Human Reproduction Vol.22, No.3 pp. 772–777, 2007 doi:10.1093/humrep/del422
Advance Access publication November 15, 2006.

Safety of sperm washing and ART outcome in 741 HIV-1-serodiscordant couples

V.Savasi[1], E.Ferrazzi, C.Lanzani, M.Oneta, B.Parrilla and T.Persico

Department of Obstetrics and Gynaecology, Sacco Clinical Sciences Institute, University of Milan Medical School, Milan, Italy

[1]To whom correspondence should be addressed at: Department of Obstetrics and Gynaecology, Sacco Clinical Sciences Institute, University of Milan Medical School, Via GB Grassi 74, 20157 Milan, Italy. E-mail: valeria.savasi@unimi.it

BACKGROUND: To evaluate the safety of sperm washing and assisted reproduction technique (ART) outcome offered to serodiscordant couples with a human immunodeficiency virus-1 (HIV-1)-positive male. METHODS: Sperm washing was performed and checked by RT–PCR on each semen sample before its fresh usage. Intrauterine insemination (IUI) or IFV/ICSI was offered according to fertility profile of each couple. Non-infected women underwent HIV testing 2 weeks before each procedure and for up to 6 months after. RESULTS: Seven hundred and forty-one couples entered the study of a possible 2011 serodiscordant couples counselled over 4 years. Superovulation and IUI were performed in 581 couples, where the pregnancy rate per cycle and pregnancy rate per couple were 19 and 78%, respectively, with multiple pregnancy rate being 4%. One hundred and sixty couples were treated by IVF/ICSI, where pregnancy rate per cycle and per couple were 22 and 41%, respectively, with multiple pregnancy rate being 10%. All female partners were still HIV-1 negative at follow-up. CONCLUSION: Sperm washing within a programme of reproductive counselling was proved to be safe in this large series of serodiscordant couples. The overall pregnancy rate (70.3%), independent of the procedure used (IUI or IVF/ICSI), justifies the effort of the medical team in setting up and implementing dedicated centres and of the individual patient in seeking a safe pregnancy.

Key words: ART/HIV/serodiscordant couples/RT–PCR/treatment outcome

Introduction

Sperm-washing techniques have substantially changed the paradigm of fathering children in serodiscordant couples for male human immunodeficiency virus (HIV) infection. The clinical value of sperm washing and its negligible or null risk were first reported in 1992 (Semprini *et al*., 1992), and since then, it has been reinforced by different articles both on methodological (Marina *et al*., 1998; Anderson, 1999; Gilling-Smith, 2000) and on clinical issues (Sauer and Chang, 2002; Ohl *et al*., 2003; Pena *et al*., 2003; Bujan *et al*., 2004; Garrido *et al*., 2004; Nicopoullos *et al*., 2004; Mencaglia *et al*., 2005). Furthermore, an estimate of the use of sperm washing in Europe (Gilling-Smith, 2000; Savasi *et al*., 2006) provided additional evidence of the clinical value of this procedure. These results brought assisted reproduction into a new era from the report of the Centers for Disease Control (CDC, 1990). The seroconversion in a woman after an artificial intrauterine insemination (IUI) from her HIV-infected husband was reported, but indeed the semen was not processed to separate lymphocytes from spermatozoa. This unwanted outcome was later reported by Ann Duerr in her review to stress the importance of sperm washing (Duerr and Jamieson, 2003).

In 2002, the American College of Obstetricians and Gynecologists (ACOG Committee on Ethics, 2001) and the American Society for Reproductive Medicine (ASRM) (Ethics Committee of the ASRM, 2002) recommended sperm washing to be offered to HIV-serodiscordant couples as a standard of care. Sauer (2005) reviewed 3019 IUI cycles after sperm washing in 1111 serodiscordant couples with 361 babies born and 543 IVF/ICSI cycles in 352 serodiscordant couples with 131 babies born.

