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ORIGINAL RESEARCH

Biological markers of fertility (inhibin-B) in HIV-infected men: influence of HIV infection and antiretroviral therapy

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Objectives

Inhibin B (IB) levels and the IB: follicle-stimulating hormone (FSH) ratio (IFR), biomarkers of global Sertoli cell function, show a strong relationship with male fertility. The aim of the study was to examine the prevalence of impaired fertility potential in HIV-infected men and the influence of antiretroviral therapy (ART) on fertility biomarkers.

Methods

A cross-sectional study with sequential sampling was carried out. A total of 169 clinically stable patients in a cohort of HIV-infected men undergoing regular ambulatory assessment in a tertiary hospital were included. The mean [± standard deviation (SD)] age of the patients was 42.6 ± 8.1 years, all were clinically stable, 61.5% had disease classified as Centers for Disease Control and Prevention (CDC) stage A, and were naıve to ART or had not had any changes to ART for 6 months (91.1%). Morning baseline IB and FSH concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) and an electrochemiluminescent immunoassay (ECLIA), respectively. A multivariate logistic regression model was used to identify factors associated with impaired fertility, defined as IB < 119 pg/mL or IFR < 23.5.

Results

The mean (± SD) IB level was 250 ± 103 pg/mL, the median [interquartile range (IQR)] FSH concentration was 5.1 (3.3–7.8) UI/L and the median (IQR) IFR was 46.1 (26.3–83.7). The prevalence of impaired fertility was 21.9% [95% confidence interval (CI) 16.3–20.7%]. Negative correlations of body mass index and waist: hip ratio with FSH and IB levels were observed ($P < 0.01$), while a sedentary lifestyle and previous nevirapine exposure were associated with a decreased risk of IB levels ≤ 25th percentile in multivariate analysis. Only older age, as a risk factor, and sedentary lifestyle, with a protective effect, were independently associated with impaired fertility in multivariate analysis.

Conclusions

Global testicular Sertoli cell function and fertility potential, assessed indirectly through serum IB levels and IB: FSH ratio, appear to be well maintained in HIV-infected men and not damaged by ART.

Keywords: antiretroviral treatment, fertility, HIV, inhibin-B, Sertoli cell

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Introduction

Most HIV infections occur early in the reproductive lives of men. Many HIV-infected men have fertility intentions [1], and so it is important to consider the impact of HIV on reproductive health and reproductive aging. Although current antiretroviral therapy (ART) has improved the

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overall health and survival of men infected with HIV, many questions remain about the impact, if any, of HIV infection and ART on fertility potential and there is no information about Sertoli cell function in HIV-infected men.

In HIV-infected men, low testosterone levels are common [2–4], but rates of infertility are unclear [5]. Previous reports have assessed the effect of HIV infection on sperm parameters, with variable results [6–9]. Compared with HIV-negative controls, HIV-infected men had less rapidly progressive sperm, more nonspermatic cells, lower ejaculate volumes and lower total sperm counts [8,9], although the majority of HIV-infected men had sperm parameters that were within the normal range defined by the World Health Organization (WHO) [9].

Inhibin B (IB) is a dimeric glycoprotein synthesized in testicular Sertoli cells and in germ cells, and it is a marker of the functional state of the seminiferous epithelium, especially Sertoli cells [10]. In adult male individuals, serum IB level is negatively correlated with serum follicle-stimulating hormone (FSH) concentration and positively correlated with sperm count, sperm concentration, Sertoli cell number and testicular volume, suggesting a paracrine role of IB in regulating spermatogenesis [10–15]. IB level is a more sensitive marker of male factor infertility than other available hormones, irrespective of the aetiology, being a direct marker of Sertoli cell function and an indirect marker of spermatogenesis [10,16–18].

These observations strongly support the potential clinical application of serum IB as a marker of spermatogenic function, and in the assessment of fertility, pregnancy-related conditions, and reproductive function [10,18]. Measurement of IB is very useful in experimental studies to improve our understanding of gonadal function and regulation of the pituitary-gonadal axis, being a very early marker of testicular damage and disorders of spermatogenesis in initial stages in populations exposed to testicular toxicants. The diagnostic power of IB as a marker of spermatogenesis is greatest when it is combined with the FSH level in an IB: FSH ratio (IFR), and evidence of a threshold supports the use of these parameters as complementary tools in the context of infertility evaluation [19,20].

