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(Article begins on next page)

Infectious burden and semen parameters

^aE. Moretti, ^bN. Figura, ^bMS Campagna, ^cF. Iacoponi, ^bS. Gonnelli, ^aG. Collodel

^aDept. of Molecular and Developmental Medicine, ^bDept. of Medical and Surgical Sciences and Neurosciences, University of Siena; ^cIstituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy

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Corresponding author: Elena Moretti, Department of Molecular and Developmental Medicine, University of Siena, Policlinico le Scotte, V.le Bracci, 53100 Siena, Italy. Phone +39 0577 233511; fax: +39 0577 233527; e-mail elena.moretti@unisi.it

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Elena Moretti: study design, semen analysis, manuscript drafting, critical revision

Giulia Collodel: study design, semen analysis, critical revision

MariaStella Campagna: enzyme-linked immunosorbent analysis

Francesca Iaconi: statistical analysis, critical revision

Stefano Gonnelli: study design, critical revision

Natale Figura: study design, manuscript drafting, critical revision

Abstract

Objectives. To investigate the relationship between chronic infections detected in serum and semen quality. The pathogen burden is a concept consisting in the observation that, in patients with heart disease, damaging effects of the coronary arteries increase concomitantly with the number of agents responsible of chronic infections to which patients mounted a serological response. Previous observations that *H. pylori* infection may reduce the semen quality prompted us to perform the present study.

Methods. Blood and semen samples were collected from 73 selected men, enrolled during January 2014 - January 2015. Semen characteristics were evaluated by light and transmission electron microscopy (TEM). TEM data were quantified with a mathematical formula providing numerical

scores, such as fertility index (FI, number of sperm free from ultrastructural defects) and the percentages of sperm apoptosis, immaturity and necrosis. Serum samples were examined by ELISA for the presence of IgG to the most common agents of chronic infections such as *H. pylori* (HP), *Mycoplasma pneumoniae* (MP) and *Chlamydomphila pneumoniae* (CP), Epstein-Barr virus (EBV), *herpes simplex virus 1* (HSV-1), cytomegalovirus (CMV).

Results. The prevalence of infections was as follows: HP 43.8%, CP 46.6%, MP 72.6%, EBV 95.9%, HSV-1 74.0% and CMV 46.6%. Concomitantly with the increased number of pathogens against which the patients mounted a significant antibody response, sperm concentration ($P<0.05$), sperm motility ($P<0.001$) and FI ($P<0.001$) were significantly reduced and the percentage of necrotic sperm was increased ($P<0.01$).

Conclusions. The higher the number of pathogens stimulating an IgG systemic response, the lower was the semen quality.

Introduction

Infertility affects approximately 15% of couples in reproductive age and in 40% *ca.* of cases the responsibility can be attributed to the male partner. The most common causes of male infertility include varicocele, endocrine diseases, cryptorchidism and genetic disorders¹. In many cases, however, the etiology of male infertility remains unknown. An important role in the development of male reproductive disorders is played by chronic infectious and inflammatory diseases in different compartments of the male genital tract, such as urethritis, prostatitis, epididymitis, which can potentially exert a negative influence on sperm function.²

Infectious agents have been implicated in the etiology of a number of chronic conditions.³ Recently, Liu et al.⁴ reported that the prevalence of *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Chlamydia trachomatis*, detected in semen, was similar in large groups of both infertile and fertile men. The authors found, however, that the presence of *U. urealyticum* in infertile men was related to a lower sperm number and vitality than in fertile men.

On the other hand, it is not known yet whether chronic infections, affecting organs that are different from the genitourinary system, could play a role in reducing the semen quality.

In previous surveys, we demonstrated that *Helicobacter pylori* chronic infection is more prevalent in infertile population⁵ and plays a negative influence on sperm motility, viability and morphology^{6 and 7} either by increasing both the systemic and the semen levels of inflammatory cytokines or by promoting autoimmunity.

In particular, these alterations were detected in the presence of strains harbouring the gene *cagA*, which encodes an immunodominant determinant protein named CagA.