Dramatic changes in life expectancy and quality of life in HIV-infected patients are putting this procedure among the expected medical care for these patients in western countries, whereas biological, clinical and epidemiological issues are still under scrutiny. Although the popularity achieved by this methodology is wide, the large number of reported cycles is still the sum of either small series or larger ones on either IUI (Vernazza *et al*., 1997; Marina *et al*., 1998) or ICSI in different centres (Ohl *et al*., 2003; Pena *et al*., 2003). Although safety issues need large multicentre follow-up, biological and clinical problems could be addressed by large series. For instance, in some centres the post–sperm-washing control is not routinely used, whereas in others frozen sperm is used just to allow time for the PCR control. Alternatively, ICSI is often used to avoid sperm washing. Ovulation induction in couples undergoing IUI is not universally adopted—some centres recommend and perform ICSI in all male serodiscordant couples, including those

with proven fertile partners (Garrido and Meseguer, 2006) and so forth. Obviously, the best answer for each issue is not represented by a single option. However, safety, efficacy and cost efficiency must be considered to rank each one of them.

We report in this article our experience on a series of 741 serodiscordant couples treated in the last 4 years in one University Centre that provided an assisted reproduction programme with a thorough diagnostic triage and a therapeutical offer from IUI to IVF to ICSI, according to the fertility profile of each serodiscordant couple. All treatments were performed with fresh spermatozoa and checked using real-time PCR, after sperm washing.

Materials and methods

Assisted reproduction technology (ART) programme was offered to serodiscordant couples, with HIV-infected male partner, seeking medical assistance. Inclusion criteria were set to protect the couple and eventually the child; partners were to engage only in protected sexual acts. HIV infection was monitored and treated, and long-term compliance was assessed by the infectious disease physician. Standard laboratory criteria were adopted: (i) $CD4^+$ lymphocytes >200/mm^3 at least twice in the 4 months before treatment; (ii) stable viral load, with no increase >0.5 log in two successive samples during the 4 months before treatment; (iii) infection by a quantifiable amplifiable strain of HIV-1. Each couple was interviewed by a psychologist at inclusion and thereafter whenever necessary.

Both members of the couple signed an informed consent for ARTs, as required by national regulations, including items specifically addressing the risk of viral transmission to the female partner and its possible consequences.

Female fertility was assessed by standard procedures. Each woman underwent a gynaecological examination, a smear test, a vaginal sample for bacteriological testing, a cervical swab for chlamydia and mycoplasma and a vaginal ultrasound examination of the uterus and ovaries combined with a sonohysterography. Assessment of tubal patency was performed either by hysterosalpingogram or sonosalpingography. FSH, LH, thyroid-stimulating hormone (TSH) and estradiol (E_2) levels were determined from blood samples on day 3 of the menstrual cycles, with prolactin (PRL) and progesterone on days 22 and 24 of the same cycles. Laparoscopy was performed in selected patients with endometriosis or unexplained pelvic pain. Semen analysis and culture were systematically performed, and treatment was given for any infection by common bacteria, mycoplasma or chlamydia. Male partners were considered fertile when the total number of motile spermatozoa after capacitation was $>10^6$/ml.

The ART laboratory used for the procedure was considered a 'viral risk' area, separated from laboratory facilities used for couples negative for HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV). The ART laboratory complied with standard recommended safety precautions (World Health Organization, 1999). Specific precautions were implemented against the risk of HIV, HCV and HBV contamination as recommended by the French decree of 10 May 2001 (Journal Officiel de la République Française, 15 May 2001), 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.

Semen processing

Semen samples were obtained by masturbation after 4–7 days of sexual abstinence. After liquefaction at room temperature, semen parameters were assessed as outlined by the WHO criteria. Semen analyses were performed, and samples were processed using a 40–80% density gradient (Pureception kit, Sage) to separate motile spermatozoa from non-sperm cells, immotile spermatozoa and seminal plasma. The ejaculate was layered over the gradient and centrifuged at 400 *g* for 30 min. After centrifugation, the supernatant was removed, and the sperm pellet recovered and resuspended in 3 ml of fresh medium (Sperm-washing medium, Sage). A washing at 400 *g* for 10 min was performed, and the supernatant was discarded, 1 ml of medium was subsequently gently layered on the pellet, and the tube was incubated at 37°C for 1 h.

