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Decreased semen volume and spermatozoa motility in HIV-1 infected patients under antiretroviral treatment

Running head: Sperm alterations in HIV infected patients

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Abstract

Inconsistent results were reported on semen quality in HIV-infected men, due to several bias in some studies. The objective of this study is to investigate semen parameters in HIV-1 infected patients and to compare their sperm characteristics with those of a control group of fertile non-infected men. Factors implicated in semen alterations in HIV-1 patients were also analyzed. One hundred and ninety HIV-infected men, of whom 91% were under antiretroviral therapy, and 218 fertile men were studied. Infertility risk factors were recorded and clinical examination was done in both groups. Records of HIV-infection history, antiretroviral treatment, and HIV-1 RNA detection in blood as well as HIV-1 genome detection in semen were obtained in infected patients.

Semen volume, percentage of progressive motile spermatozoa, total sperm count and polynuclear cell count, were decreased while pH value and spermatozoa multiple anomaly index were increased in HIV-infected patients. Even after adjustment for possible sources of bias, the decrease in semen volume and progressive motility and the increase in pH remained significant. In conclusion this study demonstrated sperm motility and ejaculate volume alterations in HIV-1 infected patients, most of whom were receiving antiretroviral therapy. In HIV-1 patients, further longitudinal studies are required in order to analyze the responsibility of treatment regimen on sperm parameters alterations.

Key Words: antiretroviral therapy / HIV-1 / mitochondrial DNA / semen quality / spermatozoa motility

Introduction

HIV-1 infection is a sexually transmitted disease that affects at least 40 million individuals in the world (UNAIDS/WHO,2005). HIV-1 may be present in the human male genital tract (Dejucq and Jegou,2001). Its transmissibility depends on the type of sexual exposure, frequency of sexual intercourse, level of infectivity of the infected partner and the susceptibility of the non-infected partner (Mayer and Anderson,1995; Royce et al.,1997). Sexual transmission is also related to blood viral load and to immunological and virological status (Lee et al.,1996; Quinn et al.,2000).

Several studies have reported the presence of HIV-1 genomes or infectious virus in seminal fluid or sperm cells from HIV-1 infected men (Anderson et al.,1992; Pasquier et al.,2000; Tachet et al.,1999; Vernazza et al.,1994). Moreover, evidence for compartmentalization of HIV-1 between semen and blood has been found (Byrn and Kiessling,1998; Coombs et al.,1998; Ghosn et al.,2004; Kiessling et al.,1998), although this is debated (van Leeuwen et al.,2004).

Currently, and particularly following the improved prognosis of HIV-1 infection thanks to highly active antiretroviral therapy (HAART), couples now request medical assistance to conceive, while reducing the risk of HIV-1 transmission to a minimum.

Several teams have developed medically assisted procreation (MAP) programmes for serodiscordant couples with an HIV-1 infected male partner to allow them to have children without infection of the female partner (Bujan et al.,2004; Garrido et al.,2004; Gilling-Smith,2000; Guibert et al.,2001; Loutradis et al.,2001; Marina et al.,1998; Marina et al.,2003;

Mencaglia et al.,2005; Nicopoullos et al.,2004; Ohl et al.,2003; Pena et al.,2002; Pena et al.,2003; Sauer and Chang,2002; Semprini,1993). In these programmes, spermatozoa are isolated from semen and usually tested for HIV-1 nucleic acid before use in MAP. Good semen parameters facilitate the different steps of MAP in these couples.

