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Original article

LEUKOCYTOSPERMIA AND SEMEN QUALITY IN MALE CANDIDATES FOR *IN VITRO* FERTILIZATION

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Abstract

Introduction: Leukocytospermia, defined as one million or more leukocytes per millilitre of semen, may indicate genital tract inflammation and contribute to oxidative sperm damage, but its significance in men undergoing *in vitro* fertilization (IVF) remains uncertain. This study aimed to determine the prevalence of leukocytospermia in male IVF candidates and its association with semen diagnosis and semen pH.

Materials and methods: This retrospective study included 208 men, aged 22–60 years, whose semen was analyzed between January and December 2024. Samples were examined according to the 2021 World Health Organization criteria; sperm concentration, motility, morphology and leukocyte concentration were assessed with a computer-assisted sperm analysis system. Groups were compared with the Mann-Whitney U and Kruskal–Wallis tests and Spearman's correlation.

Results: Leukocytospermia was found in 81 men (38.9%) and was the most frequent abnormal finding, followed by reduced total motility (37.5%). Normozoospermia was the most common diagnosis (53.4%). Leukocyte concentration differed significantly between diagnostic groups (p=0.0075): leukocytospermia was most frequent in asthenoteratozoospermia (87.5%) and asthenozoospermia (48.1%), absent in oligozoospermia, and present in 34.2% of normozoospermic men. Semen pH showed a weak positive correlation with leukocyte concentration (ρ=0.171, p=0.013).

Conclusion: Leukocytospermia affected almost two in five IVF candidates, including one third of normozoospermic men, and was most common in diagnoses involving reduced motility. Prospective studies with confirmatory leukocyte testing and IVF outcome data are needed to establish its prognostic value.

Keywords: male infertility; leukocytes; semen analysis; in vitro fertilization; sperm motility

Introduction

Infertility is defined as the failure to achieve a pregnancy after 12 months or more of regular unprotected intercourse[1]. Infertility affects approximately 15% of couples, and a male factor is involved in about half of these cases, as the sole cause in 20-30% and in combination with female factors in a further 20–30%[2].

Male infertility has heterogeneous causes, conventionally grouped as pre-testicular (hypothalamic–pituitary and other endocrine disorders, and erectile or ejaculatory dysfunction), testicular (such as varicocele, cryptorchidism, orchitis, gonadotoxic treatment and genetic abnormalities) and post-testicular (seminal tract obstruction and infection or inflammation of the accessory glands)[3], with lifestyle factors as further contributors[4]. Known genetic factors account for at least 15% of cases[5]. The remaining cases are classified as idiopathic; oxidative stress is thought to contribute in a large proportion of them[6], and asymptomatic inflammation of the genital tract in some[7].

Evaluation of male infertility requires a comprehensive andrological assessment, with semen analysis serving as the cornerstone of initial diagnosis. Conventional semen analysis assesses macroscopic and microscopic parameters, providing insights into male reproductive function. Abnormalities in these parameters may indicate impaired spermatogenesis, reproductive tract obstruction, accessory gland dysfunction, or inflammation affecting fertility[8].

Among the factors associated with impaired semen quality, leukocytospermia, defined as the presence of $\geq 1 \times 10^6$ leukocytes/mL of ejaculate, has attracted considerable clinical attention and is frequently observed in infertile men[9]. Although leukocytes are normally present in semen and contribute to immunosurveillance, elevated concentrations may indicate inflammation or infection of the male genital tract[10]. Activated leukocytes can generate excessive reactive oxygen species (ROS), resulting in oxidative stress, sperm DNA damage, and impaired motility[11].

In couples undergoing assisted reproductive techniques such as *in vitro* fertilization (IVF), semen quality is an important component of male fertility assessment, as sperm functional competence and DNA integrity may influence fertilization and embryo development[12]. Assessment of seminal leukocytes may provide additional information about genital tract inflammation and oxidative stress; however, their independent prognostic value for assisted reproductive technology (ART) outcomes remains uncertain[13].

