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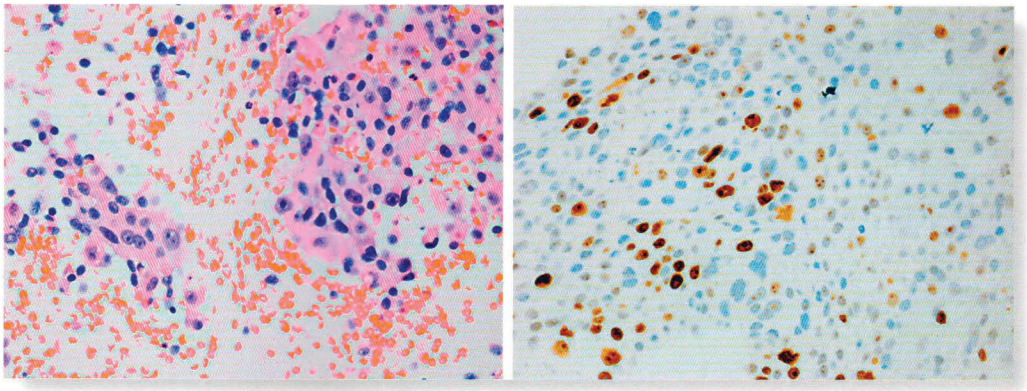

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Novel Insights from Clinical Practice

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Sperm Sediment Cytology: A New Technique for Diagnosing Occult Urologic Infections

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Established Facts

- Microscopic sperm examination is a widely used and routinely applied technique for investigating male infertility and monitoring spermatogenesis following treatment.
- However, there are no reports in the literature on sperm sedimentation and its use by cytology for diagnosing infectious agents in symptomatic urologic patients.

Novel Insights

- It is becoming increasingly clear that the seminal organs such as prostate, seminal vesicles, and their excretory duct system serve as a reservoirs for infectious agents and are therefore not detected by conventional urinalysis.
- In contrast, sperm sediment cytology (SSC) detects infectious organisms in a high number of symptomatic urologic patients and infertile males.
- The concentration by sperm sedimentation allows the identification of pathogenic agents with ease enabling subsequent specific treatment.

Keywords
Sperm sediment cytology · Urinalysis · Urologic infections · Seminal organs · Urologic cytology

Abstract

Background: Ill-defined chronic symptoms such as itching, burning, urethral discharge, and pelvic pain are not uncommon in male urologic patients. Often, microscopic urinalysis, bacterial cultures, and laboratory testing are non-contributory. We have developed a technique for sperm sedimentation and used the cytologic examination of the sediment routinely in more than 4,000 patients with urologic complaints over the last 5 years. ***Case:*** We present 3 exemplary cases, documenting the diagnostic power of sperm sediment cytology (SSC). In all 3 cases, conventional laboratory testing failed to reveal a causative agent. Case 1 is that of a 28-year-old male patient with a history of occasional swelling of the lymph nodes in the left inguinal region for 1 year. Case 2 is that of a 51-year-old male patient with a history of itching and burning of the urethra of 2 months' duration. Case 3 is that of a 22-year-old female patient with copious

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vaginal discharge after intercourse for 18 months, non-responsive to treatment. ***Conclusion:*** We were able to identify causative pathologic organisms in the sediment of all patients or their partner. Subsequent specific treatment did clear and alleviate the symptoms documenting the clinical relevance of this new technique. In our experience, SSC has proven to be a valuable technique for diagnosing occult urologic infections.
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Background