Several studies have assessed semen quality in HIV-1 infected men, with inconsistent results. Krieger *et al.* (Krieger et al.,1991) reported no difference in semen parameters in 24 HIV-1 infected men compared with 40 HIV-negative controls. Crittenden *et al.* (Crittenden et al.,1992) showed reduced motility and increased frequency of round cells in infected men. Other studies have reported decreases in motility, sperm concentration or total sperm count (Dondero et al.,1996; Dulioust et al.,2002; Muller et al.,1998; Nicopoullos et al.,2004). Three studies reported an ejaculate volume decrease (Dulioust et al.,2002; Muller et al.,1998; Nicopoullos et al.,2004). Moreover, a reduced percentage of morphologically normal spermatozoa has been reported in several studies (Dondero et al.,1996; Muller et al.,1998; Nicopoullos et al.,2004) but not in the study of Dulioust et al., (2002)**.** Correlations between blood CD4 count or viral load and motility or sperm count have been reported in some studies but not in others (Bujan et al.,2004; Gupta et al.,1997; Pasquier et al.,2000; Tachet et al.,1999; Vernazza et al.,1997).

The discrepancy between these studies could result from differences between infected populations, or from the small number of patients and control men, with the exception of the two more recent studies (Dulioust et al.,2002; Nicopoullos et al.,2004), and could also result from the choice of control group. Moreover, andrological and disease history (such as cryptorchidism, varicocele or genital infection) were not taken into account in these studies.

The present study of 190 HIV-1 infected men (i) compares their sperm characteristics with those of a control group of 218 fertile men and (ii)try to analyse the factors implicated in semen alterations in HIV-infected men.

Methods

Patients

One hundred and ninety HIV-1 infected men attending the Centre d'Etudes et de Conservation des Oeufs et du Sperme Humain (CECOS) Midi-Pyrenees, Hôpital Paule de Viguier, Toulouse, provided 190 semen samples between April 1998 and April 2004. They were all clinically asymptomatic.

Control group

Two hundred and eighteen men of proven fertility (fathers of at least one child or with a pregnant partner) were included as control group. They included candidates for sperm donation (n = 58), volunteers in a research study of men's reproductive health in France (n = 74) and men requesting vasectomy (n=86). All men provided semen samples and gave their informed consent. This study was carried out in accordance with the Declaration of Helsinki and was approved by the institutional review board.

Clinical investigations

Medical and particularly andrological history including past genital (urethritis, orchiepididymitis, prostatitis) or urinary infections, cryptorchidism, varicocele, and tobacco habits were obtained from the patients and the control group. In addition, patients underwent a clinical examination in which the position and trophicity of the epididymis and scrotal vas deferens were assessed. The length (L) and width (W) of each testis were measured with calipers according to our previously published method (Mieusset et al.,1989). Testicular volume was calculated according to the following formula: volume = 0.71 x L x W^2 where L represents the length and W the width. Presence of a varicocele, abnormal position or trophicity of the epididymis, deferent absence, were recorded as andrological clinical abnormalities..

Semen samples

Semen samples from patients and from the control group were collected at the CECOS laboratory into sterile containers by masturbation after a recommended 3-day period of sexual abstinence. Samples were processed at the laboratory within two hours of ejaculation according to WHO recommendations (WHO,1999) and to our previously published method (Bujan et al.,2004). Semen pH was measured using pH-indicator strips (pH range 6.5-10.0 and if necessary 4.0-7.0; Merck, Darmstadt, Germany). Motility was classified according to WHO criteria in four groups: *a* = rapid progressive spermatozoa, *b* = slow progressive spermatozoa, *c* = non-progressive spermatozoa and *d* = immotile spermatozoa. Rounds cells in semen were assessed according the WHO recommendations. Sperm vitality was assessed after eosin Y-nigrosin staining and was exprimed as percentage of viable spermatozoa. Total sperm count was equal to sperm count (10^6/mL) x ejaculate volume (mL). Total motile sperm count was equal to

total sperm count x percentage of motility $a + b$. Sperm morphology was analyzed according to David's classification as modified by Jouannet *et al.* (Jouannet et al.,1988). This classification allows calculation of the multiple anomaly index (MAI) which is the mean number of anomalies per abnormal spermatozoa. Semen polymorphonuclear granulocyte count was performed using peroxydase staining method recommended by WHO.

