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BEYOND THE COUNT: A PARAMETRIC ASSESSMENT REVEALING ASTHENOZOOSPERMIA AS THE PRIMARY DRIVER OF MALE INFERTILITY IN ISLAMABAD

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Abstract

Male infertility is a major global concern, primarily linked to declining semen quality with age. In Pakistan, a limited dataset is published regarding the parametric assessment of semen among patients diagnosed with either primary or secondary infertility, notably in the urban population, such as that of Islamabad, the capital territory. This study aims to evaluate the contribution of total sperm count, motility and morphology to determine the cause of infertility among males in Islamabad. A descriptive cross-sectional study was carried out at 4 different hospitals and laboratories across Islamabad from October 2025 to January 2026. A total of 150 infertile patients, with ages ranging from 18 to 54, were included in this study, and these patients were further divided into two groups: a younger group (18-32 years) and an older group (33-54 years). The total sperm count, motility, morphology, pH, liquefaction time and pus cells were analyzed against the WHO criterion with the use of SPSS version 25 by utilizing independent and one sample t-test in combination with Pearson correlation, and Chi-square analysis. The mean morphology (56.79% ± 18.86%) and mean sperm count (54.24 ± 7.6 million/mL), p value .035, were higher in comparison with the motility (27.14% ± 14.80%) and their respective reference limit as well. Out of 150 patients, 58% were found to be affected by asthenozoospermia, highlighting it as the major abnormality among the cohort. In the present study, asthenozoospermia is the main driver of infertility among the patients of Islamabad, with a significant decline in morphology and total sperm count with respect to advancing age. Our results identify younger males as a high-risk cohort for genital tract infections, linked to age-related oxidative and acidic shifts.

Keywords: *Asthenozoospermia, Male infertility, Semen analysis, Sperm morphology, Sperm motility, WHO guidelines (2021)*

INTRODUCTION

Till today, infertility among males and females alike, is affecting almost 15% of couple worldwide according to the data shared by the World Health Organization and this too vary across international borders (1). In Pakistan, a total of 21.9% population is currently affected by primary and secondary infertility, alike and this percentage is thought to reach up to 34% by the end of 2030. Majority of these infertility cases are attributed to the males (30% of the total case) because of various genetic, marriage related, and health related factors that actively destroy the molecules of testosterone (2). Among these reported cases, majority of these are idiopathic, the exact cause of the factor contributing towards infertility remains unidentified, despite the presence of advanced diagnostic tools contributing towards the challenges in reproductive health (3). Male infertility is a general term that is used to describe the situations in which a couple has difficulty in conceiving due to the faulty sperms in male partner. A lot of medical terms are associated with the dysfunction in males like, asthenozoospermia (poor motility among sperms), teratozoospermia (abnormality in sperm shape and size), and oligozoospermia (having low sperm concentration) but still all these factors combined contribute towards the male infertility (4).

Semen analysis is the primary tool that is used for accessing the fertility profile of the males as it provides a qualitative and quantitative assessment of the reproductive health. The analysis itself is conducted according to the guidelines shared by the World Health Organization in the form of laboratory manual (6th edition, 2021) (5). The main parameters highlighted in the manual are morphology, total sperm count, and motility of the sperms found in the samples of males as they don't only count cells but in fact gives a clear picture of the integrity of the male reproductive organs and the tract itself, covering the formation of sperms (in testes) to their maturation (in epididymis). Apart from these physiological markers, some pathophysiological markers like the presence of pus cells and liquefaction time of the sperms also provides a critical insight about the presence or absence of inflammatory conditions, initiated by pro-inflammatory cytokines. The presence of pus cells, accompanied by delayed liquefaction time and points out the propagation of oxidative stress and prostatic dysfunction, respectively. The initiation of oxidative stress is the silent driver that causes DNA fragmentation making the normal looking sperm unable to fertilize with egg (infertility) (6, 7).

