Diagnosis of infection-induced male infertility and the effects of therapy on the semen parameters

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Szeged

PhD Thesis

1999

Contents

Publications connected with the thesis

I. Török L, Deák J, Scultéty S, Földes J
A *Chlamydia trachomatis* jelentősége az urogenitális rendszer megbetegedéseiben
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Abbreviations

EPS	expressed prostatic secretum
FSH	folliculi stimulating hormone
LH	luteinising hormone
CFU	colony forming units
BV	bacterial vaginosis
PCR	polimerase chain reaction
B. ureolyticus	*Bacteroides ureolyticus*
C. trachomatis	*Chlamydia trachomatis*
E. coli	*Escherichia coli*
M. hominis	*Mycoplasma hominis*
N. gonorrhoeae	*Neisseria gonorrhoeae*
P. acnes	*Propionibacterium acnes*
P. anaerobius	*Peptostreptococcus anaerobius*
U. urealyticum	*Ureaplasma urealyticum*
T. vaginalis	*Trichomonas vaginalis*
In statistical analysis	
MOTILITA	motility
PATHFORM	pathological forms
Sig.	significance

Introduction

At least 50% of the cases of human infertility involve a defect in the male partner (1), and about half of male infertility cases have an idiopathic cause. Although symptomatic genitourinary infection is unusual in infertile men, clinical investigators have speculated that asymptomatic or subclinical infection or past infection may play a role in male infertility. Unfortunately, the published data concerning the role of infection in infertile men have been controversial. Although a number of methods have been developed to localize genital infection in men and to determine the aetiology of possible symptomatic infections in men, these methods have seldom been used to study infectious causes of male infertility. At the heart of the conventional diagnosis of male infertility is a descriptive analysis of the semen profile, for which the principal characteristics determined are sperm number, motility and morphology. The basic assumption underlying this approach is that pregnancy can be initiated only if there are more than a certain minimum number of normal-appearing, motile spermatozoa in the ejaculate. Some authors consider low-grade inflammation of the prostate and seminal vesicles to be an important cause of oligospermia and asthenospermia (2, 3). The role of specific infections is currently under question (4). The results of controlled studies indicate that mycoplasmal and ureaplasmal infections are as common in fertile as in infertile couples and that treatment does not improve fertility (5, 6), but Xu *et al.* found a significantly higher frequency of *Ureaplasma urealyticum* infection among infertile males than among the fertile controls (7). Chlamydial infections may be important as they are known to cause epididymitis (8, 9). Unilateral epididymal or testicular infection could affect fertility in various ways. A simple reduction in sperm count in consequence of an unilateral epididymal or vasal obstruction could lead to hypofertility in some patients. Minimal or subclinical epididymitis could also occur on the contralateral side and affect the epididymal function or cause partial occlusion. There is also certain amount of evidence that damage to one testicle may cause damage to the opposite testicle, resulting in infertility (10).

Usually, little attention is paid to anaerobic bacteria as they are sensitive to transportation and culturing, and differentiation is difficult, costly and time- consuming. Balmelli *et al.* observed that the presence of *Bacteroides ureolyticus* in the semen was coupled to an increased presence of *Enterococcus* species, to an increased number of short-tailed spermatozoa and epithelial cells, and to a decreased total fructose concentration (11). These results suggest that *B. ureolyticus* or its toxins may influence sperm morphology and function by as yet unknown mechanisms. Duerden *et al.* reported that in clinical infections *B. ureolyticus* was rarely isolated in pure cultures and its presence was most often associated with anaerobic bacteria (12). Our own examinations concentrate mainly on asymptomatic subclinical anaerobic and chlamydial infections and their relation to infertility.

Specific syndromes of genital infection in the male:

1. Urethritis

Urethritis may be divided into gonococcal and non-gonococcal varieties. Gonococcal urethritis is caused by *Neisseria gonorrhoeae. Chlamydia trachomatis* has been most firmly established in the aetiology of non-gonococcal urethritis and causes from 30-50% of all cases. Some authors suggest that *U. urealyticum* is one cause of chlamydia-negative non-gonococcal urethritis (13, 14). At least 20-30% of the patients with urethritis have neither *C. trachomatis* nor *U. urealyticum* in the urethra. These men tend not to respond well to antimicrobial therapy. *Trichomonas vaginalis*, herpes simplex virus and different kinds of bacteria have been implicated in the aetiology of this infection (15-20). Both gonococcal and non-gonococcal urethritis are often associated with symptoms of urethral discharge and dysuria. Three-quarters of men with gonorrhoea and about one-third of men with non-gonococcal urethritis complain of discharge (21). Among recent male contacts of women with either gonococcal or chlamydial infection, the infection is often asymptomatic (22). Delay in the treatment of men with urethritis or their sexual partners may therefore lead to clinical epididymitis in the male or salpingitis in the female, and result in infertility. Although urethritis *per*

se has not been shown to be a cause of infertility in men, urethritis is known to be a precursor of epididymitis.

2. Epididymitis and/or orchitis

The only known specific infectious causes of male infertility include those diseases which lead directly either to the destruction of testicular substance (*e.g.*, mumps) or to the occlusion of the transport tubules for spermatozoa. Since sperm transit through the epididymis is known to be necessary for the development of a normal sperm function, it is possible that inflammation and damage to the epididymis could ultimately lead to decreased fertility even in the absence of occlusion of the epididymal tubules. Currently, most cases of epididymitis are thought to be infectious in origin. Epididymitis and epididymorchitis have been associated with infection by bacteria, viruses (herpes simplex, cytomegalovirus, mumps and smallpox), fungi (*Histoplasma capsulatum, Coccidioides immitis, Cryptococcus neoformans and Blastomyces dermatitides*) and parasites such as *T. vaginalis, Schistosoma mansoni,* amoebae and filariae (23, 24). The route of spread of infection to the epididymis has been a subject of much debate. Some infections, such as tuberculous, pneumococcal, fungal and other infections, might well spread to the epididymis by a haematogenous route. However, this route is probably unusual for coliform or sexually transmitted organisms, since the offending organism can most often be found within the urethra or bladder. Just as pyelonephritis is usually due to the ascending spread of bacteria from an infected bladder, so epididymitis is usually due to the spread of bacteria from infection involving the urethra, bladder, or prostate. Unilateral epididymal or testicular infection could affect fertility in several ways. A simple reduction in sperm count in consequence of an unilateral epididymal or vasal obstruction could lead to hypofertility in some patients. Minimal or subclinical epididymitis could also occur on the contralateral side and affect the epididymal function or cause partial occlusion. Epididymitis not only affects the epididymis, but also involves the testicle and may lead to testicular atrophy. Wolin performed testicular biopsies on men with acute epididymitis and found that 20 out of

28 patients exhibited decreased spermatogenesis, and 9 out of 28 had testicular inflammation (25). Ludwig and Haselberger followed 46 patients with unilateral epididymitis for periods ranging from 8 days to 1 year. Two-thirds of them were found to have oligospermia, and 20% of them to have long-term infertility abnormalities reflected in the results of semen analyses (26).

3. Prostatitis

Prostatitis includes several syndromes which are often confusing even in the absence of infertility. The prevalence of clinical symptoms of prostatitis is known to be considerably higher than previously reported, afflicting between 25 and 50% of all adult men (27,28). Clinically, a widely accepted classification system has been developed on the basis of the standardized sequential bacteriological localization technique introduced by Meares and Stamey: (a) acute bacterial prostatitis, (b) chronic bacterial prostatitis, (c) non-bacterial prostatitis, and (d) prostatodynia (29). The syndrome of acute bacterial prostatitis is diagnosed on the basis of a finding of bacteriuria, an exquisitely tender prostate, and the systemic signs of acute bacterial infection. The pathogen found in the prostate can almost always be cultured from the urine. Chronic bacterial prostatitis is characterized by relapsing cystitis due to the organisms which infect the prostate. Prostatic examination may be tender or normal (30,31). Prostatic secretion obtained by transrectal massage in chronic bacterial prostatitis usually contains numerous white cells (32,33). Men with non-bacterial prostatitis have symptoms attributable to the prostate, and evidence of prostatic inflammation, but no pathogenic bacteria can be isolated from the prostate or bladder. Sexually transmitted organisms have also been implicated in the etiology of nonbacterial prostatitis.

Prostatodynia is a syndrome characterized by the symptoms of chronic non-bacterial prostatitis, but without prostatic inflammation or infection. When diagnosed clinically, chronic idiopathic prostatitis (prostatodynia), has a poor record of treatment success. The recent literature suggests that the condition referred to as chronic idiopathic prostatitis may actually have an infectious etiology (34-35). The use of antimicrobial

therapy may or may not elicit transient relief of symptoms. A number of organisms have been reported as possible causes of this syndrome: *T. vaginalis*, *C. trachomatis*, genital mycoplasmas and genital viruses (36-41).