HIV-1 genome detection

HIV-1 RNA was assessed in blood and in seminal plasma. HIV-1 RNA and DNA were assessed in native (i.e. total ejaculated cells) semen cells according to previously published methods (Bujan et al.,2004; Pasquier et al.,2000). Briefly, blood plasma HIV-1 RNA was quantified with the Amplicor HIV-1 Monitor v1.5 assay (Roche Diagnostic Systems, Meylan, France) using the ultrasensitive protocol (detection limit 20 copies/mL). HIV-1 genomes were extracted from seminal plasma or cells using the Nuclisens extraction kit (Organon Teknika S.A., Fresnes, France). For HIV-1 RNA quantification in seminal plasma, we used a modified Amplicor HIV-1 Monitor v1.5 assay (Roche Diagnostic Systems, Meylan, France). The detection limit of the assay in seminal plasma was 100 copies/mL. Finally, we used a modified HIV-1 Monitor v1.5 assay (Roche Diagnostic Systems, Meylan, France) to detect HIV-1 RNA or both HIV RNA and DNA on native semen cells. The assay detection limit in seminal cells was 20 HIV-1 genome copies/10^6 cells.

Statistical analysis

All statistical analysis used (1-β) = 80% and a level of 5% according to Stata 6.0 software (Stata Corp., College Station, Texas, USA). Sperm count, total sperm count, and total motile sperm

count distributions were normalized by logarithmic transformation. The non-parametric Mann-Whitney test was used to compare all quantitative data. Fisher's exact test was used to compare qualitative data between subgroups. Logistic regression analyses were performed to compare differences between patients and controls. These analyses were adjusted for known confounder variables, i.e. age of patient and abstinence delay, and then for medical status, i.e. medical andrology history and presence or absence of clinical anomaly.

Results

In the 190 HIV-1 infected men, all clinically asymptomatic, the mode of transmission was intravenous drug use for 34%, blood transfusion for 12%, sexual intercourse for 44% and unknown for 10% of infected men. The mean duration of HIV-1 infection was 10.2 ± 4.9 years (range: 1-20). Of infected men, 49.7% were co-infected by a hepatitis virus. Ninety-one percent of patients were under antiretroviral therapy (ART) as follows: 20 patients were receiving two nucleoside inhibitors and 152 patients were receiving three or more drugs.

Mean CD4 cell count was 581.9 ± 274.9 x 10^6/L (range 165-2246) and 129 patients had detectable HIV-1 RNA levels in blood plasma (range 3-400,000 copies/mL). Twenty seven patients had detectable HIV-1 RNA levels in seminal plasma (mean 813.1 ± 1364.0, range 3 – 5600 copies/mL). Thirty-two percent of HIV-1 infected men had a positive andrological medical history (see methods) versus 20.1% in the control group ($p<0.005$). A history of urinary or genital infection was more frequent in HIV patients than in control men (21.8% vs 10%, $p<0.01$). The frequency of clinical andrological abnormalities did not differ between groups:

24.8% and 19.5% in infected patients and control men respectively ($p>0.05$). Mean testis volumes did not differ between the two groups: 36.4 ± 10.7 mL and 35.8 ± 11.7 mL for the right and 33.8 ± 10.3 mL and 33.6 ± 11.4 mL for the left testis, in infected men and the control group respectively.

Semen characteristics for HIV-infected men and the control group are presented in Table 1. Ejaculate volume, percentage of progressive spermatozoa (motility *a*, motility *a*+*b*), total sperm motile count and polynuclear cell count were significantly decreased compared with the control group values. In contrast, pH value and MAI were increased in HIV-infected men. Since patient age and length of abstinence may modify the results, we performed further analysis after adjustment for these variables. The difference persisted after adjustment.

As andrological history or clinical abnormalities influence sperm parameters, we conducted a second analysis after adjustment for these variables. Reduced ejaculate volume, decreased percentage of progressive motile spermatozoa, reduced total motile spermatozoa and increased pH value were observed in HIV-1 infected men compared with the control group..