The *cagA* positive strains are endowed with an enhanced inflammatory and carcinogenetic potential.⁸ The association between *H. pylori* infection and decreased sperm quality has also been

supported by a recent research⁹, demonstrating that *H. pylori* antibiotic treatment significantly improved sperm motility in infertile men with asthenozoospermia and elevated anti-*H. pylori* seminal IgA.

The idea that many infectious agents, rather than any single pathogen, may be involved in the development of several pathologies introduced the concept of “infectious burden”. For example, it is known that infections by *Chlamydomphila pneumoniae*, human cytomegalovirus, *H. pylori*, influenza virus, *etc.* may contribute to the development of atherosclerosis.¹⁰

These general considerations and the possible relationship between *H. pylori* chronic infection and semen quality prompted us to investigate whether the concept of “infectious burden” could also be applicable to the field of male fertility. For this purpose, we determined the IgG seropositivity for some of the most frequent viral and bacterial agents of chronic infections, such as *H. pylori* (HP), Epstein-Barr virus (EBV), *herpes simplex virus 1* (HSV-1), cytomegalovirus (CMV), *Mycoplasma pneumoniae* (MP) and *Chlamydomphila pneumoniae* (CP) in order to explore whether the seropositivity for one or more pathogens might be associated with alterations of semen parameters. We selected these agents of chronic infections because they are obligated intracellular pathogens (EBV, HSV-1, CMV and CP) and they establish lifelong latent or persistent infections, or because they stimulate specific antibody responses that last for the entire patient’s life (HP). MP is an extracellular pathogen that may often cause repeated reinfection episodes with antibody boosts. Semen parameters were evaluated by light microscopy and sperm morphology was examined by transmission electron microscopy (TEM) analysis.

MATERIALS AND METHODS

Patients

From January 2014 through January 2015, we selected 73 male Italian subjects (aged 23-47 years) attending the Department of Molecular and Developmental Medicine, University of Siena, for semen analysis. The majority of these men only wished to control their semen, their fertility status was unknown.

The inclusion/exclusion criteria consisted in non azoospermic men with a normal 46, XY karyotype evaluated by conventional cytogenetic analysis. All subjects were negative to standard semen bacteriological analysis and showed normal serum hormonal profile: follicle stimulating hormone (FSH: 0.7-11.00 mU/ml), luteinizing hormone (LH: 0.8-8.0 mU/L) and testosterone (T: 2.7-10.9 mg/ml) evaluated by chemiluminescence using commercial kit Beckman Coulter Access for FSH, LH and T (Beckman Coulter S.p.A., Milano, Italy). They were negative also for varicocele after physical examination and scrotal Eco-color Doppler.

The patients showed BMI<25, no history of radiotherapy, chemotherapy, diabetes, medication nor autoimmune disorders. Systematic sperm defects and cryptorchidism were not observed. The evaluation of the patients included information about their medical history and lifestyle factors, such as cigarette smoking, drugs and alcohol abuse. Patients had taken neither antibiotics potentially active against considered bacteria nor proton pump inhibitors in the past three months. Their infectious status was previously unknown.

Detection of IgG to the investigated pathogens

Blood samples were collected after 12 hours of fasting between 9:00 and 10:30 AM from a cubital vein and were drawn in colour-coded Vacutainer® tubes. Blood was allowed to clot for 60 min *ca.*, separated by centrifugation at 4 °C and stored at -80 °C.

