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ORIGINAL ARTICLE

Semen quality and associated reproductive indicators in middle-aged males: the role of non-malignant prostate conditions and genital tract inflammation

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Abstract
Purpose To compare the associations between non-malignant prostate conditions, genital tract inflammation, and reproductive function in middle-aged men.
Methods Three-hundred and eighty-two voluntary male subjects who underwent the screening for prostate health were recruited for the study. Semen quality and associated reproductive indicators, seminal inflammation, and prostate-related pathologies were evaluated.
Results Sperm motility and prostate-related parameters were significantly impaired in patients with chronic prostatitis syndromes and lower urinary tract symptoms in comparison with controls. Elevated seminal markers of inflammation were in positive association with body mass index, prostate-specific antigen, and estradiol level in serum while in negative association with semen volume, total sperm count, and sperm motility. According to WHO reference limits, speculative cutoff values for WBC and IL-6 in semen to detect reduced sperm parameters were 0.342 M/mL and 56.8 ng/L, respectively.
Conclusions According to our data, one of the possible pathways for impaired reproductive quality in male subjects >45 years could be related to infection and inflammation in the genital tract with subsequent (partial) obstruction and damage of prostate and other male accessory glands.

Keywords Reproductive function · Semen quality · Middle-aged men · Benign prostate conditions · Genital tract inflammation

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Introduction

In recent decades, the increased time to conception and sub-fertility has impacted a rising number of couples. In males, the trend is related to the beginning of family planning after obtaining education and establishing a professional career, resulting in higher parental age and large economic and psychological costs at societal and individual levels.

These issues, in combination with acquired medical conditions and environmental factors in subjects >40 years, are important causes for the impaired reproductive status of the aging male [1–3].

On the other hand, there is a general consensus that reduced fertility and poorer semen quality can be the result of pathological changes and lifestyle-related factors, that is, inflammation and infection in the male genital tract [2]. On that topic, most of the prior studies have focused on male accessory gland infection (MAGI) and/or types of chronic prostatitis [4–6]. To our knowledge, there are no studies comparing the reproductive parameters in males with chronic bacterial prostatitis (CBP), chronic prostatitis/chronic prostate pain syndrome (CP/CPPS), and lower urinary tract symptoms (LUTS). In addition, there are only a two studies investigating the role of genital tract inflammation in reproductive function in the subgroup of

men >45 years [7, 8]. Also, both of the studies have been performed in infertile males.

In addition, on the basis of a recent review of the literature [4, 5], no studies are available that specifically deal with hormone levels in middle-aged subjects with CPPS, CBP, and LUTS. At the same time, the main male reproductive organs—including testicles, seminal vesicles, and prostate—are hormone dependent, which theoretically enhances the possibility of an endocrine cause for impaired semen quality in subjects with male reproductive pathologies. Therefore, we included hormonal parameters in the present study.

The main aim of the present study was to compare the reproductive and prostate-related parameters in middle-aged subjects with main non-malignant prostate conditions. Also, we specified the associations between prostate-related characteristics, semen quality, and associated reproductive indicators with markers of inflammation in the seminal tract and tried to calculate the predictive values for white blood cells (WBC) counts and interleukin-6 (IL-6) levels in semen to differentiate middle-aged subjects with and without semen pathologies [9].

Materials and methods

Study population

In the initial phase (between November 2007 and October 2010), 639 men who underwent the screening for prostate health at the Andrology Centre of Tartu University Hospital were recruited in the study. Exclusion criteria for this study included prior or current problems and/or treatment for infertility or urogenital tumors, chemo- or radiation therapy in the pelvic region, previous varicocelectomy, hernio- or vasectomy, history of undescended testicle(s), sexually transmitted disease(s), and/or abnormal findings from a digital rectal examination (DRE). In addition, none of the study subjects experienced febrile pelvic pain symptoms and acute urinary retention nor received therapy with antimicrobials, α1-blockers, or 5α-reductase inhibitors within 3 months prior.

The study group included only men who were willing to provide semen specimens ($n = 411$).

Among these subjects, 29 males with a reported incomplete semen sample [8] were excluded.

While the main goal of our study was the screening for prostate health, and the additional analyses in middle-aged subjects are mostly disinclined, the assessment of sperm parameters was performed only with a single semen sample; a similar method was described in prior studies of men >40 years [7, 10]. However, we re-evaluated the semen quality within 4 weeks after the initial examination.

Finally, the conclusive number of subjects was 382. Of them, 213 presented with CP/CPPS, 50 with CBP, 84 with LUTS, and the remaining 35 were age-matched asymptomatic controls. To identify the men with CBP and CP/CPPS, strict criteria by Nickel et al. [6] were used, including perineal and/or ejaculatory pain/discomfort within the 3 months prior and a total pain score of 4 or greater.

Clinical examination

Physical examinations included assessment of testicular size, genital pathologies, DRE, and body mass index (BMI). Participants of the study were examined by one investigator (K.A.) who had completed multiple instances of special training on standardization of clinical examinations prior to the described study. The principles of examination have been described previously [3].

Semen analysis

Routine semen analysis was performed according to WHO guidelines [9, 11] to detect semen volume, total sperm count, concentration, motility, morphology, WBC, and IL-6. Semen was obtained by masturbation and ejaculated into a sterile collection tube in a private room near the laboratory. The recommended abstinence period was a minimum of 48 h, but not longer than 7 days. The actual period of ejaculation abstinence was calculated in full days between the current and previous ejaculation as reported by the participants. The standpoints of semen analysis have been specified previously [3].

IL-6 levels in seminal plasma (100 μL of specimen was required for the assay) were measured using the Immulite automated chemiluminescence immunoassay analyzer (Immulite Siemens Healthcare Diagnostics, Inc., Deerfield, IL, USA) according to the manufacturer's instructions. The intra- and interassay coefficients of variation for IL-6 were 4.7 and 5.3 %, respectively.

All subjects were studied using the same criteria [9, 11] in the Andrology Centre and laboratories. Participants of the study were examined by one technician who had completed multiple instances of special training on laboratory standardization of semen samples prior to the study.

Microbiological investigation

Semen cultures were performed for detecting common urinary pathogens, and the colonization was confirmed within 1 week after the seminal analysis in midstream urine, applying the 4-glass test [5]. Only the subjects with a colony count of $\geq 10^5$/mL were included in the chronic bacterial prostatitis group.

Blood samples

Venous blood was obtained from the cubital vein between 8 a.m. and 11 a.m. after overnight fasting or a light morning meal. The samples were centrifuged, serum was isolated, and reproductive hormones or prostate-specific antigen (PSA) was detected within 2 h at the United Laboratories of Tartu University Hospital. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estradiol (E2), sex hormone-binding globulin (SHBG), and PSA in blood plasma were measured using the Immulite automated chemiluminescence immunoassay analyzer (Immulite Siemens Healthcare Diagnostics, Inc., Deerfield, IL, USA) according to the manufacturer's instructions. The intra- and interassay coefficients of variation were 4.2 and 8.0 % for FSH, 4.0 and 7.1 % for LH, 6.3 and 9.4 % for testosterone, 7.5 and 13.0 % for estradiol, 3.4 and 4.1 % for SHBG, and 0.8 and 2.7 % for PSA, respectively.

Prostate-related characteristics

In the initial phase of the study, all participants completed the questionnaires, including the NIH Chronic Prostatitis Symptom Index (NIH-CPSI) [12, 13] and International Prostate Symptom Score (I-PSS) [14] for LUTS.

While the main goal of our study was the screening for prostate health, clinical characteristics related to the prostate were added to the paper. All men were measured for total prostate volume (TPV) and post-voided residual urine (PVR) by trans-rectal or abdominal ultrasonography (using Logiq 5 Pro by General Electric, Milwaukee, WI, USA) and for urinary flow rates by uroflowmetry (using Urodyn 1000 by Medtronic, Minneapolis, MN, USA).

Statistical evaluation

For statistical analyses, SigmaStat (Systat Software, Chicago, IL, USA), Excel (Microsoft, Redmond, WA, USA), and R (R Foundation for Statistical Computing, Vienna, Austria) software programs were used.

In Tables 1 and 5, the median age, different reproductive parameters, and prostate-related characteristics were compared using the Kruskal–Wallis test, and the group or groups that differed from the others were isolated by a multiple comparison procedure (Dunn's method). In Table 6, the differences between the groups were compared by the Mann–Whitney test due to nonparametric distribution of variables.

The Spearman product–moment correlation was used to determine correlations between seminal inflammatory markers and age, reproductive, and prostate-related parameters.

