

# Multicenter Quality Control of the Detection of HIV-1 Genome in Semen Before Medically Assisted Procreation

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Couples in whom the man is HIV-1-positive may use medically assisted procreation in order to conceive a child without contaminating the female partner. But, before medically assisted procreation, the semen has to be processed to exclude HIV and tested for HIV nucleic acid before and after processing. The performance was evaluated of the technical protocols used to detect and quantify HIV-1 in 11 centers providing medically assisted procreation for couples with HIV-1 infected men by testing panels of seminal plasma and cells containing HIV-1 RNA and/or DNA. The performance of these tests varied due to the different assays used. False positive results were obtained in 14–19% of cases. The sensitivity for RNA detection in seminal plasma was 500–1,000 RNA copies/ml, over 500 RNA copies/10<sup>6</sup> cells in semen cells, and for DNA detection in semen cells 50–500 DNA copies/10<sup>6</sup> cells. The use of silica-based extraction seemed to increase the assay performance, whereas the use of internal controls to detect PCR inhibitor did not. This first quality control highlights the need for technical improvements of the assays to detect and quantify HIV in semen fractions and for regular evaluation of their performance. **J. Med. Virol. 78:877–882, 2006.** © 2006 Wiley-Liss, Inc.

**KEY WORDS:** HIV-1; semen; medically assisted procreation

## INTRODUCTION

Advances in antiretroviral therapy have increased the life expectancy and quality of life of individuals that are infected with HIV-1 [Al-Khan et al., 2003; Semprini and Fiore, 2004]. People living with HIV are likely to want to have children as their clinical condition improves. Discordant couples must always use condoms during intercourse since HIV-1 is primarily transmitted sexually. They are therefore unable to conceive on their own. Pioneering experiments carried out in 1992 [Semprini, 1993] led to the development of methods of medically assisted procreation and these are now available to HIV-infected couples in most European countries, particularly when the male partner is infected [Bujan et al., 2004b].

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Fertility centers perform “sperm washing” protocols before medically assisted procreation, since the excretion of HIV-1 into the semen is not predicted reliably by the blood virus load and is not necessarily prevented by potent antiretroviral treatment [Tachet et al., 1999; Pasquier et al., 2000; Bujan et al., 2004a]. Sperm washing removes cell-free plasma that can contain free HIV particles and seminal round cells (mostly white blood cells) that can be infected with HIV [Quayle et al., 1997]. It usually involves density gradient centrifugation to isolate spermatozoa and may be followed by a swim-up technique to remove non-motile cells. Fertility centers must ensure that their spermatozoa isolation method is efficient and check that the processed spermatozoa are HIV-free before commencing medically assisted procreation. This is done by testing the processed spermatozoa or other fractions obtained during processing for HIV-1 RNA or DNA. The virologists involved in this work have had to develop their own tests or modify commercial tests for use on semen since no commercial assay is available for detection of HIV-1 in seminal plasma or semen cells. These assays have been validated in each laboratory and are currently in routine use in several centers, particularly in Europe. The assays detect and usually quantify HIV-1 RNA in seminal plasma and detect HIV-1 DNA or both HIV-1 DNA and RNA in semen cells. The spermatozoa can be used for medically assisted procreation, by techniques such as intrauterine insemination (IUI), in vitro fertilization (IVF) or intracytoplasmic spermatozoa injection (ICSI) if no HIV-1 is detected in the spermatozoa fraction obtained after sperm washing.

This study was undertaken to compare the performance of protocols used presently to detect and quantify HIV-1 in centers involved in medically assisted procreation for couples with HIV-infected men.

## MATERIALS AND METHODS

### Laboratories

Eleven laboratories took part in the study. Ten were from European countries, France (6), Switzerland (1), Belgium (1), United Kingdom (1), and Italy (1) and one from the United States of America. All the centers, except the center from the United States of America, were clinical laboratories. They tested routinely semen for HIV-1 and were involved in medically assisted procreation programs for HIV discordant couples. The center numbers in the results section do not correspond to the affiliation numbers of the authors.