The aim of this study was to describe the macroscopic and microscopic semen characteristics of men referred for IVF procedures in 2024, determine the prevalence of leukocytospermia, and investigate the associations between seminal leukocyte concentration, semen diagnosis, and semen pH.

Materials and methods

A retrospective, observational study was conducted at the human ejaculate analysis laboratory of the Institute of Medical Histology and Embryology between January and December 2024. The study included 208 male candidates for IVF aged 22 to 60 years. Patients were classified into diagnostic groups according to semen analysis results: normozoospermia, oligozoospermia, asthenozoospermia, teratozoospermia, oligoasthenozoospermia, asthenoteratozoospermia, oligoteratozoospermia, oligoasthenoteratozoospermia.

Inclusion criteria: complete ejaculate analysis during the specified period. Exclusion criteria: incomplete laboratory findings, current antibiotic therapy, presence of varicocele or an improperly collected sample.

Semen samples were collected and examined according to the sixth edition of the World Health Organization (WHO) laboratory manual for the examination and processing of human semen[8]. Patients were asked to observe 2-7 days of ejaculatory abstinence; samples were obtained by masturbation into sterile containers in a private collection room close to the laboratory. In cases where collection took place outside the institution, subjects were instructed to deliver the sample to the laboratory within 30 minutes while maintaining it close to body temperature. The container was labeled with the patient's identifiers and with the date and time

of collection. Immediately after collection, the sample was placed in an incubator at 37°C and allowed to liquefy for approximately 30 minutes.

Macroscopic analysis included the assessment of ejaculate volume (reference range of 1.4-6.2 mL was used) and pH (measured with pH indicator paper, ≥7.2 was considered normal). Appearance and color were evaluated visually and classified as normal (opalescent, grey-white) or abnormal (e.g., reddish or transparent discoloration). Viscosity was assessed by attempting to aspirate the sample using a disposable plastic pipette and observing the ejaculate as it dripped under gravity. Under normal conditions, the ejaculate falls in small, discrete drops. Increased viscosity was noted by the formation of a thread-like structure exceeding 2 cm in length.

Microscopic analysis was performed using a computer-assisted sperm analysis system (Sperm Class Analyzer, SCA, Microptic S.L., Barcelona, Spain) on a phase contrast light microscope (with 10x, 40x, and 60x magnification) connected to a computer running the software. The analysis was performed on a native smear or in a counting chamber to determine sperm concentration with lower reference limits of 16×10^6 spermatozoa per mL and 39×10^6 spermatozoa per ejaculate, sperm motility classified as rapidly progressive (≥25 μm/s), slowly progressive (5 to <25 μm/s), non-progressive (<5 μm/s) or immotile. Total motility is the sum of progressive and non-progressive motility; the lower reference limits are 42% for total motility and 30% for progressive motility. Total motility was used in this study (≥42%). Also, sperm morphology was examined under fixed and stained (haematoxylin-eosin) smears, with the lower reference limit for normal forms of 4% and leukocyte concentration with leukocytospermia defined as $\geq1\times10^6$ leukocytes/mL. All parameters were interpreted according to WHO criteria[8].

Each sample was classified according to the WHO lower reference limits: normozoospermia (concentration, total motility and normal forms all at or above the reference limits), oligozoospermia (concentration $<16\times10^6$/mL), asthenozoospermia (total motility <42%), teratozoospermia (normal forms <4%), and their combinations (oligoasthenozoospermia, oligoteratozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia). Leukocytospermia was defined as an inflammatory cell concentration of 1.0×10^6/mL or more.

Statistical analysis

Statistical analysis was performed using RStudio Desktop 2026.0. Descriptive statistics included mean, SD, median, interquartile range (IQR), minimum and maximum using Microsoft Excel. Data normality was tested with the Kolmogorov-Smirnov test. Given that none of the variables was normally distributed, non-parametric tests were used. Differences between two groups were assessed with the Mann-Whitney U test, and between three or more groups with the Kruskal-Wallis test. Correlations were analyzed with Spearman's coefficient for non-normally distributed variables. A p value <0.05 was considered statistically significant.