The surprisingly high rate of male urogenital infections with *Trichomonas vaginalis* (*T. vaginalis*), *Chlamydia trachomatis* (*C. trachomatis*), human papilloma virus (HPV), cytomegalovirus (CMV), herpes simplex virus (HSV), and Candida has only recently come into focus [1–4], mainly because traditional diagnostic techniques such as urinalysis, smears, and serum testing have been shown to be inadequate [5]. With newer methods such as nucleic acid amplification [5, 6], the prevalence of Trichomonas and Chlamydia in men attending a sexually transmitted diseases clinic were shown to be as high as 17 and 19%, respectively [2]. In men with non-gonococcal urethritis, 19.9% were infected with Trichomonas [6]. Equally significant is the detection of HPV in human semen specimen in 14.9% [7]. Overall, semen seems to be the most sensitive specimen to detect Trichomonas [8]. Also, these infectious agents are highly prevalent in men having sex with men [9]. In heterosexual couples, the male partner may be the source of re-infection of his female partner [10, 11]. Alarmingly, a recent meta-analysis identified a pooled prevalence of HPV in 16% of asymptomatic sperm donors, HPV 16 being the most common subtype [12]. The authors, therefore, consider HPV-infected donor semen as a potential health risk [12]. In summary, the prevalence of infectious organisms in the male urologic system has been highly underestimated. And, semen has only recently been recognized as a valuable and sensitive diagnostic specimen.

During ejaculation, semen is produced as a concentrated suspension of spermatozoa, stored in the epididymis, mixed with and diluted by secretions of the prostate and seminal vesicles, with minor contributions from the bulbourethral (Cowper's) glands and epididymis. Traditionally, sperm analysis has been used for investigating male fertility status. For that purpose, fresh semen is examined microscopically for mobility and morphology of spermatozoa without sedimentation or staining. In our institution, the sedimentation of sperm was developed over the last couple of years in order to identify infectious pathogens. Our studies revealed semen as the most informative biological material in urologic patients [13]. Since the early 2000s, our group has developed the technique and gained considerable clinical experience [14]. In addition, we have developed a software program, Vision Cyto® Sperm Sediment software (VC® SS) with vision sperm sediment (VSS) algorithm. This program allows to perform a systematic and comprehensive macroscopic and microscopic evaluation of the sediment smear. In this communication, we are presenting some of the results obtained.

Case Reports

Case 1

Clinical Findings

This 28-year-old unmarried male with a promiscuous life style noted occasional swelling of the lymph nodes in the left inguinal region for several months. Physical examination revealed an infiltrate of 2 cm at the base of the left scrotum. There was no flushing of the skin or fluctuation. The lymph nodes in the left inguinal region were palpable and measured up to 5–6 mm. The clinical diagnosis after ultrasound examination was infiltration of the perineum of unclear etiology.

Laboratory Findings and SSC Assessment

Standard laboratory analysis of urine, blood, smears of prostatic secretions and urethral discharge were within normal limits, polymerase chain reaction (PCR) and microbiological cultures of urethral discharge were non-contributory. Examination of the ejaculate revealed astenozoospermia. Subsequently, the urologist requested SSC to exclude an infection of the lower urinary tract.

The semen was centrifuged for 20 min at 1,500 rpm, several smears were prepared, air-dried, and stained with May-Grünwald Giemsa (MGG). Cytologic examination showed a background of moderate to abundant mixed microbiota and cytoplasmic inclusions consistent with chlamydia (Fig. 1), as well as *T. vaginalis* (Fig. 2). A diagnosis of trichomoniasis and chronic chlamydial infection was made. Immunoassay supported the diagnosis of chlamydiosis with a serum anti-*C. trachomatis* IgG 1:200 and the serum antibodies to the heat shock protein 60 chlamydia were 1:400. The patient was treated accordingly for 3 months with complete resolution of the symptoms.

Case 2

Clinical Findings

This 51-year-old divorced male complained of itching and urethral burning for 2 months. He had put himself on antibiotic treatment without improvement. After worsening of the condition, he added an antifungal drug without relief.

Laboratory Findings and SSC Assessment

Conventional microscopic examination and PCR of the urethral discharge were within normal limits. SSC was performed as described in case 1. The cytologic examination showed a moderate to abundant mixed flora with Candida (Fig. 3), vegetative form of