In the previous decade the increasing cases of male infertility across international borders have raised several concerns about the male reproductive health as it is not that documented than the female reproductive profile (8). Furthermore, the concept of male biological clock has gained valuable traction as the increasing age in males is associated with a continuous decline in the motility and total sperm count ultimately leading towards the production of deformed sperms (9). The male biological clock is thought to be affected by the hormonal shifts, exposure to chemical toxins accompanied by the disruption in cellular repair mechanism of the body. In Pakistan, most of the studies conducted on the male infertility profiles are limited to either broader provinces or rural areas, leaving a consistent gap for the upper-middle and urban-middle class of Islamabad as the residents are facing many reproductive challenges due to sedentary lifestyle, and the shift towards processed food in the name of safety (10).

The main objectives of this study was to address the gap by performing parametric evaluation of key semen parameters like, total sperm count, total motility, morphology, liquefaction time and the presence of pus across two distinct age groups (18-32 and 33-54 years) as these two groups depicts the transition of men from their biological peak towards the expected potential sub-fertility to identify the age-associated trends within the local population. Such critical insights are crucial for counselling regarding fertility and to further optimize the best treatment for couples.

METHODOLOGY

This cross-sectional descriptive study was conducted at 4 different locations in Islamabad including Hazrat Bari Imam Sarkar Medical and Dental Hospital (HBS), Niazi Hospital, Al-Nafees and Ahmed Medical Centre over the course of 15th October 2025 to 15th January 2026. Due to the limited amount of time and resources, we were able to collect the sample of 150 infertile male patients within the age group of 18-50 who were married for quite a long time and were unable to conceive over the course of almost 2-3 years. The patients were selected using the technique of consecutive sampling, and the patients who met the inclusion criterion were asked to participate in this study until the sample size was achieved. All the collected specimens were initially reported by the doctors to have issues in multiple semen parameters as they were dealing with primary infertility, and the comprehensive reports were evaluated to understand the best possible cause of infertility in terms of the parameters discussed above.

INCLUSION AND EXCLUSION CRITERIA

Patients who were suspected of infertility were included based on their symptoms, patients who have abstained from ejaculation from 2-7 days before samples collections and patients who were suspected of primary infertility (unable to conceive for the first time) were included in the study.

Patients having genetic abnormalities like Klinefelter syndrome Patients with incomplete medical records, patients having recent history of testicular trauma, surgery, or systemic illness, for example radiation therapy, chemotherapy that could affect the semen parameters and the patients who used medications, or drugs which effects spermatogenesis for examples steroids, were excluded from the study.

SAMPLE COLLECTION

The research was properly carried out after taking approval from research committee IQRA University, Chak Shehzad Campus, Islamabad. The primary phase was to inform the patients to give samples by instructing them to avoid ejaculation for a period of 7-10 days to make sure the integrity in terms of semen quality and quantity. Then, the patients were instructed to give semen samples trough masturbating into a clean and sterilized container to avoid contamination. Each sample was brought to the lab within 30 to 60 minutes so it could liquefy properly. In the initial phase, the physical aspects of the semen like volume, color, thickness, and liquefaction time were accessed to ensure the quality, followed by the microscopic examination of motility, total sperm count, sperm morphology, and presence of pus cells along with red blood cells. After the microscopic examination, we allowed the sample to liquefy at room temperature for approximately 30 minutes. Once the specimen liquefied, a drop of semen was carefully placed onto a clean glass slide and gently covered with a cover slip under a light source. By using a light microscope, we initially focused on the sample under 10 X magnification to locate the field of interest and then proceeded to examine it in detail under 40 X magnification for accurate analysis.

ETHICAL CONSIDERATIONS

After the approval of study by the research committee of IQRA University, informed consent was taken from the patients to make sure the transparency of the data according to the guidelines set by MHRA, United Kingdom. The data was confidentially maintained to gain the trust of the patients who agreed in participating in our descriptive cross-sectional study. Demographic information like age, socioeconomic status and duration of infertility were taken beforehand. History of any previous surgery and sexually transmitted infection were also considered along with any family or genetic infertility that might be affecting reproductive health. The analysis of semen was performed according to the 2021 guidelines set by World Health Organization, except the semen morphology, that is though be equal or less than 4% because, most of the laboratories of Pakistan are utilizing old guidelines (1992) where the morphology should be equal or greater than 50% to be included as good. The cut off value for Total sperm count, pH, Morphology, Motility, liquefaction time and pus cells was set according to the WHO standard criterion of 42 million/mL, <7.2, 50%, 42%, <60 minutes and <5 HPF respectively.