Titers of IgG against the considered pathogens were determined by commercially available enzyme-linked immunosorbent assays (ELISA):

Helicobacter pylori IgG, HpG screen ELISA kit (sensitivity=100%, specificity=91%; inter-assay coefficient of variation <10.5%) and CagA IgG ELISA Kit (sensitivity=96%, specificity=97%, inter-assay coefficient of variation <12%) provided by Genesis Diagnostics Ltd, Littleport, UK; BEIA “EBV EBNA-1 IgG Quant” and BEIA “EBV VCA IgG Quant” (respectively: sensitivity=99.5%, specificity=100%, inter-assay coefficient of variation <10% and sensitivity=98.6%, specificity=100%, inter-assay coefficient of variation <10%), BEIA HSV 1 IgG (sensitivity=98.7%, specificity=100%, inter-assay coefficient of variation <10%), BEIA CMV IgG Quant (sensitivity=97.5%, specificity=100%, inter-assay coefficient of variation <7%) provided by Technogenetics s.r.l., Sesto San Giovanni, Milan, Italy; *Mycoplasma pneumoniae* ELISA IgG (sensitivity=98%, specificity=97%, inter-assay coefficient of variation <7%) and *Chlamydomphila pneumoniae* ELISA IgG (sensitivity=100%, specificity=83%, inter-assay coefficient of variation <7%), provided by Vircell, S. L., Santa Fe, Granada, Spain.

Tests were carried out in microplates and optical densities were assessed by the microplate reader DV990 BV 4/6 (SARIN, Florence, Italy).

Semen analysis

Semen samples were collected by masturbation after 3-4 days of sexual abstinence and evaluated according to World Health Organization guidelines.¹¹

In order to perform the electron microscopy, sperm samples were processed as reported elsewhere.⁶ Specimens were observed and photographed with a Philips CM12 transmission electron microscope (TEM; Philips Scientifics, Eindhoven, The Netherlands).

For each sample, 300 ultra-thin sperm sections *ca.* were analyzed. Major submicroscopic characteristics were recorded by trained examiners who were blind to the experiment, applying the same evaluation criteria. TEM data were elaborated using the mathematical formula based on the Bayesian technique. This formula provides the number of spermatozoa free of structural defects (fertility index, FI) and the percentages of the three main sperm pathologies: immaturity, necrosis and apoptosis.¹²

Statistical analysis

Continuous and categorical variables were expressed as median and interquartile range (IQR: 75th-25th centile) and as absolute frequency and percentage. The correlations were evaluated by Spearman's *rho* coefficient and the comparisons between positive and negative groups for each considered pathogen were calculated by Mann-Whitney U test. The prevalence of seropositivity to the pathogens in patients divided into two groups (<4 pathogens and ≥ 4 pathogens), was evaluated by two-sample test for proportions. A P value < 0.05 was considered statistically significant; the analyses were performed by SPSS v. 20 (SPSS Inc., Chicago, USA).

RESULTS

The baseline characteristics of the 73 individuals were reported in Table 1.

Median of sperm concentration resulted between the 25th and 50th centile¹¹, whereas the median of progressive motility was lower than 2.5th centile. Sperm morphology was evaluated by TEM analysis mathematically elaborated. The median of FI was reduced and the median percentages of sperm pathologies were increased in comparison with those of men considered fertile according to mathematical method¹² (Table 1). Out of the 73 analyzed cases, 32 men (43.8%) were seropositive for HP infection, 12 of whom (37.5%) were infected by HP strains expressing CagA. Seventy individuals (95.9%) were seropositive for EBV, 54 patients (74.0%) for HSV-1 and 34 men (46.6%) for CMV. Finally, 53 patients (72.6%) were seropositive for MP and 34 men (46.6%) for CP (Table 1).

Our main interest was to understand whether pathogen burden could be associated with altered semen parameters. For this reason, we grouped the patients according to the number of pathogens that stimulated a significant immune response (Table 2). In this case we did not consider CagA since it is not expressed by all HP strains.

Concomitantly with the increased number of pathogens stimulating an antibody response (Table 2), sperm concentration ($P<0.05$), sperm motility ($P<0.001$) and FI ($P<0.001$) were significantly reduced, while the percentage of sperm necrosis was increased ($P<0.01$). In the group of patients infected by 3 pathogens (19 patients, 27.4%), we observed an evident decrease in sperm concentration, motility and FI.

For this reason, we hypothesized that specific pathogens could concur to determine a decrease in sperm quality. We divided the patients into two groups: those who showed antibodies for <4 pathogens and those who were seropositive for ≥ 4 pathogens (Table 3).