Prostatic inflammation may also be present in the absence of symptoms ascribable to the prostate. O'Shaughnessy *et al.* (42) found that 30% of normal asymptomatic men in a military population undergoing routine examination had more than 50 white blood cells in clumps or masses/400x field in the expressed prostatic secretion (EPS). Shaeffer *et al.* (43) quantitated white blood cells in the EPS from asymptomatic infertile men with or without varicocele. They found infertile patients to have significantly more white blood cells in the prostatic secretions than did the fertile controls, regardless of the presence or absence of varicocele.

The methodological problems of the role of infection in infertility make the interpretation of the results of antibiotic treatment difficult. Criteria for successful treatment have included increased sperm motility, increased sperm count, decreased leukocyte counts in the semen and/or the EPS, and pregnancy. Appropriate antibiotics can be chosen on the basis of culturing and Gram staining of the examined sample. For non-feverish patients with mildly or moderately severe epididymitis, a quinolone antibiotic provides activity against most *Enterobacteriacae.* The organisms from the urine are also responsible for epididymitis, and therapy can be modified on the basis of susceptibility tests on the isolate. Antibiotic treatment may need to be prolonged in some cases. In patients in whom the aetiologic agent is a sexually transmitted pathogen, treatment of epididymitis is not complete without treatment of the sexual partner(s). Patients with chronic prostatitis are often diagnosed as having a bacterial infection, and specific aetiologic antimicrobial therapy is initiated. The class of antibiotics that seem to hold the most promise are the 5-fluoroquinolones. They have an affinity for the prostate gland and seem to accumulate there well. Additionally, there is the added benefit of a broad spectrum, covering the usual and unusual uropathogens in addition to some of the more fastidious microorganisms, such as chlamydia and mycoplasma species. There is little consensus, however, as to the length of treatment necessary to eradicate the infection. Most authors agree that 4 weeks is the minimum period of time

necessary to eradicate a prostate infection, while others extend this to 8-12 weeks. There are a number of reasons why prostatic infections tend to be refractory to the traditional courses of therapy. The first is the variable penetration of the active drug into the prostatic parenchyma for most antibiotics (44). The second is that the infection may be sequestered in a glycoprotein matrix secreted by the bacterium, which may inhibit the action of antibiotics by limiting their access to the bacteria (45). Lastly, prostatic stones may become a nidus for infection by harbouring bacteria within the interstices of the stone, thereby affording protection of the bacteria against the antibiotic that is being utilized. All of these factors complicate the treatment process for chronic bacterial prostatitis, making it difficult to decide on the appropriate time course of therapy. The technique of repetitive prostatic massage empties the prostatic ducts of inspissated secretions and presumably pockets of infection that may be harboured by obstructed regions of the gland. This treatment, combined with the concomitant use of antibiotic and non-steroidal anti-inflammatory drugs, may afford a treatment alternative for refractory cases.

Aim of the study

The present study was carried out:

- to determine the incidence of different infections in semen samples from asymptomatic men,
- to elucidate the roles of anaerobic bacteria and *C. trachomatis* as causative organisms in chronic bacterial prostatitis,
- to understand the changes in semen characteristics in the infected individuals before and after treatment, and
- to demonstrate in an *in vitro* study the significant reduction in motility associated with different anaerobic bacteria isolated from the samples.

The results of our investigations could perhaps help patients suffering from chronic subfertility.

Materials and methods

Between 1994 and 1998, 1447 infertile male patients were investigated. The distribution of the patients according to age is shown in Fig. 1. The details of the andrological examinations are to be seen in Table I.

1. Clinical methods

The methods applied to establish the clinical diagnosis included physical and ultrasonographic examinations. The transabdominal, scrotal and transrectal prostate ultrasonographic examinations were performed with Acuson 128 and Hitachi EUB-450 equipment. Transrectal sonography of the prostate and seminal vesicles is valuable for detection of the consequences of chronic urogenital infections or functional abnormalities of these organs in infertile patients.

2. Semen analysis

Semen analyses were performed at the Andrology Unit of A. Szent-Györgyi University following the guidelines of WHO (46). For this study, the following semen parameters were taken into account: total motility in 10 min after ejaculation, leukocyte counts, the total and specific head/midpiece/tail percentages of abnormal forms and fructose concentrations. Sperm counts were carried out with a Bürker chamber after dilution of the sperm.

3. Microbiological methods

The samples were transported in appropriate transport media, depending on the microorganism to be examined.

Direct smears of the EPS and ejaculates were also examined after Gram staining, and leukocytes were counted per high-power field (5 high-power fields/sample). The prostatic fluid was defined as purulent if more than 10 white blood cells were seen per high-power field at a magnification of x400.

Fig. 1. Distribution of patients according to age

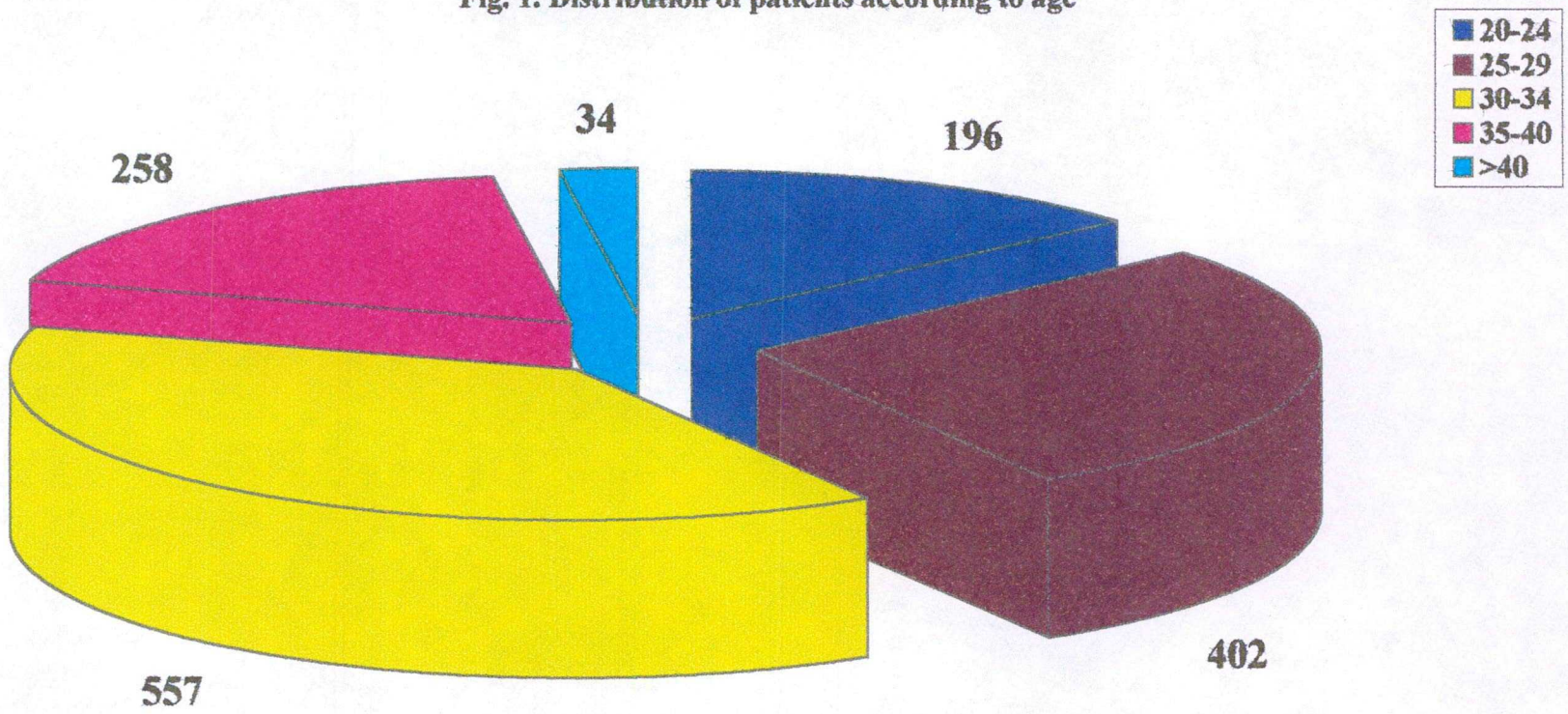


Table 1. Examination of infertile patients

Clinical examination	Laboratory tests	
Case history	urine (first voided, midstream and after prostatic massage)	
Physical examination		sediment
Transabdominal sonography		cultures for aerobic bacteria, fungi,
Scrotal sonography		*Mycoplasma /Ureaplasma, T. vaginalis*
Transrectal prostate sonography		
	expressed prosatic secretum	
		culturing for anaerobic bacteria too
Semen analysis	semen	cultures for aerobic and anaerobic bacteria, fungi,
		Mycoplasma /Ureaplasma, T. vaginalis
	urethral sample	cultures for aerobic and anaerobic bacteria,
		fungi, *Mycoplasma /Ureaplasma, T. vaginalis*
		C. trachomatis
	serum	hormone level tests (FSH, LH, testosterone, prolactin)