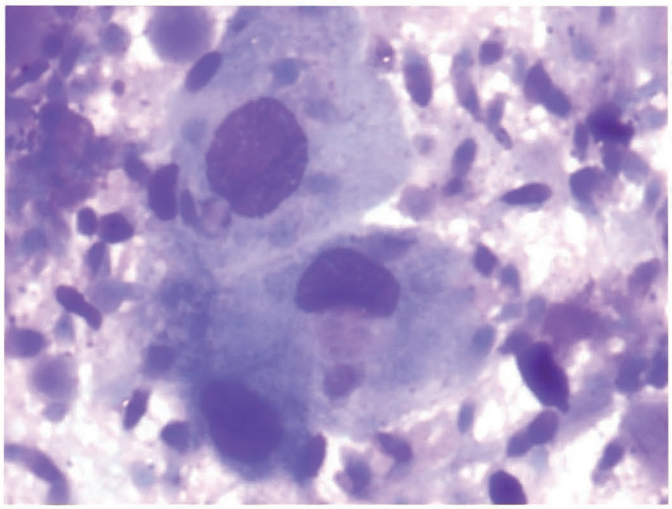

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Fig. 1. *C. trachomatis*: large epithelial cells with intracytoplasmic granules. MGG ×100 oil immersion.

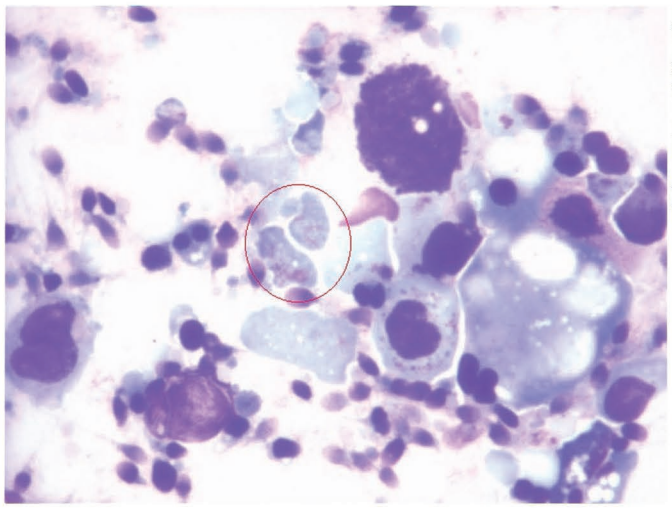

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Fig. 2. *T. vaginalis* clearly recognizable intracytoplasmic granules. MGG ×100 oil immersion.

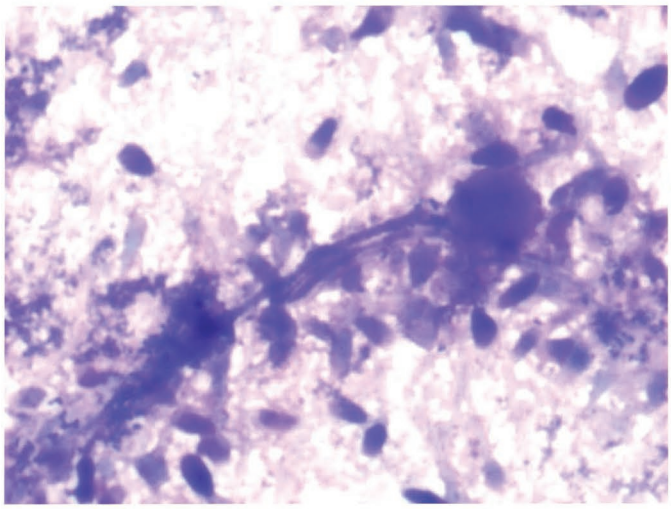

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Fig. 3. Filaments of Candida species and occasional sporozoa. MGG ×100 oil immersion.

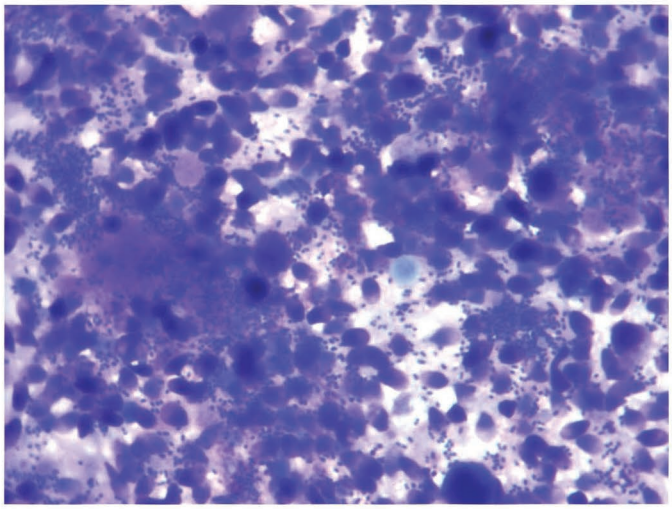

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Fig. 4. Abundant mixed microbiota. MGG ×100 oil immersion.