The patients in this study were categorized according to the WHO laboratory manual 6th edition as (11):

Oligospermic: having low concentration of sperm (< 16million/mL)

Teratoozospermic: Having abnormal sperm morphology (< 50% normal forms)

Asthenozoospermic: Having reduced motility (<42% total motility)

Normozoospermic: Having normal semen parameters

STATISTICAL ANALYSIS

The data was analyzed using SPSS version 25.0 and descriptives statistics for all the parameters were presented in the form of mean and standard deviation (±) for continuous variables. Comparison was carried out between the semen profiles of both the age groups (18-32, 33-54) conducted using independent sample t-test (continuous variables), chi square analysis (categorical variables), one sample t-test for comparing the findings against the WHO Guidelines, correlation analysis to identify the significance. The p-value of less than 0.05 was considered to be statistically significant.

RESULTS

The study comprises of 150 male infertile patients, taken across the 4 hospitals and laboratories, from the Islamabad Capital territory with their ages ranging from 18-54. These patients were divided into two distinct groups, a younger group (18-32) and older group (33-54) with a mean of 32.43 ± 7.605 years. Descriptive statistics were performed for all the semen parameters under observation. The fertility status of all the participants was assessed against the standards set by World Health Organization (6th edition, 2021) with the help of one sample t-test.

The analysis showed a relatively mixed profile for each of the semen parameters of the infertile males (Table I). Although, the mean sperm count (54.24 ± 7.6 million) was significantly higher than the reference set by the WHO reference limit of 16 million/mL (having t = 13.98 and p < 0.001), and the calculated mean morphology (56.79% ± 18.86%) was higher than the reference set limit of traditional 50% threshold, with t = 6.33 and p < 0.001 (as per the guidelines of 1992, currently practiced in Pakistani laboratories). The mean motility was calculated to be lower (27.14 ± 14.80%) than the reference limit of 42%, having t = -12.25, p < 0.001). While the remaining parameters of liquefaction time, pus cells, and pH were calculated to be (25.36 ± 9.17 minutes), (14.20 ± 14.96 /HPF), (7.78 ± 0.99) respectively and these were not different, in terms of significance, with the reference limit.

Table I. Comparison of mean semen parameters in the cohort against the WHO Guidelines for semen analysis (lower reference limit)

Parameters	Study mean (± SD)	WHO 2021 (6th edition)	t-value	p-value
pH	7.784 ± 0.996	< 7.2	0.605	0.546
Total Sperm Count (10^6)	54.244 ± 7.605	42 million	-0.274	0.035
Total Motility (%)	27.14 ± 14.800	40%	0.072	0.943
Normal Morphology (%)	56.79 ± 18.865	50% (1992 guidelines)	-0.658	0.511
Pus Cells (/HPF)	14.20 ± 14.962	< 5 per HPF	0.412	0.681
Liquefaction Time (min)	25.36 ± 9.170	< 60 minutes	-0.541	0.589

The above-mentioned reproductive health related diseases were accessed in all the 150 infertile patients (Fig, 1) and 14 patients (7%) from those fall into the category of azoospermia, meaning they have complete absence of sperm. Meanwhile, 15 (8%) from the remaining 98 patients with detectable sperms, were characterized by sperm count of less than 16 million/mL and were classified as Oligozoospermic, having less sperms than the reference limit of WHO 2021 criterion. Apart from these, 109 infertile patients (58% of the whole cohort) were characterized as Asthenozoospermic, having less sperm motility than 42% and this was studied to be the main driver of the infertility. While 52 patients (27%) were observed to be teratoozospermic, having morphology less than 50% as per the standard criterion. But still, these categories were not mutually exclusive to infertility, a large proportion of the cohort were presented with a combination of more than one defect, leading to primary infertility.