For microbiological examinations, the urethral sample, the EPS and 100 µl undiluted semen sample were inoculated onto each of the following media: 5% cattle blood agar (Columbia agar base), chocolate agar, Sabouraud agar and anaerobic blood agar consisting 5% cattle blood , supplemented with haemin and vitamin K_1. Aerobic and microaerophilic bacteria were recovered after incubation for a minimum of 48 h at 37 °C in a CO_2 -containing environment. Obligate anaerobes were recovered from the anaerobic blood agar after incubation for 6 days in an anaerobic chamber (Sheldon, Mansfield, USA) at 37 °C. The isolated aerobic and anaerobic bacteria were identified by conventional methods (Manual of Clinical Microbiology, Wadsworth Anaerobic Bacteriology Manual) (47, 48) or with the ATB System (bioMerieux, Lyon, France). Genital mycoplasmas and *U. urealyticum* were recovered by using Mycoplasma Duo kits (Sanofi Pasteur Diagnostics, Marnes-la-Coquette, France). *C. trachomatis* infection was detected via the ligase-chain reaction (Abbott, Wiesbaden-Delkenheiem, Germany).

4. Measurement of hormone levels

FSH, LH and prolactin were determined with ^{125}I-labelled IRMA kits (IZOTÓP Institute Kft, Budapet, Hungary). Testosterone was determined by a ^{3}H-labelled Radio Immuno Assay.

5. Statistical analysis

Semen parameters before and after treatment were compared by Wilcoxon matched-pairs signed-rank tests. The data on fructose levels in the semen before and after treatment were compared by means of the Student T-test. The level of significance was set at $P< 0.01$.

6. *In vitro* experiments

The effects of isolated anaerobic bacteria on the spermatozoa were investigated in *in vitro* experiments. Semen samples containing spermatozoa with normal motility were adjusted to 10^7 spermatozoa/ml. The sperm suspension was artificially infected with

anaerobic bacteria isolated from the samples of patients (*Fusobacterium nucleatum, Bacteroides fragilis, Prevotella bivia, Peptostreptococcus anaerobius* and *Propionibacterium granulosum*) in concentrations varying from 10^2 to 10^6 (CFU)/ml (colony forming units). The motility of the spermatozoa was checked following incubation for 1, 2, 3 and 18 h. BM1 synthetic medium (Bio-Media, Switzerland) was used as incubation medium for spermatozoa.

7. Andrological medication

In our patients, antibiotics were given according to the results of sperm analysis, microbiological testing and hormonal evaluations. The partner of each patient underwent a complete work-up for infertility. For the patients with chronic prostatitis, a 1-month course of selected antibiotic was applied; if this was not effective, treatment was extende to 8 weeks. The patients in whom the semen cultures demonstrated the presence of aerobic bacteria sensitive to ofloxacin or ciprofloxacin were treated with daily doses of 2 x 200 mg ofloxacin or 2 x 250 mg ciprofloxacin. In the case of quinolone-resistant isolates, the applied treatment was doxycycline at a dose of 2 x 100 mg for 10 days, and then 1 x 100 mg/day. Unusual uropathogens were treated on the basis of according their antibiotic susceptibility (azithromycin or cefuroxim). All patients who gave positive culture results for anaerobic bacteria or for mixed aerobic/anaerobic bacteria underwent 4-6 weeks of antibiotic therapy against the anaerobic bacteria found in the specimens. Amoxicillin/clavulanic acid and ampicillin/sulbactam were administered in a dose of 3 x 375 mg/day, and ampicillin/sulbactam in a dose of 2 x 375 mg/day or clindamycin in a dose of 3 x 150 mg/day. If the patient was sensitive to such antibiotics, the treatment was changed to metronidazole in a dose of 2 x 500 mg for 5 days, and then 2 x 250 mg. The caveat here is that the long-term use of broad-spectrum antibiotics can lead to side-effects that may be serious, such as pseudomembranous colitis, antibiotic-induced diarrhoea, yeast overgrowth, and the selection of resistant organisms in the intestines of the patients. Infertile patients infected by *U. urealyticum* and/or *Mycoplasma hominis* were treated with doxycycline in a dose of 2 x 100 mg for 10 days, then 1 x 100 mg/day, or

azithromycin once a day for 2 weeks, together with the sexual partner(s). If the *U. urealyticum* was associated with an anaerobic infection, doxycycline + metronidazole or azithromycin therapy was applied. *U. urealyticum* and/or *M. hominis* with aerobic bacteria were treated according to the antibiotic susceptibility results. The antibiotic treatment was combined with non-steroidal anti-inflammatory drugs (diclofenac 100 mg/day or tenoxicam 20 mg/day) and prostatic massage in cases of inspissated secretion. α-Blockers were not administered, since the patients had no symptoms of prostatitis.

Epididymitis treatment was carried out with the same antibiotics, but the duration of treatment was shorter (2-4 weeks). In each patient group, the quinolone therapy was supplemented with vitamin E, since quinolones have negative effects on the semen parameters.

During treatment, the patients were followed up and they were seen again after 4 and 6 weeks. After completion of the therapy, ultrasonographic examinations and culture procedures after prostatic massage were repeated. The semen parameters were examined again 3 months following completion of the therapy.

Results

1. Clinical diagnosis

The results of clinical examinations are shown in Tables II and III and Figs. 2 and 3. Of the 1447 patients examined, 511 had infections with or without other disorders. In these 511 patients, 596 infectious abnormalities were found. In 394 of the 511 patients with infection physical and transrectal ultrasonographic examinations demonstrated typical symptoms of prostatitis/prostatovesiculitis, but there was no perianal discomfort, no aching sensation on the inner aspects of the thighs, and no suprapubic or deep perineal discomfort. Of these 394 patients, 7 had chronic epididymitis as the leading infection and prostatitis was only a connecting infection. In 387 patients, therefore, chronic prostatitis was the leading abnormality. These patients were asked to assess their symptoms as mild, moderate or severe. A similar assessment was made after treatment. In 169 patients, physical examination and scrotal ultrasonography demonstrated typical