Knowledge of the impact of ART on fertility potential will be crucial for selecting among available antiretroviral regimens and designing fertility preservation strategies; there is no information in the literature about the effect of HIV infection or ART on Sertoli cell function as determined by measurement of IB levels.

The aims of the present study, carried out in a large cohort of ambulatory, clinically stable HIV-infected men, were: (1) to assess the prevalence of impaired fertility by measurement of IB and IFR; (2) to assess the effect of ART on fertility; (3) to explore the effect of markers of HIV infection on fertility potential.

Methods

A cross-sectional observational study was carried out in the Infectious Diseases and Endocrinology departments of a tertiary hospital in Alicante, Spain. The local Ethics Committee approved the study. All HIV-infected men belonging to a cohort of 600 HIV-infected patients, with regular assessment of endocrine parameters, were eligible to participate in this study if they were $\geq$ 18 years of age and ART-naïve or on effective ART (< 50 HIV-1 RNA copies/mL), with no changes in the last 6 months. Those with active AIDS, active illegal drug use, risk factors with potential negative effects on andrological parameters (e.g. chemotherapy and previous chemotherapy) or a psychiatric illness were excluded. All patients gave written informed consent. Participants were required to fast for 12 h prior to the blood sample being taken at between 8:00 and 9:00 AM. The samples were centrifuged and serum and plasma were stored at −30°C until determination.

Outcome variables

Inhibin-B (Sertoli cell function)

The serum IB levels were determined using an enzyme immunoassay (DSL-10-84100i Active©; DSL, Webster, TX, USA) with a sensitivity of 7 pg/mL. The intra-assay precision was as follows: mean 69 pg/mL, coefficient of variance (CV) 3.5%; mean 274 pg/mL, CV 4.6%; mean 472 pg/mL, CV 5.6%. The inter-assay precision (serum) was: mean 50.1 pg/mL, CV 7.6%; mean 188.4 pg/mL, CV 6.3%; mean 355.0 pg/mL, CV 6.2%.

Inhibin-B: FSH ratio

For each subject, an IB: FSH ratio was calculated as IB (pg/mL)/FSH (UI/L); the FSH concentration [UI/L; reference range (rr) 1–8 UI/L] was determined by electrochemiluminescent immunoassay (ECLIA) (Modular Analytics E170; Roche Diagnostics; Mannheim, Germany).

Impaired fertility potential

We defined impaired fertility potential as IB level < 119 pg/mL or IFR < 23.5 [19].

Explanatory variables

General variables included smoking, cannabinoid consumption, physical activity (sedentary lifestyle was defined as nonregular physical activity), body mass index (BMI; kg/m^2), waist: hip ratio (WHR), systolic and

diastolic blood pressure (mmHg) and testes volume (ml; measured with a Prader orchidometer).

HIV infection-related variables

HIV infection-related variables were nadir and current CD4 lymphocyte counts [cells/μL and percentage, determined using flow cytometry (BDFA Sccalibur®; Becton Dickinson, San Jose, CA, USA)], plasma viral load [HIV-1 RNA copies/ml; the lower detection limit was 39 copies/mL; determined using the COBAS® TaqMan® HIV test (Roche Diagnostics); Mannheim, Germany; Mannheim, Germany], duration of HIV infection (in years), and clinical state [Centers for Disease Control and Prevention (CDC) stage].

Antiretroviral therapy

Patients were classified into four groups: (1) those naïve to ART; (2) the nonnucleoside group naïve to protease inhibitors (PIs): currently receiving the nonnucleoside reverse transcriptase inhibitor (NNRTI) efavirenz (EFV) or nevirapine plus two or three nucleoside reverse transcriptase inhibitors (NRTIs); (3) the nonnucleoside group with previous PI exposure (but no current exposure), and (4) the PI group: receiving a ritonavir-boosted PI (PI/r) plus two or three NRTIs.

