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Assisted reproduction techniques for HIV serodiscordant couples: 18 months of experience

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BACKGROUND: Assisted reproduction techniques can minimize the risk of infection and treat possible sterility associated with serodiscordant couples. METHODS: We assessed the efficacy of these techniques in 57 couples in which at least one partner had human immunodeficiency virus (HIV-1) infection that was currently under control (47 men and 10 women). The semen of seropositive men was prepared and tested for viruses. Assisted reproduction techniques included intrauterine insemination (IUI), IVF and especially ICSI, with ovarian stimulation that used a long agonist protocol and recombinant FSH. Embryos were transferred on day 3 after oocyte retrieval. RESULTS: For couples with seropositive men, five IUI and 49 IVF or ICSI attempts were perfomed, whilst for seropositive women these numbers were three IUI and 12 IVF or ICSI. No pregnancy occurred following the eight IUI trials. Seroconversion was not observed in any partners of seropositive men. Efficacy of treatment for these couples with ICSI was good, the clinical pregnancy rate per embryo transfer was 48.8%. The results for seropositive women were disappointing, with a clinical pregnancy rate per embryo transfer of 9.1%. Fourteen babies from 47 treated couples have so far been born and no pregnancies from IUI. CONCLUSIONS: Assisted reproduction techniques and particularly ICSI provide HIV-1-seropositive men with a safe and highly effective means of fathering children. These techniques may be less effective for seropositive women.

Key words: assisted reproduction techniques/HIV/pregnancy

Introduction

The HIV epidemic currently affects 40 million people throughout the world. Of the young people who are infected, half are women. Antiretroviral medications have improved the health and life expectancy of seropositive patients, and these improvements enable them to consider having children. Nonetheless, the risk of transmitting the infection to their partner remains real. Assisted reproduction techniques, whether for a problem of sterility or to minimize the risk of virus transmission, allow couples serodiscordant for human immunodeficiency virus (HIV) to consider pregnancy by nearly eliminating the risk of infection to partner and child.

Our aim here is to present our results, in terms of pregnancy and risk of infection, of 18 months of assisted reproductive techniques indicated for HIV seropositivity. Two very different profiles emerged according to whether the man or woman was HIV^+.

History

After seeing women anxious to be pregnant who become infected with HIV through sexual activity, our team developed a multidisciplinary clinical research protocol in the year 2000, which was approved by the institutional Ethics Committee. Our experience began in January 2001 when we set up special areas in our laboratory to handle cases with viral risks. At that time, it was reserved for couples in which the man was infected by HIV. The decree of May 10, 2001 about assisted reproductive treatment of patients with viral risks (*Journal Officiel de la République Française*, May 15, 2001) made it possible to extend this treatment to serodiscordant couples in which the woman is HIV-infected and/or co-infected with hepatitis C virus (HCV) or hepatitis B virus (HBV).

Materials and methods

In compliance with the decree mentioned above, our inclusion criteria were very specific. Couples were to engage only in protected sexual

relations. Medical follow-up of the infection was essential, and the seropositive partner was required to see regularly a physician for this purpose. Control and Stability of the infection were measured by specific laboratory criteria: (i) a level of $CD4^+$ lymphocytes >200/mm^3 at least twice in the 4 months before treatment began; (ii) stable viral load, with no increase >0.5 log in two successive samples during the 4 months before treatment began; (iii) infection by an quantifiable amplifiable strain of HIV-1. The couple was interviewed by a psychiatrist or psychologist at inclusion and thereafter whenever necessary.

All patients provided written informed consent for assisted reproductive techniques. An additional consent form, specifically acknowledging comprehension of the viral risk involved, was signed by both members of the couple.

The assisted reproductive technology laboratory used for the procedure was considered a 'viral risk' area, geographically separated from the laboratory space used for the other couples who were negative for HIV and hepatitis B and C. All procedures were established in writing and validated by the hospital Hygiene Committee.