Areas under receiver operating characteristic curve (ROCC) and diagnostic test characteristics (95 % CI) for WBC and IL-6 in semen were designed using R software to estimate semen pathology with cutoff levels of semen volume <1.5 mL, sperm density <15 ($\times 10^6$ per mL), and sperm A + B motility <40 % [9].

In Tables 7 and 8, the regression analyses (using multiple linear regression models) were performed to evaluate the associations between WBC and IL-6 in semen with statistically significant correlations. Statistically, inflammatory characteristics were described as dependent variables and other assessments as independent variables.

Statistical significance was assumed at $p < 0.05$ for all parameters.

Ethical consideration

Participation in the study was voluntary. Informed consent was obtained from all study subjects. The study was approved by the Ethics Review Committee on Human Research of the University of Tartu.

Results

The general characteristics, main prostate-related, and reproductive parameters of the enrolled subjects are presented in Table 1. There were alterations in sperm motility and main prostate-related parameters in subjects with LUTS, CBP, and CP/CPPS compared to age-matched controls. According to the WHO threshold value [9], the subset of men with leukocytospermia was 5.7 % for asymptomatic controls and 4.7 and 7.5 % for subjects with LUTS and CP/CPPS, respectively, compared to 14.7 % for men with CBP ($p = 0.014$). Also, the proportions of men with three normal variables (semen volume ≥1.5 mL, sperm density ≥15 × 10^6/mL, and semen A + B motility ≥40 %) were significantly higher among the controls compared to subjects with LUTS, CP/CPPS, and CBP (91.4 vs. 79.8, 84.5, and 80.8 %, respectively, $p = 0.012$).

Associations between genital tract inflammation and age, reproductive, and prostate-related characteristics

The correlation coefficients between markers of inflammation (WBC and IL-6 levels in semen) and age, reproductive, and prostate-related parameters are shown in Table 2. For all the subjects, both markers of inflammation were in positive correlation with BMI and PSA, while in negative correlation with semen volume and total sperm count. In addition, IL-6 showed a positive correlation with age and TPV, while WBC showed a negative correlation

Table 1 Baseline characteristics of enrolled middle-aged male subjects[a]

Characteristics	Group 1 Controls $n = 35$ Median (IQR)	Group 2 LUTS $n = 84$ Median (IQR)	Group 3 CBP[b] $n = 50$ Median (IQR)	Group 4 CP/CPSS $n = 213$ Median (IQR)	Total $n = 382$ Median (IQR)
Age (years)	55.0 (52.3–59.0)	57.0 (52.0–63.0)	57 (50.0–62.0)	55.0 (50.0–61.0)	56.0 (51.0–61.0)
BMI (kg/m^2)	27.7 (25.7–29.9)	27.7 (25.3–29.8)	28.0 (24.6–31.7)	27.4 (25.1–30.4)	27.7 (25.1–30.4)
Testicular volume (right + left testicle/2, mL)	24.0 (22.0–25.0)	25.0 (22.0–25.0)	25.0 (24.0–25.0)	25.0 (22.0–25.0)	25.0 (22.0–25.0)
Basic sperm parameters					
Semen volume (mL)	3.5 (2.1–4.8)	3.3 (2.1–4.7)	3.0 (2.0–4.1)	3.2 (2.4–4.2)	3.3 (2.1–4.6)
Total sperm count (million)	257.4 (173.2–445.4)	288.9 (154.4–504.6)	297.5 (148.5–567.0)	240.0 (124.5–477.0)	264.8 (137.8–486.5)
Sperm concentration (mil/mL)	88.0 (56.8–159.0)	100.0 (54.5–146.5)	107.5 (51.0–170.0)	83.0 (40.0–143.5)	91.0 (47.8–150.3)
Sperm A + B motility (%)	58.0 (46.5–65.0)[1,12,13]	54.0 (46.0–65.0)[1,14]	53.0 (47.0–59.0)[1,12]	50.0 (39.0–60.0)[1,13,14]	52.0 (43.0–62.0)
Normal sperm (%)	6.0 (2.3–8.8)	4.5 (2.0–10.0)	5.0 (2.0–7.0)	5.0 (3.0–10.0)	5.0 (2.0–9.0)
Abstinence time (days)	5.0 (4.0–7.0)	5.0 (4.0–7.0)	5.0 (4.0–7.0)	5.0 (3.0–7.0)	5.0 (4.0–7.0)
WBC in semen (10^6/mL)	0.1 (0.0–0.5)	0.1 (0.0–0.3)	0.1 (0.0–0.4)	0.1 (0.0–0.3)	0.1 (0.0–0.3)
IL-6 in seminal plasma (ng/mL)	54.6 (29.5–93.8)	44.5 (30.9–84.5)	50.0 (31.8–125.0)	50.5 (32.3–77.0)	49.7 (31.3–84.6)
Basic prostate-related parameters					
Total prostate volume (mL)	30.0 (28.0–43.5)[2,15]	37.5 (30.0–44.5)[2,15,16]	30.5 (25.0–38.0)[2,16]	34.0 (25.0–40.0)[2]	33.0 (26.0–42.0)
Residual urine (post-voided, mL)	11.5 (3.0–35.0)[3,17]	44.0 (24.0–64.0)[3,17,18,19]	12.0 (2.0–33.8)[3,18]	17.5 (5.0–41.0)[3,19]	17.0 (5.0–41.0)
Maximum urinary flow rate (mL/s)	19.0 (15.5–27.3)[4,20,21,22]	16.3 (12.4–21.7)[4,20]	15.4 (12.3–22.6)[4,21]	17.3 (11.9–24.7)[4,22]	17.0 (12.4–23.9)
PSA (ng/mL)	1.6 (0.8–3.2)	1.8 (0.9–3.9)	1.4 (0.8–2.2)	1.4 (0.7–2.5)	1.6 (0.8–2.9)
I-PSS sub-score (irritative, 0–15)	0.0 (0.0–0.0)[5,23,24,25]	5.0 (3.0–7.0)[5,23]	4.0 (3.0–7.0)[5,24]	4.0 (2.0–7.0)[5,25]	4.0 (2.0–7.0)
I-PSS sub-score (obstructive, 0–15)	0.0 (0.0–0.0)[6,26,27,28]	5.0 (3.0–7.3)[6,26]	6.0 (3.0–9.0)[6,27]	4.0 (2.0–7.0)[6,28]	4.5 (2.0–7.0)
I-PSS sub-score (nocturial, 0–5)	0.0 0 (0.0–0.0)[7,29,30,31]	1.0 (1.0–2.0)[7,29]	1.0 (1.0–2.0)[7,30]	1.0 (1.0–2.0)[7,31]	1.0 (1.0–2.0)
I-PSS total score (0–35)	0.0 (0.0–0.0)[8,32,33,34]	11.0 (8.0–15.0)[8,32]	12.0 (8.0–18.0)[8,33]	10.0 (6.0–15.0)[8,34]	10.0 (6.0–15.0)
NIH-CPSI sub-score (pain, 0–21)	0.0 (0.0–0.0)[9,35,36]	0.0 (0.0–0.0)[9,37,38]	7.0 (5.0–9.0)[9,35,37]	7.0 (5.0–9.0)[9,36,38]	5.0 (0.0–8.0)
NIH-CPSI sub-score (quality of life, 0–6)	0.0 (0.0–0.0)[10,39,40,41]	2.0 (1.0–3.0)[10,39]	3.0 (2.0–4.0)[10,40]	3.0 (2.0–4.0)[10,41]	3.0 (1.0–4.0)
NIH-CPSI total score (0–43)	0.0 (0.0–0.0)[11,42,43,44]	7.0 (4.0–10.0)[11,42,45,46]	16.0 (13.0–21.0)[11,34,35]	16.0 (11.8–20.0)[11,44,46]	13.0 (8.0–18.0)
Basic hormonal parameters					
Testosterone (nmol/L)	16.8 (12.2–20.4)	15.5 (13.2–19.3)	14.8 (11.0–19.3)	15.1 (12.0–19.5)	15.2 (12.2–19.5)
Estradiol (pmol/L)	159.0 (130.8–186.5)	138.5 (109.5–190.0)	144.0 (114.8–185.5)	146.5 (118.0–183.0)	146.0 (116.8–186.0)
Estradiol/Testosterone	9.6 (7.4–12.4)	8.6 (6.6–11.7)	8.8 (7.5–14.0)	9.6 (7.3–12.8)	9.1 (7.2–12.4)
FSH (IU/L)	5.3 (3.2–7.5)	4.8 (3.6–6.8)	4.3 (3.3–6.9)	5.1 (3.3–7.4)	5.1 (3.4–7.2)
LH (IU/L)	3.5 (2.3–4.0)	2.5 (1.6–3.6)	2.4 (1.6–3.1)	2.7 (1.9–4.2)	2.7 (1.8–4.0)
FSH/LH (IU/L)	1.7 (1.3–2.1)	2.0 (1.4–2.8)	2.0 (1.4–2.9)	1.8 (1.3–2.5)	1.8 (1.3–2.6)
SHBG (nmol/L)	29.9 (23.3–39.8)	35.7 (27.8–44.8)	32.1 (23.2–41.1)	30.0 (23.4–41.0)	31.6 (24.0–41.7)