### Panel Constitution

The sample panels were prepared in the laboratories of spermiology and virology of the coordinating center (Toulouse University Hospital, France). On the same day, nine informed HIV-negative voluntary men provided semen samples at the spermiology laboratory. The samples were processed in the laboratory, as done routinely, within 2 hr of ejaculation [Pasquier et al., 2000; Bujan et al., 2004a]. The semen samples were

pooled to insure a sufficient seminal plasma volume and cell count. Seminal plasma and whole sperm cells were isolated by centrifugation at 11,000g and spermatozoa were prepared from whole semen by density gradient centrifugation. The processed seminal plasma (450  $\mu$ l aliquots) and harvested spermatozoa ( $5 \times 10^6$ /vial) were aliquoted in 1.5 ml Eppendorf vials and spiked with HIV.

The blood plasma from an HIV-1-infected subject was quantified (mean of two measurements) by RT-PCR (COBAS Amplicor HIV-1 Monitor v1.5; Roche Diagnostics, Meylan, France) and stored at  $-80^\circ\text{C}$ . The quantified blood plasma samples were diluted in human HIV-negative plasma to obtain adequate concentrations of HIV-1 RNA (copies/ml) in 50  $\mu$ l. The seminal plasma panel (panel A) was prepared by adding 50  $\mu$ l aliquots to 450  $\mu$ l seminal plasma to obtain final concentrations of HIV RNA of 1,000, 500, 100, and 50 copies/ml. The semen cell RNA panel (panel B) was prepared by adding 50  $\mu$ l aliquots to  $5 \times 10^6$  spermatozoa to obtain final HIV RNA concentrations of 500, 50, 10, and 5 copies/ $10^6$  spermatozoa. HIV-negative human plasma (50  $\mu$ l) was used to prepare negative samples in both panels.

The semen cell DNA panel (panel C) was prepared by adding  $8 \times 10^5$  cells containing 1 copy of HIV-1 provirus per cell to  $5 \times 10^6$  semen cells to obtain final concentrations of HIV DNA of 500, 50, 10, and 5 copies/ $10^6$  semen cells.

All samples were frozen and stored at  $-80^\circ\text{C}$ . They were shipped to the participating laboratories in dry ice and complied with the relevant legislation. They were received frozen within 48 hr. These samples were tested by each laboratory in the same way as semen samples of HIV-infected men. No specific techniques or algorithms were recommended.

### Characteristics of Assays Used to Detect and Quantify HIV-1

Eleven laboratories received the 3 panels (A, B, and C) described in Materials and Methods and sent the results of their tests to the coordinating center. All 11 laboratories tested the seminal plasma for HIV-1 RNA, but only 9 and 8 of them tested the semen cells for HIV-1 RNA and HIV-1 DNA respectively.

Of the 11 laboratories, 10 quantified HIV-1 RNA in the seminal plasma samples, and one performed a qualitative test. Six laboratories used an adapted HIV-1 Cobas<sup>®</sup> Monitor<sup>®</sup> assay (centers 1–6), one used an adapted NASBA<sup>®</sup> assay (center 7) and 4 used custom-developed techniques with real-time PCR (centers 8–9) or conventional PCR with quantification by hybridizing (centers 10–11) (Table I). The assays differed in the volume of sample extracted, the extraction technique, the volume of RNA extract that was amplified, the use of an internal control to detect PCR inhibitors (centers 1–8), the use of reverse transcription before amplification or in the amplification tube, the region of the HIV-1 genome amplified, and the quantification system used.