Considering, only the HIV-1 patients group no difference in sperm parameters was found between CD4 cell counts below or above $500x10^6$/mL (data not shown). On the other hand, detectable blood viral load was associated with reduced progressive motility *b* [7.3 ±4.2 and 6.1 ± 4.6% ($p<0.05$) in undetectable and detectable viral loads respectively]. A detectable seminal viral load (n=27) was not associated with sperm parameter modifications. However, when the HIV-1 genome was present in native semen cells (n = 22), there was a decrease in sperm count

($p<0.05$), total sperm count ($p<0.05$) and total motile sperm count ($p<0.05$), accompanied by an increase of polynuclear cells ($p<0.05$).

The mean duration of treatment was 5.7 ± 3.2 years. No statistically correlation was found between sperm parameters and treatment duration. All treated patients take at least one d-drug, 128 take 2 d-drugs, 27 take 3 and 1 patient take 4 d-drugs. Semen parameters and particularly motility were not different according the number of d-drugs. According to the treatment presence or absence no statistically differences were found in sperm parameters but the number of patient without treatment in this study was low (data not show).

Discussion

This study is the first to compare sperm parameters in 190 HIV-1 infected men with those of a large group of healthy men of proven fertility. Moreover, this study analyses sperm parameters according to the results of clinical examination and the medical andrological history, which can both represent bias in sperm parameter studies.

This study demonstrates a decrease of semen volume, of spermatozoa motility, of the total motile sperm count and an increase of pH and multiple anomaly index in HIV-infected patients.

Earlier studies were performed on a small number of patients (Crittenden et al.,1992; Dondero et al.,1996; Krieger et al.,1991) or a small number of control men (Dulioust et al.,2002; Muller et al.,1998). The first study compared 24 HIV-infected men with 40 healthy men who provided semen for other investigations during the study period and found no difference in sperm parameters between the two groups (Krieger et al.,1991). In this study, three patients with AIDS

had abnormal semen. Crittenden *et al.* found a decreased percentage of motile sperm and an increased proportion of round cells in sperm from HIV-1 infected men (n = 39) versus 51 men without HIV (Crittenden et al.,1992). Similar sperm alterations were reported by another study of 21 HIV-infected men (76% under ART) and 30 control men(Dondero et al.,1996). Moreover, the authors also reported, in infected patients, a decreased percentage of sperm with normal morphology (Dondero et al.,1996; Nicopoullos et al.,2004) and a decrease in total sperm count (Dondero et al.,1996). In another study including a large HIV-infected population (n = 250) but with a small number of fertile men (n = 38), the same sperm alterations were observed with in addition decreased ejaculate volume in HIV-infected men (Muller et al.,1998).

Two recent studies reported sperm characteristics in 105 and 189 HIV-infected men compared with 234 and 79 control men, respectively (Dulioust et al.,2002; Nicopoullos et al.,2004). It is noteworthy that in these studies, the control groups were composed of men whose women partners were undergoing in vitro fertilisation (IVF) due to tubal infertility. However, the male partners of women with tubal infertility due to a past or present genital infection could themselves have sperm or genital infection, whether past or present, that could have resulted in sperm alterations. In the studies of Nicopoullos *et al.* (Nicopoullos et al.,2004) and Dulioust *et al.* (Dulioust et al.,2002), no information was given on the andrological medical history or results of clinical examination of the control men. In the study of Dulioust *et al.* (Dulioust et al.,2002), in which 94% of HIV patients were under ART, a decrease of rapidly progressive motile spermatozoa (motility *a*) and an increase of less rapidly progressive spermatozoa (motility *b*) were reported in HIV patients. An increase of round cells and a decrease of total

sperm count were reported. However, decrease in total sperm count could be explained by the decrease in ejaculate volume, since sperm count decrease was not observed.