After swim-up, a supernatant volume of ~500 µl was recovered, and an aliquot of this volume (100 µl) was tested for detectable HIV RNA. Nucleic acid extraction and HIV-1 RNA quantification were performed using a real-time PCR assay (NucliSens EasyQ HIV-1 v1.1, bioMérieux sa. Marcy l'Etoile, France). The assay detection limit was 50 RNA copies/ml.

The remaining washed sperm (400 µl) was stored at 4°C for ~22 h and used for IUI and ICSI/IVF procedures, if the PCR test for HIV was negative.

IUI

Fertile couples were offered IUI with ovulation induction using gonadotrophins. A low dose (50–75 IU) (Gonal F®, Ares-Serono, UK or Puregon®, Organon, France) of recombinant FSH was given from day 3 to the day of ovulation induction (HCG 5000 IU) based on follicle ultrasound monitoring from day 8 to a dominant follicle of 18 mm mean diameter. Insemination was performed 36 h after HCG administration. For the IUI, we use only fresh sperm, with a PCR control of semen sample within 12 h.

IVF/ICSI

Ovarian stimulation protocols were chosen according to clinical data, patients' age and hormonal profile and the result of any previous stimulation. The standard long protocol was adopted for most IVF cycles. The 'short protocol' or 'flare-up protocol' was used for older or poor-responder patients. A GnRH analogue (Decapeptyl®, Ipsen, France; or Suprefact®, Aventis Pharma, Germany) was given s.c. each day. Recombinant FSH (Gonal F® or Puregon®) 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.

Cycles were monitored every other day by serial transvaginal sonography and E_2 assays. Serum LH was measured to improve ovulation induction timing. Oocytes were retrieved under ultrasound guidance by flushing ovarian follicles with ASP medium (Vitrolife), incubated in G-FERT medium (Vitrolife) and subsequently fertilized by conventional IVF or ICSI and cultured in G-1 medium (Vitrolife) until the day of transfer (day 2 or 3). 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). We have cryopreserved 51 embryos before the Italian act on March 2004, which banned embryo cryopreservation. After March 2004, and in accordance with Italian law, we fertilized three oocytes per cycle only after careful oocyte selection, whereas no embryo selection was allowed.

A sample of PCR-negative frozen semen was obtained in all couples treated by IVF/ICSI. This was used only in cases where fresh semen collected at the time of oocyte retrieval tested positive for HIV after sperm washing.

The status of the female partner was confirmed by HIV antibody testing and viral load measurements in the 2 weeks before and 2–3 weeks after each ART attempt. These tests were repeated 3 and 6 months after treatment and again at delivery. The children born were tested once after birth for the presence of HIV-1 antibodies.

Statistical analysis

Descriptive, parametric and non-parametric statistics were used where appropriate. Skewness was checked before using parametric tests.

A multivariate analysis was performed to assess independent and significant variables associated to IUI outcome after categorical transformation. All statistical analysis was performed using STATA 9 software (Statacorp, TX, USA).

Results

From January 2002 to January 2006, 2011 couples serodiscordant for HIV-positive male partner were counselled for ART at our centre. Seven hundred and forty-one of these couples completed the immuno-virological and fertility triage, met the inclusion criteria and were treated according to our protocols. Three hundred and ninety-four additional couples completed the same workup and signed the informed consent to be allocated on a waiting list. One hundred and ninety-four couples were not eligible according to our criteria. Six hundred and eighty-two couples declared their wishes to undergo ART procedures but either have not yet completed the diagnostic triage or were lost to follow-up. Table I summarizes demographic data and the immuno-virological profile of the male patients of this series.

Efficacy of sperm-washing procedures

The total number of negative sperm-washing procedures was 2871. All procedures were performed on the day of semen collection (2400 procedures in patients undergoing IUI and 283 in patients undergoing IVF/ICSI) In addition, we performed 188 sperm-washing procedures for a prudent cryopreservation of semen samples in couples undergoing IVF/ICSI.