The techniques used to detect HIV-1 RNA in semen cells were all very similar to those used for seminal plasma. All the assays detected HIV-1 RNA and DNA in

TABLE I. Main Characteristics of Assays Used to Quantify HIV-1 RNA in Seminal Plasma and Cells

	Center										
	1	2	3	4	5	6	7	8	9	10	11
Test used	Monitor	Monitor	Monitor	Monitor	Monitor	Monitor	NASBA	—	—	—	—
Volume tested (µl)	100	200	250	250	>500	500	100	200	200	125	100
Internal control	+	+	+	+	+	+	+	+	+	+	+
Extraction technique	Nuclisens	Nuclisens	Nuclisens	Qiagen	Monitor	Monitor	Nuclisens	Qiagen	High pure	Trizol	Trizol
Amplified region	gag	gag	gag	gag	gag	gag	gag-pol	LTR	LTR	gag	pol
Quantification method	Hybridation	Taqman	Taqman	Hybridation	Agarose gel						
Estimated sensitivity (c/ml)	200	200	50	200	?	100	100	200	200	100	40
Estimated sensitivity* (c/10 <sup>6</sup> cells)	200	200	125	na	20	na	100	na	200	50	5

na, not applicable.

\*Sensitivity estimated by each laboratory using its own protocol.

the absence of DNase treatment. Two laboratories used the HIV-1 Cobas<sup>®</sup> Monitor<sup>®</sup> to detect HIV-1 DNA in semen cells (centers 4–6), one used the HIV-1 AmpliCor<sup>®</sup> assay (center 3), and five used a home-made assay (two real-time PCR (centers 4–8–9) and two conventional PCR (centers 10–11)) (Table II).

The Chi-square test was used to compare detection frequencies for the various assays.

## RESULTS

### HIV-1 RNA Detection in Seminal Plasma

Samples containing no HIV RNA were all negative except for three samples from two laboratories (Table III). They were positive with fewer than 300 RNA copies/ml. The overall specificity was 86%. HIV-1 RNA was detected in 6/11 (55%) samples containing 50 copies/ml, 7/11 (64%) samples containing 100 copies/ml, 7/9 (78%) samples containing 500 copies/ml, and in 11/11 (100%) samples containing 1,000 copies/ml. The overall sensitivity (95% detection) was estimated to be 500–1,000 RNA copies/ml. Quantifications of the 1,000 copies/ml sample were all within the 3-fold range, except for three that were under-evaluated (88, 134, and 255 copies/ml). Only one laboratory using an internal control detected a PCR inhibitor in the 500 RNA copies sample of this panel. There were no significant differences between the detection frequencies of positive samples of assays with (22/30, 73%) or without (9/12, 75%) internal control checking for PCR inhibitors. The Monitor<sup>®</sup> assays with adapted silica based extraction gave 20/24 (83%), correct results for positive and negative samples, the Monitor<sup>®</sup> assays without adapted extraction gave 8/12 (67%) correct results, the NASBA<sup>®</sup> gave 4/6 (67%) correct results, the real-time PCR gave 7/12 (58%) correct results and the conventional RT-PCR assays gave 11/11 (100%) correct results. The detection frequencies of the adapted Monitor<sup>®</sup> assays using silica based extraction and those not using it were similar ( $P = 0.25$ ).

### Detection and Quantification of HIV-1 RNA in Semen Cells

All the tests on samples containing no HIV RNA were negative except for three positive results for samples from three laboratories, two gave fewer than 60 RNA copies/10<sup>6</sup> cells, for an overall specificity of 82% (Table IV). HIV-1 RNA was detected in 5/9 (55%) of the samples containing 5 HIV-1 RNA copies/10<sup>6</sup> cells, in 6/8 (75%) of those containing 10 HIV-1 RNA copies/10<sup>6</sup> cells, in 7/9 (78%) of samples containing 50 HIV-1 RNA copies/10<sup>6</sup> cells and in 7/8 (88%) of those containing 500 copies/10<sup>6</sup> cells. The overall sensitivity (95% detection) was estimated at over 500 RNA copies/10<sup>6</sup> cells. Results of quantitation of the 500 copies/10<sup>6</sup> semen cells samples were within the 3-fold range, except for three that were over-estimated (2,580, 2,060, and 12,000 copies/10<sup>6</sup> cells). PCR inhibitors were detected in three samples from this panel; all by the same center (center 6). There were no significant differences between detection

TABLE II. Main Characteristics of Assays Used to Quantify HIV-1 DNA in Seminal Cells