In the study of Nicopoullos *et al.* (Nicopoullos et al.,2004), where 56% of HIV patients were under ART, a decrease of progressive motility (*a*+*b*), a decrease in sperm count and total sperm count and a reduction of ejaculate volume were reported. Furthermore, the percentage of sperm with normal morphology was also reduced in HIV-infected men. In contrast with these findings, no difference was reported in ejaculate volume, sperm concentration or sperm motility in an other study of 70 HIV-infected patients (70% of whom were under ART) and 73 healthy seronegative male partners of women with tubal infertility (Garrido et al.,2005).

Differences in the results of published studies (table2) could be due to differences in i) recruitment of the HIV-infected men and the fertility status of men in the control group, ii) methodological variations in semen analysis, iii) proportion of men under ART. Moreover, andrological medical history (i.e. genital or urinary infections, cryptorchidism, varicocele, …) and results of clinical andrological examination were not reported in these studies. Our study take in consideration several points. First, we had a similar number of men in our HIV-infected and our control groups. Second, our control group was composed of fertile men recruited by three different methods. Third, andrological medical history was evaluated and a clinical andrological examination was performed for men in both groups. Four, all semen probes were collected and analysed in our laboratory according to the same procedure. Finally, age and length of abstinence (Auger et al.,1995; Chen et al.,2003) and andrologic data (Comhaire,2000;

Schlegel and Hardy,2006) were integrated in the analyses since they can influence semen parameters.

We observed in HIV-infected men semen alterations such as a decrease of ejaculate volume, in agreement with the three largest studies of HIV-infected men (Dulioust et al.,2002; Muller et al.,1998; Nicopoullos et al.,2004), and an increase of pH value. A decrease of progressive motile spermatozoa was also found, in agreement with other studies (Crittenden et al.,1992; Dondero et al.,1996; Dulioust et al.,2002; Muller et al.,1998; Nicopoullos et al.,2004). In contrast with three previous studies, we did not observe a decrease of sperm count. A decrease of total sperm count in the ejaculate was reported in two published studies (Dulioust et al.,2002; Nicopoullos et al.,2004) but in our study this decrease did not persist after adjustment for the subject's andrological history and result of andrological evaluation. It is noteworthy that the most frequently altered semen parameters in the largest studies of HIV-infected men (Dulioust et al.,2002; Muller et al.,1998; Nicopoullos et al.,2004) were the ejaculate volume and the percentage of progressive motility.

The mechanism of semen alterations in HIV-infected men is still unclear, although several hypotheses have been put forward. Men with advanced HIV infection and particularly those with AIDS status had abnormal sperm (Krieger et al.,1991) or abnormal spermatogenesis (Dejucq-Rainsford and Jegou,2004), but in our study all patients were asymptomatic.

The CD4 cell count, which reflects HIV infection immune status, was positively correlated with percentage of motile spermatozoa (Crittenden et al.,1992; Dondero et al.,1996; Lasheeb et

al.,1997; Muller et al.,1998; Nicopoullos et al.,2004) and sperm count (Lasheeb et al.,1997; Nicopoullos et al.,2004; Politch et al.,1994), and negatively correlated with ejaculate volume (Dulioust et al.,2002). The duration of HIV infection was negatively correlated with ejaculate volume in one study (Dulioust et al.,2002). In this last study, blood viral load was negatively correlated with motility, in agreement with our findings, and positively correlated with the percentage of morphologically normal spermatozoa, in contrast with the study of Nicopoullos *et al.* (Nicopoullos et al.,2004).

It is very difficult to reach a conclusion concerning the relationship between immunological and virological HIV status and sperm parameters in asymptomatic HIV-infected patients. No study has examined the sperm characteristics of several patients before and after HIV contamination. Nevertheless, it is interesting to note that a case report has shown a decrease of sperm motility and morphologically normal spermatozoa and an increase of sperm concentration after HIV-1 contamination in a man participating in a semen donation programme (van Leeuwen et al.,2004).