Characteristics	Group 1 Controls $n = 35$ Mean ± SD	Group 2 LUTS $n = 84$ Mean ± SD	Group 3 CBP[b] $n = 50$ Mean ± SD	Group 4 CP/CPSS $n = 213$ Mean ± SD	Total $n = 382$ Mean ± SD
Age (years)	56.1 ± 5.1	56.9 ± 6.7	56.2 ± 7.2	55.3 ± 6.7	56.0 ± 6.7
BMI (kg/m^2)	28.0 ± 4.2	28.1 ± 3.9	28.4 ± 4.5	28.1 ± 4.0	28.1 ± 4.0
Testicular volume (right + left testicle/2, mL)	23.1 ± 4.1	23.6 ± 4.1	24.0 ± 2.6	23.6 ± 3.5	23.5 ± 3.8
Basic sperm parameters					
Semen volume (mL)	3.4 ± 1.7	3.7 ± 2.1	3.2 ± 1.5	3.6 ± 2.0	3.5 ± 1.9
Total sperm count (million)	368.7 ± 328.1	372.3 ± 331.0	380.9 ± 41.7	343.1 ± 309.2	357.4 ± 313.6
Sperm concentration (mil/mL)	124.3 ± 93.7	114.8 ± 86.8	121.1 ± 85.0	102.6 ± 80.2	109.7 ± 83.8
Sperm A + B motility (%)	56.5 ± 16.8[1,12,13]	53.3 ± 15.8[1,14]	52.3 ± 14.6[1,12]	48.7 ± 17.6[1,13,14]	51.1 ± 16.8
Normal sperm (%)	6.6 ± 5.4	6.2 ± 5.5	5.7 ± 4.6	6.5 ± 5.3	6.3 ± 5.2
Abstinence time (days)	5.3 ± 1.4	5.8 ± 2.5	6.6 ± 4.7	5.9 ± 3.8	5.9 ± 3.5
WBC in semen (10^6/mL)	0.3 ± 0.5	0.3 ± 0.6	0.5 ± 0.9	0.3 ± 0.6	0.3 ± 0.6
IL-6 in seminal plasma (ng/mL)	72.6 ± 63.6	84.7 ± 108.1	148.8 ± 310.7	77.7 ± 111.3	88.1 ± 151.2
Basic prostate-related parameters					
Total prostate volume (mL)	36.8 ± 13.2[2,15]	39.3 ± 14.4[2,15,16]	33.7 ± 13.1[2,16]	34.7 ± 14.1[2]	35.8 ± 14.1
Residual urine (post-voided, mL)	23.3 ± 30.2[3,17]	43.5 ± 65.9[3,17,18,19]	31.7 ± 49.3[3,18]	31.0 ± 38.3[3,19]	33.3 ± 47.1
Maximum urinary flow rate (mL/s)	22.2 ± 9.6[4,20,21,22]	17.2 ± 7.3[4,20]	17.9 ± 8.9[4,21]	18.9 ± 9.6[4,22]	18.7 ± 9.1
PSA (ng/mL)	2.8 ± 2.2	2.8 ± 2.7	2.3 ± 2.3	2.3 ± 6.8	2.4 ± 3.4
I-PSS sub-score (irritative, 0–15)	0.0 ± 0.0[5,23,24,25]	4.9 ± 3.2[5,23]	5.0 ± 3.4[5,24]	4.6 ± 3.5[5,25]	4.5 ± 3.4

Table 1 continued

Characteristics	Group 1 Controls $n = 35$ Mean ± SD	Group 2 LUTS $n = 84$ Mean ± SD	Group 3 CBP[b] $n = 50$ Mean ± SD	Group 4 CP/CPSS $n = 213$ Mean ± SD	Total $n = 382$ Mean ± SD
I-PSS sub-score (obstructive, 0–15)	0.0 ± 0.0[6,26,27,28]	5.6 ± 3.0[6,26]	6.1 ± 3.7[6,27]	5.0 ± 3.6[6,28]	4.9 ± 3.6
I-PSS sub-score (nocturial, 0–5)	0.0 ± 0.0[7,29,30,31]	1.4 ± 1.0[7,29]	1.6 ± 1.2[7,30]	1.4 ± 1.2[7,31]	1.4 ± 1.1
I-PSS total score (0–35)	0.0 ± 0.0[8,32,33,34]	12.0 ± 4.8[8,32]	12.8 ± 6.9[8,33]	11.1 ± 6.9[8,34]	10.7 ± 6.7
NIH-CPSI sub-score (pain, 0–21)	0.0 ± 0.0[9,35,36]	0.0 ± 0.0[9,37,38]	7.2 ± 3.0[9,35,37]	7.2 ± 3.0[9,36,38]	5.0 ± 4.2
NIH-CPSI sub-score (quality of life, 0–6)	0.0 ± 0.0[10,39,40,41]	2.2 ± 1.4[10,39]	2.9 ± 1.4[10,40]	3.1 ± 1.4[10,41]	2.7 ± 1.5
NIH-CPSI total score (0–43)	0.0 ± 0.0[11,42,43,44]	7.4 ± 4.1[11,42,45,46]	16.7 ± 5.9[11,34,35]	16.2 ± 6.5[11,44,46]	13.1 ± 7.5
Basic hormonal parameters					
Testosterone (nmol/L)	17.1 ± 6.2	16.8 ± 5.4	16.0 ± 7.2	16.0 ± 5.8	16.3 ± 6.0
Estradiol (pmol/L)	158.1 ± 39.6	154.2 ± 61.2	154.8 ± 57.8	153.8 ± 51.5	154.2 ± 53.4
Estradiol/Testosterone	10.4 ± 4.6	9.9 ± 5.3	10.5 ± 4.9	10.6 ± 4.9	10.4 ± 5.0
FSH (IU/L)	5.7 ± 2.6	5.7 ± 3.5	5.3 ± 3.1	5.8 ± 3.8	5.7 ± 3.3
LH (IU/L)	3.2 ± 1.2	2.8 ± 1.6	2.7 ± 1.5	3.2 ± 5.8	3.1 ± 1.6
FSH/LH (IU/L)	1.8 ± 0.9	2.5 ± 2.7	2.2 ± 1.0	2.4 ± 5.0	2.3 ± 3.9
SHBG (nmol/L)	33.1 ± 12.5	36.4 ± 13.4	33.5 ± 17.3	33.9 ± 15.2	34.3 ± 14.9

LUTS lower urinary tract symptoms, *CBP* chronic bacterial prostatitis, *CP/CPPS* chronic prostatitis/chronic pelvic pain syndrome, *IQR* interquartile range (25th–75th ‰), *BMI* body mass index, *WBC* white blood cells, *IL-6* interleukin 6, *PSA* prostate-specific antigen, *I-PSS* International Prostate Symptom Score, *NIH-CPSI* National Institute of Health Chronic Prostatitis Symptom Index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *SHBG* sex hormone-binding globulin

[a] $p < 0.05$ was considered statistically significant

[b] Semen culture positive ($\geq 10^5$ per mL) for *Escherichia coli*, *Enterococcus species*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Klebsiella* species, and *Staphylococcus aureus*

[1] $p = 0.021$; Kruskal–Wallis test

[2] $p = 0.004$; Kruskal–Wallis test

[3] $p = 0.031$; Kruskal–Wallis test

[4] $p = 0.035$; Kruskal–Wallis test

[5] $p < 0.001$; Kruskal–Wallis test

[6] $p < 0.001$; Kruskal–Wallis test

[7] $p < 0.001$; Kruskal–Wallis test

[8] $p < 0.001$; Kruskal–Wallis test

[9] $p < 0.001$; Kruskal–Wallis test

[10] $p < 0.001$; Kruskal–Wallis test

[11] $p < 0.001$; Kruskal–Wallis test

[12–46] $p < 0.05$; Dunn's method

with sperm motility. Also, there was a positive correlation between IL-6 and WBC count in semen ($r = 0.185$, $p < 0.001$). The statistically significant correlations between semen and prostate-related parameters are summarized in Fig. 1.