	Center											
	3	4	5	6	8	9	10	11				
Test used	Amplitor	—	Monitor	Monitor	—	—	—	—	—	—	—	—
Volume tested (µl)	250	200	>500	250	200	200	200	200	200	200	200	100
Internal control	+	+	+	+	+	—	—	—	—	—	—	—
Extraction technique	Qiagen	Qiagen	Monitor	Qiagen	Qiagen	High pure	Lysis proteinase K					
Amplified region	gag	LTR	gag	gag	LTR	LTR	LTR	LTR	LTR	LTR	LTR	gag
Quantification method	Hybridation	Tagman	Hybridation	Hybridation	Tagman	Tagman	Tagman	Tagman	Tagman	Tagman	Tagman	Agarose gel
Estimated sensitivity* (c/10 <sup>6</sup> cells)	5	?	5	?	5	5	5	5	5	5	5	5

\*Sensitivity estimated by each laboratory using its own protocol.

frequencies for positive samples by assays with (16/22, 73%) and without (8/12, 67%) internal control checking for PCR inhibitors. The Monitor<sup>®</sup> assays with adapted extraction gave correct results for 15/18 (83%) positive and negative samples, the Monitor<sup>®</sup> assays without adapted extraction gave correct results for 8/12 (67%) samples, the NASBA<sup>®</sup> for 4/6 (67%) samples, the real-time PCR for 2/6 (33%) samples, and conventional RT-PCR for 11/12 (92%) samples.

**HIV-1 DNA Detection in Semen Cells**

All tests on samples containing no HIV DNA were negative, except for three (that were positive), giving an overall specificity of 81%. Tests were positive for 1/8 (12.5%) samples containing 5 HIV-1 DNA copies/10<sup>6</sup> cells, 1/8 (12.5%) samples containing 10 HIV-1 DNA copies/10<sup>6</sup> cells, 7/8 (78%) samples containing 50 HIV-1 DNA copies/10<sup>6</sup> cells and 8/8 (100%) for samples containing 500 HIV-1 DNA copies/10<sup>6</sup> cells. The overall sensitivity (95% detection) was estimated at 50–500 DNA copies/10<sup>6</sup> cells. Quantification of the 500 copies/10<sup>6</sup> semen cells samples were within the 3-fold range. No PCR inhibitor was detected for this panel. The detection frequencies of assays with (9/20, 45%) and without (8/12, 67%) internal control checking for PCR inhibitors were similar (*P* = 0.23). The Monitor<sup>®</sup> assays gave correct results for 9/18 (50%) positive and negative samples, the Amplicor<sup>®</sup> assays gave correct results for 4/6 (67%), the real-time PCR for 9/12 (75%), and the conventional RT-PCR for 8/12 (67%) of samples (Table V).

**DISCUSSION**

This study was done to assess the performance of assays used to check the semen of HIV-infected men before medically assisted procreation. They are used to assess safety before processing and/or to validate the sperm washing process. These tests on semen are required legally before medically assisted procreation using semen from HIV infected men in some countries, including France. We prepared three panels of samples to mimic observed or plausible situations. Panel A was designed to detect and quantify HIV-1 RNA in seminal plasma, panel B to detect HIV particles that may remain adsorbed onto spermatozoa and panel C to detect HIV-infected cells remaining among processed spermatozoa. Panel C could have been used to detect RNA, but would not have permitted evaluation of assay sensitivity because the number of HIV RNA copies/infected cell was unknown.