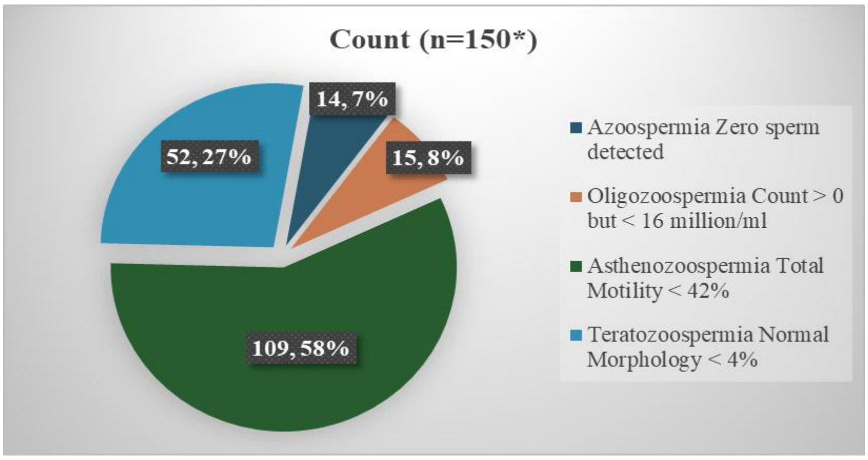

Fig. 1. Distribution of sperm related abnormalities across the cohort. The data suggests asthenozoospermia as the primary driver of infertility among patients of Islamabad

A significant difference was observed when the patients were categorized based on age groups. The age group of younger participants (18-32 years) showed remarkable mean sperm count (59.68 ± 29.39

million/mL) than the older group (33-54) with a total mean of (46.05 ± 27.60 million/mL) with a p value of 0.035. Moreover, the sperm morphology was quite good in the younger group (62.71% ± 17.71%) than in the corresponding older group (55.38% ± 19.34%0 with p value of 0.017. Furthermore, the younger group have lower pus cells (12.13 ± 12.94/HPF) and high level total motility (28.72% ± 15.16%) compared to the older groups but still the differences were not that much significant and as the data for pus cells and normal morphology was not normally distributed (Fig. 4), Mann Whitney-U test was performed to access the level of significance between the two age groups resulting in the p values of .575 and .515 respectively (Fig. 2).