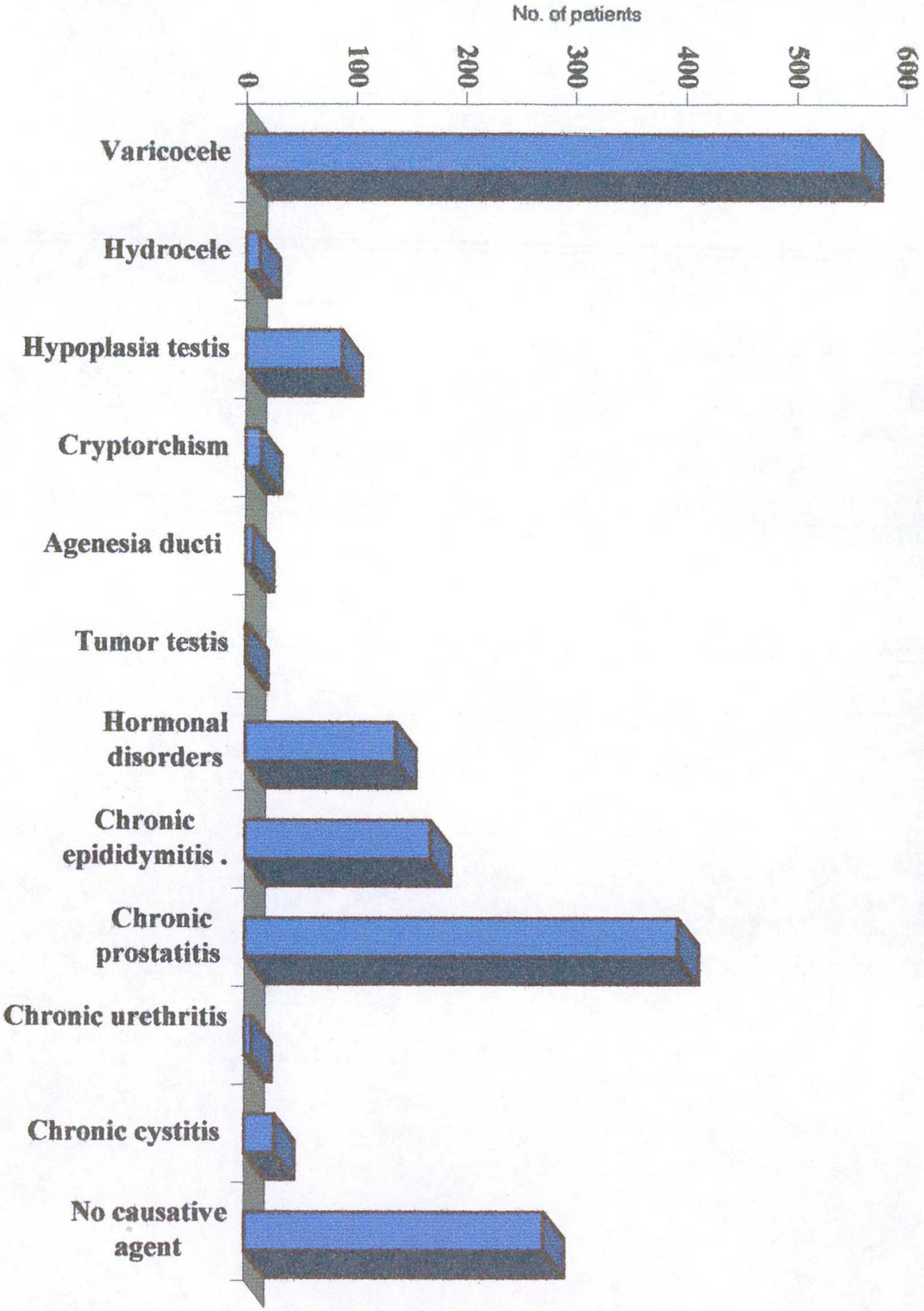


Fig. 2 Distribution of abnormalities among infertile patients (1447)

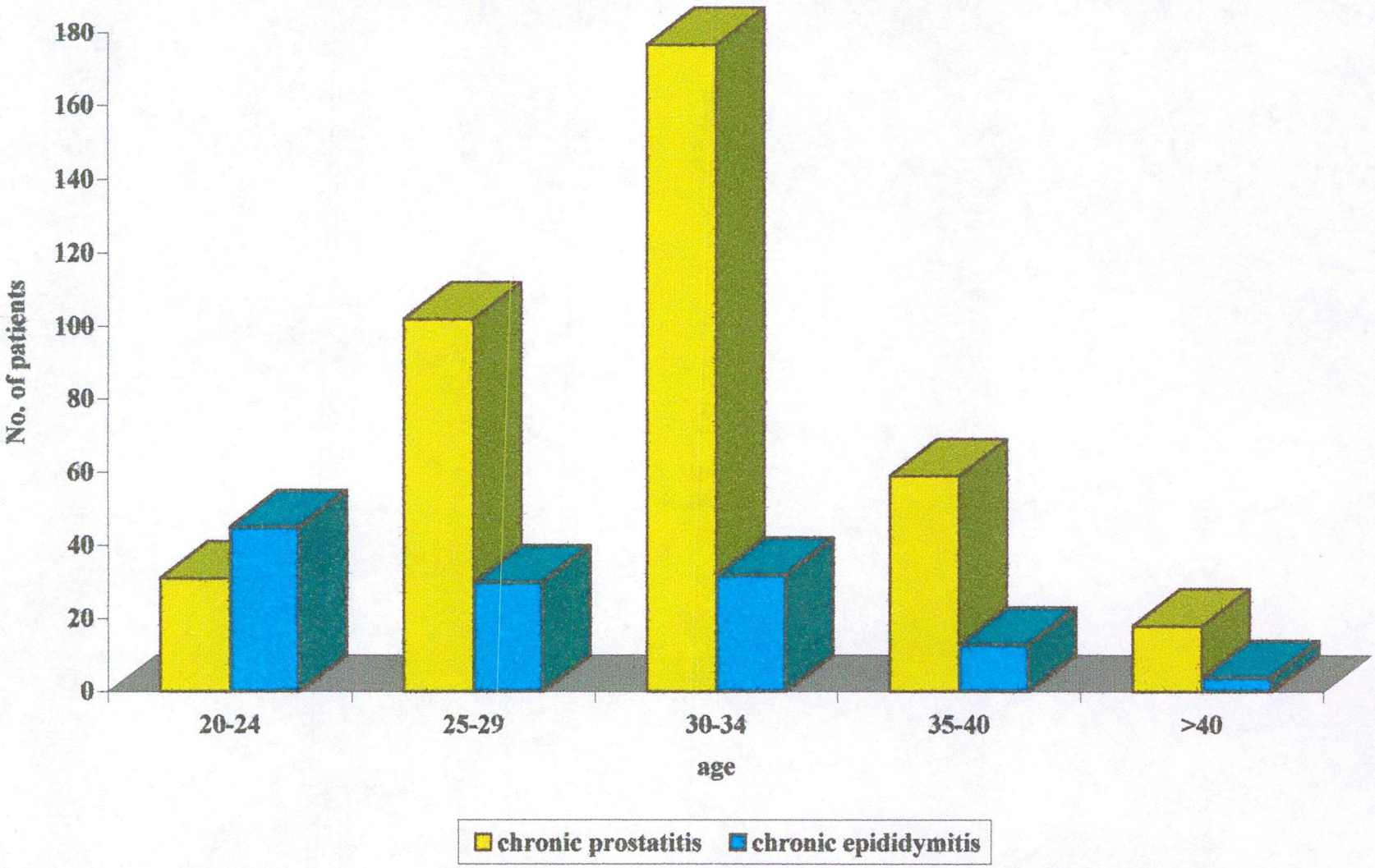
Table II. Distribution of infectious abnormalities according to age

Age	No. of patients	Disorder	Chronic prostatitis/ prostatoves.	Chronic epididymitis	Chronic urethritis	Chronic cystitis
20-24	76	98	31	61	4	2
25-29	132	153	102	49	2	0
30-34	209	221	181	33	0	7
35-40	72	99	62	22	0	15
>40	22	25	18	4	0	3
Total	511	596	394	169	6	27

Table III Distribution of patients according to leading abnormality

Age	No. of patients	No. with infection	No. with			
			prostatitis	epididymitis	urethritis	cystitis
20-24	196	76	31	45	0	0
25-29	402	132	102	30	0	0
30-34	557	209	177	32	0	0
35-40	258	72	59	13	0	0
>40	34	22	18	4	0	0
Total	1447	511	387	124	0	0

Fig. 3 Frequency of chronic prostatitis and chronic epididymitis in different age group of examined patients

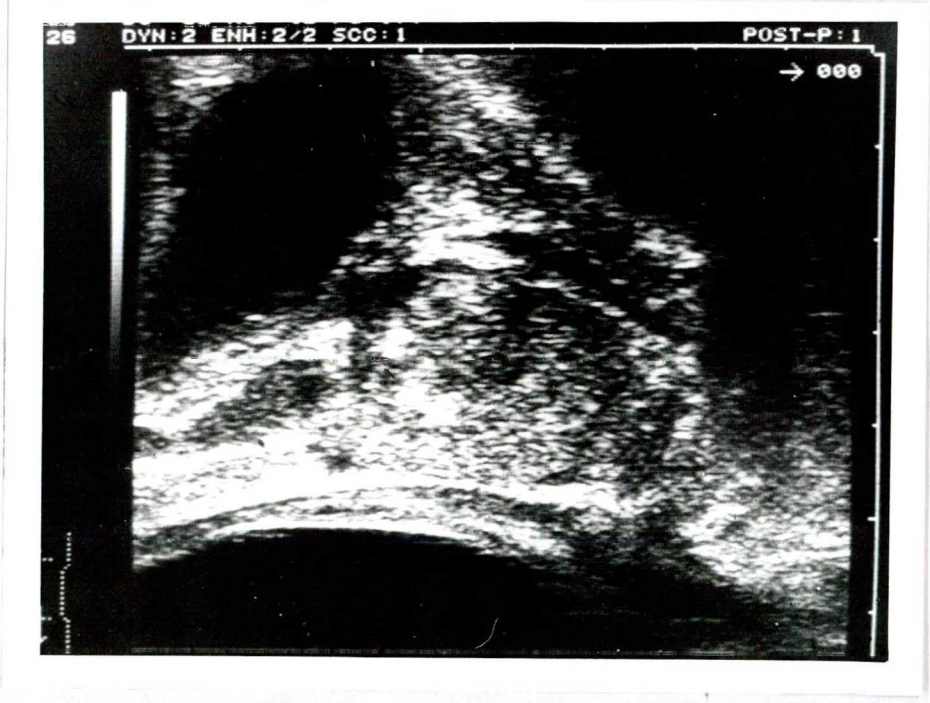


symptoms of chronic epididymitis. Of these 169 patients 45 displayed chronic prostatitis as the main infection, and they were therefore included in the prostatitis group. The remaining 124 patients were included in the epididymitis group. Neither urethritis nor cystitis was detected as leading symptom, but only as symptoms accompanying other disorders. 14 of the 511 patients had varicocele or a hormone disorder, and were therefore excluded from the study (10 from the prostatitis group and 4 from the epididymitis group). Thus 497 patients had infections without any other abnormality. 273 patients had neither infections nor other disorders, but the semen parameters were decreased. The disorders revealed by ultrasonography are shown in Figs 4 and 5. Of the 497 infectious patients included in this study, 14% had normal sperm counts, 60% were oligozoospermic, 23% had oligozoospermia maxima and 3% azoospermia (Table IV). An increased frequency of positive seminal cultures was found in patients with abnormal semen as compared with those with normal semen. The majority of patients with male infertility have oligospermia and asthenospermia, and are subfertile rather than sterile.