The assisted reproductive technologies laboratory follows recommended safety precautions (World Health Organization, 1999). Specific precautions were added against the risk of HIV, HCV and HBV contamination (Decree of May 10, 2001). General measures protected the staff (use of hand, face, and eye protection), and the potentially infected gametes and embryos were handled separately. A special biosafety cabinet workstation was used for all tasks that involved handling sperm, oocytes and embryos.

In all cases, a standard vaginal sample for bacteriological testing was taken several days before the assisted reproductive technique procedures. Semen was systematically cultured and treatment given for any infection by common bacteria, mycoplasma or chlamydiae.

The ovarian stimulation protocol was chosen according to the clinical data, the patients' hormone profile, and the result of any earlier stimulations. For IVF we used the standard long protocols. A GnRH analogue (Decapeptyl®; Ipsen, France, or Suprefact®; Aventis Pharma, Germany) was given s.c. each day. Recombinant FSH (Gonal F®; Ares-Serono, UK or Puregon®; Organon, France) was given together after desensitization. The GnRH analogue was continued up to the day that hCG 5000 IU (Gonadotrophine Chorionique Endo 5000®; Organon) was administered. Only a few follicles were stimulated for the intrauterine insemination; the pure protocol used recombinant gonadotrophin (Gonal F® or Puregon®) and induction by 5000 IU hCG.

Cycles were monitored with serial transvaginal ultrasonography and serum LH and estradiol (E_2) assays by the assisted reproductive technologies center nearest the couple's home. For IVF, oocytes were retrieved transvaginally under ultrasound guidance. The oocyte retrieval, embryo transfer and insemination procedures all took place at our centre. Supernumerary embryos on day 3 were frozen and thawed according to the standard technique involving 1,2-propanediol and sucrose as cryoprotectants (Testart *et al*., 1986).

The status of the partners of seropositive men was confirmed by HIV antibody testing and viral load measurements in the 2 weeks before and 2–3 weeks after each assisted reproductive technique attempt. These tests were repeated 3 and 6 months after assisted reproductive techniques and at any delivery.

The principle of this technique involved verification by virological testing that the sperm population had no detectable HIV-1 provirus or genome. Only such samples, negative for HIV-1 RNA and DNA, could be used.

Sperm preparation used two successive methods: a two-step 40–80% discontinuous gradient (PureSperm® 100; Nidacon International AB, Sweden) and the swim-up technique performed for 30–60 min at 37°C. At the end of the incubation period, the supernatant containing the motile sperm constituted the final fraction.

Table I. Assisted reproductive technique indicated according to HIV-1 level in sperm

HIV RNA (copies/ml) in seminal plasma	Proviral DNA and HIV RNA in final fraction of sperm	Assisted reproductive technique to use
<1000	Undetectable	IUI, IVF or ICSI
Between 1000 and 10 000	Undetectable	ICSI
>10 000	–	None

A modified Amplicor HIV-1 Monitor v1.5 assay (Roche Diagnostic Systems, France) was used to detect HIV-1 RNA in seminal plasma or RNA and DNA in seminal cells. The internal quality standard (IQS) was added to 500 µl of seminal plasma or to frozen pellets of 2×10^6 cells before acid nucleic extraction. Extraction was performed using Nuclisens NASBA Diagnostics extraction kit (Organon Teknika SA, France). All extracted samples were then processed using the ultrasensitive Monitor assay protocol. One negative and three positives controls were included in each run. The assay detection limit was 50 RNA copies/ml in seminal plasma and 50 copies of RNA or DNA in 1×10^6 seminal cells.

In compliance with the May 10, 2001 decree, the results of the virologic testing of the seminal plasma and the final fraction determined the choice of assisted reproductive technologies technique (Table I).

For men co-infected with HCV and HIV, the seminal plasma and the final fraction of seminal cells were also tested for HCV RNA. The Cobas Amplicor HCV kit (Roche Diagnostic Systems, France) was used to detect HCV RNA. The internal control (IC) was added to 200 µl seminal plasma or to 2×10^6 sperm before HCV RNA extraction. RNA was extracted with the Nuclisens kit, as recommended by the manufacturer before being processed using the Amplicor assay protocol. The detection limit of the assay was 100 copies/ml in seminal plasma and 20 copies/2×10^6 seminal cells/2×10^6 sperm. Assisted reproductive techniques did not proceed when the final fraction was positive.