HP and MP were more prevalent (respectively $P=0.034$ and $P=0.002$) in the group of patients seropositive for ≥ 4 pathogens; the prevalence of seropositivity for HP CagA positive strains, EBV and HSV-1, was similar in the two groups and differences did not reach statistical significance.

It is noteworthy that the seropositivity for CMV and CP appeared only in the group of ≥ 4 pathogens.

To explore the impact of each pathogen upon the different semen characteristics, we ranked our patients according to the presence *versus* the absence of IgG to the single pathogen considered (Table 4). In this case, the variables of patients infected by HP strains expressing CagA have been shown in the table and considered in the statistical calculation. EBV and HSV-1 infections did not influence the semen quality. HP positive patients showed reduced sperm concentration ($P<0.05$), motility ($P<0.001$) and FI ($P<0.001$) in comparison with HP negative patients. In the presence of HP strains expressing CagA, the percentage of sperm motility was reduced ($P<0.001$) and the percentage of necrosis was increased ($P<0.05$, Table 4).

Patients infected by CMV showed reduced sperm motility ($P<0.001$) and FI ($P<0.05$), as well as increased necrosis ($P<0.05$) compared to individuals negative for CMV. Decreased sperm motility ($P<0.01$), FI ($P<0.05$) and increased sperm necrosis ($P<0.01$) were observed also in MP infected patients in comparison with those negative for MP. Sperm motility ($P<0.01$) and FI ($P<0.05$) were reduced and the sperm necrosis was increased ($P<0.05$) in patients infected by CP compared to those who were uninfected.

COMMENTS

In the present study, we have reported the first observation that pathogen burden affects semen characteristics. Two main considerations prompted us to perform the present study:

1- the demonstrated relationship between infectious agents and chronic conditions³. The pathogen burden has been linked to the progression of coronary artery disease (CAD)¹³, the risk of myocardial infarction or death among CAD patients¹⁴ and atherosclerosis lesions.¹⁰ In children, the pathogen burden was associated to a decrease in height-for-age and an increase in the likelihood of asthma.¹⁵

2- HP infection is putatively associated with many extra-digestive disorders.¹⁶ We demonstrated that HP infection, especially if caused by strains expressing CagA, may negatively influence the sperm quality.^{6 and 7}

In this study, we investigated whether the infectious burden might exert a deleterious effect on semen quality. In addition to HP, we determined the immune response against EBV, HSV-1, CMV, MP and CP, which are common agents of chronic infections in humans.

In the selected group of patients, the prevalence of HP infection was 43.84%; in a previous study we found that the HP prevalence in a group of males in reproductive age was 25.1% and 42.8% of infected men were seropositive for CagA.¹⁷

EBV is one of the most successful viruses identified, resulting in lifelong infection in >95% of the Earth's adult population.¹⁸ Smith and Robinson¹⁹ reported an HSV-1 prevalence of 85% in a sample of Italian male military draftees and Suligoj et al.²⁰ found that 61.4% of Italian adolescents had antibodies against HSV-1. CMV is a very common human herpesvirus with a worldwide seroprevalence ranging from 45% to 100%.²¹ Our results were partly in agreement with those reported above. MP and CP cause respiratory tract infections that are common throughout the

world. In the male population of deployed U. S. service members, the seroprevalence of CP and MP was respectively 65.1% and 21.9%.²² Choroszy-Krol et al.²³ reported that approximately 40%-70% of population showed specific antibodies against CP. In our study, the prevalence of CP chronic infection was similar to that one reported in the literature, whereas the prevalence of MP chronic infection was increased.

As regards the considered viruses, it is known that DNA from EBV, HSV-1 and CMV was directly detected in semen samples and its presence in semen was associated with infertility²⁴⁻²⁹. On the contrary, to the best of our knowledge, HP, MP and CP were never found in human semen.

The present study had another perspective: we were interested in determining whether the burden of common chronic infections serologically detected might have an effect upon the semen quality.