The positive rate after sperm washing (assessed using real-time PCR with a threshold at 500 copies/ml) was 4%. This high positive rate could be due to contamination during RT–PCR analysis. In additional 2% of cases, it was not possible to give a results because the test failed.

Table I. Characteristic profile of human immunodeficiency virus (HIV)-positive male partners at admission to assisted reproduction technology (ART) procedures

	HIV-positive male partners included in ART procedures
Number of patients (*n*)	741
Age, mean ± SD	41 ± 4.4
Use of drugs, *n* (%)	422 (57)
Sexual transmission, *n* (%)	245 (33)
Blood product transmission, *n* (%)	30 (4)
Unknown, *n* (%)	44 (6)
Married, *n* (%)	511 (69)
Antiretroviral therapy, *n* (%)	638 (86)
No antiretroviral therapy, *n* (%)	103 (14)
CD4 count × 10^6/l (median, interquartile range)	510 (341–675)
Viral load < 50 copies/ml, *n* (%)	267 (36)
Viral load > 50 copies/ml, *n* (%)	824 (64)
Viral load: interquartile range	<50–5958
Co-infected with HCV, *n* (%)	437 (59)
Co-infected with HBV, *n* (%)	296 (40)

HBV, hepatitis B virus; HCV, hepatitis C virus.

Couples treated by IUI

Five hundred and eighty-one (78%) couples underwent superovulation and IUI. The mean age (±SD) was 38 ± 4 for male and 33.9 ± 4.1 for female partners. Basal FSH in women was 6.9 ± 2.9 IU/l. Basal FSH in women who conceived (6.9 ± 1.9) and did not conceive (6.5 ± 2.5) after IUI was not significantly different (*P* = n.s.). The mean numbers of treatments per couple was 4.13. The results of our IUI programme are summarized in Table II.

Seventy per cent of pregnancies were conceived in the first three cycles. Eighteen (4%) pregnancies were of multiple orders: 14 twins, 2 triplets and 2 quadruplets.

Table III summarizes the clinical outcome of IUI stratified for maternal age, antiretroviral therapy and semen characteristics. A multivariate analysis proved that maternal age was the only significant and independent predictor of IUI success (*P* = 0.002; CI 0.54–0.87). Semen total motile cell count inseminated was of borderline significance (*P* = 0.07).

All HIV-1 blood tests performed by PCR assays from the inseminated women were negative 3 months after the last IUI. Women who delivered infants were tested again for HIV-1

Table II. Outcome of intrauterine insemination (IUI) cycles in 581 couples

Couples (*n*)	581
Number of IUI cycles (*n*)	2400
Clinical pregnancies (*n*)	456
Clinical pregnancy rate per IUI cycle (%)	19
Clinical pregnancy rate per couple (%)	78
Miscarriages (*n*)	54
Miscarriage rate in total number of pregnancies (%)	12
Tubal pregnancy (*n*)	5
Ongoing pregnancy (*n*)	72
Number of deliveries (*n*)	325*
Multiple pregnancy rate (%)	4
Maternal seroconversion (*n*)	0
Congenital seroconversion (*n*)	0

*337 newborn babies.

Table III. Pregnancy rate in intrauterine insemination (IUI)-treated couples, stratified for maternal age, antiretroviral therapy and total motile sperm count inseminated

	Clinical pregnancy rate	*P**	AOR	95% CI
Age of female partner (years)				
<30	71/290 (24.5)	0.002	0.69	0.54–0.87
30–34	224/1036 (21.6)			
35–39	149/851 (17.5)			
≥40	12/223 (5.4)			
Antiretroviral therapy				
Yes	332/1902 (17.4)	0.105	1.42	0.92–2.19
No	124/498 (25)			
Total motile count inseminated (×10^6)				
≥1<2	86/449 (19.2)	0.07	1.17	0.98–1.41
2–5	80/610 (13.1)			
5–10	126/628 (20.1)			
>10	164/713 (23)			

AOR, adjusted-OR logistic regression.
Pregnant couples for each group of patients according to stratification, percentage in brackets.
*The *p* value is a result of an ANOVA analysis and has an overall significance for all ages and all TMC.

after delivery. The results were negative in all cases and for all the children.