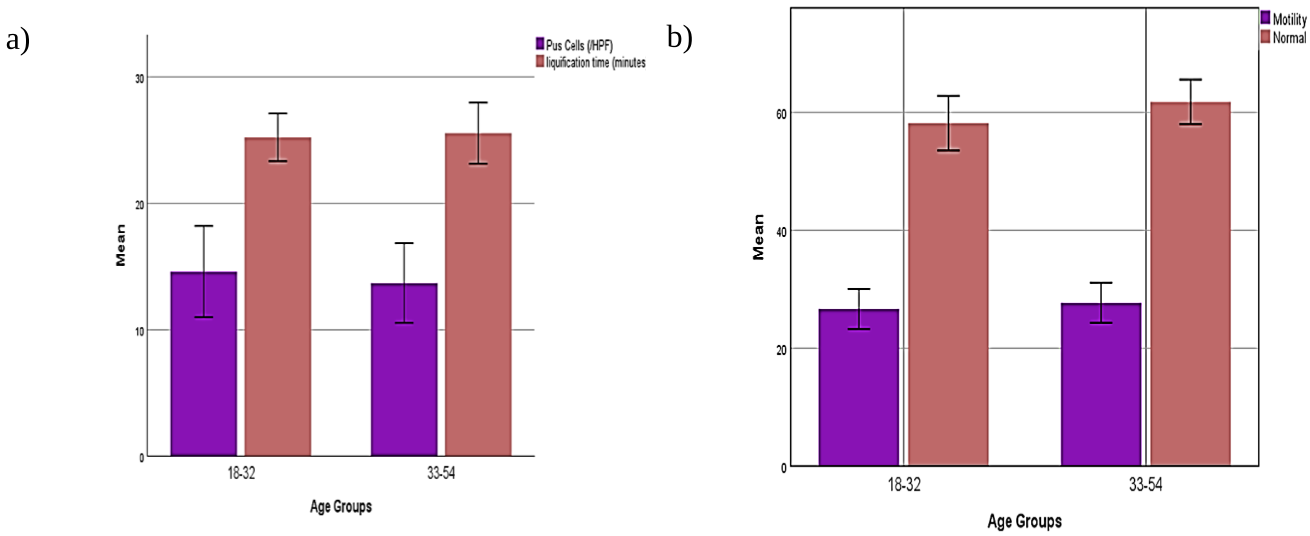

Fig. 2. Comparison of mean semen parameters and inflammatory markers across both age groups. **(a)** Younger group exhibits lower pus cell counts than the older group. **(b)** The motility and morphology were better in the older group, suggesting that acidic pH affects the motility in younger population

More insights were confirmed from the correlation analysis of the semen parameters (Table III). A positive correlation was observed between the sperm count and motility in younger and older groups alike with values of r = 0.33, p = 0.002 and r = 0.30, p = 0.014 respectively. This showed that the higher sperms counts were associated with better sperm motility. In the meanwhile, unique negative correlation was observed in younger group, taking into account the difference between pH and total sperm motility (r = -0.27, p = 0.013) as well between the pus cells and total sperm motility (r= -0.22, p = 0.040) highlighting the fact that acidic pH along with minor infections, both were responsible for affecting sperm movements in younger groups than the older ones.

Table II. Pearson correlation analysis stratified by the age groups. There is a negative correlation between the pus cells and motility among the younger group (18-32 years)

Variables	Younger group (18–32)	Older group (33–55)	Comparison
Count vs. Motility	r = 0.33, p = 0.002	r = 0.30, p = 0.014	Strong in both groups
Motility vs. pH	r = -0.27, p = 0.013	Not significant	Unique to young men: Acidity significantly reduces motility in the younger group
Motility vs. Pus cells	r = -0.22, p = 0.040	Not significant	Unique to young men: Infections hurt sperm movement more in younger patients

Finally, chi-square analysis was conducted to assess the classifications of infertility with that of the presented age groups and the results were quite clear as there was no significance among the parameters including morphology (0.277), total sperm count (0.208), liquefaction time (0.361), pus cells (0.575) and motility (0.273). These indications highlighted the fact that, although the mean values were declining with age (Fig. 3), both the groups did not show any statistical difference in pathological categories, although the Phi and Cramer's V values were quite rigorous.

