor motility 17.8%) and "Grade c motility" (Very poor motility 0.6%). Monthly percentages of each Grade of motility available in the table I of supplementary file. "Grade b" percentage was high in age groups 31-35 followed by 26-30 years with $n=287$ (34.5%) and $n=258$ (31.0%) respectively. "Grade c"

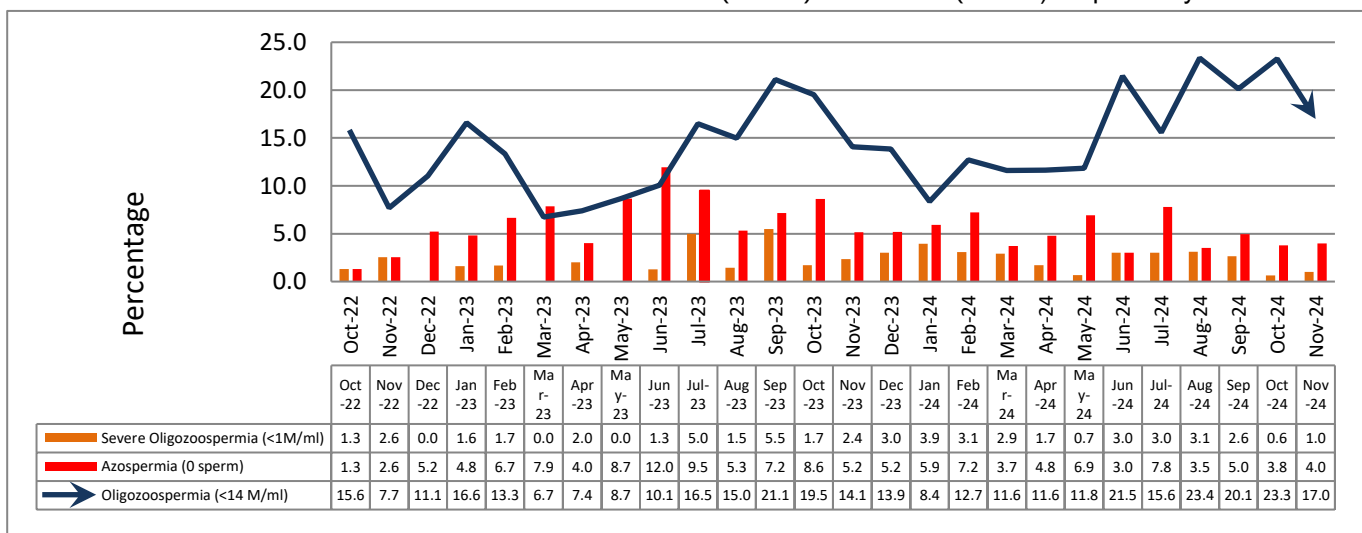


Figure 1. Oligozoospermia, Severe Oligozoospermia and Azoospermia pattern in District Sialkot, Punjab Pakistan

Table I: Semen abnormalities spectrum across age groups of District Sialkot.

Age groups	Oligospermia percentage	Sever Oligospermia Percentage	Azzospermia Percentage	Asthenozoospermia Percentage	Teratozoospermia Percentage	Grade motility (Poor) Percentage	Grade motility (Very Poor) Percentage
<19	3(0.6%)	-	1(0.6%)	7(0.4%)	5(0.6%)	6(0.7%)	-
20-25	63(13.3%)	7(10.4%)	32(20.8%)	215(13.5%)	124(14.5%)	92(11.1%)	5(16.7%)
26-30	171(36.2%)	35(52.2%)	60(39.0%)	551(34.5%)	181(21.2%)	258(31.0%)	9(30.0%)
31-35	154(32.6%)	14(20.9%)	39(25.3%)	497(31.1%)	305(35.7%)	287(34.5%)	10(33.3%)
36-40	61(12.9%)	6(9.0%)	13(8.4%)	216(13.5%)	139(16.3%)	127(15.3%)	2(6.7%)
41-45	14(3.0%)	4(6.0%)	7(4.5%)	88(5.5%)	84(9.8%)	48(5.8%)	1(3.3%)
46-50	4(0.8%)	-	2(1.3%)	15(0.9%)	12(1.4%)	7(0.8%)	3(10.0%)
51-55	2(0.4%)	-	-	6(0.4%)	5(0.6%)	5(0.6%)	-
56-60	-	1(1.5%)	-	3(0.2%)	-	2(0.2%)	-