Couples treated by IVF/ICSI

One hundred and sixty couples were treated by second-level ART procedures (mean number of cycles per couple 1.8). The mean age (±SD) was 40 (±4) for male and 36 (±4) for female partners. Basal FSH in women who conceived was 7.2 ± 2.2 and in women who did not conceive was 7.4 ± 2.3 (P = n.s.). The results of our IVF/ICSI programme are summarized in Table IV.

Discussion

This article reports the largest single series examining ART outcome in serodiscordant couples with an HIV-1-positive male. According to their fertility profile and the criteria adopted by our centre, 581 couples underwent IUI achieving a pregnancy rate per couple of 78% (19% per IUI cycle), with multiple pregnancy rate being 4%, and 160 couples underwent IVF/ICSI achieving a pregnancy rate per couple of 41% (24% per embryo transfer) with multiple pregnancy rate being 10%. As expected, maternal age and FSH were lower in the IUI group. Male partners were ~4 years older than female partners in both groups. The semen of all male HIV-infected partners was treated by the sperm-washing procedure (Persico *et al*., 2006). After a 6-month follow-up, no female partner proved positive for HIV1.

In this experience, pregnancy rate per couple by IUI was higher than the average 45% overall pregnancy rate reviewed on different series by Sauer (2005), and the pregnancy rate per IUI, as well, was higher than the average 14% briefly reported on the European experience by Gilling-Smith (2000). These results could be explained by the routine adoption of ovulation induction with low doses of recombinant FSH and timing of ovulation with recombinant LH according to Marina *et al*. (1998), the standard usage of fresh sperm after real-time PCR and possibly a good selection of IUI cases, with an average of four attempts per couple. The ovulation induction policy was not a standard of care in those reported series, and the number of attempts was not homogeneously reported by each author.

Table IV. Outcome of IVF/ICSI cycles in 160 couples

Couples (*n*)	160
IVF/ICSI cycles (*n*)	283
Cancelled cycles (*n*)	5
Clinical pregnancies (*n*)	65
Duration of stimulation (days, mean ± SD)	11 ± 2
Gonadotrophin dose (IU, mean ± SD)	2200 ± 780
Fertilization rate, IVF (%)	65
Fertilization rate, ICSI (%)	88
Oocytes/retrieval (mean ± SD)	8 ± 5.6
Metaphase II oocytes/retrieval (mean ± SD)	6 ± 4.2
Embryos transferred (mean ± SD)	2 ± 1
Pregnancy rate (%)	23
Pregnancy rate/embryo transfer (%)	24
Pregnancy rate/couple (%)	41
Multiple pregnancy rate (%)	10
Maternal seroconversion	0
Delivered offspring seroconversion	0

In addition to this, the use of frozen semen (Marina *et al*., 1998; Ohl *et al*., 2003; Bujan *et al*., 2004; Manigart *et al*., 2006) might have impacted negatively on the number of available motile sperm after freezing, as already reported (Leruez-Ville *et al*., 2002; Marcus-Braun *et al*., 2004; Oneta *et al*., 2004; Desrosiers *et al*., 2006) and on the pregnancy rate per IUI. Multiple pregnancy rate was 4% with two triplets and two quadruplets. All these couples conceived at their second, third or fourth attempt. These couples were counselled on the use of superovulation and its risks. Yet, they decided to accept these risks and face the possible consequences including multiple pregnancies.

In socioeconomic areas where HIV infection has turned into a chronic illness for the vast majority of patients (Englert *et al*., 2001), the desire for fathering a child has become a legitimate ethical and medical issue (Klein *et al*., 2003; Sauer, 2005). All couples seeking medical counselling in our centre were engaged in long-term relationships, and 69% of these were married. Counselling unprotected intercourses on the day of ovulation to fertile couples reduces the risk of horizontal transmission of HIV, but this 'reduction' would condemn ~5% of women to be infected by their partners (Mandelbrot *et al*., 1997). Highly active antiretroviral therapy could further reduce this risk but does not guarantee an undetectable virospermia. We still do not support the idea that these couples should be allowed to try to conceive naturally, just focusing on the best ovulation window (Barreiro *et al*., 2004). This is an unacceptable option when considered on an epidemiological scale and not on a single medical practice risk perception. Adoption and heterologous insemination could be an alternative (Bujan *et al*., 2002). However, in our counselling experience, these options may clash with personal or ethical values. In Italy adoption can be very difficult, and heterologous insemination is not legally acceptable since 2004.