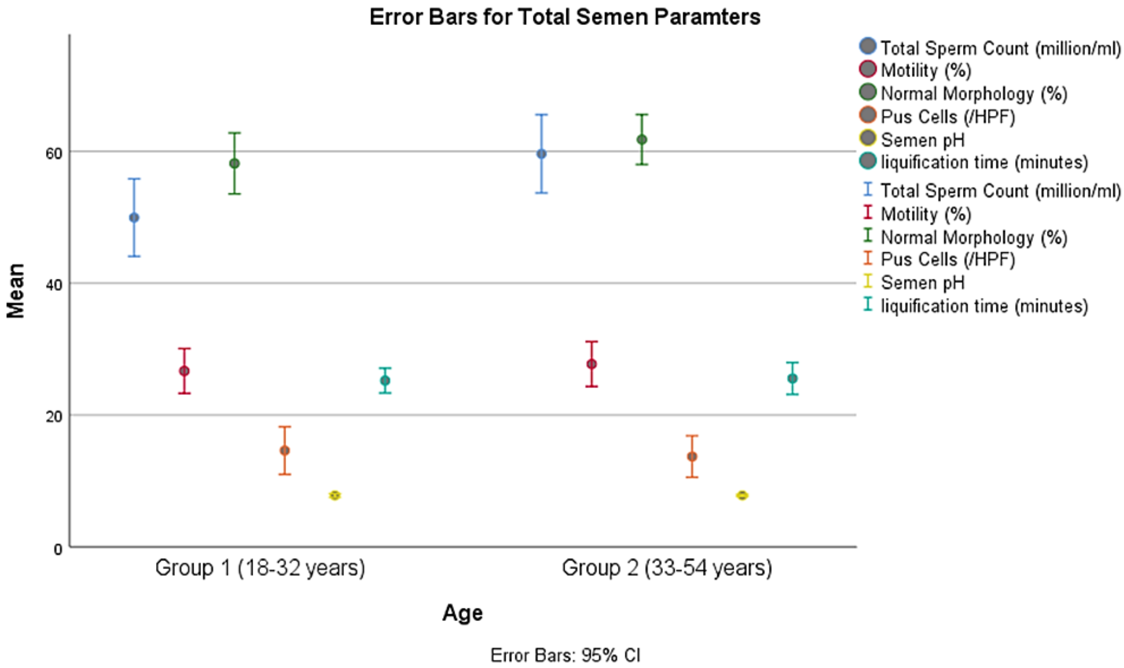

Fig. 2. Comparative error bars highlight the distribution of total sperm count, motility, normal morphology, pus cells, pH, and liquefaction time. Although the mean values for count and morphology were higher in younger group, no statistical difference was observed