Table II: District Sialkot spectrum of semen abnormalities.

Spectrum of semen abnormalities	N (%)
Oligozoospermia	472 (9.7%)
Severe Oligozoospermia	67 (2.3%)
Azoospermia	154 (3.5%)
Teratozoospermia	35.82%
Head Defects	15.88%
Middle Piece (Neck) Defects	11.18%
Tail Defects	8.85%
Asthenozoospermia	1598 (31.3%)
Grade a motility	Good 2781 (59.6%)
	Fair 1027 (22.0%)
Grade b motility	Poor 832 (17.8%)
Grade c motility	Very poor 30 (0.6%)

percentage was also high in age group 31-35 years with n=10 (33.3%) followed by age group 26-30 years n=9 (30.0%) as its detail and along with other age groups shown in table I. The average for the entire time period shows that (59.6%) of sperm has good/fair progressive motility. However, a combined almost 40.4% (22.0% Fair + 17.8% poor + 0.6% very poor) have impaired movement, aligning with the 31.3% asthenozoospermia rate, summarized detail of all spectrum of semen abnormalities shown in table II.

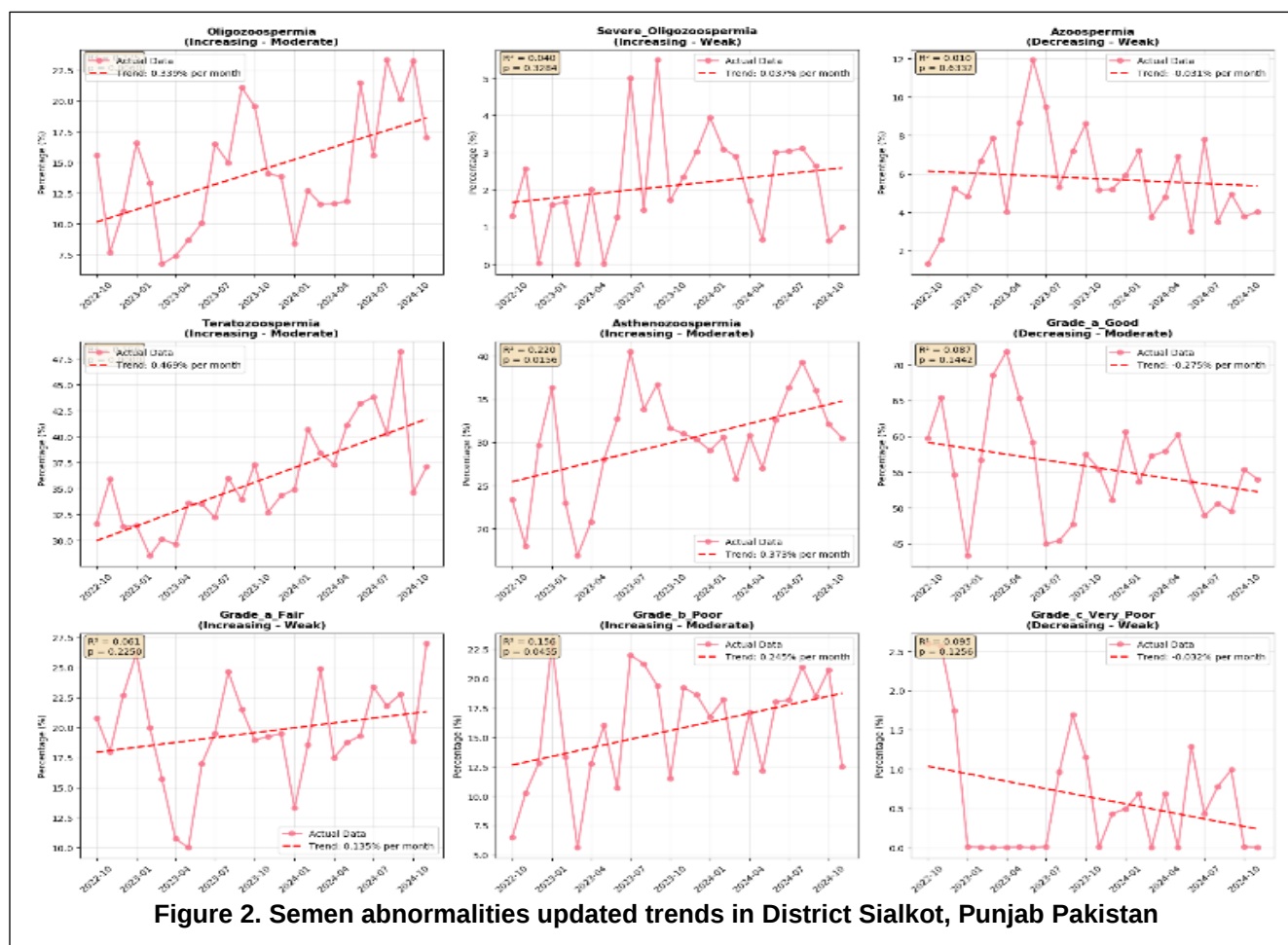
In our analysis the most significant per month increasing trends ($p < 0.05$) were found in Oligozoospermia, Teratozoospermia,