Results

Descriptive statistics and normality testing for all semen parameters are presented in Table 1. The Kolmogorov–Smirnov test showed that none of the six parameters were normally distributed ($p < 0.05$); therefore, data are described primarily by median and interquartile range, and non-parametric tests were used for further analyses.

Table 1. Descriptive statistics and normality testing of semen parameters

Parameter	Ref. (WHO 2021)	Mean ± SD	Median (IQR)	Range	Below ref., n (%)	Above ref., n (%)	K-S D	p
Volume (mL)	1.4–6.2	2.54±1.18	2.30(1.20)	0.40-7.50	29(13.9)	2(1.0)	0.142	<0.001
pH	≥ 7.2	7.49±0.20	7.50(0.00)	6.40-8.00	8(3.8)	–	0.393	<0.001
Concentration (×10⁶/mL)	≥ 16	88.07±97.01	48.43(95.20)	5.51-494.62	30(14.4)	–	0.208	<0.001
Motility (%)	≥ 42	55.95±27.41	53.77(47.47)	4.76-100.00	78(37.5)	–	0.097	0.037
Morphology (%)	≥ 4	12.23±8.29	10.43(10.00)	0.00-49.50	22(10.6)	–	0.111	0.011
Inflammatory cells (×10⁶/mL)	< 1	1.34±2.18	0.78(1.22)	0.00-19.82	–	81(38.9)	0.269	<0.001

SD, standard deviation; IQR, interquartile range; K-S D, Kolmogorov–Smirnov test; p < 0.05 indicates non-normal distribution. Below/Above ref., patients with values below/above the WHO 2021 reference value (N = 208).

The median semen volume was 2.30 mL, with 29 patients (13.9%) below the lower reference limit and 2(1.0%) above the upper limit. The median pH was 7.50, and only 8 patients (3.8%) had a pH below 7.2. Sperm concentration showed the greatest variability (median 48.43×10⁶/mL; range 5.51-494.62×10⁶/mL), with 30 patients (14.4%) below the reference value. Reduced total motility was the most frequent abnormality among the conventional semen parameters, present in 78 patients (37.5%; median 53.77%), whereas abnormal morphology was found in 22 patients (10.6%; median 10.43% normal forms). Leukocytospermia (≥1×10⁶/mL) was identified in 81 patients (38.9%), with a median inflammatory cell concentration of 0.78×10⁶/mL making it the most common single abnormal finding in the cohort.

The overview of the distribution of diagnoses and the presence of leukocytospermia by diagnostic group is presented in Table 2. Normozoospermia was the most frequent diagnosis (111 men, 53.4%), followed by asthenozoospermia (52 men, 25.0%) and oligoasthenozoospermia (14 men, 6.7%). The remaining diagnostic categories together accounted for 31 men (14.9%).

Table 2. Distribution of semen diagnoses and inflammatory cell concentration by diagnosis

Diagnosis	n (%)	Mean±SD	Median (IQR)	Range	Leukocytospermia n (%)
Normozoospermia	111(53.4)	1.20±2.23	0.49(1.22)	0.00-19.82	38(34.2)
Asthenozoospermia	52(25.0)	1.72±2.42	0.91(1.41)	0.06-13.99	25(48.1)
Oligoasthenozoospermia	14(6.7)	0.97±0.68	0.85(0.62)	0.11-2.66	6(42.9)
Oligozoospermia	9(4.3)	0.46±0.29	0.49(0.56)	0.08-0.78	0(0.0)
Asthenoteratozoospermia	8(3.8)	2.08±1.46	1.53(0.72)	0.65-4.92	7(87.5)
Teratozoospermia	7(3.4)	2.18±3.75	0.39(2.27)	0.00-10.10	2(28.6)
Oligoasthenoteratozoospermia	4(1.9)	1.12±0.76	1.05(1.15)	0.42-1.97	2(50.0)
Oligoteratozoospermia	3(1.4)	0.90±0.44	0.82(0.43)	0.51-1.37	1(33.3)
Total	208(100)	1.34±2.18	0.78(1.22)	0.00-19.82	81(38.9)