Age as a risk factor for prostate and reproductive pathologies showed a statistically significant positive correlation with TPV ($r = 0.356$, $p < 0.001$), PVR ($r = 0.114$, $p = 0.041$), and PSA ($r = 0.434$, $p < 0.001$), while there was a negative correlation with maximum urinary flow rate ($r = -0.180$, $p = 0.001$), NIH-CPSI total score ($r = -0.140$, $p = 0.011$), testicular size ($r = -0.140$, $p = 0.011$), and sperm parameters, that is, semen volume ($r = -0.226$, $p < 0.001$) and total sperm concentration ($r = -0.131$, $p = 0.017$).

We did not find any correlation between hormonal and seminal inflammatory markers in all investigated subjects. Instead, the hormonal parameters were associated with age, sperm parameters, and BMI (data not shown). In addition, BMI was in correlation with markers of seminal inflammation (Table 2) and main hormonal markers, PSA, and sperm motility (data not shown).

The cutoff values for IL-6 and WBC to detect semen pathologies

To detect the influence of seminal inflammation on sperm pathologies, the ROCC for IL-6 and WBC in semen were devised and presented in Tables 3 and 4. In particular, semen volume showed the highest area under the curve (0.716 and 0.596 for IL-6 and WBC in semen, respectively) with a high negative prognostic value (NPV). A similar NPV showed WBC and IL-6 to detect abnormal sperm concentration ($<15 \times 10^6$ per mL) and motility (<40 %). The results of the combined ROCC for WBC and IL-6 in semen to detect semen abnormalities are presented in Figs. 2 and 3. According to WHO reference limits [9], speculative cutoff values for WBC and IL-6 in semen to

Table 2 Correlation coefficients between the seminal inflammatory markers, age, reproductive, and prostate-related parameters in middle-aged subjects

Characteristics ($n = 332$)	Seminal inflammatory markers			
	WBC in semen r value[1]	p value	IL6 in semen r value[1]	p value
Age (years)	0.045	0.377	0.342	**<0.001**
Testicular volume (right + left/2, mL)	−0.002	0.971	−0.046	0.371
BMI (kg/m^2)	0.112	**0.030**	0.112	**0.030**
Basic semen parameters				
Semen volume (mL)	−0.164	**0.001**	−0.255	**<0.001**
Total sperm count (million)	−0.124	**0.034**	−0.112	**0.030**
Sperm concentration (mil/mL)	−0.014	0.786	0.076	0.140
Sperm A + B motility (%)	−0.195	**<0.001**	−0.067	0.191
Total count of normal sperm (%)	−0.039	0.443	−0.086	0.093
Abstinence time (days)	−0.038	0.456	0.098	0.078
Basic prostate-related parameters				
Total prostate volume (mL)	0.009	0.855	0.149	**0.004**
Residual urine (post-voided, mL)	0.001	0.989	0.013	0.810
Maximum urinary flow rate (mL/s)	0.054	0.299	−0.092	0.076
PSA (ng/mL)	0.168	**0.004**	0.234	**<0.001**
I-PSS sub-score (irritative, 0–15)	0.070	0.172	0.009	0.854
I-PSS sub-score (obstructive, 0–15)	0.019	0.713	0.011	0.826
I-PSS sub-score (nocturial, 0–5)	0.040	0.411	0.004	0.929
I-PSS total score (0–35)	0.065	0.204	0.004	0.946
NIH-CPSI sub-score (pain, 0–21)	0.020	0.718	−0.011	0.844
NIH-CPSI sub-score (quality of life, 0–6)	−0.020	0.714	−0.025	0.764
NIH-CPSI total score (0–43)	0.020	0.702	0.013	0.798
Basic hormonal parameters				
Testosterone (nmol/L)	0.031	0.547	−0.092	0.073
Estradiol (pmol/L)	0.072	0.197	0.063	0.262
Estradiol/testosterone	−0.001	0.998	0.046	0.399
FSH (IU/L)	0.061	0.289	0.011	0.843
LH (IU/L)	0.057	0.323	−0.026	0.650
FSH/LH (IU/L)	0.018	0.758	0.051	0.380
SHBG	0.027	0.641	0.053	0.358

Bold values indicate statistically significant correlation ($p < 0.05$)

WBC white blood cells, *IL-6* interleukin 6, *BMI* body mass index, *PSA* prostate-specific antigen, *I-PSS* International Prostate Symptom Score, *NIH-CPSI* National Institute of Health Chronic Prostatitis Symptom Index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *SHBG* sex hormone-binding globulin

[1] Spearman rank correlation coefficient

detect reduced sperm parameters were 0.342 mil/mL and 56.8 ng/L, respectively.

We subsequently grouped the subjects according to WBC and IL-6 levels in semen (Tables 5, 6).

In Table 5, the subjects were divided into three groups—two of them were separated according to the cutoff level of our ROCC analysis (males with mild and moderate WBC count, i.e., WBC <0.35 mil/mL and 0.35–0.99 mil/mL, respectively), whereas the third group included subjects with leukocytospermic values that exceeded the WHO threshold (WBC count in semen ≥1 mil/mL). The separation of subjects based on IL-6 in semen was made using the cutoff level of our ROCC analysis (IL-6 <57.0, ≥57.0 ng/L). For all described groups, semen volume and total sperm count decreased along with the increase in PSA and estradiol level in serum. On the basis of the WBC count in semen, we also found statistical differences between the groups in sperm motility.

In addition, a multiple regression analysis was subsequently performed to uncover the significant effects of seminal inflammation in semen on significant reproductive and prostate-related parameters (Tables 7, 8). The results

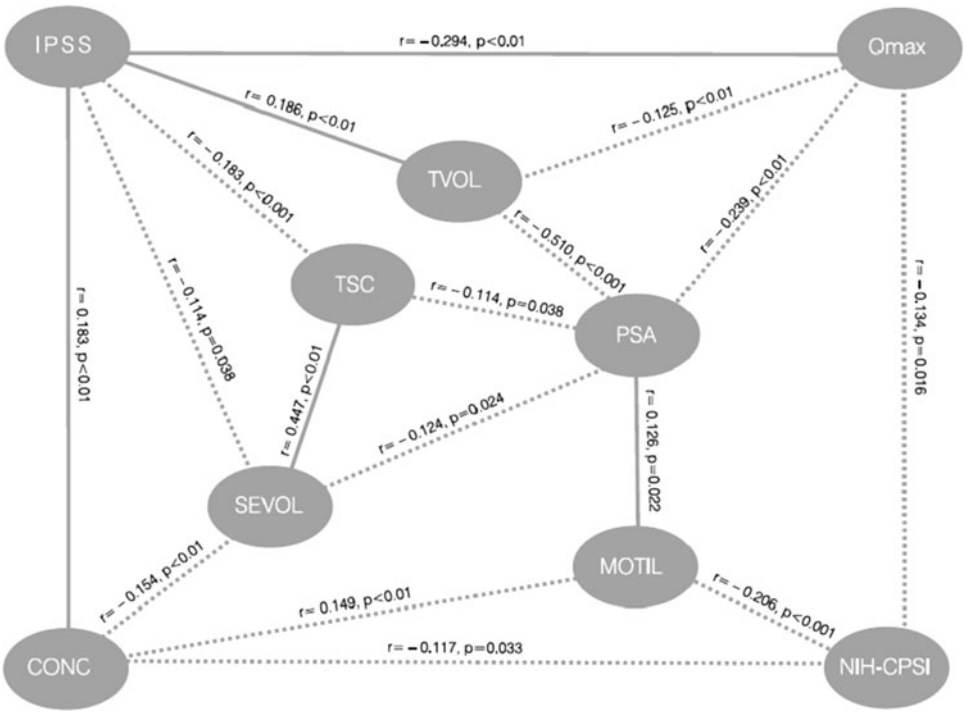

Fig. 1 The correlations between semen and prostate-related parameters in middle-aged males. *SEVOL* semen volume, *TSC* total sperm count, *CONC* sperm concentration, *MOTIL* sperm motility, *PSA* prostate-specific antigen, *I-PSS* International Prostate Symptom Score, *NIH-CPSI* National Institute for Health related Chronic Prostatitis Symptom Score, Q_{max} maximum urinary flow, *TVOL* total prostate volume

confirmed the associations of the WBC count with the IL-6 level and sperm motility in semen.

