Safety and efficacy of sperm washing in HIV-1-serodiscordant couples where the male is infected: results from the European CREAThE network

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Objective: To examine the safety and effectiveness of assisted reproduction using sperm washing for HIV-1-serodiscordant couples wishing to procreate where the male partner is infected.

Design and methods: A retrospective multicentre study at eight centres adhering on the European network CREAThE and involving 1036 serodiscordant couples wishing to procreate. Sperm washing was used to obtain motile spermatozoa for 3390 assisted reproduction cycles (2840 intrauterine inseminations, 107 in-vitro fertilizations, 394 intra-cytoplasmic sperm injections and 49 frozen embryo transfers). An HIV test was performed in female partners at least 6 months after assisted reproduction attempt. The outcome measures recorded were number of assisted reproduction cycles, pregnancy outcome and HIV test on women post-treatment.

Results: A total of 580 pregnancies were obtained from 3315 cycles. Pregnancy outcome was unknown in 47 cases. The 533 pregnancies resulted in 410 deliveries and 463 live births. The result of female HIV testing after assisted reproduction was known in 967 out of 1036 woman (7.1% lost to follow-up). All tests recorded were negative. The calculated probability of contamination was equal to zero (95% confidence interval, 0–0.09%).

Conclusion: This first multicentre retrospective study of assisted reproduction following sperm washing demonstrates the method to be effective and to significantly reduce HIV-1 transmission risk to the uninfected female partner. These results support the view that assisted reproduction with sperm washing could not be denied to serodiscordant couples in developed countries and, where possible, could perhaps be integrated into a global public health initiative against HIV in developing countries.

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Introduction

According to the latest UNAIDS/World Health Organization (WHO) update (December 2006), the total number of people living with HIV had reached 39.5 million. As the AIDS epidemic continues to take a massive and global toll on human lives, the call for comprehensive strategies to prevent HIV transmission has never been higher. A fundamental policy in halting the spread of HIV has been the encouragement and facilitation of condom use in vaginal and anal sexual contacts. This approach however raises a major dilemma for serodiscordant couples, where the male partner is infected, who wish to conceive. These couples form an increasing part of the HIV population in developed countries, where most individuals infected with HIV are of reproductive age and able to access effective antiretroviral treatment. Improved life expectancy and quality has enabled these individuals to reanalyse their future and consider parenting as a socially and ethically acceptable option and many now express the desire for parenthood [1–3] as a fundamental part of healthy family life and return to normality. Recently published guidelines from the American Society for Reproductive Medicine (ASRM) and ethics recommendations concerning assisted reproduction on people infected by virus have been modified to allow assisted reproduction in HIV-serodiscordant couples [4].

The desire to conceive could counteract preventive barrier methods such as condom use if couples do not receive sufficient information on reproductive options to reduce viral transmission risk. Adoption and assisted reproduction from donor sperm are two reproductive options available to couples which totally eliminate the risk of HIV transmission. However, both methods are not equally available throughout Europe and both deny the infected person a genetic link to his child and could reinforce feelings of stigma and discrimination. Unprotected intercourse restricted to the time of ovulation may limit the risk of HIV transmission but from the limited data available, transmission has been shown to occur under these conditions [5]. One way of allowing HIV-serodiscordant couples, where the male is infected, to conceive their biological children while limiting the risk of HIV transmission is the use of assisted reproduction after semen processing using the sperm washing method. Sperm washing, as a means of preventing female infection in HIV-1-serodiscordant couples where the male partner is infected, was first proposed 17 years ago at a time when no methods were available to detect HIV-1 nucleic acids in sperm and before the introduction of highly active antiretroviral treatment (HAART) [6]. Since then, several publications have reported the results of different sperm washing programmes in such couples [7–18].

The mean risk of sexual HIV-1 transmission for a single act of penile–vaginal intercourse appears to be on the order of 0.1% (see review by Bartin and Overbaugh [19]). Therefore, it is not easy to establish whether sperm washing and assisted reproduction actually reduces the risk of transmission in a significant way.