Hypothalamic–pituitary–gonadal (HPG) axis

The concentration of free testosterone was calculated from the concentrations of Total testosterone (TT) TT (ng/mL; rr 3–10 ng/mL), Sex Hormone Binding Globulin (SHBG) SHBG [nmol/L; rr 4–72 nmol/L; determined using a chemiluminescent immunoassay (Unicell DXI; Beckmann Coulter, California, USA)] and albumin [rr 480–803 mmol/L; 3170–5300 mg/dL; determined using kinetic nephelometry (immunochemical systems IMMAGE; Beckmann Coulter; California, USA)], using the equation described by Vermeulen *et al.* [21]. Hypogonadism was defined as calculated free testosterone (CFT) < 0.22 nmol/L (< 6.36 ng/dL), lower than the normal range for healthy young men [4,22]. The concentration of luteinizing hormone (LH) (UI/L; rr 2–11.2 UI/L) was determined by ECLIA (Modular Analytics E170; Roche Diagnostics).

Lipodystrophy

Lipodystrophy was determined using a standard questionnaire based on physical examination [23].

Systemic inflammatory markers

We measured high-sensitivity C-reactive protein (hsCRP) (using turbidimetry kinetics; IMMAGE; Beckmann Coulter), plasminogen activator inhibitor-1 (PAI-1), tumour necrosis factor-alpha (TNF-α), soluble forms of the TNF1 and TNF2 receptors (sTNFR1 and sTNFR2, respectively), and interleukin-6 (IL-6) (using an enzyme immunoassay; Quantikine; R&D Systems, Abingdon, UK). Two samples for each inflammatory marker were obtained; one was immediately processed, and the other was incubated for 24 h at 37°C with an accelerator [10 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma, St Louis, MO, USA) and 500 ng/mL ionomycin (Calbiochem; Novabiochem, La Jolla, CA, USA)], after which they were processed as the initial sample becoming part of the biobank clinical study.

Statistical analysis

Qualitative variables are expressed as relative and absolute frequencies. Parametric variables are expressed as mean ± standard deviation (SD) and nonparametric variables as median and 25−75th percentile (P25−75). For the study of correlations between IB and quantitative variables, Pearson and Spearman tests were used as appropriate, whereas a multiple linear regression model was constructed to investigate factors independently associated with IB, adjusting for potential confounders (all variables yielding statistical significance in the bivariate analysis).

Separate multivariate, unconditional logistic regression models were used to identify factors independently associated with the presence of IB levels ≤ P25 and impaired fertility (as dependent variables), using all variables yielding $P < 0.1$ in the bivariate analysis, and those considered clinically relevant (age, lifestyle, duration of HIV infection, CD4 count, CDC stage, ART group and time of exposure). In all cases, a P-value of < 0.05 was considered statistically significant. The statistical package SPSS version 19.1 (SPSS Inc., Chicago, IL, USA) was used throughout.

Results

A total of 183 men were asked to participate in the study. Fourteen patients refused to give their consent, so 169 subjects were included in the study (Table 1), all of whom were Caucasian, with a mean (± SD) age of 42.6 ± 8.1 years; 59.6% were smokers, 27.4% used cannabis, 43.2% had a sedentary lifestyle and 56.4% were men who have sex with men; the median duration of HIV infection was 9 [interquartile range (IQR) 4–15] years; 61.5% and 23.1% of patients were in clinical stages A and C, respectively, and 81% had an undetectable viral load (≤ 39 copies/mL). Only nine patients had a CD4 count < 200 cells/μL. One hundred and fifty-four patients (91.1%) were receiving ART. There were no differences in clinical characteristics between patients who did and did not participate in the study (data not shown).

The mean (± SD) IB level was 250 ± 103 pg/mL (median 234 pg/mL; P2.5 95.9 pg/mL; P10 130 pg/mL; P25 179 pg/mL; P97.5 497.8 pg/mL), the median (IQR) FSH concentration was 5.1 (3.3–7.8) UI/L (P2.5 1.8 UI/L; P10 2.5 UI/L; P97.5 15.1 UI/L) and the median (IQR) IFR was 46.1 (26.3–83.7) (P2.5 10.5; P10 14.7; P97.5 205.8).