Laboratory constraints made it impossible to obtain the virological results for fresh sperm during the same day. The final fraction was therefore cryopreserved for subsequent use. The sperm preparation was diluted drop by drop with the freezing medium (Spermfreeze; Irvine Scientific®, USA) was aliquoted in 0.25 ml CBS high-security straws™ (Cryo Bio System, Group IMV Technologies, France). A programmable freezer was used to control the cooling rate. The samples were then taken to –196°C by plunging them directly into the liquid nitrogen. The straws were then stored in separate tanks dedicated to gametes with viral risks.

To thaw the sperm, the straws were taken out of the liquid nitrogen tank and warmed slowly at room temperature. After washing with culture medium, the contents were then expelled into a centrifuge tube, gently diluted with 2 volumes of culture medium and centrifuged at 600 *g* for 10 min. The pellet was resuspended in 0.3 ml of culture medium, and the concentration and motility of the thawed sperm were assessed in a Makler chamber.

When the woman was seropositive for HIV, HCV and/or HBV, the follicular fluid was handled in the at-risk area.

Statistical analysis was performed according to the Mann–Whitney *U*-test.

Results

From January 1, 2001 to July 30, 2002, 101 couples with at least one partner seropositive for HIV requested assisted

Table II. Immuno-virological characteristics of couples included

	Man HIV+	Woman HIV+
No. of patients	47	10
Immuno-virological examinations	50	16
Plasma HIV viral load (VL) at initial record with VL <50 copies/ml (%)	31 (62)	8 (50)
Median VL if ⩾50 copies/ml (range)	645 (50–29 100)	1155 (98–32 000)
Plasma HIV viral load at second record with VL <50 copies/ml (%)	35 (70)	8 (50)
Median VL if ⩾50 copies/ml (range)	355 (50–52 500)	740 (200–33 000)
Median CD4+ count at initial record $\times 10^6$/l (range)	508 (214–1346)	430 (210–648)
Median CD4+ count at second record $\times 10^6$/l (range)	570 (212–1557)	438 (295–653)
HIV-1 viral load in seminal plasma with VL <50 copies/ml (%)	39 (79.6)	
Median VL if ⩾50 copies/ml (range)	209 (51–2630)	
HIV-1 final fraction <50 copies/ 1×10^6 sperm (%)	50 (100)	
Patients co-infected with HCV (%)	13 (27.7)	1 (10)
HCV viral load in semen		
Positive (%)	2 (13.3)	
Uninterpretable (%)	2 (13.3)	
HCV final fraction <5020 copies/2×10^6 sperm (%)	15 (100)	
Patients co-infected with HBV (%)	2 (13.3)	0

reproductive techniques at our centre. These included 81 cases in which the man was HIV+; in 22, he was co-infected with HCV and in two with HBV. There were also 18 cases of women with HIV, one co-infected with HCV. These 18 couples had infertility problems as many self-inseminations had already failed. Finally both members of two couples were HIV+, with one of the men also co-infected with HCV.

Seven couples were determined to be ineligible: immuno-virological stability not obtained (three men and one woman), inadequate sperm semen quality with $<2\times 10^6$ motile sperm recovered (two cases), not amplifiable HIV-2 (one man). Fifty-seven couples were included (47 infected men and 10 women). The 37 remaining couples were still in the process of being treated at the time of writing.

The immuno-virological characteristics of the 57 couples included are summarized in Table II.

Group of couples with HIV-1 infected male

Forty-two men were receiving highly active antiretroviral therapy (HAART). Two men were receiving dual antiretroviral therapy and three men were receiving none. Fifty semen samples have been analysed for 47 patients. Repetition of assisted reproductive technology attempts required the constitution of a new batch of semen straws for three patients. Partners of seropositive men, aged (mean ± SD) 32.7 ± 4.5 years showed a mean FSH level of 7.0 ± 2.9 IU/l. Table III summarizes the characteristics of the assisted reproductive technique attempts. Thirty-nine couples were treated through 54 assisted reproductive technique attempts. Fourteen children were already born for 12 couples so 30.8% of the treated couples became parents.