Discussion

Although some recent studies have described the risk of MAGI and inflammation for seminal pathologies in middle-aged infertile males [4, 7, 8], little is known about common associations between reproductive function, seminal fluid inflammation, and prostate pathologies in males of that age. According to a recent review [5], there is only one study comparing the semen quality in subjects with NIH type II and type III prostatitis. To our knowledge, there are no prior reports comparing reproductive function in men with LUTS, CBP, and CP/CPPS.

While the symptoms of prostatitis, chronic pelvic pain, and LUTS may overlap—and it could be difficult to determine whether one syndrome preceded the other(s) or whether these syndromes may exist concomitantly—we found that sperm motility was differentiated between the middle-aged subjects with CP/CPPS, CBP, and LUTS, and age-matched controls.

Interestingly, there were no changes between the groups in hormonal levels, testicular size, and/or seminal inflammatory markers. However, we found differences between the subjects in prostate-related characteristics.

An additional substantial finding of our study was that the increased level of seminal inflammatory markers, WBC count, and IL-6 are associated with reduced semen volume, total sperm count, and sperm motility, and with elevated PSA and estradiol levels in middle-aged men.

Although the leukocytospermia, defined as the presence of $\geq 1 \times 10^6$ peroxidase-positive WBCs per mL in ejaculate [9], has been evaluated since the mid-1960s [16], and an increased number of leukocytes in seminal fluid is generally accepted as an indicator of inflammation, the clinical influence of WBC on semen parameters is still debatable and controversial [5]. For example, improved sperm quality, decreased seminal leukocytosis, and increased pregnancy rates after the initial antimicrobial treatment were found in infertile subjects [4]. Otherwise, a higher leukocyte rate in semen has been detected in a previous report of fertile men [17]. Therefore, it is suggested to confirm seminal inflammation using different markers, such as WBC count, and pro-inflammatory cytokines [5]. In our study, we used IL-6 as the well-known and leading cytokine measured by inflammatory diseases in the seminal tract since the mid-1990s [18]. Although previous findings in this field are controversial [19, 20], IL-6 as a pro-inflammatory cytokine may be associated with prostatitis [21, 22], BPH [19], the development of prostate cancer [23], as well as spermatogenesis [4]. Whereas IL-6 in our study was correlated with age, TPV, semen volume, and total sperm count, the cytokine may provide an additional link between aging, prostate-related conditions, semen quality, and reproductive diseases.

A recent article has shown that IL-6 was significantly increased in subjects with CP/CPPS and BPH, compared to healthy controls [21]. In the present study, we did not see

Table 3 Areas under the ROCC and diagnostic test characteristics (95 % CI) for white blood cells in semen as characteristic used to estimate sperm pathology with cutoff levels of semen volume <1.5 mL, sperm density <15 ($\times 10^6$ per mL), and sperm A + B motility <40 % [9]

Characteristics ($n = 322$)	SEVOL	CONC	MOTIL	VOL + CONC	VOL + MOTIL	CONC + MOTIL	VOL + CONC + MOTIL
Sensitivity (95 % CI)	43.6 (27.8–60.4)	33.3 (14.6–57.0)	51.4 (34.4–68.1)	38.6 (26.0–52.4)	38.0 (26.8–50.3)	34.1 (20.5–49.9)	37.2 (26.5–48.9)
Specificity (95 % CI)	77.8 (73.0–82.1)	76.1 (71.4–80.4)	61.4 (56.1–66.6)	78.1 (73.2–82.5)	78.8 (73.8–83.2)	76.9 (72.1–81.3)	78.9 (73.9–83.4)
Positive predictive value (95 % CI)	18.3 (11.0–27.6)	7.5 (3.1–14.9)	12.5 (7.7–18.8)	23.7 (15.5–33.6)	29.0 (20.1–39.4)	16.1 (9.3–25.2)	31.2 (22.0–41.6)
Negative predictive value (95 % CI)	92.4 (88.7–95.2)	95.1 (92.0–97.3)	92.2 (87.9–95.3)	87.8 (83.5–91.4)	84.8 (80.1–88.7)	90.0 (85.9–93.2)	83.0 (78.2–87.2)
AUC for WBC in semen	59.6 (49.6–69.6)	52.1 (37.9–66.3)	52.6 (42.1–63.1)	55.4 (46.8–64.1)	56.0 (48.2–63.8)	52.5 (43.0–62.1)	55.7 (48.2–63.2)

ROCC receiver operating characteristic curve, *CI* confidence interval, *AUC* area under the curve, *WBC* white blood cells, *SEVOL* semen volume, *CONC* sperm concentration, *MOTIL* sperm motility

Table 4 Areas under the ROCC and diagnostic test characteristics (95 % Cl) for IL-6 in semen as characteristic used to estimate sperm pathology with cutoff levels of semen volume <1.5 mL, sperm density <15 ($\times 10^6$ per mL), and sperm A + B motility <40 % [9]

Characteristics ($n = 322$)	SEVOL	CONC	MOTIL	VOL + CONC	VOL + MOTIL	CONC + MOTIL	VOL + CONC + MOTIL
Sensitivity (95 % CI)	73.7 (56.9–86.6)	80.0 (56.3–94.3)	80.6 (64.0–91.8)	52.7 (38.8–66.3)	63.8 (51.3–75.0)	83.3 (68.6–93.0)	64.0 (52.1–74.8)
Specificity (95 % CI)	65.1 (59.8–70.2)	37.0 (32.0–42.3)	34.7 (29.7–40.0)	77.8 (72.9–82.2)	63.9 (58.2–69.2)	35.3 (30.2–40.7)	64.5 (58.8–69.9)
Positive predictive value (95 % CI)	19.0 (13.0–26.3)	6.6 (3.8–10.5)	11.5 (7.8–16.0)	28.7 (20.1–38.6)	28.2 (21.3–36.0)	13.8 (9.8–18.7)	30.8 (23.6–38.6)
Negative predictive value (95 % CI)	95.7 (92.2–97.9)	97.1 (92.7–99.2)	94.4 (88.9–97.7)	90.6 (86.6–93.8)	88.8 (83.9–92.6)	94.4 (88.9–97.7)	87.9 (82.9–91.9)
AUC for IL-6 in semen	71.6 (62.4–80.7)	54.8 (41.8–67.7)	54.2 (44.9–63.6)	67.8 (60.0–75.6)	65.6 (58.4–72.7)	57.6 (48.9–66.3)	67.0 (60.2–73.8)

ROCC receiver operating characteristic curve, *CI* confidence interval, *AUC* area under the curve, *IL-6* interleukin-6, *SEVOL* semen volume, *CONC* sperm concentration, *MOTIL* sperm motility

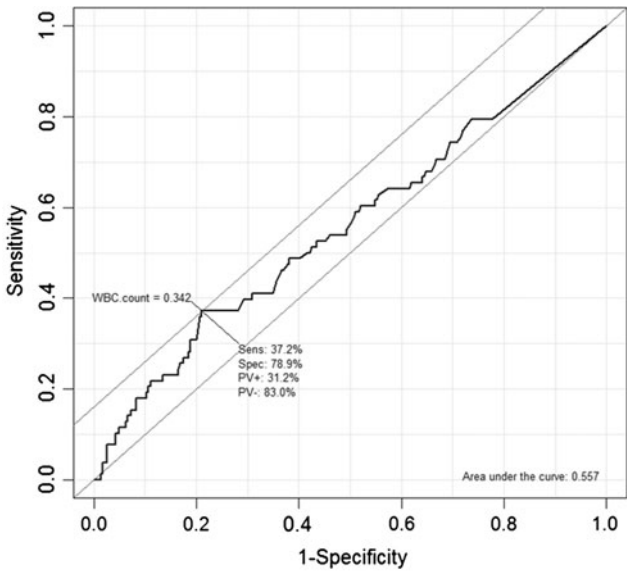

Fig. 2 The receiver operating characteristic (ROC) curve for WBC count semen as characteristic used to estimate sperm pathology with cutoff levels of semen volume <1.5 mL, sperm concentration <15.0 ($\times 10^6$ per ejaculate), and sperm A + B motility <40 % [9]