Asthenozoospermia and "Grade b" (Poor motility) with slop (per month increasing percentage) of (0.339), (0.469), (0.373) and (0.245) respectively whereas decreasing trends were observed in "Grade a" (Good motility) (-0.275), "Grade c" (Very Poor motility) (-0.032) and Azoospermia (-0.031) per month as shown in table III and figure 2.

Monthly trend slopes comparison is shown in figure 3 of supplementary file; in this bar chart Positive slopes (green bars) indicate an increasing trend over time whereas Negative slopes (red bars) indicate a decreasing trend over time. "Grade b" (Poor motility), "Grade a" (Fair motility), Asthenozoospermia, Teratozoospermia, Oligozoospermia and Severe Oligozoospermia all show positive increasing trends on monthly basis (up to ~0.45%). Which mean that in District Sialkot sperm quality (count, motility, morphology) is decreasing over time. "Grade c" (Very Poor motility), "Grade a" (Good motility) and Azoospermia shows negative trends. Deceasing trend in Azoospermia (0 sperm) did not mean that male infertility decreasing over time in District Sialkot on the other side other parameters like low sperm count, poor motility and abnormal morphology trend are increasing and good motility is decreasing that's are major factors to increasing male primary infertility with poor sperm quality. Model Fit (R-squared/ R^2 Values) indicates how well the model explains the observed variation (0=poor fit, 1=perfect fit). Teratozoospermia trend has the

Table III: Trend Analysis (Linear Regression Slopes)

Parameter	Slope	R ²	p-value	Trend	Strength	Sig.
Oligozoospermia	0.3386	0.275	0.006	Increasing	Moderate	Yes
Severe Oligozoospermia	0.0369	0.040	0.328	Increasing	Weak	No
Azoospermia	-0.0308	0.010	0.633	Decreasing	Weak	No
Teratozoospermia	0.4695	0.556	<0.001	Increasing	Moderate	Yes
Asthenozoospermia	0.3732	0.220	0.016	Increasing	Moderate	Yes
Grade a Good Motility	-0.2752	0.087	0.144	Decreasing	Moderate	No
Grade a Fair Motility	0.1348	0.061	0.225	Increasing	Weak	No
Grade b Poor Motility	0.2445	0.156	0.045	Increasing	Moderate	Yes
Grade c Very Poor Motility	-0.0319	0.095	0.126	Decreasing	Weak	No



strongest fit ($R^2 \approx 0.55$) followed by the Asthenozoospermia and Oligozoospermia these two parameters also have moderate fits ($R^2 \approx 0.2-0.3$).