No seroconversion has occurred from the use of sperm we tested.

Group of couples with HIV-1 infected female

At inclusion, seven women were receiving HAART and three no antiretroviral therapy at all. After consultation with virus disease specialists our assisted reproductive technique team decided to continue HAART for four women (seven attempts) and to stop it for the other three (five attempts). The treatment was resumed at the 21st week of pregnancy for the one who became pregnant; treatment was resumed for the second woman after four attempts failed, and was not resumed for the third.

Comparison between FSH level of seropositive women and partners of seropositive men showed a significant difference (9.0 ± 2.4 and 7.0 ± 2.9 IU/l respectively, $P < 0.001$). Seropositive women were significantly older than partners of seropositive men (35.9 ± 4.1 and 32.7 ± 4.5 years respectively, $P < 0.001$). Nine couples were treated through 15 assisted reproductive technique attempts. One pregnancy occurred which was still ongoing.

Discussion

Legitimacy of the desire for pregnancy

More than half the people infected by HIV are of child-bearing age. The progress of treatments has substantially modified the prognosis for infected patients; the infection often does not progress and can be considered to be chronic (Anderson, 1999).

The increase in heterosexual transmission has led to the infection of young women from every socioeconomic category. At the same time, the reduction in viral load before delivery has considerably diminished the risk of maternal–fetal transmission in the industrialized countries. Among patients who receive good follow-up and treatment and who deliver by Caesarean section, this frequency is <2% (Mandelbrot *et al.*, 1998).

Patients can thus make life plans and even envisage having children. This pressing desire for children is legitimate and any other conclusion would discriminate against these patients. The fight against discrimination, social, occupational or familial, has from the beginning of the epidemic been a priority for the National Ethics Committee for the Life Sciences and Health (CCNE) and for the National AIDS Council (CNS) (opinion dated February 10, 1998). A cultural revolution is taking place, especially in the medical profession (Balet *et al.*, 1998).

Table III. Assisted reproductive technology features

	Man HIV+	Woman HIV+
Women's age (years, mean ± SD)	32.7 ± 4.5	35.9 ± 4.1
Basal FSH in women (IU/l, mean ± SD)	7.0 ± 2.9	9.0 ± 2.4
Intrauterine inseminations	5	3
Sperm inseminated ($\times 10^6$/ml, mean ± SD)	1.3 ± 1.0	8.9 ± 2.2
Pregnancy after IUI	0	0
IVF or ICSI	49	12
Pregnancy after IVF and ICSI	20	1
Pregnancy rate/embryo transfer (%)	48.8	9.1
Duration of stimulation (days, mean ± SD)	11.5 ± 1.4	12.8 ± 1.8
Gonadotrophin dose (IU, mean ± SD)	1972 ± 885	2893 ± 1098
Oocytes/retrieval (mean ± SD)	9.0 ± 5.5	8.6 ± 4.5
Metaphase II oocytes/retrieval (mean ± SD)	6.4 ± 3.9	6.5 ± 3.2
Fertilization rate (%)	60.4	71.4
Embryos transferred (mean ± SD)	1.7 ± 0.5	1.9 ± 0.6
Pregnancy rate/assisted reproductive technology attempt (%)	37.0	6.7
Multiple pregnancy rate (%)	15	0
Transfers after thawed embryos	3	0
Pregnancy with thawed embryos	0	0
Children born	14	0

Legitimacy of assisted reproductive technologies

Antiretroviral treatments help significantly in reducing the viral load in semen but it is far from clear that undetectable levels can be reached (Zhang *et al*., 1998). In patients receiving treatment who have an undetectable plasma viral load, the frequency of viral RNA detection in seminal fluid is reported to be 2% (Vernazza *et al*., 1998). HIV levels in blood and semen are not well correlated (Kim *et al*., 1999). Decontamination of the sperm and isolation of the sperm from the seminal fluid are therefore essential and thus legitimize assisted reproductive technologies. Moreover, no intracellular penetration by the virus in the separated sperm have been reported in seropositive patients receiving treatment (Pudney *et al*., 1998). Sperm do not express significant levels of HIV receptors, so they are unlikely to be major targets for HIV infection (Kim *et al*., 1999). Isolation of the sperm from the seminal fluid is therefore essential and thus legitimizes assisted reproductive technologies which use only the final fraction.