Table 1 Clinical characteristics of the whole cohort (n = 169)

	% (n)	Mean ± SD	Median (P_{25}–P_{75})
Age (years)		42.6 ± 8.1	
CDC stage			
A	61.5 (104)		
B	15.4 (26)		
C	23.1 (39)		
Duration of HIV infection (years)			9.3 (4–15)
Nadir CD4 count (cells/μL)			204 (101–293)
Current CD4 count (cells/μL)			533 (384–705)
Viral load (copies/mL)*			872 (142–24125)
Group			
Naïve	8.4 (14)		
NN (naïve to PIs)	38 (65)		
NN (noncurrent PIs)	12 (20)		
PI	41.6 (70)		
Duration of exposure (months)			
Total ART		87 ± 60	
NNRTI		50 ± 40	
PI		60 ± 48	
Smoking status			
Never smoked	33.7 (57)		
Ex-smoker	6 (10)		
Smoker	60.3 (102)		
Sedentary lifestyle			
No	56.8 (96)		
Yes	43.2 (73)		
BMI (kg/m^2)		24.8 ± 3.5	
WHR		0.93 ± 0.1	
Lipodistrophy†			
No	73.3 (107)		
Yes	26.7 (39)		
Testes volume (mL)		20.6 ± 4.7	
TT (ng/mL)		5.2 ± 1.7	
CFT (ng/dL)		9 ± 3.5	
FSH (UI/L)	–	–	5.1 (3.3–7.7)
LH (UI/L)	–	–	6 (4.6–8.3)
Inhibin B (pg/mL)		250 ± 103	
IFR	–	–	46.1 (26.3–83.7)

ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SD, standard deviation; NN (naïve to PIs), current ART with two or three nucleoside reverse transcriptase inhibitors (NRTIs) plus a nonnucleoside reverse transcriptase inhibitor (NNRTI) and never received protease inhibitors (PIs); NN (noncurrent PIs), current ART with two or three nucleoside reverse transcriptase inhibitors (NRTIs) plus a nonnucleoside reverse transcriptase inhibitor (NNRTI) and previous PI exposure (but no current exposure) P_{25}, 25th percentile; PI, current ART with two or three NRTIs plus an enhanced PI; BMI, body mass index; WHR, waist-to-hip ratio; TT, total testosterone; CFT, calculated free testosterone; IFR, inhibin B: FSH ratio.
To convert to the International System of Units: TT (ng/mL × 3.467) nmol/L; CFT (ng/dL × 0.03467) nmol/L; inhibin B (pg/mL × 1) ng/L.
*Excluding patients with undetectable viral load.
†No data available in 23 patients.

Impaired fertility

Impaired fertility potential was observed in 21.9% [95% confidence interval (CI) 16.3–20.7%] of subjects. Factors associated with impaired fertility in the univariate analysis are shown in Table 2. When we analysed the effect of ART exposure on fertility potential, we detected no differences related to the antiretroviral drug or exposure time, except for lamivudine exposure and current lamivudine-based treatment.

Cannabis use, insulin resistance, hypertension, waist > 102 cm, metabolic syndrome National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) criteria: metabolic syndrome is present if three or more of the following five criteria are met: waist circumference over 40 inches (men) or 35 inches (women), blood pressure over 130/85 mmHg, fasting triglyceride (TG) level over 150 mg/dl, fasting high-density lipoprotein (HDL) cholesterol level less than 40 mg/dl (men) or 50 mg/dl (women) and fasting blood sugar over 100 mg/dl, higher hsCRP level, hypogonadism, CD4 count < 200 cells/μL and higher viral load (in naïve patients) were not associated with impaired Sertoli cell function (data not shown).

In multivariate analysis, only older age, as a risk factor [odds ratio (OR) 2 (95% CI 1.03–3.8) for every 10-year increase in age; P = 0.04], and sedentary lifestyle, with a protective effect (OR 0.3; 95% CI 0.1–0.8; P = 0.01), were independently associated with impaired fertility (Fig. 1).

Inhibin-B, clinical variables and HIV infection

Negative correlations between BMI (r = −0.21), WHR (r = −0.2) and IB level (P < 0.01) were observed, while a sedentary lifestyle (OR 0.22; 95% CI 0.08–0.6; P = 0.005) was associated with a decreased risk of IB level ≤ P25. In the regression analysis, no association of IB level ≤ P25 with HIV infection-related variables was detected. The multivariate logistic regression analysis confirmed an independent inverse association between IB level ≤ P25 and sedentary lifestyle (OR 0.27; 95% CI 0.08–0. 9; P = 0.03).