Entamoeba histolytica (*E. histolytica*), signs of CMV, and foreign body cells. A diagnosis of a mixed bacterial, viral, fungal infection was made (amebiasis, CMV, and candidosis).

Express immunoassay against *E. histolytica* in sperm sediment specimen confirmed the diagnosis of urogenital amebiasis. Serum analysis revealed positive anti-CMV IgG and anti-CMV IgM confirming a persistent CMV infection. Unfortunately, follow-up information on the further clinical course was not available.

Case 3

Clinical Findings

A 22-year-old female patient complained of abundant vaginal discharge for 18 months. She had only one male partner, no use of condom. The gynecologic examination showed cervical erosion and hyperemia, microscopic examination revealed mixed microbiota, and leukocytosis. *Enterococcus faecalis* and streptococcus were identified by microbiologic culture. PCR showed Gardnerella and *Candida albicans*. A diagnosis of cervicitis and dysbiosis was made. Antibacterial and antifungal treatment had no effect. The gynecologist recommended condom use and SSC of the sexual partner.

Result of SSC of the Sexual Partner

The sperm sediment showed degenerate forms of *T. vaginalis* as well as *E. histolytica* cysts, confirmed by immunochromatography for antigens of *E. histolytica*. There was a moderate to abundant macrophage reaction and abundant mixed microbiota (Fig. 4).

Treatment of both partners led to complete resolution of the symptoms illustrating that the male partner may be the source of repeated re-infections.

Discussion

Infections are the main cause for urologic complaints in men [1]. However, conventional laboratory testing has diagnostic value only in patients with acute disease, whereas in men with an indolent course with poorly defined urogenital symptoms it is often non-diagnostic. Yet, in these patients, infectious organisms such as Trichomonas, Chlamydia, and Candida are often the causative agent for the symptoms [10]. But traditional diagnostic methods are highly inadequate. A recent study from the Cleveland Clinic in September 2016 [15] showed a detection rate of Trichomonas in urinary cytology as only 0.1% (73 cases of 60,000 specimens) over a 30-year period. This contrasts with reported rates of up to 20% trichomoniasis in patients attending sexually transmitted diseases clinics [2, 6]. Also, male partners of women with trichomoniasis were infected in surprising 72%, most of them being asymptomatic [11]. Thus, re-infections are common, if only one partner is being treated. Clearly, for a successful management, partner diagnosis and treatment have to be included, as convincingly documented by case 3 of this report. Table 1 shows the summary of the most common violation of the vaginal microbiota in 2,068 female sexual partners of the patients with urologic complaints. The data from table highlight the suffering of both men and women. Such situation can induce reproductive problem.

Table 1. Summary of the most common violation of the vaginal microbiota in 2,068 female sexual partners of the patients with urologic complaints

Cytological features	Female sexual partners, *n* (%)
Candidosis	850 (41.10)
Bacterial vaginosis	448 (21.66)
Trichomoniasis	140 (6.76)
Nothing special	630 (30.3)

It is becoming more and more evident that it is not the urinary bladder which harbors the infectious organisms in symptomatic male urologic patients but the seminal organs such as prostate, seminal vesicles, and epididymis. It has been speculated that the lack of glycogen in urethra and bladder and the toxicity of urine adversely affect the biologic survival and morphologic characteristics particularly of Trichomonas. Therefore, semen appears to be a much more appropriate specimen for examination since it mirrors much more closely the microbiologic status (microbiota [10]) of the male urogenital system.