Our findings on safe pregnancy with no sexual transmission of HIV-1 to the female partner after sperm washing add up to >3000 cases so far reported and to the consistent biological results published on the efficiency of sperm washing (Sauer, 2005). According to the experience of our laboratory, this definition should be applied only to procedures where the sperm washing is completed by the swim-up phase of sperm cell selection (Hanabusa *et al*., 2000; Persico *et al*., 2006).

The efficiency of IUI and its relatively low cost make this first-level procedure the technique of choice in serodiscordant couples with an HIV-positive male partner, except for women over 40 years of age and when no other infertility problems are involved including borderline number of total motile sperm cells. These findings and recommendations are well in agreement with data and opinions reported, among others, by Nicopoullos and Bujan (Nicopoullos *et al*., 2004; Bujan *et al*., 2006).

When the female partner was suffering from infertility factors or the male partner had $<1 \times 10^6$ total motile cells in the final fraction after sperm washing or both partners had a combination of sub-fertility conditions, we performed IVF/ICSI. The pregnancy rate per embryo transfer was in agreement with both similar smaller series (Ohl *et al*., 2003) and larger non-HIV series (ESHRE, 2006). Other markers of outcome were as

good in these couples treated after sperm washing as in other infertility series of comparable age: fertilization rate was 65% by IVF and 88% by ICSI. IVF/ICSI results are lower in terms of pregnancy rate when compared with those series in which fertile serodiscordant couples underwent ICSI (Garrido *et al*., 2004). However, pregnancy rate achieved by IUI and IVF/ICS altogether in our series sums up to 70.3% which is higher than the results obtained by ICSI in fertile couples. The problem with ICSI as first-line therapy in all serodiscordant couples is also the high multiple pregnancy rate [14% (Garrido *et al*., 2004) and 57.1% (Pena *et al*., 2003)] and the possible obstetrical and neonatal complications associated with these pregnancies. Finally, we should also consider the cost issue determined by ICSI to all serodiscordant couples. In fact, in addition to procedure related costs, we should also consider the possible additional costs determined by prenatal and neonatal care in multiple pregnancies (Oliviennes, 2000; Nakhuda and Sauer, 2005). These costs do not even benefit from a higher pregnancy rate.

The answer to the question posed by Sauer (2005) as to which is the preferred technique, between IUI and IVF/ICSI, is just to be found in the fertility profile of the serodiscordant couple seeking medical advices. The safety issue definitely requires large multicentre observational trials and until then 'participants need to understand that no procedure is risk free, as all carry a possibility for transmitting infection'.

The persistence of this remaining area of uncertainty on safety is probably the reason to extend prudent testing for HIV-1 by PCR after sperm washing not only to IUI and IVF but also to ICSI until final biological evidence is provided. In our experience on 48 samples (Persico *et al*., 2006), HIV-1 RNA tested positive in 13% of seminal plasma and 3% non-sperm cell, and HIV-1 DNA was tested positive in 15% of non-sperm cell. So far, we agree with Garrido (2006) and Gilling-Smith (2000) that to protect patient from technical errors during semen washing, viral detection sampling before ART is the method of choice. We could also argue that this procedure accounted for a negligible fraction of costs in ICSI, and this could temporarily settle the discussion on clinical protocols notwithstanding ongoing research on biological issues (Garrido and Meseguer, 2006; Piomboni *et al*., 2006).