2. Microbiological results

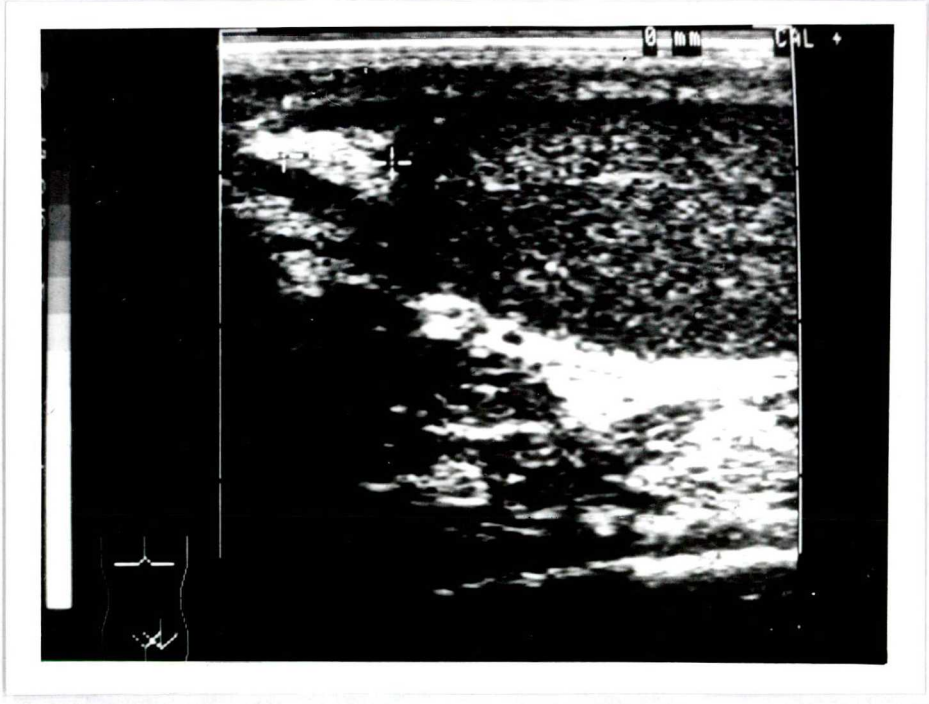
Of the 387 patients with prostatitis, 126 exhibited high colony counts of Gram-positive and Gram-negative anaerobic bacteria (10^7 CFU/ml) (Fig. 6). This mixed anaerobic flora was similar to the flora found in women with bacterial vaginosis (Fig 7). 5% were positive for both aerobic and anaerobic bacteria. The most frequently isolated anaerobic, Gram-positive bacteria were *Peptostreptococcus* spp. and *Propionibacterium* spp., and the most frequent Gram-negative bacteria were *B. ureolyticus*, *Prevotella* spp. and *Porphyromonas* spp. Altogether, 673 anaerobic bacteria were isolated from 126 patients, which means an average of 5.34 anaerobic bacteria/positive patient. *C. trachomatis* was isolated in 1%, *M. hominis* in 5%, *U. urealyticum* in 3% and *Candida* spp. in 3%. In 36% of the patients, only aerobic bacteria were cultured. The most frequently isolated aerobic bacteria were *Escherichia coli* and other *Enterobacteriacae*, *Enterococcus faecalis*, *Proteus* spp. and *Providencia* spp. The remaining 19% were completely negative for both aerobic and anaerobic bacteria. Among the epididymitis

Fig. 4 Chronic prostatitis examined by transrectal ultrasonography



The examination was carried out with Hitachi 315 equipment with a 5 mHz biplan transducer

Fig. 5 Chronic epididymitis examined by scrotal ultrasonography



The examination was carried out with Ultramark 4 Plus equipment

Table IV

Distribution of abnormal density of semen in 497 examined patients

	No. of patients with infection
Normospermia	69 (14%)
Oligozoospermia	298 (60%)
Oligozoospermia max.	115 (23%)
Azoospermia	15 (3%)

Fig. 6

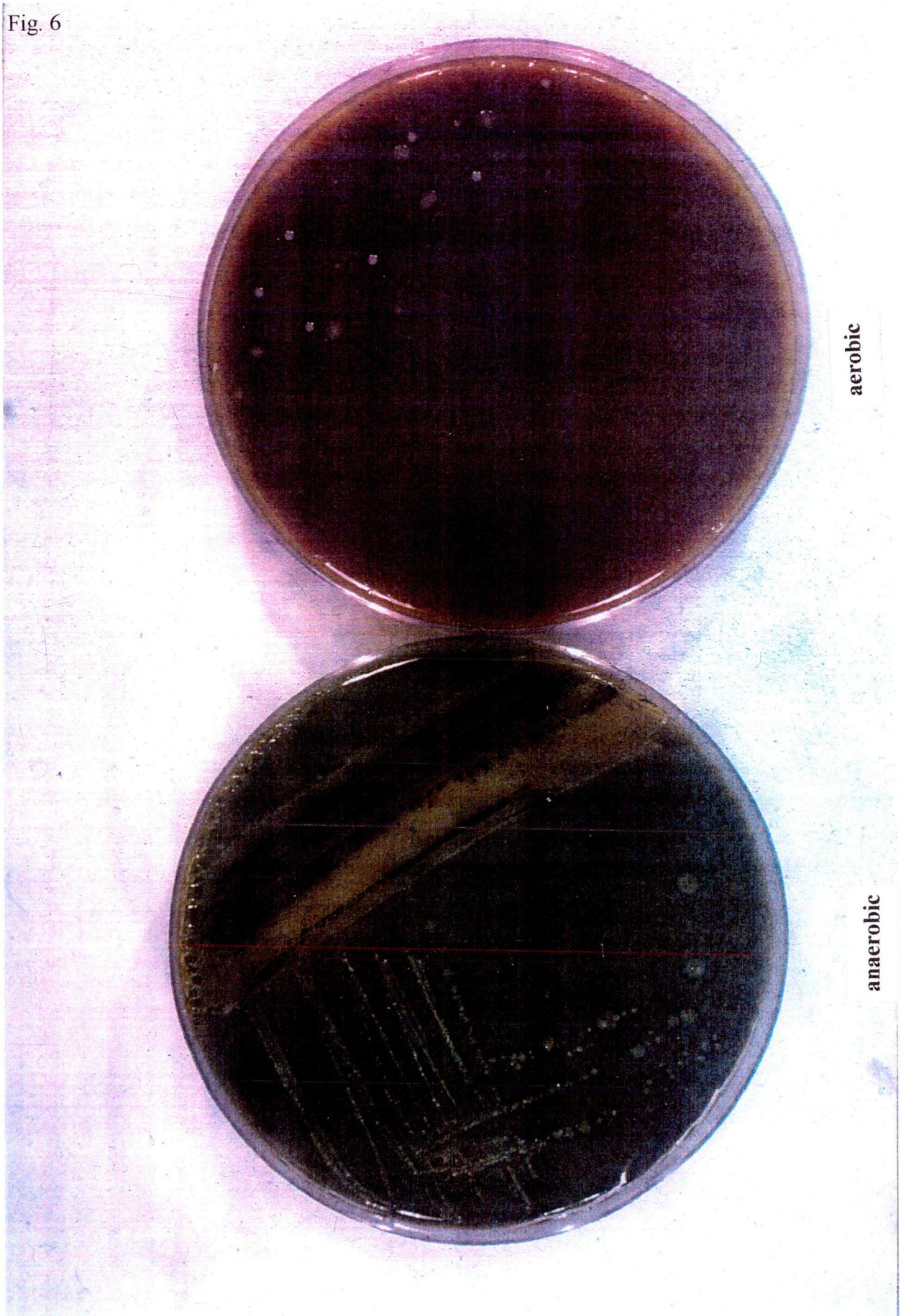
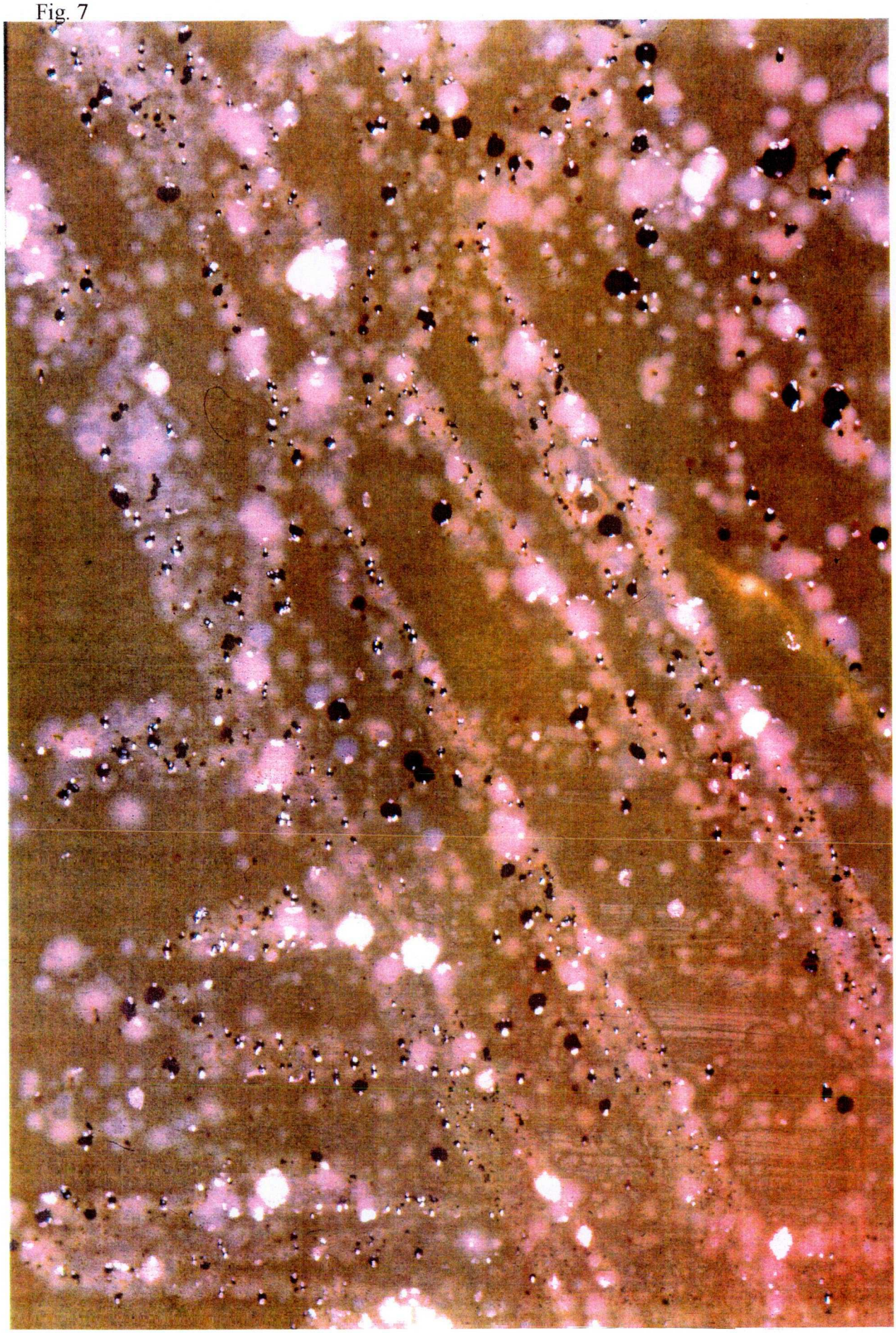