The SSC findings are summarized in Table 2 which was found in more than 4,000 patients with urologic complaints over the last 5 years. The high rate of diagnostic valuable findings deserves attention. An overwhelming percentage of patients favored 98% (4,126 of 4,210 = 98%) impaired microbiological status of sperm with Candida (124 of 4,126 = 3%), Gardnerella (2,352 of 4,126 = 57%), Mobiluncus (371 of 4,126 = 9%), and other mixed flora (1,279 of 4,126 = 31%). The majority of cases indicates virus/bacterial-associated cells (1,979/4,210 = 47%) with HPV (534/1,979 = 27%), CMV (752/1,979 = 38%), HSV (237/1,979 = 12%), and *C. trachomatis* (455/1,979 = 23%). Fewer cases (1,053 of 4,210 = 25%) were presented by different protozoa with *T. vaginalis* (611 of 1,053 = 58%), *E. histolytica* (421 of 1,053 = 40%), *Lamblia intestinalis* (21 of 1,053 = 2%).

Another special issue is the detection of HPV in the semen. In fact, there are only few reports in the worldwide literature which have investigated this challenge in men. In a series of reports, the authors refer to a prevalence of human papillomas viruses in semen. The studies of recent decades note that the HPV DNA in semen was detected in 14.9–23.4% of cases with predominance of high-risk HPV (hrHPV) types [3, 7, 12]. Some of the authors provide new insight into this emerging topic by confirmation about absence significant association between HPV presence and impairment of semen quality [7, 16], unless there is slight decrease of pH borderline in HPV DNA-positive semen

Table 2. Summary of SSC findings in 4,210 patients with urologic complaints over the last 5 years

Results, *n* (%)	4,126 (98) Mixed flora		1,053 (25) Protozoa		1,979 (47) Virus/bacterial-associated cells	
Type of findings, *n* (%)	Candida	124 (3)	*T. vaginalis*	611 (58)	HPV	534 (27)
	Gardnerella	2,352 (57)	*E. histolytica*	421 (40)	CMV	752 (38)
	Mobiluncus	371 (9)	*Lamblia intestinalis*	21 (2)	HSV	237 (12)
	Other cocci	1,279 (31)			*C. trachomatis*	455 (23)

(7.4 vs. 7.5) [16]. According to the data, HPV is present in the semen of asymptomatic men of populations seeking fertility evaluation/treatment of 16 vs. 10% in other populations. In contrast, another study does not confirm high HPV prevalence in semen without detectable lesions: in 48% of subjects with urethral lesions, in 22% of patients with penile lesions, in 2% of patients without HPV-associated lesions [3]. Authors provide the key role of semen contamination by HPV of recipient, especially in the era of assisted reproductive technology [3, 7, 12, 16].

As far as our experience, HPV-associated cells were found by SSC technique simultaneously with investigating and monitoring spermatogenesis in infertile men while they presented normozoospermia. These SSC-HPV findings were correlated in 6% of cases (8 of 138 = 6%) with hrHPV DNA in the male patients. Hopefully, the SSC could also be recommended together with routine semen examination as a first triage to identify the correct urological management.

Conclusion

Sperm sedimentation allows to concentrate and enrich the diagnostic material as we have shown in the past years [13]. SSC is a minimally invasive, quick, and inexpensive technique which could be applied widely in urologic clinics and offices. Routinely, we have used the MGG stain which had been developed specifically for the identification of infectious organism and has stood the test of time for more than 100 years. In order to standardize the evaluation of the sperm sediment, we have developed a software (VC® SS software with VSS algorithm) that allows to obtain the necessary accuracy and completeness of the examination [14].

In summary, we believe that SSC is a necessary step in the evaluation of symptomatic urologic patients. Together with the clinical information, it is indispensable in our opinion for planning the appropriate clinical management.

Statement of Ethics

The authors are responsible for the ethical and scientific nature of the study (case reports). Consent from the patients has been received for the use of the data presented (case reports).

Disclosure Statement

There were no conflicts of interest of which the authors were aware in the conduct of this review. The authors did not receive any payments and does not anticipate any future payments, compensation, financial or beneficial considerations, or favor for the conduct of the review or the writing of this paper.

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