Vernazza (Vernazza *et al*., 2006) added further advice on how to reduce the risk of transmission beyond this problem of testing semen after sperm washing for ART procedures. It is a minor limitation of his letter that the large series quoted from a private Milan centre has never been presented in detail. In his letter, Vernazza *et al*. (2006) addressed the issue every centre is facing nowadays: the long waiting lists that tempt some couples to try for a spontaneous conception by unprotected intercourses. In our centre, ~400 couples are on the waiting list, with an average 6-month delay at the time of writing. In our opinion, although large comprehensive follow-up studies should be undertaken to assess the relative risks of the possible scenarios described by Vernazza, healthcare policymakers should be made aware of the changing paradigm of HIV-infected patients in western countries to allocate resources to preventing HIV transmission within serodiscordant couples which, like other more lucky ones, are just trying to live a possible significant part of human life: childbearing.

Acknowledgements

We acknowledge the role of Giorgio Pardi, Chairman at the DMCO San Paolo Institute where he first encouraged and funded the first researches on sperm washing started in Milan at San Paolo DMCO in the early 1990s, Dr Enrico A. Semprini and Dr Paolo Levi-Setti, for the pioneer-dedicated researches and clinical work throughout a decade at the same Institute.

References

American College of Obstetricians and Gynecologists Committee on Ethics (2001) ACOG committee opinion. Human immunodeficiency virus: ethical guidelines for obstetricians and gynecologists. Obstet Gynecol 97(Suppl 1), 1–l6.

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

Barreiro P (2004) Benefit of antiretroviral therapy for serodiscordant couples willing to be parents. Late breakers Seventh International Congress on Drug Therapy in HIV Infection, 14–18 November, Abstract number *PL*13.3, Glasgow, Scotland.

Bujan L, Daudin M and Pasquier C (2002) Reproductive options for HIV-serodiscordant couples. Perspect Sex Reprod Health 34,104.

Bujan L, Pasquier C, Labeyrie E, Lanusse-Crousse P, Morucci M and Daudin M (2004) 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.

Bujan L, Daudin M and Pasquier C (2006) Choice of ART programme for serodiscordant couples with an HIV infected male partner. Hum Reprod 21,1332–1333.

Centers for Disease Control (CDC) (1990) HIV-1 infection and artificial insemination with processed semen. MMWR Morb Mortal Wkly Rep 249,255–256.

Decree of May 10 (2001). Prise en charge en assistance médicale à la procréation des patients à risque viral. Page 7735. Arrêté du 10 mai 2001. Journal Officiel de la République Française, 15 mai 2001.

Desrosiers P, Legare C, Leclerc P and Sullivan R (2006) Membranous and structural damage that occur during cryopreservation of human sperm may be time-related events. Fertil Steril 85,1744–1752.

Duerr A and Jamieson D (2003) Assisted reproductive technologies for HIV-discordant couples. Am J Bioeth 3,45–47.

Englert Y, Van Vooren JP, 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.

ESHRE (2006) Assisted reproductive technology in Europe, 2002. Results generated from European registers by ESHRE. Hum Reprod 21,1680–1697.

Ethics Committee of the American Society for Reproductive Medicine (2002) Human immunodeficiency virus and infertility treatment. Fertil Steril 77,218–222.

Garrido N and Meseguer M (2006) Use of washed sperm for assisted reproduction in HIV-positive males without checking viral absence. A risky business? Hum Reprod 21,567–568 (Letter).

Garrido N, Meseguer M, Bellver J, Remohì J, Simòn C and Pellicer A (2004) Report of the results of a 2 year programme of sperm wash and ICSI treatment human immunodeficiency virus and hepatitis C virus serodiscordant couples. Hum Reprod 19,2581–2586.

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

Hanabusa H, Kuji N, Kato S, Tagami H, Kaneko S, Tanaka H and Yoshimura Y (2000) An evaluation of semen processing methods for eliminating HIV-1. AIDS 14,1611–1616.

Klein J, Pena JE, Thornton MH and Sauer MV (2003) Understanding the motivations, concerns, and desires of human immunodeficiency virus 1-serodiscordant couples wishing to have children through assisted reproduction. Obstet Gynecol 101,987–994.