Decrease in ejaculate volume and semen pH increase could be due to reduced accessory gland secretions or to ejaculatory dysfunction. Dysfunction of the prostate and seminal vesicles, which are responsible for about 90-95% of ejaculate volume, could be due to past or silent inflammation or infection, virus cell gland colonisation, or perhaps to the effects of ART drugs which were present in the genital tract (Eron et al.,1998; Henry et al.,1988; Taylor et al.,2001).

Decreased motility may be due to the viral effect on spermatozoa, to abnormal seminal plasma composition or to changes in spermatozoa metabolism due to ART. HIV RNA has been

evidenced in several genitourinary secretions (Coombs et al.,2006). Whether HIV has an impact on gland function and/or on biochemical seminal parameters is not known in asymptomatic patients.

Studies have demonstrated the relationship between mitochondria and sperm motility (Donnelly et al.,2000; Folgero et al.,1993; May-Panloup et al.,2003; Ruiz-Pesini et al.,1998). As several ART have mitochondrial toxicity (Brinkman et al.,1998; Johns,1995; Lewis and Dalakas,1995), the observed changes in motility could be due to the ART itself. A report of increased frequency of multiple DNA deletion in the sperm of patients receiving HAART for more than 12 months supports this hypothesis (White et al.,2001). In a small study in treated HIV patients, a negative correlation between duration of treatment with drugs known to be strong inhibitors of mtDNA replication and the mtDNA content of spermatozoa was reported, but with no difference in sperm count, motility or mtDNA according to type of antiretroviral drug(Diehl et al.,2003). On the other hand, Robbins *et al.* (Robbins et al.,2001) did not find sperm or lymphocyte chromosomal changes after the start of nucleoside-containing antiretroviral therapy. They observed improved sperm motility for men with CD4 cell counts up to 200 cells/mm^3 at study entry and no improvement for men with under 200 cells/mm^3, but this study was performed in a small number of patients and the authors considered that chronic long-term effects could have been missed by the sampling method used. Unfortunately, as in our study ninety one percent of the HIV-patients were under antiretroviral treatment neither statistical difference between treated and not treated patients, nor correlations between d-drugs use and sperm motility were found.

In conclusion, our findings demonstrate sperm alterations in HIV-infected men. Long-term prospective studies in men starting antiretroviral therapy are needed to evaluate the impact of

ART on semen parameters and more particularly on the male gamete genome. Indeed, because of the mitochondrial toxicity of several antiretroviral drugs and the possible incorporation of these molecules in lymphocyte or testis cell DNA in men or monkeys (Olivero et al.,2002; Olivero et al.,2001; Olivero et al.,2000; Olivero et al.,1999), it is of paramount importance to answer these questions.

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Table 1: Comparison of age, body mass index, testis volume and semen characteristics between the HIV group and the control group