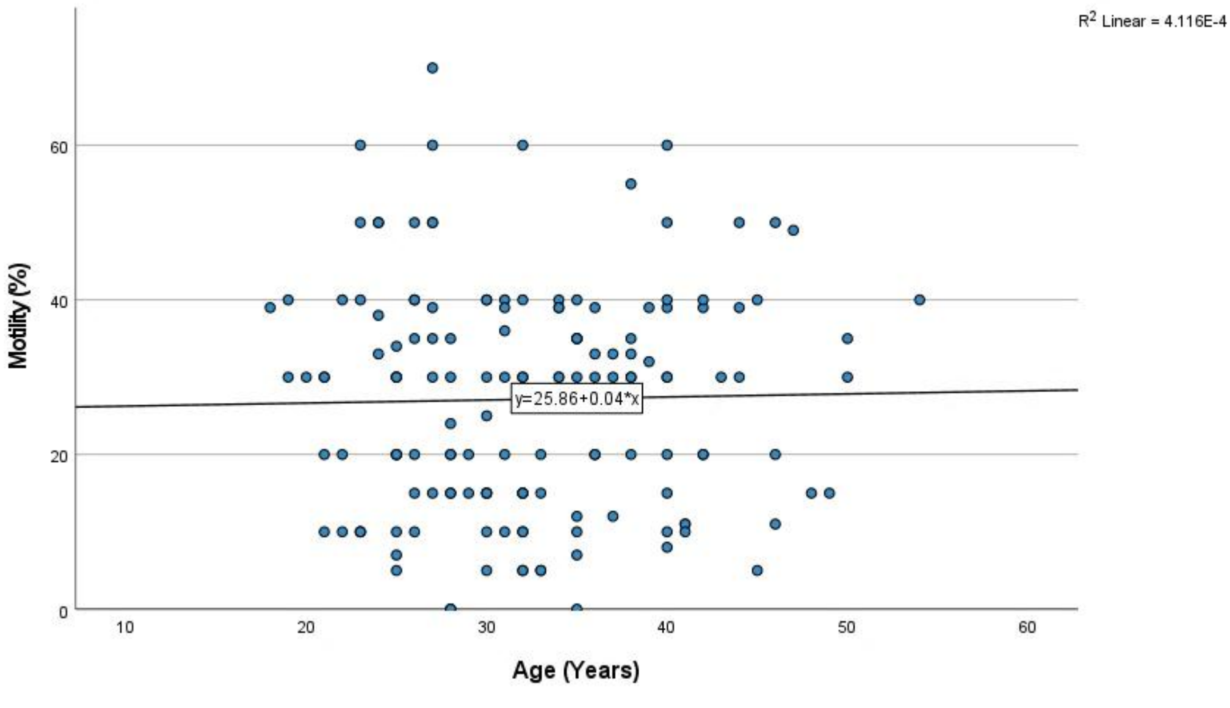

Fig. 4. Illustration of age with motility among the whole cohort (n = 150). The regression line indicates a negative correlation highlighting the decline in sperm motility with advancing age. Each individual data point represents the declining shift from younger to older patients

DISCUSSION

The current study highlights the basic parametric assessment of basic semen profiles across a cohort of Islamabad capital territory, revealing a complex impairment in sperm motility along with the preservation of sperm concentration and morphology. The results showed that semen quality goes down with age. This problem was seen more in younger men.

One of the predominant findings is the differentiation between the qualitative and quantitative semen parameters, studied across both the age groups and on everyone alike. The average sperm count was 54 million. This is higher than the WHO limit. But motility was only 27%, which is lower than normal. This shows that sperm movement is the main problem among the patients under consideration. The mean of the sperm count (54.24 ± 7.6 million/mL) and the mean morphology (56.79 ± 18.86%) was also observed to be higher than 50% (rather than 4%), the threshold for total morphology still practiced in many laboratories of Pakistan due to the lack of specific tools. However, this robustness in the two factors was contradicted by the total motility, whose mean (27.14 ± 14.80) was significantly lower than the threshold (42%). These results highlighted the primary pathological conditions that might be associated with the infertility of the patients, and the most dominant one was not the production of sperms but their maturation, dysfunction in ependymal environment or may be the disruption in their energy metabolism. This aligns with the results of previous studies where they discovered that the phenomenon of oxidative stress is responsible for damaging the DNA midpiece, ultimately resulting in the disruption in metabolism of energy along with production and utilization of ATP at the post-maturation site of spermatogenesis (12, 13). Furthermore, Asthenozoospermia was studied to be the main factor contributing towards the progressive infertility among the patients.

When the data were accessed by grouping the patients into age groups, groups with younger patients (18-32 years) showed a relatively higher sperm count (59.68 ± 29.39 million/mL) along with superior morphology (62.71% ± 17.71%) than the older groups with a mean sperm count and mean morphology of 46.05 ± 27.60 million/mL and 55.38% ± 19.34% respectively. Other studies also found that sperm health gets worse with age where the researchers documented that the advancing age is very well associated with the decline in spermatogenesis due to the damage done by oxidative stress, low antioxidant capacity, strengthened by the lack of cell repair mechanism (14-16). In addition to this, the higher age group have lower motility and higher pus cells accumulation in their sperms but still there was not a significant difference between these two parameters towards the infertility.

The key finding of this study is the abnormality in total motility (asthenozoospermia), affecting a total of 58% of the total cohort as, that was way less (27.14%) than that of the standard reference (42%) set by the WHO as accessed by one sample t-test. This findings is plausible as the motility alone is highlighted to be the most crucial factor in conception, as it not only depends on numbers but the capacity as well (17). Unlike the sperm count, which reflects sperm quantity, motility is essential for initiating fertilization as the sperms are of no use if they are not viable and strong enough to penetrate the egg cells, after evading their surroundings (uterus, fallopian tube and cervical mucus). Therefore, while factors like sperm count and morphology contribute to the semen profile, the low motility is studied to be the rate-limiting step in achieving conception (18, 19). Correlation analysis between motility and sperm count among both age groups further strengthened these findings, suggesting that the production of functionally competent and abundant sperms cells is due to the robust and healthy seminiferous epithelium. According to the data, a negative distinct correlation was observed in younger age group, showing remarkable pathophysiological mechanisms with a p value of 0.013 and 0.040 for pH and pus cells, respectively. These findings give a clear insight into the risk imposed by oxidative stress and ionic imbalances on the younger spermatozoa, that is primly caused by leukocyte targeting reactive oxygen species inside the body as already evidenced (13, 20). This shows the urgent need for the treatment of infections in young men, related to their reproductive health, as inflammation is suspected to be the main reversible factor that can drive sub-fertility among the population. In contrast to this, chi-square analysis does not show any significance association between semen parameters and age groups with a p value greater than 0.05 for each parameter. Although a clear decline was observed in the mean values for total morphology and sperm count, the portion of the participants among abnormal and normal groups remained the same as per the results of previous studies (21, 22). These findings suggests that as the men progress in age, there is a chance for them to maintain their viability above the pathological thresholds, further highlighting the utilizing of continuous data to detect subtle changes in the age-related trends of reproductive health.