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, Heard I, Henrion-Geant E and Henrion R (1997) Natural conception in HIV-negative women with HIV-infected partners. Lancet 349,850–851.

Manigart Y, Rozenberg S, Barlow P, Gerard M, Bertrand E and Delvigne A (2006) ART outcome in HIV-infected patients. Hum Reprod 21,2935–2940.

Marcus-Braun N, Braun G, Potashnik G and Har-Vardi I (2004) Effect of cryopreservation on quality and fertilization capacity of human sperm. Eur J Obstet Gynecol Reprod Biol 10,63–66.

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.

Mencaglia L, Falcone P, Lentini GM, Consigli S, Pisoni M, Lofiego V, Guidetti R, Piomboni P and De Leo V (2005) ICSI for treatment of human immunodeficiency virus and hepatitis C virus-serodiscordant couples with infected male partner. Hum Reprod 20,2242–2246.

Nakhuda GS and Sauer MV (2005) Addressing the growing problem of multiple gestations created by assisted reproductive therapies. Semin Perinatol 29,355–362.

Nicopoullos JD, Almeida PA, Ramsay JWA and Gilling-Smith C (2004) The effect human immunodeficiency virus on sperm parameters and the outcome of intrauterine insemination following sperm washing. Hum Reprod 19,2289–2297.

Ohl J, Partisani M, Wittemer C, Schmitt MP, Claudine C, Stoll-Keller F, Rongieres C, Bettahar-Lebugle K, Lang JM and Nisand I (2003) Assisted reproduction techniques for HIV serodiscordant couples: 18 months of experience. Hum Reprod 18,1244–1249.

Oliviennes F (2000) Double trouble: yes a twin pregnancy is an adverse outcome. Hum Reprod 15,1663–1665.

Oneta M, Persico T, Savasi V, Colasante C, Crivelli M, Leone F and Ferrazzi E (2004) Over-night incubation after "sperm washing" does not affect IVF outcome in HIV-1 serodiscordant couples. Hum Reprod 19(Suppl 1),0–253; Berlin, Germany.

Pena JE, Thornton MH and Sauer MV (2003) Assessing the clinical utility of in vitro fertilization with intracytoplasmic sperm injection in human immunodeficiency virus type 1 serodiscordant couples: report of 113 consecutive cycles. Fertil Steril 80,356–362.

Persico T, Savasi V, Ferrazzi E, Oneta M, Semprini AE and Simoni G (2006) Detection of human immunodeficiency virus-1 RNA and DNA by extractive and in situ PCR in unprocessed semen and seminal fractions isolated by semen-washing procedure. Hum Reprod 21,1525–1530.

Piomboni P, Mencaglia L and De Leo V (2006) Reply to: Use of washed sperm for assisted reproduction in HIV-positive males without checking viral absence. A risky business? Hum Reprod 21,568 (Letter).

Sauer MV (2005) Sperm washing techniques address the fertility needs of HIV-seropositive men: a clinical review. Reprod Biomed Online 10,135–140.

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

Savasi V, Oneta M, Lanzani C, Ratti M and Ferrazzi E (2006) Reproductive assistance in HIV-1 serodiscordant couplet for male HIV infection. Hum Reprod 21(Suppl 1),i135; Prague, Czech Republic.

Semprini AE, Levi-Setti P, Bozzo M, Ravizza M, Taglioretti A, Sulpizio P, Albani E, Oneta M and Pardi G (1992) Insemination of HIV-negative women with processed semen of HIV-positive partners. Lancet 340,1317–1319.

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

Vernazza PL, Gillian BL, Dyer J, Fiscus SA, Eron JJ, Frank AC and Cohen MS (1997) Quantification of HIV in semen: correlation with antiviral treatment and immune status. AIDS 11,987–993.

Vernazza PL, Hollander L, Semprini AE, Anderson DJ and Duerr A (2006) HIV-discordant couples and parenthood: how are we dealing with the risk of transmission? AIDS 20,635–636 (Letter).

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

Submitted on August 2, 2006; resubmitted on September 19, 2006; accepted on September 27, 2006