Statistical significance (p-values) chart evaluates statistically significant slopes, here Green bars mean these parameters are significant ($p < 0.05$) and Red bars mean not significant whereas vertical dashed line showing the significance threshold ($p = 0.05$). According to this chart Teratozoospermia, Oligozoospermia, Asthenozoospermia and “Grade b” (Poor motility) show statistically significant results (green) vice-versa for remaining parameters as shown in figure 3 of supplementary file. Distribution of Trend Strengths, pie chart categorizes the overall strength of trends. Moderate trends mean trends are increasing or decreasing with moderate pattern instead of sharp or strong trend with 55.6% cases. Weak trends mean trends are increasing or decreasing in weak or slow pattern with 44.4% of cases.

Discussion

In the present district-level analysis of semen parameters in Sialkot, the majority of samples demonstrated normal sperm concentration (46.7%), while abnormalities included oligozoospermia (9.7%), severe oligozoospermia (2.3%), and azoospermia (3.5%). These findings indicate that although high percentage show normal semen count whereas significant portion exhibits abnormalities that may compromise fertility potential. The observed percentage of oligozoospermia and azoospermia appears low than that previous regional reported studies, like in 2018 a study was conducted in Lahore with $n=200$ sample size concluded higher percentage of Oligospermia (23.2%) and Azoospermia (19.2%) in infertile males.¹⁷ In 2022 investigated in Sialkot city, reported Oligozoospermia (8%) and Azoospermia (28%) of with $n=260$ number of patients.¹⁸ The discrepancies between these findings and present studies can be explained by methodological differences. In previous studies Azoospermia percentage was high because they used purposive sample technique mean almost all patients who were

selected have some infertility issues. Such sampling methods sometimes tend to overestimate semen abnormalities parameters. In contrast, this analysis utilized large district level dataset including large male population undergoes semen analysis which provides good representative estimation of semen abnormalities in general population of this region. Recent study was conducted in Sialkot city with 1393 patients, they concluded the prevalence rate 15.4% for oligospermia and 3.1% for Azoospermia.¹⁹ These prevalence percentages are relatively comparable with present findings and suggested that a degree of regional consistency present when large and less selective population are studied.

Azoospermia with or without other semen analysis parameters is the major cause of male primary infertility factor in this analysis age specific patterns observed in this analysis that highlight the complexity of male infertility. Azoospermia was mostly observed in 26-30 years of age group with (39.0%) that indicated male infertility issue are not limited to the older age group. Although male reproductive capacity generally declines gradually with age, several environmental and lifestyle factors including smoking, occupational exposure to chemicals, environmental pollutants, and metabolic disorders have been shown to negatively affect spermatogenesis even in younger men.²⁰ These factors may contribute to the relatively high burden of infertility related abnormalities in the reproductive age group observed in this study.

Morphological abnormalities also represented a significant component of semen impairment in the study population. The average prevalence of teratozoospermia in District Sialkot was 35.82%, with head defects (15.88%) occurring more frequently than middle (11.18%) and tail defects (8.85%). The highest prevalence of teratozoospermia rate was observed in the 31-35 age group (35.7%). Age related morphological abnormalities have been reported in many studies that cumulative environmental exposures, DNA fragmentation, oxidative stress effected normal spermatogenic process.²⁰ these finding suggest that morphological impairments prominently present in advancing age and may play considerable role in reducing male infertility.