The Kruskal-Wallis test showed a statistically significant difference in inflammatory cell levels between the diagnostic groups (H=19.23, p=0.0075), indicating that inflammatory cell levels vary according to the semen diagnosis. The highest median values were found in asthenoteratozoospermia (1.53×10⁶/mL) where leukocytospermia was present in 7 of 8 patients (87.5%), oligoasthenoteratozoospermia (1.05×10⁶/mL, 50%), asthenozoospermia (0.91×10⁶/mL, 25 of 52 patients, 48.1%), and the lowest in teratozoospermia (0.39×10⁶/mL), normozoospermia and oligozoospermia (both 0.49×10⁶/mL), whereas none of the 9 patients with oligozoospermia had leukocytospermia (median 0.49×10⁶/mL).

In the teratozoospermia group, the mean value (2.18×10⁶/mL) was considerably higher than the median, reflecting a small number of samples with high leukocyte concentrations (maximum 10.10×10⁶/mL).

Mean semen pH by diagnostic category is shown in Figure 1. The median semen pH was 7.5 in every diagnostic category, and mean values ranged from 7.43 in oligoasthenozoospermia to 7.57 in oligoteratozoospermia; all means were above the WHO lower reference limit. Of the 8 samples with pH below 7.2, four were from men with asthenozoospermia, two with normozoospermia, one with asthenoteratozoospermia and one with oligoasthenozoospermia.

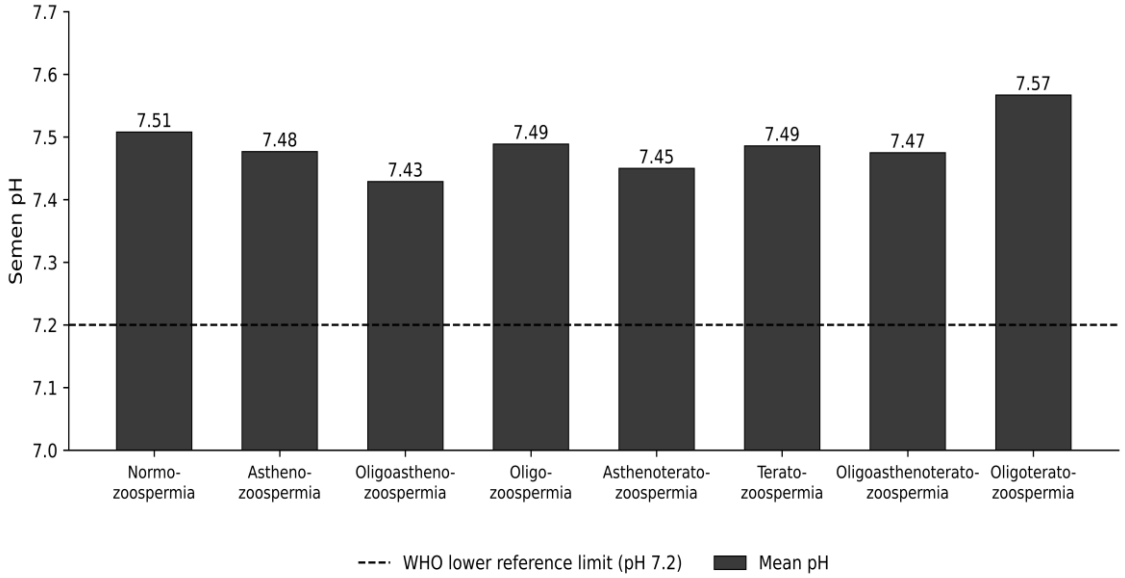

Fig. 1. Mean semen pH by diagnosis. The dashed line marks the WHO 2021 lower reference limit (pH 7.2).