Semen samples were analyzed following WHO¹¹ guidelines and the sperm morphology was explored by TEM. In clinical practice, the light microscopic method plays a central role in the assessment of semen quality. TEM examination, however, enables a more detailed evaluation of sperm alterations than that provided by light microscope. In addition, TEM results can be quantified applying the mathematical formula based on probability calculation able to provide FI and the percentages of sperm apoptosis, immaturity and necrosis.¹² By means of this method, we observed that infectious burden negatively influences sperm parameters. We detected a consistent decrease in sperm concentration, motility and FI in patients with antibodies against at least three pathogens. We also observed a significant increased prevalence in HP and MP infections in patients seropositive for ≥ 4 pathogens. In addition, seropositivity for CMV and CP was detected only in the group with antibodies for ≥ 4 pathogens. EBV and HSV cause widespread infections and are likely to be more common than either HP, MP and CP. Moreover, viral infections may predispose to other bacterial infections particularly in the lungs, since the increased T cell response and subsequent cytokine production related to inflammation can lead to the increased adhesion and penetration of bacteria.

We were also interested in exploring the impact of each pathogen on the semen characteristics and we ranked patients according to the presence *versus* the absence of a single pathogen. The presence of an antibody response to EBV and HSV-1 did not influence the considered sperm characteristics, although we did not investigate the occurrence of the viral DNA in semen samples. Kapranos et al.²⁴ found that HSV-1 played a significant role in male infertility; this discordance might be due to the different methods applied to detect the virus, *i.e.* viral DNA in the semen *versus* serological method used in this study. Another research²⁶ reported the lack of association between the presence in the semen of HSV-1 and EBV DNA and the alteration of semen parameters.

In this study, we observed that HP infection influenced sperm concentration, FI and motility, whereas a positive CagA status was associated with decreased sperm motility and increased sperm necrosis, as previously demonstrated by our group.^{6,7 and 17} Patients with anti-CMV IgG at significant titer showed decreased sperm motility and FI, as well as increased necrosis. Naumenko et al.²⁹ reported that the presence of CMV DNA was increased in a group of infertile patients with chronic inflammatory disease of the genitourinary tract, but also in this case the diagnosis of infection was performed by the detection of the viral DNA in the semen. In the present study, both MP and CP chronic infections negatively influenced sperm motility and FI, as well as causing an increase of sperm necrosis.

It is worthwhile to speculate the possible mechanisms underlying the effect of pathogen burden upon the semen parameters. The first hypothesis involves the inflammatory response to the infection. For examples, it is known that HP strains expressing CagA stimulate a local and systemic overproduction of IL-8, IL-1 β , IL-6 and TNF- α .^{6 and 30} We recently detected increased levels of some of these inflammatory cytokines directly in the semen of HP infected individuals.¹⁷ We then supposed that high concentrations of potentially harmful proinflammatory cytokines can be

responsible for sperm alterations, as we observed a positive correlation between IL-6 and TNF- α concentrations and sperm necrosis.¹⁷

Another mechanism through which infectious agents may play a role in altering semen parameters could consist in phenomena of molecular mimicry: the infection may induce an immune response against pathogens that may cross react with the host's tissues. In the past, we investigated the existence of structural homology between HP epitopes and human sperm. We observed that certain HP antigens shared partial homology with human beta-tubulin⁵ and several enzymes involved in glycolysis and oxidative metabolism pathways. The existence of such homology-based structures may help explain reduced sperm motility observed in the individuals infected by HP strains expressing CagA.⁷

These mechanisms involving cytokine production and antigenic mimicry were also considered to explain the link between pathogen burden and atherosclerosis, as well as premature coronary heart disease.^{10 and 13}

In addition to the described mechanisms, a direct negative influence of different virus on sperm could be hypothesized in our cases too, since viral DNA was found in other studies in the semen samples of infected people.²⁴⁻²⁹

CONCLUSIONS

We reported the first observation that pathogen burden may affect sperm quality. Further studies, enrolling a larger number of patients, are necessary to confirm these preliminary data. Investigations on possible mechanisms of sperm damage are also mandatory.