The technical protocols used to detect and/or quantify HIV-1 in semen varied considerably and were all different. This was due mainly to the way in which the assays were developed from assays used to detect/quantify HIV-1 in blood, the availability of techniques and the laboratory expertise. Home-made tests seemed to be less practical for routine use than commercially available assay adapted to semen. Previous versions of commercial kits have already been tested on seminal plasma [Dyer et al., 1996; Shepard et al., 2000; Dunne

TABLE III. Results of HIV-1 RNA Quantification in Seminal Plasma Panel (Panel A)

Target value	Center										
RNA copies/ml	1	2	3	4	5	6	7	8	9	10	11
0	<200	<200	<50	<200	<200	113	<100	268	<200	<100	<40
0	<200	<200	<50	<200	<200	277	<100	<200	<200	<100	<40
50	<200	100	33	<200	<200	123	<100	646	<200	102	+
100	<200	200	54	<200	53	48	300	<200	<200	57	+
500	142	400	519	639	64	ini	<100	787	<200	440	nt
1,000	1,355	600	517	255	88	134	1,500	831	577	953	+

nt, not tested; ini, inhibitors detected.

et al., 2003] or diluted 1:1 seminal plasma panels [Fiscus et al., 2000]; they showed the presence of some false positive and negative results.

The assays tested in the present study also produced false positive results. This is a well-known caveat of PCR, particularly when the PCR assay used has a very low detection limit. False positive results have been suspected by some [Quayle et al., 1998] and proven by others [Fiscus et al., 2000]. They may explain, at least partly, detection observed when testing highly purified motile spermatozoa obtained using both density gradient and swim-up [Marina et al., 1998; Meseguer et al., 2002]. False positive results can increase control tests, the number of semen samples required, cost and the complexity of medically assisted procreation management.

By contrast, false negative results can lead to safety problems. The use of internal controls can help to detect any PCR inhibitors in seminal plasma. They are not included systematically in home-made techniques that rely only on the efficacy of extraction to avoid potential inhibitors. The results of center 9 for the RNA panels may thus be due to PCR inhibitors or insufficient sensitivity. Extraction technique using silica or resin beads are therefore preferred because of better washing. PCR inhibitors were detected by only one center in this study. This center was also one of the centers not using silica for RNA extraction, although this gives better overall results, as shown for HCV detection in semen [Bourlet et al., 2003]. All the assays assessed on semen had similar sensitivities to those obtained with blood plasma or cells.

The safety of prepared semen from HIV infected men for use in AMP is checked in two ways: (1) By detecting HIV in the prepared spermatozoa, this will always be restricted by the limit of detection of the assay used and (2) by quantifying HIV in semen before processing to ensure that it is not too high for effective sperm washing. Studies on semen spiked with HIV-1 have shown that HIV cannot be detected in spermatozoa fractions when the semen contain less than 10<sup>6</sup> HIV RNA copies before processing, and thus by whatever the sperm washing technique used [Politch et al., 2004]. This artificial HIV load is much higher than those usually found in the semen (5,000–15,000 copies/ml) of untreated men [Vernazza et al., 1997]. These investigators found no HIV genome in the semen of HIV-infected men after its washing by density gradient centrifugation and swim-up [Hanabusa et al., 2000], even when the virus load was high [Kim et al., 1999; Bujan et al., 2002; Bujan et al., 2004a]. The processing used in this trial thus effectively removed HIV. HIV genome was detected rarely in the prepared spermatozoa, although it is impossible to guarantee that all HIV particles have been removed because of the detection threshold. Ultra-sensitive PCR could be useful [Meseguer et al., 2002], but this technique is time consuming and needs large volumes of sample, which is often incompatible with the subsequent medically assisted procreation techniques. This is why no medically assisted procreation can be performed in France if the HIV virus load of the seminal plasma is greater than 10,000 RNA copies/ml. This threshold was determined using sperm washing by density gradient

TABLE IV. Results of HIV-1 RNA Quantification in Seminal Cells Panel (Panel B)

Target value	Center										
RNA copies/ 10 <sup>6</sup> cells	1	2	3	5	6	7	9	10	11		
0	<200	<200	6	<200	ini	<100	<200	<100	+		
0	<200	<200	<50	<200	58	<100	<200	<100	<40		
5	<200	<200	131	11	<200	680	<200	16	+		
10	85	33	179	7	ini	<100	<200	14	+		
50	70	190	1467	114	131	<100	<200	56	+		
500	2,580	2,060	1,288	515	ini	12,000	<200	800	+		

nt, not tested; ini, inhibitors detection.