Fig. 7



patients, the anaerobic bacteria were isolated less frequently (14%), *i. e.* altogether 119 anaerobic bacteria from 48 patients, but *U. urealyticum* was observed in higher frequency (9%) (Fig 8). The average was 2.47 anaerobic bacteria/positive patient. All aerobes and anaerobes present in the semen were identified (Table V). The motility of the spermatozoa was decreased in all cases when bacteria were isolated from the semen. The number of pathological forms was extremely high (66%) in the cases of anaerobic infection (Tables VI-IX). Among the sexual partners of male patients who were positive to anaerobic bacteria, bacterial vaginosis was diagnosed in 101 cases (Fig. 9). Comparisons of the isolated anaerobes demonstrated that the same bacteria were present in the vaginal sample and the EPS and/or urethral sample (Table X). This finding suggests that anaerobic bacteria may be sexually transmitted pathogens and treatment must also be carried out on with the sexual partner(s).

3. Results of treatment

After completion of the therapy, the physical and ultrasonographic examinations, semen analyses and culture procedures were repeated. In parallel, vaginal samples from the sexual partners were cultured repeatedly. In some cases, the ultrasonographic examination revealed residual abnormalities in the prostatic glands (moderate inhomogeneous structure) during a period of weeks after the treatment. Naturally, in those cases where the initial ultrasonographic examinations did not indicate anatomical abnormalities, sonography was not repeated.

The anti-anaerobic therapy was effective, since patients previously positive for anaerobic bacteria were then negative, or only Gram-positive anaerobic bacteria of the normal flora were growing in low numbers (< 10^2 CFU/ml). After 4 - 8 weeks, the therapy applied against other causative organisms resulted in negative culture results in 297 patients (76%), while in the remaining 24% the bacteria were found in much lower CFU. In these latter cases, the administration of the antibiotics was continued for another 4 weeks. 23 patients (4.6%) gave positive culture results of 10^2 CFU/ml after treatment for 3 months, but the sexual partners had negative culture results, and their treatment was therefore terminated. They were recalled after 3 weeks in order for the

Fig. 8 Culture results on the samples of patients

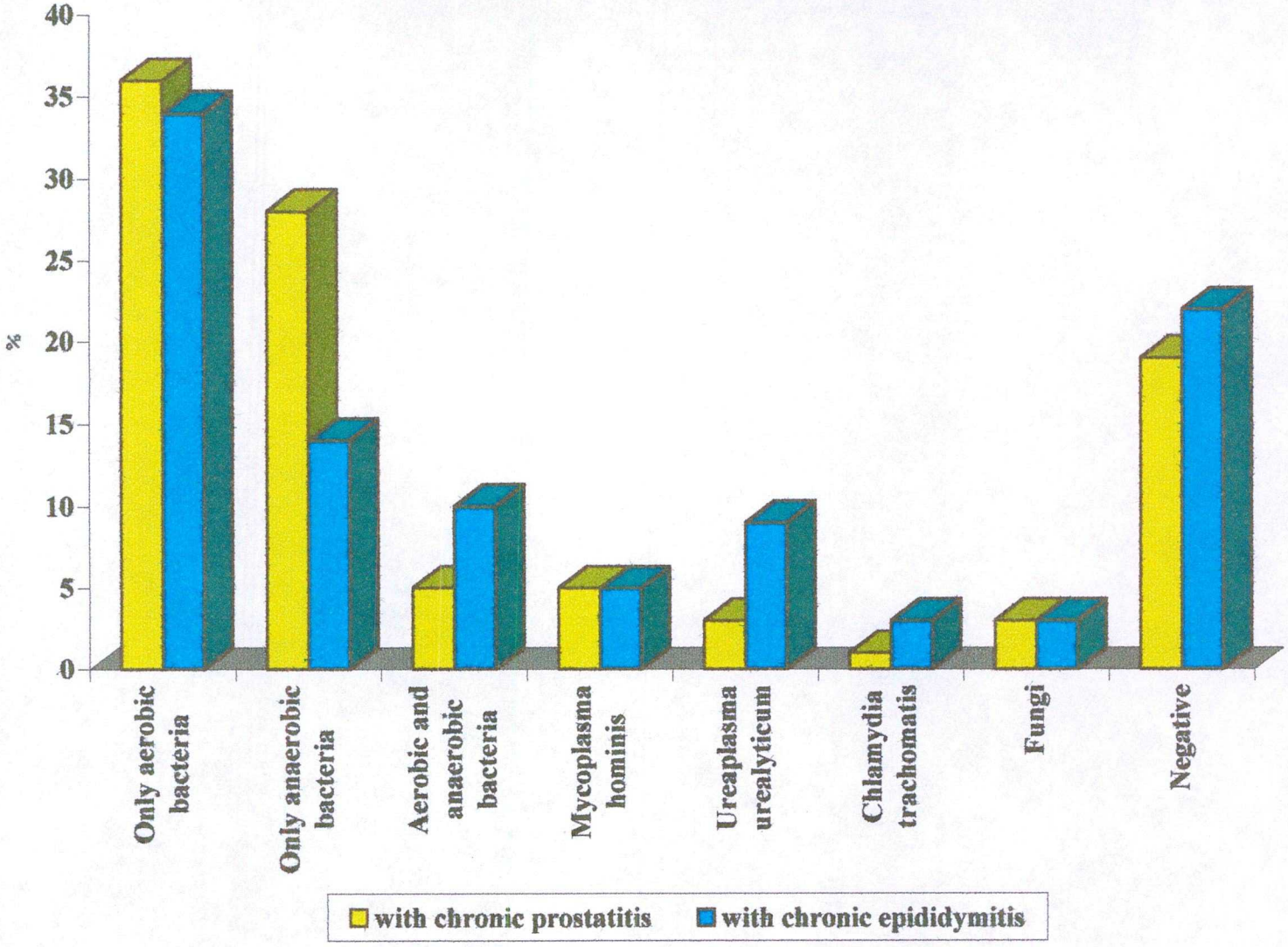


Table V. Distribution of aerobic and anaerobic species isolated from EPS/semen of chronic prostatitis and epididymitis patients