Inhibin-B and antiretroviral therapy

When we analysed the effect of ART on the IB level, we observed no difference between naïve patients and patients in any ART combination group, and no effect of duration of ART exposure or duration of ART subtype use in the linear regression model. Nevertheless, we observed higher IB levels in patients with past TDF use *vs.* those without such treatment (mean ± SD: 261 ± 113 *vs.* 216 ± 67 pg/mL, respectively; P = 0.002).

Table 2 Risk factors associated with impaired testicular Sertoli cell function and fertility potential

Factor	Impaired fertility potential* (n = 37)	No impaired fertility potential (n = 132)*	OR (95% CI)	P value
Age (years) (mean ± SD)	43.2 ± 7.5	42.3 ± 8.1	1.01 (0.97–1.06)	0.5
CDC stage [% (n)]				
A	70.3 (26)	59.1 (78)	1	
B	2.7 (1)	18.9 (25)	0.58 (0.26–1.3)	0.18
C	27 (10)	22 (29)	Versus B/C	
Duration of HIV infection (years) (mean ± SD)	10.3 ± 7.3	10.1 ± 6.4	1.08 (0.61–1.89)	0.78
Nadir CD4 count (cells/μL) [median (P_{25}–P_{75})]	205 (156–278)	202 (99–296)	1 (0.99–1.002)	0.83
Current CD4 count (cells/μL) [median (P_{25}–P_{75})]	517 (399–641)	536 (382–729)	1 (0.99–1.001)	0.9
Detectable viral load [% (n)]	4.3 (7)	15.3 (25)	1.03 (0.4–2.6)	0.9
Viral load (copies/mL) [median (IQR)]†	415 (116–13300)	981 (144–29700)	1 (1–1)	0.4
Group [% (n)]				
Naïve	8.1 (3)	9.1 (12)	1	
NN (naïve to PIs)	40.5 (15)	37.1 (49)	1.2 (0.3–4.9)	0.77
NN (noncurrent PIs)	10.8 (4)	12.9 (17)	0.94 (0.17–4.9)	0.94
PI	40.5 (15)	40.9 (54)	1.1 (0.27–4.4)	0.88
Duration of exposure (months) (mean ± SD)				
Total ART	85.9 ± 59.4	87.6 ± 60.9	1 (0.99–1.01)	0.8
NNRTI	43.9 ± 36.3	51.9 ± 41.2	0.99 (0.98–1.01)	0.58
PI	64.4 ± 44.4	59.4 ± 49.4	1.02 (0.99–1.01)	0.68
Individual ART [% (n)]				
Lamivudine exposure (yes)	56.3 (18)	72.5 (87)	0.5 (0.22–1.091)	0.08
Lamivudine current (yes)	18 (6)	36.5 (44)	0.4 (0.15–1.04)	0.06
Smoker [% (n)]	17.8 (19)	32.1 (17)	0.57 (0.27–1.2)	0.14
Sedentary lifestyle [% (n)]	25.7 (9)	48.3 (26)	0.37 (0.16–0.86)	0.02
BMI (kg/m^2) (mean ± SD)	25.1 ± 3.5	24.8 ± 3.6	1.02 (0.92–1.14)	0.65
WHR (mean ± SD)	0.94 ± 0.07	0.93 ± 0.07	3.6 (0.02–604.2)	0.6
Lipodystrophy [% (n)]	20.5 (8)	20.6 (22)	0.99 (0.4–2.5)	0.99
Testes volume¶ (mL) (mean ± SD)	17.5 ± 4.4	21.5 ± 4.4	0.82 (0.72–0.94)	0.002
TT (ng/mL) (mean ± SD)	5.1 ± 1.5	5.3 ± 1.8	0.93 (0.75–1.16)	0.54
CFT (ng/dL) (mean ± SD)	8.5 ± 2.5	9.1 ± 3.8	0.94 (0.84–1.06)	0.36
LH (UI/L) (mean ± SD)	8 ± 3.2	6.8 ± 5.1	1.05 (0.97–1.12)	0.19

ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; CI, confidence interval; LH, luteinizing hormone; SD, standard deviation; NN (naïve to PIs), current ART with two or three nucleoside reverse transcriptase inhibitors (NRTIs) plus a nonnucleoside reverse transcriptase inhibitor (NNRTI) and never received protease inhibitors (PIs); NN (noncurrent PIs), current ART with two or three nuceloside reverse transcriptase inhibitors (NRTIs) plus a nonnucleoside reverse transcriptase inhibitor (NNRTI) and previous PI exposure (but no current exposure); OR, odds ratio; P_{25}, 25th percentile; PI, current ART with two or three NRTIs plus an enhanced PI; BMI, body mass index; WHR, waist-to-hip ratio; TT, total testosterone; CFT, calculated free testosterone.
To convert to the International System of Units: TT (nmol/L) = TT(ng/mL) × 3.467; CFT (nmol/L) = CFT (ng/dL) × 0.03467; inhibin B (ng/L) = inhibin B (pg/mL) × 1.
*Defined as inhibin B (IB) level < 119 pg/mL and/or IB: FSH ratio (IFR) < 23.5.
†Excluding patients with undetectable viral load.
¶no data available in 23 patients.

Also, previous tenofovir (TDF) exposure (OR 0.28; 95% CI 0.08–0.94; P = 0.03) and nevirapine (NVP) exposure (OR 0.37; 95% CI 0.14–0.96; P = 0.04) were protective against IB level ≤ P25. Multivariate logistic regression models that included NVP or TDF exposure and relevant clinical variables confirmed an independent association with NVP exposure (coefficient B, 0.28; 95% CI 0.08−0.95; P = 0.04).

Inhibin-B and the pituitary–gonadal axis

There was a negative correlation between FSH and IB levels (r = −0.36; P < 0.001), whereas a positive correlation between testis volume and IB level (r = 0.42; P < 0.001) was observed. Hypogonadism was present in 21.9% of patients (95% CI 16.3–28.7%). There was no relationship between hypogonadism, CFT or LH and IB.

Inhibin-B and systemic inflammatory markers

In a subpopulation of 74 patients, we evaluated the association of systemic inflammatory markers with IB level. IB was negatively correlated with incubated PAI-1 (r = −0.44; P < 0.001) and incubated sTNFR1 (Spearman's rho = −0.23; P = 0.04). Also, patients with IB ≤ P25 *vs.* IB ≥ P75 had higher concentrations of incubated PAI-1

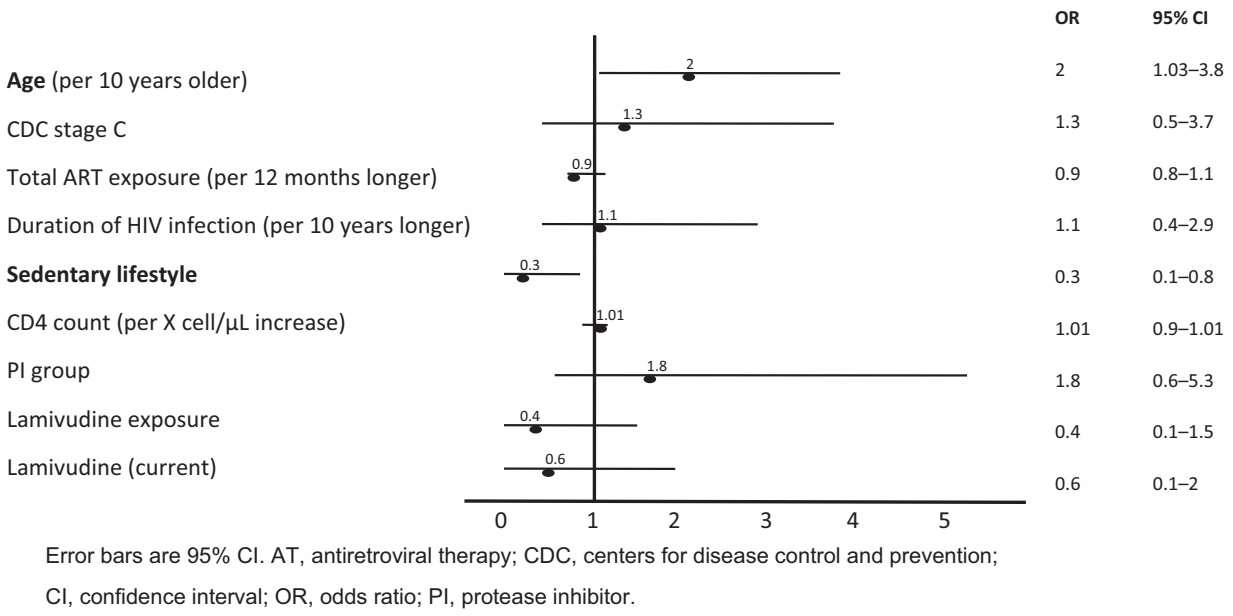

Fig. 1 Risk factors associated with impaired fertility potential in the multivariate logistic regression model. Error bars are 95% confidence intervals. ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; CI, confidence interval; OR, odds ratio; PI, protease inhibitor.