Since both variables were non-normally distributed, Spearman's rank correlation was applied. Semen pH showed a weak but statistically significant positive correlation with leukocyte concentration (Table 3). When pH was analyzed categorically, leukocytospermia ($\geq 1\times10^6$/mL) was present in 79 of 200 men (39.5%) with pH≥7.2 and in 2 of 8 men (25.0%) with pH<7.2, and leukocyte concentration did not differ significantly between the two groups (Table 3).

Table 3. Association between semen pH and leukocyte concentration (N = 208)

Test	Coefficient	p	Interpretation
Spearman correlation (ρ)	0.171	0.013	Weak positive correlation
Mann-Whitney U (pH≥7.2 vs.<7.2)	U=730	0.677	No significant difference between groups

P-Spearman's rank correlation coefficient, U-Mann-Whitney U test, $p<0.05$ was considered statistically significant

Discussion

In this retrospective analysis of 208 men referred for IVF, leukocytospermia was the most common single abnormal finding (38.9%), followed closely by reduced total motility (37.5%), while abnormalities in volume, concentration, morphology and pH were each present in fewer than 15% of men. Leukocyte concentration differed significantly between semen diagnostic categories, with the highest values in groups characterised by impaired motility, and showed a weak positive correlation with semen pH. More than half of the men were normozoospermic. This is not unexpected in an IVF population, since a male factor is the sole cause of infertility in only 20-30% of couples[2], and many men are therefore referred because of female-factor or unexplained infertility.

The prevalence observed in our cohort (38.9%) is higher than the 10-20% traditionally reported in infertile men[13] and the up to 25-30% reported in more recent series[9,14]. A similar

prevalence (33.9% of cycles) was reported by Gill *et al.,* in 5,435 IVF/intracytoplasmic sperm injection (ICSI) cycles[15]. However, direct comparison is limited, because leukocytes in that study were identified by peroxidase staining, whereas in our study inflammatory cells were counted by the SCA system. Leukocytes are difficult to distinguish from immature germ cells on routine microscopy, and round cells counted without a confirmatory peroxidase or immunocytochemical test may overestimate the true leukocyte concentration[8,16]. In addition, the classification in our study was based on a single semen sample, and seminal leukocyte concentrations are known to vary between consecutive ejaculates.

Leukocyte concentration varied significantly across diagnostic categories. The highest median values and the highest proportions of leukocytospermia were found in asthenoteratozoospermia (87.5%), oligoasthenoteratozoospermia (50.0%) and asthenozoospermia (48.1%), i.e. in categories that include reduced motility, whereas none of the men with isolated oligozoospermia had leukocytospermia. A similar pattern was described by Younas *et al.*, who found the highest leukocyte counts among asthenozoospermic samples and none in oligozoospermic samples[17]. This distribution is biologically plausible. Activated leukocytes, predominantly neutrophils, are an important source of ROS in semen, and excessive ROS can damage the sperm plasma membrane through lipid peroxidation and impair flagellar function, leading to reduced motility[18,19]. Agarwal *et al.,* showed that even low-level leukocytospermia (0.1-1.0×10^6/mL) was associated with higher seminal ROS levels and greater sperm DNA fragmentation than in men without seminal leukocytes, although conventional semen parameters did not differ between the groups[20].

In contrast, none of the men with isolated oligozoospermia had leukocytospermia, and this group had the lowest mean leukocyte concentration (Table 2), in agreement with the findings of Younas *et al.*[17]. This suggests that reduced sperm output in these men is more likely of non-inflammatory origin, such as impaired spermatogenesis, which remains unexplained in most cases [4,21], than the result of an active inflammatory process in the genital tract.

Notably, leukocytospermia was also present in 34.2% of normozoospermic men, who would be classified as normal by all WHO reference criteria. A normal basic semen analysis therefore does not exclude genital tract inflammation. Because leukocyte-derived ROS can damage sperm DNA without affecting conventional parameters[20] and increased sperm DNA fragmentation has been associated with a higher risk of early pregnancy loss[22], this subgroup may merit further evaluation.