Species	No. of isolates from patients with prostatitis (n=126)	epididymitis (n=48)
Gram-positive aerobes		
Staphylococcus epidermidis	5	7
Streptococcus alfa-haemolyticus	6	2
Corynebacterium spp.	5	0
Enterococcus faecalis	9	5
Gram-negative aerobes		
Escherichia coli	18	8
Klebsiella spp.	15	3
Providencia spp.	7	1
Proteus spp.	5	1
Citrobacter freundii	1	0
Total aerobes	71	27
Gram-positive anaerobes		
Peptostreptococcus spp.	115	48
Propionibacterium spp.	71	12
Bifidobacterium spp.	24	9
Eubacterium spp.	20	11
Actinomyces spp.	19	0
Clostridium spp.	10	2
Gram-negative anaerobes		
Bacteroides ureolyticus	63	3
Prevotella spp.	84	12
Porphyromonas spp.	79	9
Veillonella spp.	51	11
Bacteroides spp.	72	2
Fusobacterium spp.	63	0
Tissierella spp.	1	0
Mobiluncus spp.	1	0
Total anaerobes	673	119

Table VI
Semen parameters in 377 patients with chronic prostatitis

	Mean value of parameters		
	Before therapy	**After therapy**	**Normal value**
Density (M/ml)	22,9	26,17	>20
Motility (%)	44,25	52,23	>60
Pathological form (%)	61	51,53	<40
Fructose level (mg/ml)	1,31	1,51	1,19-2,2

Table VII
Semen parameters in 126 patients with prostatitis caused by anaerobic infection

	Mean value of parameters		
	Before therapy	**After therapy**	**Normal value**
Density (M/ml)	21	24,6	>20
Motility (%)	44,51	53,35	>60
Pathological form (%)	66,76	53,73	<40
Fructose level (mg/ml)	1,31	1,47	1,19-2,2

Table VIII
Semen parameters in 120 patients with chronic epididymitis

	Mean value of parameters		
	Before therapy	**After therapy**	**Normal value**
Density (M/ml)	21.75	24.94	>20
Motility (%)	44.68	53,6	>60
Pathological form (%)	61.92	52,78	<40
Fructose level (mg/ml)	1.45	1,49	1,19-2,2

Table IX
Semen parameters in 48 patients with epididymitis caused by anaerobic infection

	Mean value of parameters		
	Before therapy	**After therapy**	**Normal value**
Density (M/ml)	19,89	24,2	>20
Motility (%)	40,75	51,02	>60
Pathological form (%)	66,1	54,02	<40
Fructose level (mg/ml)	1,32	1,4	1,19-2,2

Fig. 9 Frequency of bacterial vaginosis (BV) in sexual partners of examined patients with anaerobic infection

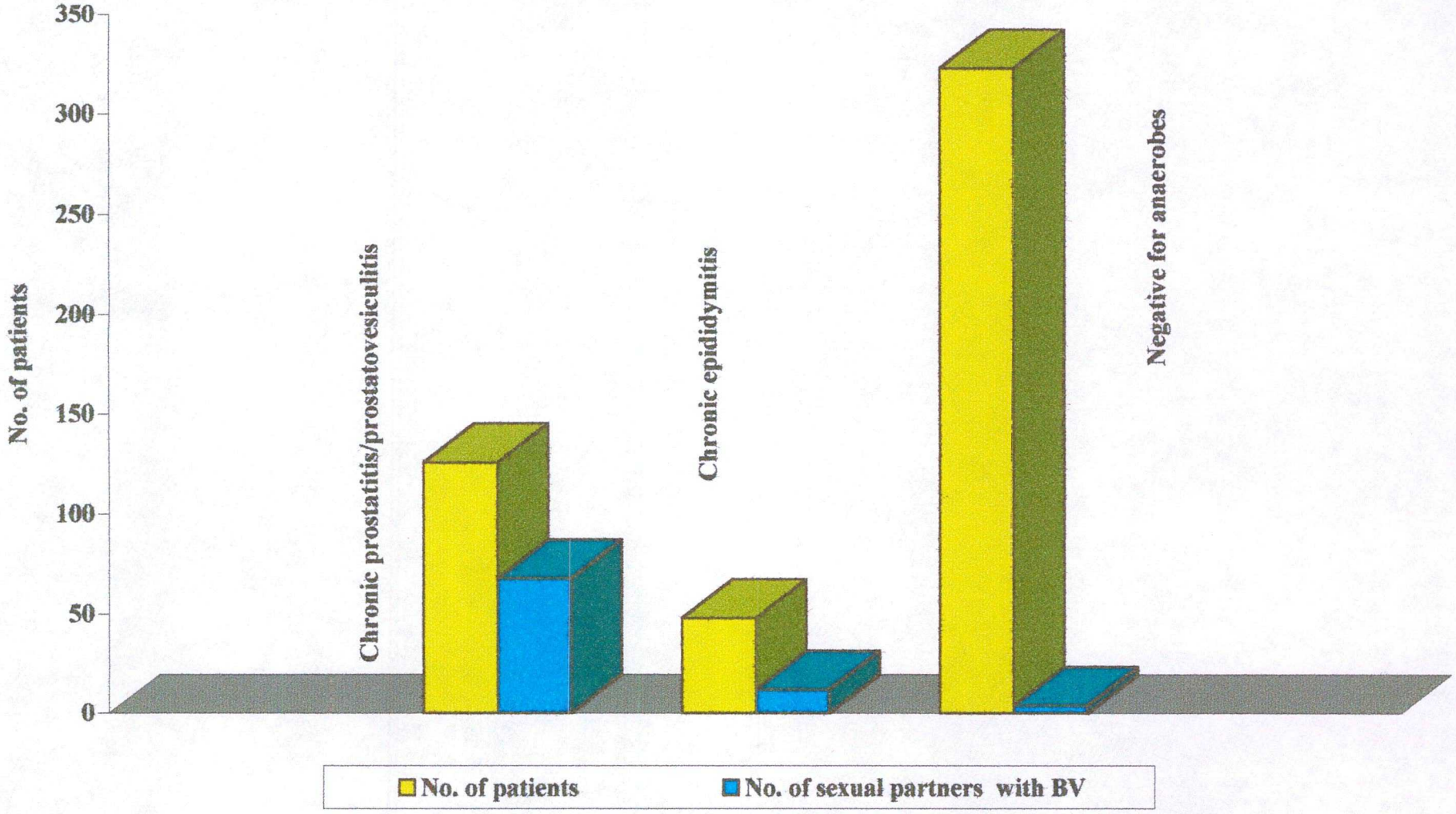


Table X.
Presence of anaerobic bacteria in samples of one chosen couple

Isolated bacteria		
Male		Female
+	*B. ureolyticus*	–
+++	*Porphyromonas* spp.	+++
+	*Bacteroides* spp.	++
+	*Mobiluncus* spp.	++
–	*Fusobacterium* spp.	+
+	*Lactobacillus* spp.	+
+++	*P. anaerobius*	+++
+	*Actinomyces* spp.	–
+	*P. acnes*	–

presence of bacteria in the semen/urethral sample to be examined. In 19 patients the microbiological results were unchanged, while in 4 cases the CFU level of the cultured bacteria was increased. In these cases, repeated antibiotic treatment was begun in accordance with the antibiotic susceptibility results. The antibiotic treatment caused a transitory deterioration in semen quality, but 2 or 3 months later the parameters had returned nearly to normal. Semen characteristics after completion of the therapy are shown in Tables VI-IX. We found an increased sperm motility and decreased pathological forms of spermatozoa. The extremely high number of pathological forms in cases of anaerobic infection (about 66%) had decreased to 53% in both groups of examined patients. The density of the semen had reached the normal range or improved. The fructose level had risen from the low value. Statistical analysis demonstrated that the differences between the data before and after treatment were significant. (Tables XI-XIV).