TABLE V. Results of HIV-1 DNA Quantification in Seminal Cells Panel (Panel C)

Target value	Center							
	3	4	5	6	8	9	10	11
DNA copies/10 <sup>6</sup> cells								
0	–	–	5	–	–	–	20	–
0	–	–	21	–	–	–	–	–
5	–	–	–	–	–	–	80	–
10	–	–	–	–	–	+	–	–
50	+	+	10	–	+	+	10	+
500	+	+	456	+	+	+	700	+

nt, not tested.

centrifugation alone [Leruez-Ville et al., 2002]. Apart from measures to check that the processed spermatozoa are HIV-free, basic hygiene and safety rules must be respected, particularly during semen sampling, processing and freezing to avoid contaminating other semen samples or the manipulators. Certain medically assisted procreation techniques may also reduce the risk by requiring fewer spermatozoa.

It was found that the performance of the HIV detection assays used before medically assisted procreation varied greatly. The use of silica-based nucleic acid extraction seems to enhance HIV detection, as HCV. The inclusion of an internal control to detect PCR inhibitors does not seem to increase significantly the sensitivity of the assays tested, but is probably a safe and reassuring practice. We have assessed only the basic performance of assays. A future study should assess a combination of assays regarding the algorithm used by each laboratory. For example, semen fraction may be tested using only one or two assays, on only one semen fraction or more. The overall safety of the procedure may then be difficult to evaluate further; factors such as cost and feasibility of assays are also important. Lastly, a commercial kit or a consensus protocol could be a great help in providing uniform tests performances on semen. This study is the first quality control evaluation organized for detecting HIV nucleic acid in semen fractions before medically assisted procreation. Regular quality control will help laboratories to improve their practice.