The balance between the importance of the message of prevention and help for patients who want a child has tilted towards medical intervention (Englert *et al*., 2001). Assisted reproduction should be adopted as public policy in this situation (Drapkin Lyerly *et al*., 2001). Clear legal changes in France have translated this conclusion into rights.

HIV+ women are reported to have a frequency of upper genital tract infections and their sequelae 10-fold greater than the general population (Sobel, 2000). Physicians are thus often asked to treat tubal infertility in seropositive women. Assisted reproductive technologies also allow the treatment of possible male sterility at the same time as it prevents transmission to the partner at conception.

Choice of technique

The choice of technique depended on the overall medical picture, as it does for all couples in assisted reproductive technologies. Nonetheless because of the viral specificity of these couples specificity of the indication, other considerations played a role.

For seropositive men, the decree dated May 10, 2001 requires that sperm be prepared whenever possible with two successive techniques (density gradient, then swim-up), which results in use of most if not all of the sperm in the ejaculate. Adequate testing requires $\geqslant 2\times 10^6$ sperm in the final fraction. Below this threshold, even ICSI cannot be proposed. This was initially a problem for several patients in our centre but, except for two, was most often transient or managed by pooling two ejaculates produced the same day. The seminal plasma and final fraction were tested virologically and the quantity of virus found in seminal plasma also determined the type of assisted reproductive techniques (Table I).

Because virological findings could not be obtained the same day for fresh sperm in our laboratory, they therefore had to be frozen in straws. This combination of constraints as well as the distance from home of some couples had to be considered. ICSI rapidly became the favoured method, not for safety reasons but because freezing dramatically decreases the number of available motile sperm as already reported (Leruez-Ville *et al*., 2002). Despite the high cost of the ICSI process, the efficiency of this technique avoids the expensive repetitive virological semen testing and numerous cycles of ovarian stimulation.

For seropositive women, IUI was initially considered possible in the absence of severe infertility factors but was rapidly replaced by IVF to optimize the probability of success and diminish the number of attempts necessary. When interruption of the antiretroviral treatment was recommended because of the planned pregnancy, we used ICSI to avoid unexpected fertilization failure and thus to reduce the delay of conception. For one patient with Von Willebrand's disease, insemination was proposed to avoid the possible risk of haemorrhage associated with the oocyte retrieval.

Virological aspects

In this study, the sperm preparation procedure effectively led to an undetectable HIV-1 genome in the semen of seropositive

men. Virus was detected in the seminal fluid of 20.4% of the sperm samples tested but no final fraction was positive for HIV. The infection of the men under treatment was well controlled. Moreover, although viral levels in sperm could have been increased by genital tract infection, the systematic sperm cultures and the treatment of possible infections enabled control of this additional risk. No seminal fluid was infected to the point of having a viral load >10 000 copies/ml, the threshold that would have excluded assisted reproductive techniques (Table I). A study of 32 clinically asymptomatic men (Pasquier *et al*., 2000) found similar results: HIV-1 RNA was detected in 30% of the seminal plasma samples, HIV-1 genomes were found in 18% of the semen samples, but in none of the motile sperm after the density gradient and swim-up. On the other hand, Marina *et al*. (1998) had to eliminate 5.6% of the final fractions tested because of detectable viral loads in a cohort of 63 seropositive patients.

None of these studies, as well as others performed using IUI, IVF or ICSI (Semprini *et al*., 1997; Weigel *et al*., 2001; Sauer *et al*., 2002) or our study reported any cases of seroconversion in either mother or child. Assisted reproductive techniques therefore appear reliable and safe.

Efficacy of assisted reproductive technologies

For the men with HIV infection, the ANRS 096 (Agence Nationale de Recherche sur le SIDA) protocol used IUI, with sperm prepared by two successive techniques (Daudin *et al*., 2001). A total of 174 cycles of insemination in 54 couples led to 31 pregnancies and an 18% pregnancy rate per cycle. Half the pregnancies occurred during the first two cycles.