In Europe, centres involved in sperm washing programmes have created the CREAThE network (Centre for REproductive Assisted Techniques for HIV in Europe) whose primary objective is to pool knowledge, experience and results in order to improve the service offered to HIV-serodiscordant couples.

The present retrospective study analysed pooled data, from eight centres involved in the CREAThE network, to study the safety and efficacy of assisted reproduction with sperm washing in the largest series of sperm washing cases ever performed in serodiscordant couples where the male partner was HIV-1 positive.

Materials and methods

Eight centres from six European countries participated in retrospective data collection on assisted reproduction cycles performed between 1989 to 2003 (Table 1). Serodiscordant couples ($n = 1036$) in which the male

Table 1. Patients and assisted reproduction cycles performed according to the centres involved on CREAThE network.

		Patients		Assisted reproduction cycles				
Country	Centre	Couples	Follow-up lost* *n* (%)	IUI	IVF	ICSI	FET	Total
Belgium	Brussels	10	0	31	9			40
France	Paris	149	0	252	0	134	4	390
	Strasbourg	55	0	6	1	90	12	109
	Toulouse	83	0	298	0	1	0	299
Germany	Mannheim	29	0	62	1	13	1	77
Italy	Milan	588	74 (12.6)	1883	73	133	30	2119
United Kingdom	Londres	57	0	115	23	23	2	163
Switzerland	St Gallen	65	0	193	0	0	0	193
Total		**1036**	**74 (7.1)**	**2840**	**107**	**394**	**49**	**3390**

IUI, intra-uterine insemination; IVF, in-vitro fertilization; ICSI, intra cytoplasmic sperm injection; FET, frozen embryo transfer.
*Patients for whom the result of HIV test was unknown at least 6 months after the last assisted reproduction attempt.

partner was infected with HIV-1, attended the centres to follow a sperm washing programme. Variables concerning HIV-1 infection in the man, age of the woman at the time of treatment, and past gynaecological history were recorded from the patients' data in each centre. A fertility screen was performed for both partners. Sperm washing was performed according to published methods [6,9] with minor adaptations in each centre. Briefly, sperm washing has been previously reported using three steps: step 1, a spermatozoa migration on density gradient; step2, repeated washing of the migrated pellet, step 3, a spermatozoa swim up. Sperm washing was performed on one or more semen samples according to the protocol used in each centre. Briefly the sperm obtained after a recommended 3–7 days of abstinence was submitted to a separation following density gradient centrifugation. The 90% fraction was centrifuged and washed (method with two steps only) or submitted to a swim up method (method with three steps). The London, Strasbourg, Mannheim and Toulouse centres systematically performed the preparation with three steps and the Brussels and Milan centres also performed three steps except when there was a fertility issue where only the two-step method was performed. The St Gallen and Paris centres used only the two-step method and St Gallen used a specific double tube technique that has been reported [20]. The aim of all these sperm washing methods was to select only motile spermatozoa free of seminal plasma and semen cells such as leukocytes.

After its introduction into clinical use (1995–1997), HIV-1 genome detection was performed according to published methods [21] on the final fraction of spermatozoa obtained after sperm washing, except in the St Gallen centre where a more stringent separation method was used [20]. If HIV-1 RNA or DNA were not detected in the isolated spermatozoa fraction, fresh spermatozoa (Brussels, Milan, London) or frozen–thawed spermatozoa (Brussels, Milan, London, Paris, Strasbourg, Mannheim, Toulouse) fractions were used. Washed samples with detectable HIV-genomes were not used for assisted reproduction.

The assisted reproduction procedure choice (intra-uterine insemination (IUI), in-vitro fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI)) was based on the result of the couple's fertility screen and each centre's protocols. Supernumerary embryos obtained after IVF or ICSI were frozen with the possibility of being transferred in a subsequent cycle (frozen embryo transfer; FET) if pregnancy did not occur after the first embryo transfer. Following each assisted reproduction cycle with washed sperm, HIV screening was performed on the female partners. The result of a HIV-test performed at least 6 months after the last assisted reproduction treatment was recorded in the database for this study.