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Table 1

Descriptive analysis of considered variables. Age, volume, sperm concentration, percentage of motility, fertility index, percentages of sperm immaturity, apoptosis and necrosis are reported as median and interquartile range in brackets. The percentages of individuals seropositive for the selected pathogens are also reported.

	Synthesis index
Age	33 (6)
Volume (ml)	3 (2)
Concentration (Sp/mlx10 ⁶)	44 (80.75)
% Motility (a+b)	25 (21)
Fertility index (FI)	247838 (1065053)
% Sperm immaturity	71.0 (14.7)
% Sperm apoptosis	7.5 (7.01)
% Sperm necrosis	35.4 (19.0)
HP	43.8%
CagA+ status	37.5%
EBV VCA/ EBNA-1	95.9%
HSV 1	74.0%
CMV	46.6%
MP	72.6%
CP	46.6%

*Referring values for TEM analysis¹²:

- lowest FI found in fertile individuals was 1,603,886 of sperm free of ultrastructural defects;
- sperm immaturity: median 47.3, 25th centile 38.6, 75th centile 58.2;
- sperm apoptosis: median 4.1, 25th centile 3.6, 75th centile 4.7;
- sperm necrosis: median 34.6, 25th centile 24.7, 75th centile 40.1.

Table 2

Median and interquartile range (in brackets) of the considered variables from patients grouped according to the number of pathogens against which they had produced serum IgG at significant titers. Correlation coefficients and P values are also reported.

Seropositivity	Patients	Age	Volume (ml)	Concentration (Sp/ml x10 ⁶)	% Motility	FI	% Apoptosis	% Immaturity	% Necrosis
1 pathogen	2 (2.7%)	34 (0)	3.75 (0.5)	156.6 (2.75)	61.0 (6)	2355201 (441208)	8.7 (9.6)	58.3 (25.25)	27.5 (10.85)
2 pathogens	12 (16.4%)	35 (9)	2.9 (1.75)	113.75 (134.75)	45.5 (17)	1214420 (1376191)	4.2 (9.9)	70.3 (25.32)	31.9 (7.14)
3 pathogens	19 (27.4%)	32 (5)	3.0 (2)	36.0 (92.75)	25.0 (23)	328206 (1729255)	7.2 (6.8)	68.15 (19.28)	35.2 (12.77)
4 pathogens	17 (21.9%)	33 (4)	3.0 (1.5)	55.0 (81.5)	24.0 (10)	247838 (862541)	7.2 (6.6)	71.0 (12.85)	37.2 (15.77)
5 pathogens	12 (16.4%)	35 (8)	4.5 (2.1)	33.1 (40.65)	23.0 (17)	181618 (299527)	7.8 (8.1)	73.8 (5.0)	50.5 (33.41)
6 pathogens	11 (15.1%)	35 (9)	3.0 (1.3)	53.0 (47)	17.0 (13)	45234 (271323)	7.9 (5.5)	70.5 (14.2)	51.1 (36.69)
Spearman rho		0.06	0.13	-0.24	-0.52	-0.46	0.16	0.11	0.33
P value		0.59	0.274	<0.05	<0.001	<0.001	0.17	0.34	<0.01

Table 3 Prevalence of seropositivity into two group of patients: those seropositive for <4 pathogens and those seropositive for ≥ 4 pathogens.