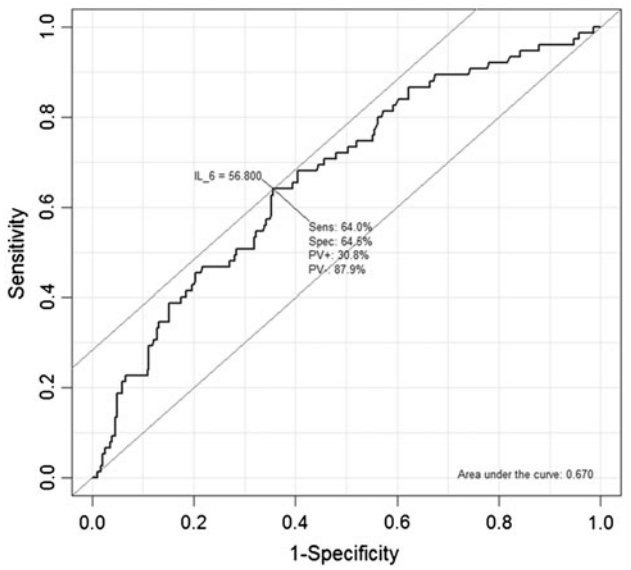

Fig. 3 The receiver operating characteristic (ROC) curve for IL-6 in semen as characteristic used to estimate sperm pathology with cutoff levels of semen volume <1.5 mL, sperm concentration <15.0 ($\times 10^6$ per ejaculate), and sperm A + B motility <40 % [9]

the differences in IL-6 level between those groups. Some possible rationales for that are the similar subsets of men with leukocytospermia in our study groups and the use of a non-age-matched control group in the study by Penna et al. [21] (mean age 34.9 vs. 54.8 years in Penna's and our study, respectively). Therefore, in agreement with Engeler [4], it is very relevant to have an age-matched control group in clinical reproductive studies.

In addition, the WBC and IL-6 levels showed a significant positive correlation with the PSA rate in serum. Although prior studies are controversial [24], similar data have been demonstrated in our previous studies for younger asymptomatic subjects in semen [25] and for aging men with LUTS in expressed prostatic secretion [26]. Also, the present study confirmed the prior positive correlation of the PSA level in serum with age, maximum urinary flow rate, and total volume of the prostate [15]. But, based on our findings, the PSA level in serum also correlated with sperm parameters like semen volume, sperm motility, and total sperm count. Therefore, according to an initial speculative view, an increased serum level of PSA in middle-aged males could give information not only about prostate-related conditions, but may be an additional indicator for impaired reproductive quality. In that case, one of the possible pathways could be related to inflammation in the genital tract with subsequent (partial) obstruction and damage of the prostate gland.

One of the striking findings in our study was the difference in E2 levels and the estradiol-to-testosterone ratio between the groups based on the level of seminal inflammatory markers.

To our knowledge, no prior reports are available that specifically deal with hormone levels in subjects with genital inflammation. However, there is evidence from animal models that prostatitis may change the balance of sexual hormones. For example, Stoker et al. [27] demonstrated the role of perinatal estrogenic exposure in the alterations in prostate volume and an increased rate of prostatitis in rats. Also, treatment with 17β-estradiol showed estrogen-induced tissue damage close to human prostatitis [4]. Otherwise, a decrease in testosterone level may have a critical role in affecting experimental prostatitis and suppression of immune function in rat models [28].

In humans, variations in hormonal status could be associated with overweight and elevated BMI. Also, it has been suggested that increased weight and abdominal obesity in males may have a negative impact on fertility and sperm quality, that is, for semen volume, sperm concentration, motility [29], and the formation of reactive oxygen species (ROS) [30].

Therefore, one cannot exclude the associations between higher BMI and genital tract inflammation. In our study, BMI was in correlation with testosterone, estradiol, PSA, sperm motility, and markers of seminal inflammation.

The present study has a few limitations. First, this study was a one-center study and therefore related to some organizational problems. While the main goal of the study was the screening for prostate health, the most sophisticated problem was to compose an optimal and operative protocol for the study to assess all parameters related to reproductive

Table 5 The associations between age, reproductive function, and prostate-related parameters according to WBC count in semen[a]

Characteristics	Group 1 0–0.34 × 10^6 WBC/mL $n = 292$ Median (IQR)	Group 2 0.35–0.99 × 10^6 WBC/mL $n = 64$ Median (IQR)	Group 3 ≥1.0 × 10^6 WBC/mL $n = 26$ Median (IQR)
Age (years)	55.0 (50.0–61.0)	57.0 (53.0–61.0)	57.0 (53.0–61.3)
BMI (kg/m^2)	27.6 (25.2–30.0)	27.4 (24.8–31.0)	28.5 (25.1–32.2)
Testicular volume (right + left testis/2, mL)	25.0 (22.0–25.0)	25.0 (22.3–25.0)	25.0 (22.0–25.0)
Basic sperm parameters			
Semen volume (mL)	3.5 (2.5–4.8)[1,9,10]	2.5 (1.7–3.4)[1,9]	2.7 (1.5–4.2)[1,10]
Total sperm count (million)	281.0 (153.3–502.1)[2,11,12]	210.9 (96.8–478.0)[2,11,13]	175.4 (75.8–362.9)[2,12,13]
Sperm concentration (mil/mL)	94.0 (49.3–145.0)	91.0 (43.3–178.8)	77.0 (27.0–140.00)
Sperm A + B motility (%)	54.0 (44.0–63.5)[3,14,15]	47.0 (38.5–56.0)[3,14]	50.0 (36.0–58.0)[3,15]
Normal sperm (%)	5.0 (3.0–10.0)	5.0 (3.0–8.8)	5.0 (1.0–7.5)
Abstinence time (days)	5.0 (4.0–7.0)	5.0 (4.0–7.0)	5.0 (4.0–7.0)
WBC in semen (10^6/mL)	0.0 (0.0–0.1)[4,16,17]	0.5 (0.4–0.7)[4,16,18]	1.5 (1.2–2.2)[4,17,18]
IL-6 in seminal plasma (ng/mL)	45.1 (30.3–71.8)[5,19]	55.1 (37.1–98.3)[5,20]	124.0 (58.1–277.0)[5,19,20]
Basic prostate-related parameters			
Total prostate volume (mL)	33.0 (26.0–42.0)	33.5 (27.0–42.5)	32.5 (27.0–42.0)
Residual urine (post-voided, mL)	16.0 (5.0–40.0)	23.0 (6.0–56.3)	16.5 (7.0–50.0)
Maximum urinary flow rate (mL/s)	16.7 (11.8–23.9)	18.2 (13.7–24.1)	16.6 (13.3–24.1)
PSA (ng/mL)	1.3 (0.8–2.7)[6,21,22]	1.7 (0.7–3.2)[6,21,23]	2.1 (1.6–3.8)[6,22,23]
I-PSS sub-score (irritative, 0–15)	4.0 (2.0–7.0)	3.0 (1.0–6.0)	3.0 (2.0–8.0)
I-PSS sub-score (obstructive, 0–15)	5.0 (2.0–7.0)	5.0 (2.0–7.0)	4.0 (1.0–6.0)
I-PSS sub-score (nocturial, 0–5)	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–2.0)
I-PSS total score (0–35)	10.0 (6.0–15.0)	10.0 (6.0–14.5)	8.0 (4.0–15.0)
NIH-CPSI sub-score (pain, 0–21)	5.0 (0.0–8.0)	4.5 (0.0–7.0)	5.5 (0.0–8.0)
NIH-CPSI sub-score (quality of life, 0–6)	3.0 (2.0–4.0)	2.5 (1.0–4.0)	3.0 (1.0–4.0)
NIH-CPSI total score (0–43)	13.0 (8.0–17.0)	12.0 (7.0–17.5)	14.5 (8.0–19.0)
Basic hormonal parameters			
Testosterone (nmol/L)	15.2 (12.2–19.3)	15.6 (12.1–20.5)	14.9 (13.9–22.5)
Estradiol (pmol/L)	145.0 (116.0–182.0)[7,24]	139.0 (110.00–195.0)[7,25]	189.5 (147.0–215.5)[7,24,25]
Estradiol/testosterone	9.3 (7.3–12.3)[8,26]	8.7 (6.4–12.3)[8,27]	10.5 (7.6–14.0)[8,26,27]
FSH (IU/L)	4.8 (3.3–7.3)	5.4 (3.7–7.2)	4.9 (3.3–6.6)
LH (IU/L)	2.7 (1.8–3.9)	2.4 (1.9–3.9)	2.9 (1.9–4.1)
FSH/LH (IU/L)	1.8 (1.3–2.4)	1.9 (1.4–3.0)	1.6 (1.3–2.6)
SHBG (nmol/L)	30.8 (23.4–41.6)	34.5 (27.7–43.8)	29.9 (23.2–40.7)