A European experience of >2000 inseminations with washed and tested sperm has been reported (Gilling-Smith, 2000). The pregnancy rate per insemination was 14%. Our centre has minimal experience (five attempts without pregnancy) with IUI.

For the men with HIV infection, our results for IVF/ICSI were encouraging: the clinical pregnancy rate per embryo transfer was 48.8%. These results are similar to those from other protocols. The French ANRS 092 protocol concerned a pilot study where IVF/ICSI was chosen for its efficacy and to prevent contact between the female genital tract and any sperm (Guibert *et al*., 2001). In 97 cycles of assisted reproductive techniques, 34 pregnancies occurred giving a 35.1% pregnancy rate and 22.7% delivery rate per oocyte retrieval. Seven additional pregnancies occurred after the transfer of frozen embryos, thereby raising the pregnancy rate per transfer by 11%. Overall, 39.7% of the couples gave birth.

Another study used ICSI for 34 serodiscordant couples in which the man was HIV-positive (Sauer *et al*., 2002). Treatment efficacy was good, in terms of oocytes retrieved (15.8 ± 1.3), fertilization rate (64.9%), clinical pregnancies per embryo transfer (45.5%) and ongoing or delivered pregnancies per embryo transfer (30.9%). A recent study has presented preliminary data for 36 couples treated by ICSI for male HIV infection and poor semen quality (Semprini *et al*., 2002). Mean number of oocytes collected per retrieval was 9.2 (SD: 5.49) and pregnancy rate per retrieval was 53.5%. Possible explanations for this excellent performance include the absence of a clinical indication for ICSI and especially the absence of infertility among the female partners.

Little work has been done with assisted reproductive techniques in seropositive women. The clinical and biological course of HIV disease during pregnancy is nonetheless well known. In asymptomatic women whose infection is well controlled, pregnancy does not aggravate HIV disease (Weisser *et al*., 1998; Saada *et al*., 2000). In the industrialized countries, prospective studies confirm the efficacy of HAART in preventing mother–child transmission (Mandelbrot *et al*., 2001; Cooper *et al*., 2002). Knowing that this transmission occurs late in pregnancy due to fetal–maternal exchanges at the end of pregnancy and especially during labour (Rouzioux *et al*., 1995), here assisted reproductive techniques should cause no harm at all.

Couples in which the woman is seropositive have often attempted self-insemination at home, before turning to assisted reproductive techniques (Delfraissy *et al*., 2002). The sterility factor is predominant here, unlike in the couples with HIV^+ men. The assisted reproductive technique results are accordingly more disappointing.

Moreover, the seropositive women seeking assisted reproductive techniques were significantly older than the partners of the seropositive men. Some patients had waited a long time for this treatment made possible by medical progress and by the recent legislative changes in France. Their hormone levels, with elevated FSH, confirm the trend towards ovarian depletion. The respective impacts on ovarian function of both infection and HAART are still not well-known. Some studies suggested a negative effect of the infection on menstrual cycle length (Harlow *et al*., 2000) and on ovulation (Clark *et al*., 2001).

In conclusion, treatment progress and clear changes in social attitudes now make it possible for assisted reproductive techniques to be offered to HIV serodiscordant couples. Assisted reproductive techniques are performed with the double benefit of controlling the risk of viral transmission and of treating possible sterility. Our results suggest that screening and washing sperm are a safe risk-reduction option for couples who want to conceive. Assisted reproductive techniques are efficacious for HIV^+ men, when their primary indication is the presence of the virus. The pregnancy rates were not as good among the seropositive women, who were often older and had other infertility problems.

References

Anderson, D.J. (1999) Assisted reproduction for couples infected with the human immunodeficiency virus type 1. *Fertil. Steril.*, **72**, 592–594.

Balet, R., Lower, A.M., Wilson, C., Anderson, J. and Grudzinskas, J.G. (1998) Attitudes towards routine human immunodeficiency virus (HIV) screening and fertility treatment in HIV positive patients—a UK survey. *Hum. Reprod.*, **13**, 1085–1087.