All couples were required to systematically use condoms during intercourse throughout the period of treatment. They were informed about the assisted reproduction procedure and the fact that sperm washing may not totally eliminate transmission risk. All couples gave their informed consent to the assisted reproduction with sperm washing and the procedure was done in accordance with the declaration of Helsinki. In accordance with each country's laws, an agreement by an institutional review board was obtained. A limited number of anonymized clinical data for each patient from each centre were recorded in a central database and statistical analysis was performed using STATA software version 8.0 (StataCorp LP, College Station, Texas, USA). The probability and the 95% confidence intervals (95% CI) of HIV-contamination risk in the female partners were calculated according to Clopper and Pearson.

Results

In the eight centres involved in this study, a total of 3390 assisted reproduction cycles were performed for 1036 couples. The number of cycles per couple varied from 1 to 19 (3.24 ± 2.58). Intra-uterine insemination was the most frequently used procedure (84%) (Table 2). The average age at the time of treatment was 32.3 years

Table 2. Results of assisted reproduction attempts according to the different procedures used.

Procedures	IUI	IVF	ICSI	FET	Total	P[d]
Couples	853	76	262	40	1231[a]	
Cycles	2840	107	394	49	3390	
Pregnancy per cycle (%)[b]	15.1	29.0	30.6	20.4	17.5	<0.001
Multiple pregnancy rate (%)	4.9	17.2	20.8	20.0	9.12	<0.01
Delivery per cycle (%)[c]	11.5	20.8	15.8	14.3	12.3	<0.05
Pregnancy per couple (%)	42.7	38.2	43.1	25.0	41.9	>0.05
Delivery per couple (%)	35.1	26.3	21.0	17.5	30.9	<0.01

FET, frozen embryo transfer; ICSI, intra cytoplasmic sperm injection; IUI, intra-uterine insemination; IVF, in-vitro fertilization.
[a]The total was over 1036 couples as a couple could be have different assisted reproduction procedures (for example four IUI + two IVF).
[b]Missing information in 66 IUI and seven IVF cycles.
[c]Missing information in 91 IUI, 11 IVF and 40 ICSI cycles.
[d]*P*-value for comparison between procedure groups.

(range, 19–49) for female partners and 35.4 (range, 24–66) for the male partners.

Previous pregnancies were reported for 219 (27%) of the 803 women for which this information was collected. These 219 women reported 272 pregnancies resulting in 149 terminations, 52 miscarriages and 65 deliveries. The latter were in 60 women. Previous pregnancy outcome was unknown for six women. Among the women who had previously delivered, 45 had 48 children with their current partner and these couples were now requesting assisted reproduction for a new pregnancy.

Three thousand, three hundred and ninety cycles of assisted reproduction with sperm washing were performed. In 75 cases the outcome of treatment was not known. Pregnancy resulted in 580 of the 3315 cycles where outcome was known (clinical pregnancy rate per cycle 17.5%; 95% CI, 16.2–18.8). Among the 1036 treated couples, 499 women were pregnant at least once (48.2%; 95% CI, 45.2–51.3). Three hundred and seventy were singleton pregnancies, 28 twin pregnancies and 25 were higher order multiple pregnancies for which two embryonic reductions were performed. The pregnancy order was not known for 157 pregnancies. Final outcome of pregnancy was not specified in 47 cases. Five hundred and thirty-three pregnancies resulted in 410 deliveries (80%): 368 singletons, 29 sets of twins (two neonatal deaths), 13 sets of triplets, resulting in 463 live births. One hundred and twelve miscarriages, eight extra-uterine pregnancies, two terminations of pregnancy and one intrauterine death occurred.

Nine hundred and sixty-seven out of 1036 women had a negative HIV test at least 6 months after their last assisted reproduction attempt with washed sperm. In 74 cases, the results of HIV testing in the female partner were unknown. Those lost to follow-up had attended the Italian centre amounting to 7.1% of all couples entered on the database.