Characteristics	Group 1 0–0.34 × 10^6 WBC/mL $n = 292$ Mean ± SD	Group 2 0.35–0.99 × 10^6 WBC/mL $n = 64$ Mean ± SD	Group 3 ≥1.0 × 10^6 WBC/mL $n = 26$ Mean ± SD
Age (years)	55.7 ± 6.8	57.0 ± 6.3	57.1 ± 6.1
BMI (kg/m^2)	28.1 ± 3.9	28.2 ± 4.4	28.4 ± 4.4
Testicular volume (right + left testis/2, mL)	23.4 ± 4.1	23.9 ± 2.7	23.9 ± 2.4
Basic sperm parameters			
Semen volume (mL)	3.8 ± 1.9[1,9,10]	2.8 ± 1.6[1,9]	3.2 ± 1.9[1,10]
Total sperm count (million)	365.4 ± 304.1[2,11,12]	325.0 ± 325.9[2,11,13]	280.1 ± 277.3[2,12,13]
Sperm concentration (mil/mL)	106.1 ± 77.2	125.3 ± 101.2	111.9 ± 101.0
Sperm A + B motility (%)	52.6 ± 16.9[3,14,15]	45.6 ± 15.9[3,14]	46.0 ± 19.0[3,15]
Normal sperm (%)	6.5 ± 5.3	6.4 ± 5.3	5.2 ± 4.9
Abstinence time (days)	5.9 ± 3.5	6.2 ± 4.2	5.7 ± 2.3

Table 5 continued

Characteristics	Group 1 0–0.34 × 10^6 WBC/mL $n = 292$ Mean ± SD	Group 2 0.35–0.99 × 10^6 WBC/mL $n = 64$ Mean ± SD	Group 3 ≥1.0 × 10^6 WBC/mL $n = 26$ Mean ± SD
WBC in semen (10^6/mL)	0.1 ± 0.1[4,16,17]	0.5 ± 0.2[4,16,18]	1.9 ± 1.3[4,17,18]
IL-6 in seminal plasma (ng/mL)	66.3 ± 69.0[5,19]	99.3 ± 121.3[5,20]	278.8 ± 429.4[5,19,20]
Basic prostate-related parameters			
Total prostate volume (mL)	35.7 ± 14.2	36.3 ± 14.4	35.5 ± 11.6
Residual urine (post-voided, mL)	29.3 ± 37.8	49.2 ± 74.2	36.0 ± 48.8
Maximum urinary flow rate (mL/s)	18.6 ± 9.5	19.1 ± 7.8	18.3 ± 7.2
PSA (ng/mL)	2.2 ± 1.9[6,21,22]	2.4 ± 2.7[6,21,23]	3.0 ± 2.1[6,22,23]
I-PSS sub-score (irritative, 0–15)	4.4 ± 3.3	4.2 ± 3.6	4.9 ± 4.3
I-PSS sub-score (obstructive, 0–15)	4.9 ± 3.6	5.0 ± 3.6	4.1 ± 3.2
I-PSS sub-score (nocturial, 0–5)	1.4 ± 1.1	1.2 ± 1.0	1.4 ± 1.1
I-PSS total score (0–35)	10.7 ± 6.6	10.4 ± 6.6	10.5 ± 7.6
NIH-CPSI sub-score (pain, 0–21)	5.0 ± 5.2	4.4 ± 8.1	5.6 ± 4.5
NIH-CPSI sub-score (quality of life, 0–6)	2.7 ± 1.4	2.6 ± 1.6	2.8 ± 1.8
NIH-CPSI total score (0–43)	13.1 ± 7.5	12.3 ± 7.8	13.6 ± 7.8
Basic hormonal parameters			
Testosterone (nmol/L)	16.0 ± 5.3	16.9 ± 6.8	17.5 ± 7.8
Estradiol (pmol/L)	152.5 ± 52.4[7,24]	151.2 ± 53.5[7,25]	189.4 ± 55.6[7,24,25]
Estradiol/testosterone	10.5 ± 5.0[8,26]	9.6 ± 4.2[8,27]	11.8 ± 6.1[8,26,27]
FSH (IU/L)	5.6 ± 3.3	6.1 ± 3.4	5.2 ± 2.1
LH (IU/L)	3.1 ± 1.6	2.9 ± 1.7	3.2 ± 1.7
FSH/LH (IU/L)	2.1 ± 1.6	3.5 ± 8.8	1.9 ± 0.9
SHBG (nmol/L)	33.1 ± 12.9	39.0 ± 18.6	36.4 ± 22.3

IQR interquartile range (25th–75th ‰), *WBC* white blood cells, *SD* standard deviation, *BMI* body mass index, *IL-6* interleukin-6, *PSA* prostate-specific antigen, *I-PSS* International Prostate Symptom Score, *NIH-CPSI* National Institute of Health Chronic Prostatitis Symptom Index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *SHBG* sex hormone-binding globulin

[a] $p < 0.05$ was considered statistically significant

[1] $p < 0.001$; Kruskal–Wallis test

[2] $p = 0.032$; Kruskal–Wallis test

[3] $p = 0.005$; Kruskal–Wallis test

[4] $p < 0.012$; Kruskal–Wallis test

[5] $p < 0.001$; Kruskal–Wallis test

[6] $p = 0.028$; Kruskal–Wallis test

[7] $p = 0.002$; Kruskal–Wallis test

[8] $p = 0.037$; Kruskal–Wallis test

[9–27] $p < 0.05$; Dunn's method

quality and seminal markers in males >45 years. Therefore, we tried to use well-known and current parameters. For example, prior reports have demonstrated that different altered immunological markers may indicate changes in semen parameters [4]. Our option was IL-6 as the leading cytokine measured since the mid-1990s [18].

Also, at the beginning of the study, we tried to minimize all the possible weaknesses. For example, part of the previously published studies did not use the accepted classification of prostatic diseases [4], and almost three-quarters of the previous studies on reproductive function in middle-aged men did not consider the duration of abstinence before semen analysis [31].

In our study, the recommended period of abstinence [9] was no shorter than 48 h and no longer than 7 days for all participants. In the initial phase of the study, the subjects with a reported incomplete semen sample [9] were excluded.

The leading drawback of most prior studies has been the lack of an age-matched control group [4]. In our study, that

Table 6 The associations between age, reproductive function, and prostate-related parameters according to IL-6 level in semen[a]

Characteristics	Group 1 IL-6 <57 mL $n = 195$ Median (IQR)	Group 2 IL-6 ≥57 mL $n = 135$ Median (IQR)
Age (years)	53.0 (49.0–59.0)	55.0 (51.0–61.0)
BMI (kg/m^2)	27.4 (24.9–30.2)	27.8 (25.6–31.0)
Testicular volume (right + left testis/2, mL)	25.0 (22.0–25.0)	25.0 (22.0–25.0)
Basic sperm parameters		
Semen volume (mL)	3.6 (2.6–5.0)[1]	2.7 (1.7–4.1)[1]
Total sperm count (million)	299.0 (160.5–519.0)[2]	218.7 (115.1–435.3)[2]
Sperm concentration (mil/mL)	88.0 (41.0–145.5)	96.0 (53.0–164.3)
Sperm A + B motility (%)	53.0 (44.0–62.0)	51.0 (40.0–61.0)
Normal sperm (%)	6.0 (3.0–10.0)	4.0 (2.0–9.0)
Abstinence time (days)	5.0 (4.0–7.0)	6.0 (4.0–7.0)
WBC in semen (10^6/mL)	0.1 (0.0–0.2)	0.1 (0.0–0.5)
IL-6 in seminal plasma (ng/mL)	34.2 (26.6–43.9)[3]	97.9 (71.8–162.8)[3]
Basic prostate-related parameters		
Total prostate volume (mL)	32.0 (25.0–40.0)	34.0 (28.0–44.0)
Residual urine (post-voided, mL)	17.5 (5.0–41.0)	17.0 (5.0–40.8)
Maximum urinary flow rate (mL/s)	18.2 (12.4–24.3)	16.5 (12.4–22.3)
PSA (ng/mL)	1.2 (0.7–2.2)[4]	2.1 (1.0–3.8)[4]
I-PSS sub-score (irritative, 0–15)	3.0 (2.0–7.0)	4.0 (2.0–7.0)
I-PSS sub-score (obstructive, 0–15)	5.0 (2.0–7.0)	4.0 (2.0–7.0)
I-PSS sub-score (nocturial, 0–5)	1.0 (1.0–2.0)	1.0 (1.0–2.0)
I-PSS total score (0–35)	10.0 (6.0–15.0)	9.0 (5.00–15.0)
NIH-CPSI sub-score (pain, 0–21)	5.0 (0.0–8.0)	5.0 (0.0–8.0)
NIH-CPSI sub-score (quality of life, 0–6)	3.0 (2.0–4.0)	2.0 (1.0–4.0)
NIH-CPSI total score (0–43)	14.0 (9.0–18.0)	12.0 (7.5–17.0)
Basic hormonal parameters		
Testosterone (nmol/L)	15.4 (12.2–20.0)	14.9 (12.2–19.0)
Estradiol (pmol/L)	141.5 (115.0–177.0)[5]	155.0 (123.0–192.0)[5]
Estradiol/testosterone	8.9 (7.0–12.1)	9.6 (7.7–13.0)
FSH (IU/L)	5.1 (3.3–7.5)	4.8 (3.5–6.8)
LH (IU/L)	2.6 (1.9–3.9)	2.7 (1.7–4.0)
FSH/LH (IU/L)	1.8 (1.3–2.6)	1.9 (1.4–2.6)
SHBG	30.1 (23.1–40.9)	33.7 (25.1–42.6)