Asthenozoospermia showed an average prevalence of 31.3% in the region, with the 26-30 age group being the most affected (34.5%). Across the study period nearly 40.4% (22.0% Fair + 17.8% poor + 0.6% very poor)

exhibited impaired movement, which is consistent with the observed 31.3% asthenozoospermia rate. Across studies conducted in Lahore and Sialkot over different time periods, asthenozoospermia consistently emerged as the most prevalent semen abnormality. In Lahore, study reported asthenozoospermia as the predominant semen abnormality (31.3%), followed by teratozoospermia (20.2%).¹⁷ In Sialkot, investigation identified asthenozoospermia as the leading contributor to male infertility, accounting for 37.3% of cases. Additionally, in 2022 study from Sialkot, documenting asthenozoospermia in 39% of patients, whereas teratozoospermia was comparatively infrequent at 1.7%. Collectively, these findings highlight the persistent predominance of asthenozoospermia in male infertility across different regions and study periods in Pakistan.¹⁸⁻¹⁹

Impaired sperm motility, for sperm to reach and penetrate the oocyte may represent male infertility possible including factors increasing oxidative stress, environment pollution, sedentary lifestyle and nutritional deficiencies responsible for it. Infertility is not just azoospermia, any abnormal semen pattern including individually or collectively low sperm count, poor motility, abnormal morphology, or low volume analysis are also responsible of infertility.²¹ Therefore, comprehensive semen analysis remains essential for identifying the full spectrum of male infertility factors.

In 2022 an analysis was don on male primary infertility, to find out regional trends in notable differences emerged across global settings. In Central Europe, Eastern Europe, and Central Asia, the infertility showed a significant increasing trend, with a slope of 5.4 ($p<0.001$), reflecting a gradual increase over recent decades. Conversely, high-income countries demonstrated a significant decline, with a negative slope of 9.3 ($p<0.001$), suggesting improvements in male reproductive health or effective interventions targeting infertility risk factors in these regions. The North Africa and Middle East region demonstrated the steepest rise in male primary infertility (slope 19.0), reflecting a rapidly growing burden, while South Asia showed a similarly strong upward trend (slope 16.5), indicating increasing vulnerability in male reproductive health across this densely populated region.²² As in global male semen abnormalities spectrum increasing with very high percentages in our analysis same increasing trends were founded. In this analysis the most significant per month increasing trends ($p<0.05$) were found in Oligozoospermia, Teratozoospermia,

Asthenozoospermia and “Grade b” (Poor motility) with slop (0.339), (0.469), (0.373) and (0.245) respectively as shown in table III and figure 2. These findings suggest a progressive shift in the prevalence of semen abnormalities, with mild abnormalities increasing and extreme situations like azoospermia remaining stable or slightly declining. Such changes may reflect shifting environmental exposures, lifestyle factors, and advances in early diagnostic methods.