(mean ± SD: 10.6 ± 2.1 *vs.* 7.6 ± 0.9 ng/mL, respectively; $P < 0.001$) and incubated sTNFR1 [median (IQR): 1404.5 (990.7–1688.7) *vs.* 952.5 (781.7–1210.2) pg/mL, respectively; $P = 0.004$].

Discussion

To our knowledge, this is the first study that has evaluated Sertoli cell function and fertility potential by means of IB levels and IFR in a large group of HIV-infected men. Our results for IB, FSH and IFR are similar to published reference values for non-HIV-infected men whose fertility had been proven and for a nonselected population in previous studies [19,24]. Although our population had a higher mean age, we found that these fertility parameters were not impaired, so we could infer that neither ART nor HIV infection compromised the Sertoli cell function and fertility potential of these individuals. Only the age of the patients, as a risk factor, and sedentary lifestyle, with a protective effect, were independently associated with impaired fertility.

The prevalence of impaired fertility potential, which was 21% in our series, will of course depend on the definition employed. There are no published data on impaired fertility in nonselected populations with IB or IFR cut-off levels as suggested by Andersson *et al.* [19] and Jørgensen *et al.* [18], but we can deduce from their published data, for populations younger than 45 years, that the prevalence is about 14–15%. Minor differences compared with our results are mainly explained by the age-associated increase in FSH.

Inverse relationships between age and IB and IFR have been reported [20,24], and these associations are stronger in subjects older than 30 years. Despite the paucity of information, deleterious effects of long-term exercise on reproduction and IB level have also been described in the general population [25]; testicular heating, increases in free radicals and reactive oxygen species, and gonadotropin suppression are all contributing factors to IB and semen changes. These changes in hormone levels seem to be short-lived, with hormone levels returning to baseline values in weeks following the cessation of intense physical activity [25].

In accordance with previous data in the general population, our study confirmed the direct relationship of IB level to testicular volume [11,19,26,27] as a consequence of the number of Sertoli cells in the testis [28], an inverse relationship to BMI and WHR and a strong and negative correlation with FSH [20].

There is a lack of agreement on the impact of HIV infection on fertility [5]. Differences in the populations studied and available ART, methodological variations in semen analysis and low numbers of studied subjects probably explain these discrepancies. Data supporting some impact of HIV infection on fertility [8,9] are limited to seminal parameters in selected populations evaluated

in assisted conception units, and these studies lack adequate evaluation of ART (drugs, current regimen, background and exposure time). Moreover, and in agreement with our findings, where HIV infection parameters did not compromise Sertoli cell function, it was reported that prolonged exposure to asymptomatic, untreated HIV infection did not affect semen quality [29] and that HIV-related parameters (CD4 cell count, viral load, CDC stage, duration of disease, duration of ART, and number and type of antiretroviral drugs) were not significantly correlated with any sperm parameter [30].

Regarding the effect of ART exposure on fertility potential, we detected no differences related to the antiretroviral drug or exposure time, except for lamivudine exposure and current lamivudine-based treatment, which were near statistical significance. Lamivudine is a potent NRTI, and is recommended as part of first-line once-daily triple-drug therapy for treating and preventing HIV infection; it has not shown evidence of impairment fertility in male/female animal studies. There are no previous data on the effect of lamivudine on male fertility. As the multivariate analysis did not confirm the existence of an independent association between current or past exposure to lamivudine and fertility, we cannot assume that it has protective role in preserving HIV-infected male fertility; further studies are needed to address this issue.