4. Results of *in vitro* studies

Incubation of the spermatozoa with 10^2 CFU/ml of isolated anaerobic bacteria (*Bacteroides* spp., *Prevotella* spp. or *Fusobacterium* spp.) resulted in no change in motility after 2 h, but after 18 h there were only 1% moving spermatozoa. 10^6 CFU/ml led to 50%, 20% and no spermatozoa moving after 1, 2 and 3, 4 of incubation, respectively. The effects of Gram-positive anaerobes (*Peptostreptococcus* spp. and *Propionibacterium* spp.) at 10^6 CFU/ml were slower: a decrease in motility was first observed only after 3, 4 h of incubation, and after 18 h 30% of the spermatozoa were still moving. These results suggest, that, in high concentrations, anaerobic bacteria (mainly Gram-negative anaerobes) reduce the motility of spermatozoa similarly to *E. coli*, the effect of which was examined earlier (49).

Discussion

There is disagreement regarding the influence of urogenital infections on male fertility. Different authors report different causative organisms, and the various sperm examinations do not always seem to have the necessary bacteriological precision. In

Table XI. Statistical analysis of semen parameters in 377 patients with chronic prostatitis

Test Statistics(a)

	DENSITY	MOTILITA	PATHFORM
Mann-Whitney U	60632,500	55938,500	54295,000
Wilcoxon W	138447,500	133753,500	132110,000
Z	-5,323	-6,788	-7,302
Asymp. Sig. (2-tailed)	,000	,000	,000
a Grouping Variable: CSP			

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	FRUKTOZB - FRUKTOZA	-,2014	,1936	9,752E-03	-,2205	-,1822	-20,650	3	,000

Table XII. Statistical analysis of semen parameters in 120 patients with chronic prostatitis caused by anaerobic infection

Test Statistics(a)

	DENSITY	MOTILITA	PATHFORM
Mann-Whitney U	5827,500	5571,000	4558,000
Wilcoxon W	13828,500	13572,000	12559,000
Z	-3,652	-4,093	-5,844
Asymp. Sig. (2-tailed)	,000	,000	,000
a Grouping Variable: CSP			

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	FRUKTOZB - FRUKTOZA	-,1599	,1708	1,521E-02	-,1900	-,1298	-10,511	1	,000

Table XIII. Statistical analysis of semen parameters in 126 patients with chronic epididymitis

Test Statistics(a)

	DENSITY	MOTILITA (٢)	PATHFORM
Mann-Whitney U	10986,000	9854,500	10246,000
Wilcoxon W	25351,000	24219,500	24611,000
Z	-3,672	-4,930	-4,493
Asymp. Sig. (2-tailed)	,000	,000	,000
a Grouping Variable: CSP			

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	FRUKTOZB - FRUKTOZA	-3,7278E-02	,1361	1,047E-02	-5,7946E-02	-1,6610E-02	-3,561	1	,000

Table XIV. Statistical analysis of semen parameters in 47 patients with chronic epididymitis caused by anaerobic infection

Test Statistics(a)

	DENSITY	MOTILITA	PATHFORM
Mann-Whitney U	719,500	752,500	719,000
Wilcoxon W	1895,500	1928,500	1895,000
Z	-3,175	-2,930	-3,174
Asymp. Sig. (2-tailed)	,001	,003	,002
a Grouping Variable: CSP			

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	FRUKTOZB - FRUKTOZA	-8,8542E-02	,1018	1,469E-02	-,1181	-5,8984E-02	-6,026	47	,000

some cases of prostatitis, considered to be non-bacterial because of the negative culture results, it is difficult to know whether only suboptimal laboratory methods are responsible for the diagnostic failures. As concerns the detection in chronic prostatitis of bacteria that are difficult to culture or non-culturable, not only the morphologic and metabolic diversity of the organisms must be taken into account, but also the consequences of their interactions with other organisms and their host. The interactions of these organisms within the host can lead to either enhancement or depression of their individual properties (production of extracellular slime or biofilms). Bacteriological advances, which include the use of specialized culture media and stains, electron microscopy, and PCR for amplifying microbial sequences in tissues and body fluids, have revealed an increasing number of previously unidentifiable organisms in a variety of pathologic conditions (50, 51).

In the present study, a careful culturing technique was used to isolate aerobic, facultative anaerobic and strict anaerobic bacteria. It revealed that anaerobic and aerobic bacteria appear to be the microorganisms that are most frequently encountered in cultures from the semen of infertile men. The changes caused by these bacteria cause may be direct effects, the numbers of spermatozoa themselves being diminished besides their motility, morphology and ability to fertilize from this aspect, the connection between the concentration of bacteria and the concentration of gametes could be important, and they may be indirect effects, the infection altering the constituent qualities of the seminal fluid, and thereby exerting a secondary effect on the spermatozoa.

Therapeutic trials, which might have helped solve the problem, do not yet seem to have been decisive. The great variety in the clinical and bacteriological aspects, and the particular biological features of the organs involved, such as the prostate, make treatment a difficult problem to solve, with particular regard to the choice of an effective antibiotic whose pharmacokinetics must be suitable both for the microorganisms and for the tissue of the infection site.

This study has concentrated on asymptomatic infection and its relation to infertility. The diagnosis of the imflammation of male accesory genital organs is difficult and the

influence on fertility is debatable. There is a great variability in the different studies as concerns the incidence of bacterial isolation in the semen of fertile and infertile men (52, 53). We attempted to delineate the effects of bacterial infection on the basis of the seminal characteristics. Anaerobic bacteria were recovered from 28% of the patients, as compared with 16-19% in other studies (54, 55). Our results suggest that anaerobic culturing methods involving prolonged incubation may be required for the laboratory diagnosis. The results of the present study indicate that bacterial infection causes alteration in seminal characteristics such as volume, motility and viability. The decrease in sperm motility may be due to the immobilization or death of the spermatozoa. In some patients (19%), we could find no infectious agent or anatomical abnormality, but the semen parameters were pathological. These may involve chronic idiopathic prostatitis or prostatitis caused by fastidious organisms. Both molecular and specialized culture findings designed to detect fastidious bacteria that are difficult to culture in prostatic tissue and fluids point to a possible aetiologic role for these microorganisms (56, 57). *C. trachomatis* is suspected of being responsible for chronic non-bacterial inflammation of the prostate, besides *U. urealyticum* and *Mycoplasmae.* The undiagnosed and untreated diseases may lead to infertility (58). Our earlier examinations on *C. trachomatis* (59, 60) demonstrated a high frequency of this organism in patients with urethritis and symptomatic chronic prostatitis. In the group of patients with asymptomatic chronic prostatitis, we found *C. trachomatis* in only 1%, in contrast with a study in Japan, which suggested that *C. trachomatis* is a frequent causative organism in chronic idiopathic prostatitis (61). The uncertain role of this microorganism in chronic prostatitis warrants further research. The reason for the difference in prevalence of *C. trachomatis* in the examined patient groups may be the difference in diagnostic methods, or the upper respiratory infection or other infection of the patients may have been treated with antibiotics effective against *Chlamydia* (e. g. azithromycin) before the patients attended the Andrologic department. Future studies should be directed toward more nucleic acid-based experimentation in order to define the microbiology of the prostate gland and to determine the relationship of these bacteria to chronic idiopathic prostatitis. The identification of such factors would be

helpful in devising effective treatment strategies. Our plan for the future is to use a PCR method for the direct detection of the infectious agents in the semen. The treatment of different infections in the genitourinal tract can result in improvements in the semen parameters, and spontaneous fertility may return or assisted reproduction procedures may become much safer.

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Acknowledgements

I wish to express my special thanks to all those without whom this task would have never been fulfilled

My thanks are due to,

Professor László Kovács, Head of the Department of Gynecology, and **Dr János Szöllősi** reader of the Department of Gynecology, for their untiring efforts and constant enthusiasm and professionalism in the supervision on this study.

I am very grateful to **Professor László Pajor**, Head of the Department of Urology, for his help, understanding, and encouragement.

Special thanks to **Professor Sándor Scultéty** his help throughout my professional life.

I wish to thank to **members of the staff at the Department of Urology** for their considerable cooperation and creatinga peaceful working atmosphere.

I wish to express my appreciation to **Professor Elizabeth Nagy, Dr Judith Deák, Dr Ildikó Szőke** and other members of the staff of the Department of Clinical Microbiology, for their knowledge, advice and handling the laboratory part of this study.

I am very grateful to **Dr Tibor Nyári** for his help in statistical analysis of my results.

My thanks goes to members of **the staff of Andrological Department** of Gynecology of Albert Szent-Györgyi Medical University, and **Kaáli Institute**.