Characteristics	Group 1 IL-6 <57 mL $n = 195$ Mean ± SD	Group 2 IL-6 ≥57 mL $n = 135$ Mean ± SD
Age (years)	54.2 ± 6.3	55.3 ± 6.4
BMI (kg/m^2)	27.8 ± 4.0	28.6 ± 3.9
Testicular volume (right + left testis/2, mL)	23.5 ± 3.8	23.3 ± 4.1
Basic sperm parameters		
Semen volume (mL)	4.0 ± 2.0[1]	3.0 ± 1.8[1]
Total sperm count (million)	376.3 ± 321.2[2]	325.4 ± 306.6[2]
Sperm concentration (mil/mL)	99.8 ± 73.4	107.8 ± 89.7
Sperm A + B motility (%)	51.9 ± 16.9	49.0 ± 16.8
Normal sperm (%)	6.7 ± 5.3	6.1 ± 5.4
Abstinence time (days)	5.7 ± 3.4	6.0 ± 3.2
WBC in semen (10^6/mL)	0.2 ± 0.2	0.4 ± 0.8

Table 6 continued

Characteristics	Group 1 IL-6 <57 mL $n = 195$ Mean ± SD	Group 2 IL-6 ≥57 mL $n = 135$ Mean ± SD
IL-6 in seminal plasma (ng/mL)	35.0 ± 11.9[3]	142.8 ± 143.8[3]
Basic prostate-related parameters		
Total prostate volume (mL)	34.2 ± 12.6	39.1 ± 15.9
Residual urine (post-voided, mL)	31.9 ± 39.7	35.7 ± 56.0
Maximum urinary flow rate (mL/s)	19.4 ± 9.7	18.0 ± 8.3
PSA (ng/mL)	1.9 ± 2.1[4]	3.3 ± 4.3[4]
I-PSS sub-score (irritative, 0–15)	4.4 ± 3.3	4.3 ± 3.5
I-PSS sub-score (obstructive, 0–15)	4.9 ± 3.5	4.5 ± 3.6
I-PSS sub-score (nocturial, 0–5)	1.4 ± 1.1	1.3 ± 1.1
I-PSS total score (0–35)	10.6 ± 6.3	10.1 ± 7.1
NIH-CPSI sub-score (pain, 0–21)	4.8 ± 4.1	4.4 ± 4.4
NIH-CPSI sub-score (quality of life, 0–6)	2.9 ± 1.5	2.4 ± 1.5
NIH-CPSI total score (0–43)	13.1 ± 7.4	11.8 ± 7.9
Basic hormonal parameters		
Testosterone (nmol/L)	16.4 ± 5.9	16.1 ± 5.6
Estradiol (pmol/L)	148.7 ± 49.7[5]	162.6 ± 56.3[5]
Estradiol/Testosterone	10.0 ± 4.8	11.0 ± 5.2
FSH (IU/L)	6.0 ± 3.7	5.4 ± 2.5
LH (IU/L)	3.2 ± 1.8	3.0 ± 1.5
FSH/LH (IU/L)	2.6 ± 5.4	2.0 ± 0.9
SHBG	33.2 ± 14.4	36.0 ± 14.4

IQR interquartile range (25th–75th ‰), *IL-6* interleukin-6, *BMI* body mass index, *WBC* white blood cells, *PSA* prostate-specific antigen, *I-PSS* International Prostate Symptom Score, *NIH-CPSI* National Institute of Health Chronic Prostatitis Symptom Index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *SHBG* sex hormone-binding globulin

[a] $p < 0.05$ was considered statistically significant

[1] $p < 0.001$; Mann–Whitney rank sum test

[2] $p = 0.028$; Mann–Whitney rank sum test

[3] $p < 0.001$; Mann–Whitney rank sum test

[4] $p = 0.013$; Mann–Whitney rank sum test

[5] $p = 0.046$; Mann–Whitney rank sum test

criterion is in accordance with the requirements. In addition, all the subjects were studied using the same criteria [9, 11], in the same center and laboratories. No patient had a DRE within a 1-week period prior to sampling. Also, none of the study subjects received antimicrobial, α1-blockers, or 5α-reductase inhibitor therapy within 3 months prior to the study.

Finally, as has been the case with most previous semen quality studies, the present group included only men who attended a screening in the outpatient clinic and were willing to provide semen specimens, and therefore, they do not represent the general population of males aged >45 years. While the main goal of our study was the screening for prostate health in middle-aged males, and the subjects of that age mostly do not wish to perform additional analyses, the assessment of sperm parameters was performed only with a single semen sample.

A similar method is described in prior studies of men >40 years [7, 8, 10, 21]. However, according to our study protocol, we re-evaluated the semen quality newly within 4 weeks after initial examination. In fact, most of the participants showed similar results compared with the initial semen analysis (data not shown).

In conclusion, our data demonstrate that sperm motility and the proportion of men having normal semen variables are significantly higher among the age-matched controls compared to subjects with non-malignant prostate pathologies. Also, the elevated seminal inflammatory markers in

Table 7 Multiple regression analysis indicating relationship on white blood cells count in semen[1,2]

Characteristics ($n = 322$)	WBC ($\times 10^6$/mL)	
	β[3]	p value[4]
Semen volume (mL)	−0.020	Ns
Total sperm count ($\times 10^6$)	−0.001	Ns
Sperm motility (A + B, %)	−0.003	0.047
IL-6 in seminal plasma (pg/mL)	0.002	<0.001
BMI (kg/m^2)	−0.006	Ns
PSA (ng/mL)	0.002	Ns

WBC white blood cells, *IL-6* interleukin-6, *BMI* body mass index, *PSA* prostate-specific antigen

[1] Adjusted R^2 0.3208

[2] The values are given only in statistically significant correlations

[3] The β-coefficient indicates the association of the variable with WBC in semen

[4] p value gives the probability of this association; $p < 0.05$ is considered as statistically significant

Table 8 Multiple regression analysis indicating relationship on interleukin-6 in semen[1,2]

Characteristics ($n = 322$)	IL-6 (ng/mL)	
	β[2]	p value[3]
Age (years)	1.522	Ns
Semen volume (mL)	−2.483	Ns
Total sperm count ($\times 10^6$)	−0.034	Ns
WBC ($\times 10^6$/mL)	133.882	<0.001
BMI (kg/m^2)	−0.006	Ns
Total prostate volume (mL)	0.751	Ns
PSA (ng/mL)	−0.139	Ns

IL-6 interleukin-6, *WBC* white blood cells, *BMI* body mass index, *PSA* prostate-specific antigen

[1] Adjusted R^2 0.3266

[2] The values are given only in statistically significant correlations

[3] The β-coefficient indicates the association of the variable with IL-6 in semen

[4] p value gives the probability of this association; $p < 0.05$ is considered as statistically significant

middle-aged males are associated with decreased semen volume, total sperm count, and sperm motility, as well as elevated PSA and estradiol level in serum.

While there is no consensus about the causes for impaired reproductive quality in male subjects >45 years, one of the possible pathways could be related to infection and inflammation in genital tract with subsequent (partial) obstruction and damage of prostate and other male accessory glands.

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Conflict of interest None.

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