Clark, R.A., Mulligan, K., Stamenovic, E., Chang, B., Watts, H., Andersen, J., Squires, K. and Benson, C. (2001) Frequency of anovulation and early menopause among women enrolled in selected adult AIDS clinical trials group studies. *J. Infect. Dis.*, **184**, 1325–1327.

Cooper, E., Charurat, M., Mofenson, L., Hanson, C., Pitt, J., Diaz, C., Hayani, K., Handelsman, E., Smeriglio, V. and Hoff, R. *et al*. (2002) Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission. *J. AIDS*, **29**, 484–494.

Daudin, M., Pasquier, C., Izopet, J., Kallman, M., Lachendowier, C., Lanusse, P., Morucci, M., Mercadier, B., Labeyrie, E. and Seguela, G. et al. (2001) Le protocole ANRS 096: prise en charge en assistance médicale à la procréation des couples sérodifférents dont l'homme est infecté par le VIH. Résultats préliminaires de Toulouse. *Reprod. Hum. Horm.*, **14**, 365–369.

Delfraissy, J.F., Rouzioux, C, Bujan, L., Faucher, J.M., Heard, I., Jouannet, P., Le Mercier, Y., Leruez, M., Meier, A. and Prestel, T. (2002) Désir d'enfant et assistance médicale à la procréation. In *Prise en charge thérapeutique des personnes infectées par le VIH. Paris Rapport 2000. Ministère de l'emploi et de la solidarité, Secrétariat d'Etat à la santé et à l'action sociale*. Médecine-Sciences, Flammarion, p. 282.

Drapkin Lyerly, A. and Anderson, J. (2001) Human immunodeficiency virus and assisted reproduction: reconsidering evidence, reframing ethics. *Fertil. Steril.*, **75**, 843–858.

Englert, Y., Van Vooren, J.P., Place, I., Liesnard, C., Laruelle, C. and Delbaere, A. (2001) ART in HIV-infected couples: has the time come for a change of attitude? *Hum. Reprod.*, **16**, 1309–1315.

Gilling-Smith, C. (2000) HIV prevention. Assisted reproduction in HIV-discordant couples. *AIDS Read*, **10**, 581–587.

Guibert, J., Merlet, F., Le Dû, A., Mandelbrot, L., Leruez-Ville, M., Costagliola, D., Kunstmann, J.M., De Almeida, M., Heard, I. and Dulioust, E. et al. (2001) Prise en charge des couples séro-différents pour le VIH. Résultats du protocole NECO (ANRS 092) à Paris. *Reprod. Hum. Horm.*, **14**, 363–364.

Harlow, S., Schuman, P., Cohen, M., Ohmit, S., Cu-Uvin, S., Lin, X., Anastos, K., Burns, D., Greenblatt, R. and Minkoff, H. et al. (2000) Effect of HIV infection on menstrual cycle length. *J. AIDS*, **24**, 68–75.

Kim, L.U., Johnson, M.R., Barton, S., Nelson, M.R., Sontag, G., Smith, J.R., Gotch, F.M. and Gilmour, J.W. (1999) Evaluation of spermatozoa washing as a potential method of reducing HIV transmission in HIV-discordant couples. *AIDS*, **13**, 645–651.

Leruez-Ville, M., de Almeida, M., Tachet, A., Dulioust, E., Guibert, J., Mandelbrot, L., Salmon, D., Jouannet, P. and Rouzioux, C. (2002) Assisted reproduction in HIV-1-serodifferent couples: the need for viral validation of processed semen. *AIDS*, **16**, 2267–2273.

Mandelbrot, L., Le Chenadec, J., Berrebi, A., Bongain, A., Benifla, J.L., Delfraissy, J.F., Blanche, S. and Mayaux, M.J. (1998) Perinatal HIV-1 transmission: interaction between zidovudine prophylaxis and mode of delivery in the French Perinatal Cohort. *J. Am. Med. Assoc.*, **280**, 55–60.