	HIV	Control	HIV	Control		
	n		Mean [sd.]		*p*	*p***
Age (year)	190	216	37.2 [5.8]	37.9 [6.2]	*NS*	*NS*
BMI	128	160	22.9 [3.0]	24.7 [2.8]	*<0.01**	*<0.01*
Testis characteristics						
Right testis volume (mL)	137	124	36.4 [10.7]	35.8 [11.7]	*NS*	*NS*
Left testis volume (mL)	137	124	33.8 [10.3]	33.6 [11.4]	*NS*	*NS*
Length of abstinence (days)	189	206	5.1 [3.2]	6.2 [7.8]	*NS*	*NS*
Semen characteristics						
Ejaculate volume (mL)	190	218	**3.3 [1.6]**	**3.9 [1.9]**	*<0.01**	*<0.01*
pH	190	217	**8.2 [0.3]**	**7.9 [0.3]**	*<0.01**	*<0.01*
Motility a (%)	190	218	**32.8 [17.2]**	**37.4 [14.1]**	*<0.05**	*<0.05*
Motility b (%)	190	218	6.4 [4.5]	6.2 [3.6]	*NS*	*NS*
Progressive motility a+b (%)	190	218	**39.2 [16.2]**	**43.6 [13.8]**	*<0.05**	*<0.01*
Motility c (%)	190	218	**7.0 [3.2]**	**6.3 [2.9]**	*<0.05**	*NS*
Motility d (%)	190	218	**53.8 [15.6]**	**50.1 [13.4]**	*<0.05**	*<0.05*
Vitality (%)	189	218	68.8 [15.3]	69.7 [13.3]	*NS*	*NS*
Sperm count (10^6.mL^{-1})	190	218	108.3 [96.8]	96.7 [88.2]	*NS*	*NS*
Total sperm count (10^6 per ejaculate)	190	218	330.9[287.7]	353.8[317.9]	*NS*	*NS*
Total motile sperm count (10^6 per ejaculate)	190	218	**127.9[120.7]**	**150.7[127.1]**	*<0.05*	*<0.05*
Morphologically normal spz (%)	97	107	27.0 [11.4]	28.6 [14.3]	*NS*	*NS*
Multiple anomaly index	97	107	**1.8 [0.2]**	**1.7 [0.2]**	*<0.05**	*NS*
Round cells (10^6.mL^{-1})	188	218	2.3 [3.2]	2.0 [2.3]	*NS*	*NS*
Polynuclear cells (10^6.mL^{-1})	139	30	**0.1 [0.4]**	**0.8 [2.4]**	*<0.01**	*NS*

* Significant difference persisted after adjustement on age and length of abstinence
** *p*-value with adjustment on age, length of abstinence, medical andrology history and result of andrological examen
NS, Not statistically Signifiant

Table2: Published studies comparing sperm parameters between HIV-patients and control group.

Studies	**Krieger 1991**	**Crittenden 1992**	**Dondero 1996**	**Muller 1998**	**Dulioust 2002**	**Nicopoullos 2004**	**Garrido 2004**	**This study**
HIV-Patients n (%under ART)	**24** (50)	**39** (48)	**21** (76)	**250** ⊗ (?)	**189** (94)	**105** (55)	**73** (70)	**190** (91)
Control group	Healthy men	Sperm donor or Hemophiliac men	Fertile or pre-marriage men	Fertile men	PWTI	PWTI	PWTI	Fertile men *
n	**40**	**51**	**30**	**38**	**79**	**234**	**73**	**218**
Adjustement for :								
Age	no	no	no	no	yes	no	yes	yes
Abstinence delay	no	no	no	no	yes	no	yes	yes
Andrological history	no	no	no	no	no	no	no	yes
Andrological examen	no	no	? x	no	no	no	no	yes
Significant changes in HIV-patients compared with control group								
Volume (ml)	no	no	no	**reduced**	**reduced**	**reduced**	no	**reduced**
pH	no	nd	nd	no	no	nd	no	**increased**
Motility a (%)	no	nd	nd	**reduced**	**reduced**	no	no	**reduced**
Motility a+b (%)	no	**reduced**	**reduced**	**reduced**	no	**reduced**	no	**reduced**
Sperm count 10^6/ ml	no	no	**reduced**	**reduced**	no	**reduced**	no	no
Total sperm count 10^6/ ejaculate	no	no	nd	nd	**reduced**	**reduced**	no	no
Normal sperm morphology (%)	no	nd	reduced	no	no	**reduced**	nd	No but **MAI increase**

ART: antiretroviral treatment, PWTI : partner of women with tubal infertility included in In Vitro Fertilization programme, MAI: multiple anomalies index,

* from three different mode of recruitment (see methods), nd: not done, no: no significant changes between HIV-patients and control group, x: "man with andrological diseases excluded", ⊗ sperm probes were performed outside the laboratory.