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## REFERENCES

- Al-Khan A, Colon J, Palta V, Bardeguet A. 2003. Assisted reproductive technology for men and women infected with human immunodeficiency virus type 1. *Clin Infect Dis* 36:195–200.
- Bourlet T, Levy R, Laporte S, Blachier S, Bocket L, Cassuto G, Chollet L, Leruez-Ville M, Maertens A, Mousnier F, Pasquier C, Payan C, Pellegrin B, Schvoerer E, Zavadzki P, Chouteau J, Duverlie G, Izopet J, Lunel-Fabiani F, Pawlotsky JM, Profizi N, Rouzioux C, Stoll-Keller F, Thibault V, Watre P, Pozzetto B. 2003. Multicenter quality control for the detection of hepatitis C virus RNA in seminal plasma specimens. *J Clin Microbiol* 41:789–793.
- Bujan L, Daudin M, Alvarez M, Massip P, Puel J, Pasquier C. 2002. Intermittent human immunodeficiency type 1 virus (HIV-1) shedding in semen and efficiency of sperm processing despite high seminal HIV-1 RNA levels. *Fertil Steril* 78:1321–1323.
- Bujan L, Daudin M, Matsuda T, Righi L, Thauvin L, Berges L, Izopet J, Berrebi A, Massip P, Pasquier C. 2004a. Factors of intermittent HIV-1 excretion in semen and efficiency of sperm processing in obtaining spermatozoa without HIV-1 genomes. *AIDS* 18:757–766.
- Bujan L, Pasquier C, Labeyrie E, Lanusse-Crousse P, Morucci M, Daudin M. 2004b. Insemination with isolated and virologically tested spermatozoa is a safe way for human immunodeficiency type 1 virus-serodiscordant couples with an infected male partner to have a child. *Fertil Steril* 82:857–862.
- Dunne AL, Mitchell F, Allen KM, Baker HW, Garland S, Clarke GN, Mijch A, Crowe SM. 2003. Analysis of HIV-1 viral load in seminal plasma samples. *J Clin Virol* 26:239–245.
- Dyer JR, Gilliam BL, Eron JJ, Jr., Grosso L, Cohen MS, Fiscus SA. 1996. Quantitation of human immunodeficiency virus type 1 RNA in cell free seminal plasma: Comparison of NASBA with Amplicor reverse transcription-PCR amplification and correlation with quantitative culture. *J Virol Methods* 60:161–170.
- Fiscus SA, Brambilla D, Coombs RW, Yen-Lieberman B, Bremer J, Kovacs A, Rasheed S, Vahey M, Schutzbank T, Reichelderfer PS, Group A. 2000. Multicenter evaluation of methods to quantitate human immunodeficiency virus type 1 RNA in seminal plasma. *J Clin Microbiol* 38:2348–2353.
- Hanabusa H, Kuji N, Kato S, Tagami H, Kaneko S, Tanaka H, Yoshimura Y. 2000. An evaluation of semen processing methods for eliminating HIV-1. *AIDS* 14:1611–1616.
- Kim LU, Johnson MR, Barton S, Nelson MR, Sontag G, Smith JR, Gotch FM, Gilmour JW. 1999. Evaluation of sperm washing as a potential method of reducing HIV transmission in HIV-discordant couples wishing to have children. *AIDS* 13:645–651.
- Leruez-Ville M, de Almeida M, Tachet A, Dulioust E, Guibert J, Mandelbrot L, Salmon D, Jouannet P, Rouzioux C. 2002. Assisted reproduction in HIV-1-serodifferent couples: The need for viral validation of processed semen. *AIDS* 16:2267–2273.
- Marina S, Marina F, Alcolea R, Exposito R, Huguet J, Nadal J, Verges A. 1998. Human immunodeficiency virus type 1-serodiscordant couples can bear healthy children after undergoing intrauterine insemination. *Fertil Steril* 70:35–39.
- Meseguer M, Garrido N, Gimeno C, Remohi J, Simon C, Pellicer A. 2002. Comparison of polymerase chain reaction-dependent methods for determining the presence of human immunodeficiency virus and hepatitis C virus in washed sperm. *Fertil Steril* 78:1199–1202.
- Pasquier C, Daudin M, Righi L, Berges L, Thauvin L, Berrebi A, Massip P, Puel J, Bujan L, Izopet J. 2000. Sperm washing and virus nucleic acid detection to reduce HIV and hepatitis C virus transmission in serodiscordant couples wishing to have children. *AIDS* 14:2093–2099.
- Politch JA, Xu C, Tucker L, Anderson DJ. 2004. Separation of human immunodeficiency virus type 1 from motile sperm by the double tube gradient method versus other methods. *Fertil Steril* 81:440–447.
- Quayle AJ, Xu C, Mayer KH, Anderson DJ. 1997. T lymphocytes and macrophages, but not motile spermatozoa, are a significant source of human immunodeficiency virus in semen. *J Infect Dis* 176:960–968.
- Quayle AJ, Xu C, Tucker L, Anderson DJ. 1998. The case against an association between HIV-1 and sperm: Molecular evidence. *J Reprod Immunol* 41:127–136.
- Semprini AE. 1993. Insemination of HIV-negative women with processed semen of HIV-positive partners. *Lancet* 341:1343–1344.
- Semprini AE, Fiore S. 2004. HIV and reproduction. *Curr Opin Obstet Gynecol* 16:257–262.
- Shepard RN, Schock J, Robertson K, Shugars DC, Dyer J, Vernazza P, Hall C, Cohen MS, Fiscus SA. 2000. Quantitation of human immunodeficiency virus type 1 RNA in different biological compartments. *J Clin Microbiol* 38:1414–1418.
- Tachet A, Dulioust E, Salmon D, De Almeida M, Rivalland S, Finkielstzajn L, Heard I, Jouannet P, Sicard D, Rouzioux C. 1999. Detection and quantification of HIV-1 in semen: Identification of a subpopulation of men at high potential risk of viral sexual transmission. *AIDS* 13:823–831.
- Vernazza PL, Gilliam BL, Dyer J, Fiscus SA, Eron JJ, Frank AC, Cohen MS. 1997. Quantification of HIV in semen: Correlation with antiviral treatment and immune status. *AIDS* 11:987–993.