Although the study has potential limitations such as focus on a single region and cross-section design, the study still provides robust and enhanced data for the population of Islamabad. The increased prevalence of overlapping abnormalities in this population highlights the abundance of etiologies like environmental pollution, processed foods (rare but still affect the maturity of immune system) and high stress. Future studies should prioritize data collection from interventional trials and data collection from various cities of Pakistan, entirely focused on modifications of lifestyle and therapies that utilize antioxidants. Ultimately, a detailed diagnostic approach must be used in Pakistan to access the total and progressive motility along with the accurate assessment of diagnostic markers in combination with the traditional approaches for reproductive health.

CONCLUSION

The present study concludes that the male infertility profile in Islamabad, Pakistan is characterized based on multiple and distinct semen profile, notably the motility of sperms that was far below the limit despite the preservation of sperm morphology and total sperm count. More than half of the population, under evaluation, was studied to be affected by asthenozoospermia, the primary driver of the male infertility as per the study along with the potential decline in normal morphology and total sperm concentration by with respect to the age. These findings support the concept of making biological clock, but still the negative correlation in case of counted pus cells and the pH of semen suggest that the younger population is more likely at the risk of acidic pH and inflammation related to the genital tract, ultimately affecting the reproductive health in a negative way. The present results also highlight the importance of comprehensive analysis of semen profile, rather than the traditional sperm counting alone to better understand and access the motility along with inflammatory markers in younger population to aggressively investigate the etiological causes to reverse the damage done to the reproductive health. The male infertility should be accessed primarily by the regular analysis of semen in such patients that are at higher risk and further investigations should be carried out in the future to better target the oxidative species that might leads towards inflammation of genital tract. To conclude, early diagnosis and better modifications in lifestyle are the main considerations that can significantly reduce the risks associated with the progression of male infertility, ultimately providing us with pathways and techniques to further strengthen the reproductive health.

Limitations:

The study has several limitations, one of which is the cross-sectional study design that only captured the data in a single frame of time as it fails to percale a causal relationship among the different semen parameters as it requires a larger portion of dataset (greater than 150). In addition to this, the study is limited to semen parameters rather than explaining the confounding variables like diet, exposure to environmental toxins and body mass index, the key variables that are directly linked with fertility. The lack of data on the primary and secondary infertility renders us to stratify the data with fertility history of the patients under consideration and the geographical limitations (study in Islamabad only) reduce the generalizability of our findings to other cities of Pakistan.

Future Recommendation:

Futuristic studies should target larger population across different geographical locations in Pakistan to further strengthen the causative agents, their effects on semen parameters and their effects on the male reproductive health. Integration of karyotyping and hormonal profiling (Testosterone and Follicle stimulating hormone) will provide us a deeper insight into the reproductive health. Such interdisciplinary approaches will further help us in navigating the region-specific guidelines for diagnosis and treatment of infertility.

Conflict of interests:

The authors don't share any competing interests in this manuscript

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Authors' contribution:

MD, MA, FH & HK Conducted the experimental work and data collection; AS & IH Supervised the study and reviewed the manuscript; MAJ & BA Performed data analysis and interpretation; AA, HA, AT & SB Assisted in laboratory assays; MD Contributed to sample collection and processing; MSK Participated in manuscript drafting and editing.

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