Seropositivity	<4 pathogens		≥ 4 pathogens		P value
	<i>N cases</i>	<i>Prevalence %</i>	<i>N cases</i>	<i>Prevalence%</i>	
HP	10/33	30.3	22/40	55.0	0.034
CagA	5/10	50.0	7/22	31.8	0.324
EBV	31/33	93.9	39/40	97.5	0.447
HSV 1	24/33	72.7	30/40	75.0	0.825
CMV	0/33	0	34/40	85.0	/
MP	18/33	54.5	35/40	87.5	0.002
CP	0/33	0	34/40	85.0	/

Table 4

Mean (interquartile range) of considered variables stratified following presence/absence of IgG at significant titer to the selected pathogens. *P<0.05; **P<0.01; ***P<0.001

Table IV	HP		HP/CagA status		EBV	
	<i>negative</i> (n=41)	<i>positive</i> (n=32)	<i>negative</i> (n=12)	<i>positive</i> (n=20)	<i>negative</i> (n=3)	<i>positive</i> (n=70)
Age	33.0 (6.0)	33.0 (6.0)	32.5 (5.5)	35.0 (8.0)	34.0 (24.0)	33.0 (6.0)
Volume	3.0 (1.5)	3.75 (2.5)	4.4 (2.0)	3.25 (1.9)	4.0 (2.5)	3.0 (2.0)
Concentration	55.0 (123)*	38.0 (50.9)	42.5 (55.4)	33.4 (43.5)	116.0 (128.25)	42.5 (71.5)
% Motility	34.0 (22.0)***	17.0 (11.0)	22.5 (13.5)***	10.5 (7.0)	30.0 (45.0)	25.0 (21.0)
FI	901420 (1672685)***	103390 (230356)	195358 (386499)	41122 (96347)	1834567 (2375088)	241212 (971650)
% Apoptosis	7.2 (6.7)	7.7 (5.5)	7.4 (6.8)	9.2 (5.1)	0.8 (3.2)	7.7 (7.5)
% Immaturity	71.9 (16.6)	69.7 (14.2)	73.1 (11.6)	63.5 (15.8)	72.1 (5.4)	70.8 (16.9)
% Necrosis	33.2 (19.0)	37.3 (18.1)	36.0 (10.5)*	50.9 (28.0)	22.1 (16.95)	36.0 (19.85)

Table IV	HSV1		CMV		MP		CP	
	<i>negative</i> (n=19)	<i>positive</i> (n=54)	<i>negative</i> (n=39)	<i>positive</i> (n=34)	<i>negative</i> (n=20)	<i>positive</i> (n=53)	<i>negative</i> (n=39)	<i>positive</i> (n=34)
<i>Age</i>	32.0 (7.0)	33.0 (7.0)	33.0 (5.0)	34.0 (11.0)	33.5 (7.5)	33.0 (6.0)	33.0 (5.0)	34.0 (11.0)
<i>Volume</i>	3.5 (2.0)	3.0 (2.0)	3.0 (2.0)	3.35 (1.9)	3.25 (2.15)	3.0 (2.0)	3.0 (2.0)	3.35 (1.9)
<i>Concentration</i>	54.0 (127.0)	42.0 (75.0)	44.0 (107.0)	43.0 (48.5)	52.25 (134.6)	44.0 (54.75)	44.0 (107.0)	43.0 (48.5)
<i>% Motility</i>	32.0 (21.0)	24.5 (18.0)	32.0 (31.0) ^{***}	23.0 (11.0)	31.0 (29.5) ^{**}	24.0 (13.0)	32.0 (31.0) ^{**}	23.0 (11.0)
<i>FI</i>	275032 (1742598)	229258 (971650)	666926 (1799965) [*]	218641 (451613)	999133 (1896824) [*]	223930 (622978)	666926 (1799965) [*]	218641 (451613)
<i>% Apoptosis</i>	9.8 (7.5)	7.3 (6.8)	7.2 (7.2)	7.85 (5.85)	8.45 (8.15)	7.3 (6.7)	7.2 (7.2)	7.85 (5.85)
<i>% Immaturity</i>	68.75 (23.1)	71.4 (13.6)	68.75 (35.2)	72.7 (11.8)	69.8 (13.3)	72.0 (14.8)	68.75 (19.0)	72.7 (11.8)
<i>% Necrosis</i>	35.2 (8.75)	36.9 (21.3)	35.2 (11.5) [*]	37.0 (28.1)	32.3 (5.4) ^{**}	37.95 (18.8)	35.2 (11.5) [*]	37.0 (28.1)