Mandelbrot, L., Landreau-Mascaro, A., Rekacewicz, C., Berrebi, A., Benifla, J.L., Burgard, M., Lachassine, E., Barret, B., Chaix, M.L. and Bongain, A. (2001) Lamivudine–zidovudine combination for prevention of maternal–infant transmission of HIV-1. *J. Am. Med. Assoc.*, **285**, 2083–2093.

Marina, S., Marina, F., Alcolea, R., Exposito, R., Huguet, J., Nadal, J. and Verges, A. (1998) Human immunodeficiency virus type 1-serodiscordant couples can bear healthy children after undergoing intrauterine insemination. *Fertil. Steril.*, **70**, 35–39.

Pasquier, C., Daudin, M., Righi, L., Berges, L., Thauvin, L., Berrebi, A., Massip, P., Puel, J., Bujan, L. and Izopet, J. (2000) Spermatozoa 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.

Pudney, J., Nguyen, H., Xu, C. and Anderson, D.J. (1998) Microscopic evidence against HIV-1 infection of germ cells or attachment to spermatozoa. *J. Reprod. Immunol.*, **41**, 301–306.

Rouzioux, C., Costagliola, D., Burgard, M., Blanche, S., Mayaux, M.J., Griscelli, C. and Valleron, A.J. (1995) Estimated timing of mother-to-child human immunodeficiency virus type I transmission by use of a Markov model. *Am. J. Epidemiol.*, **142**, 1330–1337.

Saada, M., Le Chenadec, J., Berrebi, A., Bongain, A., Delfraissy, J.F., Mayaux, M.J. and Meyer, L. (2000) Pregnancy and progression to AIDS: results of the French prospective cohorts. *AIDS*, **14**, 2355–2360.

Sauer, M.V. and Chang, P.L. (2002) Establishing a clinical program for human immunodeficiency virus 1-seropositive men to father seronegative children by means of in vitro fertilization with intracytoplasmic spermatozoa injection. *Am. J. Obstet. Gynecol.*, **186**, 627–633.

Semprini, A.E., Fiore, S. and Pardi, G. (1997) Reproductive counselling for HIV-discordant couples. *Lancet*, **349**, 850–851.

Semprini, A.E., Vucetich, A., Oneta, M., Rezek, D., Rubino, P., Scarselli, F. and Hollander, L.H. (2002) Spermatozoa washing and ICSI in HIV discordant couples: >50% pregnancy rate. *Hum. Reprod.*, **17** (Abstract Book 1), P-340.

Sobel, J.D. (2000) Gynecologic infections in human immunodeficiency virus-infected women. *CID*, **31**, 1225–33.

Testart, J., Lassalle, B., Belaisch-Allart, J., Hazout, A., Forman, R., Rainhorn, J.D. and Frydman, R. (1986) High pregnancy rate after early human embryo freezing. *Fertil. Steril.*, **46**, 268–272.

Vernazza, P.L., Troiani, L., Flepp, M., Cone, R., Shock, J. and Roth, F. (1998) Potent antiretroviral treatment (ART) results in marked suppression of seminal HIV-RNA and DNA sheddings. AIDS European Meeting. Glasgow (abstract). *Drug Ther. HIV Infect.*, **15**, 3–6.

Weigel, M.M., Gentili, M., Beichert, M., Friese, K. and Sonnenberg-Schwan, U. (2001) Reproductive assistance to HIV-discordant couples—the German approach. *Eur. J. Med. Res.*, **6**, 259–262.

Weisser, M., Rudin, C., Battegay, M., Pfluger, D. and Kully, C. (1998) Does pregnancy influence the course of HIV infection? Evidence from two large Swiss cohort studies. *J. Acquir. Immun. Defic. Syndr. Hum. Retrov.*, **17**, 404–410.

World Health Organization (1999) *WHO Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interaction*, 4th edn. Cambridge University Press, Cambridge.

Zhang, H., Dornadula, G., Beumont, M., Livornese, L., Van Uitert, B., Henning, K. and Pomerantz, R.J. (1998) Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. *N. Engl. J. Med.*, **339**, 1803–1809.

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