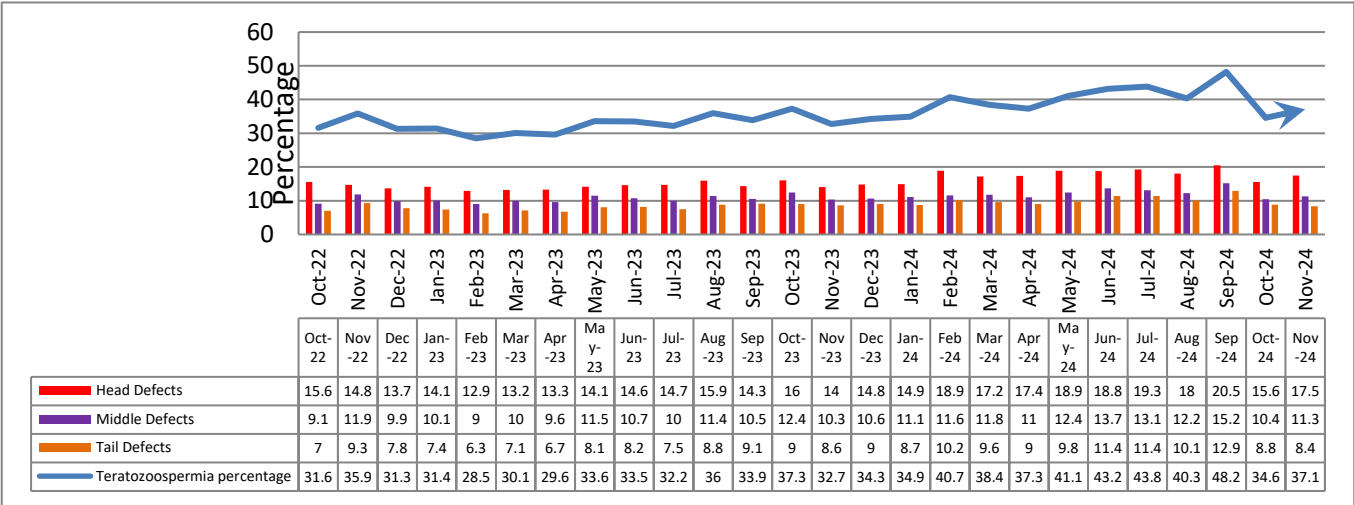
Conclusion

This study offers valuable epidemiological insights on the distribution and patterns of sperm abnormalities at the district level in Pakistan. This study uses large dataset and district-wide analysis to gain a deeper understanding of semen quality patterns in the general population. This study adds to the existing literature on male infertility trends in Pakistan by providing region-specific data and could help in future reproductive health policy, raise public awareness and clinical management strategies.

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Figure 3 of Supplementary file



Teratozoospermia along with Head, middle and tail defects tendency in District Sialkot

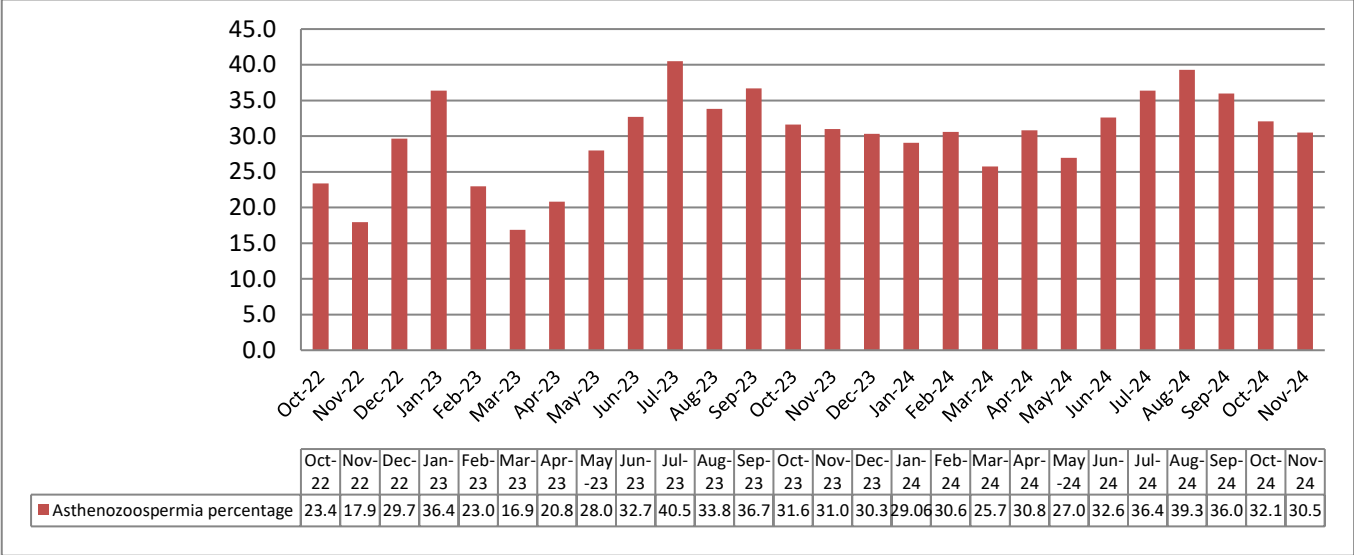


Figure 2: Monthly average percentage of Asthenozoospermia in District Sialkot, Punjab Pakistan