Nevertheless, the literature is not uniform. In a meta-analysis, the lower sperm concentration and progressive motility in leukocytospermic samples disappeared when studies including men with signs of genital tract infection were excluded [23]. Another study found that the association between leukocytes and sperm quality was concentration dependent, with better motility and morphology at leukocyte levels below 1 × 10^6/mL [24]. Several of our diagnostic subgroups were small (n=3-9), and hence, the proportions in these categories should be interpreted with caution.

Semen pH reflects the balance between alkaline seminal vesicle secretions and acidic prostatic secretions, and low pH combined with low volume is classically associated with ejaculatory duct obstruction[8]. In our cohort, pH was remarkably uniform, with a median of 7.5 in every diagnostic category, and only eight men had a pH below 7.2. We found a weak positive correlation between pH and leukocyte concentration (ρ=0.171), but no difference in leukocyte levels between men with normal and low pH. One possible explanation is that inflammation of the accessory glands, particularly the prostate, alters their secretory function; in a large cross-sectional study, prostatitis was independently associated with leukocytospermia[25]. However, the correlation explained only about 3% of the variation, pH was measured with indicator paper, which produces many tied values, and the low-pH group

was very small. This finding should therefore be regarded as hypothesis-generating rather than conclusive.

The clinical significance of leukocytospermia in the context of assisted reproduction remains uncertain [26]. Sperm preparation techniques used in IVF, such as swim-up and density gradient centrifugation, remove most seminal leukocytes[27] and neither the meta-analysis by Castellini *et al*.,[23] nor the large cohort study by Gill *et al*.,[15] found a negative effect of leukocytospermia on ART outcomes. Castellini *et al.* found no differences in fertilization or clinical pregnancy rates in men without genital tract infection[23], and Gill *et al.,* reported comparable fertilization, blastulation and live birth rates in ICSI cycles with preimplantation genetic testing for aneuploidy[15]. Because IVF outcomes were not available in our study, the impact of the high prevalence of leukocytospermia observed in our cohort on reproductive success could not be assessed.

Furthermore, leukocytospermia is a poor predictor of bacterial infection, as most leukocytospermic samples are culture-negative[28]. Taken together, these data suggest that leukocytospermia in asymptomatic IVF candidates should be interpreted as a marker of possible genital tract inflammation and oxidative stress rather than as an automatic indication for antibiotic treatment, and that further evaluation should be guided by clinical findings.

The strengths of this study include a well-defined cohort of IVF candidates examined over a full calendar year in a single laboratory, with all samples analyzed according to WHO 2021 criteria using the same protocol and SCA system.

This study has several limitations. Its retrospective, single-center design limits the generalizability of the results. Each man was classified on the basis of a single semen sample, although at least two samples taken several weeks apart are recommended[9]. Inflammatory cells were counted by the SCA system without confirmatory peroxidase or immunocytochemical staining, which may have overestimated leukocyte concentration. Semen culture was not performed, so the relationship between leukocytospermia and infection could not be assessed; this is relevant because leukocytospermia is not a reliable predictor of a positive culture [28]. Seminal ROS and sperm DNA fragmentation were not measured, hence the proposed oxidative mechanism could not be confirmed directly. Data on potential confounders such as smoking, body mass index and age were not included in the analysis. Finally, IVF/ICSI outcomes were not available, therefore, the association between leukocytospermia and reproductive success remains a subject for future research.

Conclusion

Leukocytospermia was the most frequent abnormal semen finding in men referred for IVF, present in almost two in five men, including one third of those with normozoospermia. Leukocyte concentration differed between semen diagnoses: leukocytospermia was most common in diagnoses involving reduced motility and absent in isolated oligozoospermia, suggesting that seminal inflammation may be linked to impaired sperm motility rather than reduced sperm production. Its association with semen pH was weak and of uncertain clinical relevance. Because a normal semen analysis does not exclude genital tract inflammation, assessment of seminal leukocytes may be a useful part of the routine evaluation of IVF candidates. Prospective studies with confirmatory peroxidase testing, microbiological evaluation and IVF outcome data are needed to determine whether leukocytospermia affects reproductive outcomes.

Conflict of interest statement. None declared.

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