The only study that compared all standard semen parameters in HIV-infected patients under stable ART to WHO 2010 reference values showed that median values of all assessed semen parameters were within the normal range [30]. However, for each semen variable, about 25% of patients had values below the fifth percentile of the WHO 2010 reference group; the authors concluded that the study provides evidence of impaired conventional semen parameters and altered sperm protein composition in HIV-infected men, but also stated that HIV surrogate parameters (including ART) are not suitable for predicting semen quality. It should be pointed out that 46.5% of the study population were in clinical stage C (23.1% in our population). The main weakness of this work was the lack of an adequate evaluation of ART (assessment of the influence of ART on semen parameters was limited to ART exposure time, the number of drugs in ART and the seminal penetration score of ART drugs).

Analysis of the influence of ART on IB level in a multivariate logistic regression model confirmed that NVP exposure was protective against IB level $\leq$ P25. As already mentioned above, there are no previous data on the effect of ART on IB level. As regards NVP and sperm count, in a retrospective cross-sectional study in which there were no observed differences in sperm parameters between NRTI, NNRTI and PI regimens, in the NNRTI subpopulation, NVP was associated with better sperm quality than TDF [31]. Nevertheless, the lack of consistency of our findings, with no association between IB level and the duration of NVP exposure in linear regression analysis, and no difference in mean IB level between patients with past use of NVP and those without, prevents us from drawing definite conclusions about the role of NVP in the fertility of HIV-infected men, but certainly justifies the carrying out of prospective studies to address the protective effect of NVP-containing ART regimens in reproduction.

The role of subclinical chronic inflammation in the pathogenesis of Sertoli cell dysfunction was supported by the findings that the proinflammatory cytokine IL-1 modulates IB secretion [10], and addition of IL-1 to Sertoli cell cultures results in a reduction in IB levels [32]. Nonetheless, our data did not support this notion: IB levels were not influenced by most of the inflammatory biomarkers measured.

The strengths of our study include the evaluation of a large number of nonselected ambulatory HIV-infected men in a stable clinical condition; the fact that the study design allows examination of the effect of drug history and limits the effects of confounding factors; the use of IB and IFR as reproducible and reliable biomarkers of Sertoli cell function and fertility potential.

Results of laboratory measurements of semen quality depend on many factors, with large biological variation in semen quality [33], explaining the well-known intraindividual variation in semen composition [34,35]. Such variability has consequences for the interpretation of semen analyses, as it is impossible to characterize a man's semen quality from the evaluation of a single semen sample, whereas day-to-day variations in IB level are relatively low in men and do not seem to be influenced by seasonal factors, and a single blood sample can provide a reliable measurement of reproductive hormones over both short and long time-periods in population studies [36]. Finally, the results produced by commercially available IB immunoassays have a positive linear correlation of > 0.97 between them [37].

This study has some limitations. It was a cross-sectional study; prospective studies may be useful to identify temporal risk factors. There were no semen analyses and no data regarding family status (whether a man had fathered children). There was no control group to directly compare the prevalences and predictors of Sertoli cell dysfunction; however, we used the cut-off values proposed by Andersson *et al.* [19], because fertile individuals proved to be the most appropriate reference group in the evaluation of reproductive hormone levels; moreover, the use of another cut-off also proposed in fertile man, IB < 150 pg/mL [18], did not change the results.

Recently, Barbotin *et al.* [38] published a new reference range for serum IB in normozoospermic patients in a selected population. Despite the limitation that the reference population were men who did not have proven fertility, their IB and FSH levels were in agreement with data for fertile men published by Andersson *et al.* [19], which were used in our study; nevertheless, with the combination of cut-off values of FSH > 7.8 IU/L and IB < 92 pg/mL proposed by Barbotin *et al.* [38], the prevalence of impaired fertility potential in our series was 24%, and neither ART nor HIV infection compromised the Sertoli cell function and fertility potential of these individuals.

In summary, we describe for first time that fertility potential measured using IB level and IFR was not influenced by ART or HIV infection. Our data suggest that fertility in ambulatory nonselected HIV-infected men in a stable clinical condition is preserved. Older age and moderate/intense physical activity were independent factors associated with impaired fertility. NVP-containing ART regimen could exert a protective effect on IB level.

More research is needed to confirm our data. Prospective studies will probably provide some answers and contribute to the identification of new pathogenic factors involved in Sertoli cell dysfunction in the impaired fertility subpopulation.

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