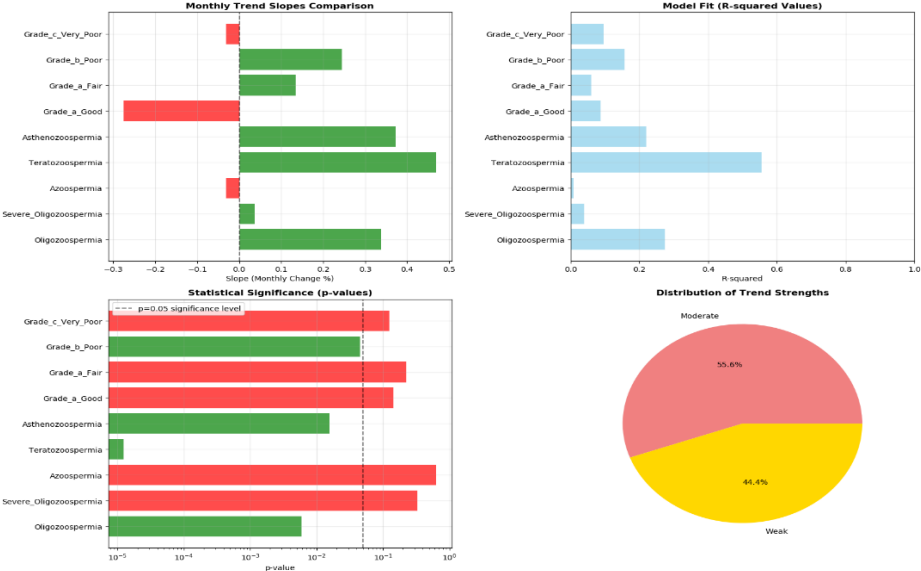


Figure 3: Linear Regression analysis of Spectrum of semen abnormalities by Jupyter Notebook, Python

Forward Progression/ Progressive motility average percentages from October 2022 to November 2024				
Months	Grade a		Grade b	Grade c
	GOOD	FAIR	POOR	VERY POOR
Oct-22	59.7	20.8	6.5	2.6
Nov-22	65.4	17.9	10.3	2.6
Dec-22	54.7	22.7	12.8	1.7
Jan-23	43.3	26.2	23.0	0.0
Feb-23	56.7	20.0	13.3	0.0
Mar-23	68.5	15.7	5.6	0.0
Apr-23	71.8	10.7	12.8	0.0
May-23	65.3	10.0	16.0	0.0
Jun-23	59.1	17.0	10.7	0.0
Jul-23	45	19.5	22	0
Aug-23	45.4	24.6	21.3	1.0
Sep-23	47.7	21.5	19.4	1.7
Oct-23	57.5	19.0	11.5	1.1
Nov-23	55.4	19.2	19.2	0.0
Dec-23	51.1	19.5	18.6	0.4
Jan-24	60.6	13.3	16.7	0.5
Feb-24	53.6	18.6	18.2	0.7
Mar-24	57.3	24.9	12.0	0.0
Apr-24	57.9	17.5	17.1	0.7
May-24	60.2	18.8	12.2	0.0
Jun-24	53.6	19.3	18.0	1.3
Jul-24	48.9	23.4	18.2	0.4
Aug-24	50.6	21.8	21.0	0.8
Sep-24	49.5	22.8	18.5	1.0
Oct-24	55.3	18.9	20.8	0.0
Nov-24	54.0	27.0	12.5	0.0