In terms of treatment cycles performed, a negative HIV test was recorded 6 months post treatment in 3272 (96.5%) and no HIV test was recorded following the 124 (3.5%) cycles from 74 cases. No female seroconversion occurred following treatment in the 3272 cycles for which the results were known, allowing us to calculate the probability of contamination risk to be zero (95% CI, 0–0.09%).

Discussion

Prevention of HIV sexual transmission requires systematic use of condoms during intercourse. The consequence is to induce sterility in the couples. For this reason, numerous couples of reproductive age now seek medical assistance to have a child while minimizing the risk of infecting the HIV-negative partner.

For HIV-1-serodiscordant couples with an infected male partner, unprotected intercourse on the day of ovulation has been proposed and studies have analysed its safety. In 1997, Mandelbrot *et al.* reported four seroconversions, not related to the unprotected intercourse during ovulation, but occurring 7 months later or postpartum in a series of 92 natural conceptions in 92 HIV-1-negative women with an HIV-1-infected partner [5]. A more recent study [22] reported pregnancy and HIV-1 incidence in 178 married couples with discordant HIV-1 status. Women who became pregnant had a mean number of 8.4 acts of penetrative sex/month with 43% protected intercourse in comparison with a mean of 2.5 acts with 95% protected in women who did not become pregnant. One out of 14 women (7%) who became pregnant seroconverted and five out of 78 women who did not become pregnant seroconverted. It should be noted, however, that these two studies were done before the introduction of HAART or in a country were the HAART was not universally available.

In 1992, assisted reproduction with sperm washing was proposed in order to reduce the risk of female HIV contamination in HIV-1-serodiscordant couples [6]. Eight European centres offering assisted reproduction with sperm washing to HIV-1-serodiscordant couples have combined their results in this study. A total of 1036 couples underwent 3390 assisted reproduction cycles resulting in 580 pregnancies. No transmission of HIV to the female partner was observed after 3272 cycles with complete follow-up information. The upper level of the 95% CI of the transmission risk was thus 0.09%.

Several serodiscordant-couples studies have estimated that the risk of sexual HIV-1 transmission for a single act of penile–vaginal intercourse in a stable sexual partnership appears to be on the order of 0.1% (see Baeten and Overbaugh [19]). According to the De Vencenzi study [23] the calculated probability of women seroconverting was 0.1% (95% CI, 0.05–0.15) per natural intercourse [23]. Taking into account this hypothesis of contamination probability of 0.1% and that, according to the method published by Hanley and Lippman-Hand [24] we need 2275 cycles to demonstrate that the risk of female contamination during assisted reproduction is lower than during natural intercourse. Accordingly, the number of assisted reproduction cycles in the present retrospective study without female contamination allows us to demonstrate the safety of the proposed assisted reproduction methods.

From a medical aspect, one must remember that couples search for a risk-reducing strategy in order to conceive. Moreover, a contrast exists between the calculated

transmission risk during one act of sexual intercourse and the rate of female HIV-seroconversion reported for HIV-1-serodiscordant couples where the male partner is HIV-positive: four out of 92 couples (4.3%) in the Mandelbrot study [5], six out of 92 (6.5%) in the Ryder study [22]: and eight out of 74 (10.8%) in the De Vincenzi study [23] representing a total of 18 female seroconversions in a population of 268 (6.7%). In our study, we reported no seroconversions in the 967 women ($P < 0.01$) treated with washed sperm during assisted reproduction programmes.

Recently a case of seroconversion was reported in a woman preparing for assisted reproduction whose infected male partner was not under antiretroviral therapy [25].

It is noteworthy that the studies on transmission risk during unprotected intercourse were done either before HAART was available or in countries where it was unobtainable due to restricted resources. Blood viral load is known to increase the risk of seroconversion and HAART is known to reduce such risk [26]. It must, however, be remembered that several additional factors are associated with HIV-1 genome excretion in semen such as genital infection and inflammation and that HIV-1 genome has been found in a small proportion of men with undetectable blood viral load under HAART [27,28] while infectiousness in these cases was unknown. The debate over the real risk of timed intercourse when the infected male has an undetectable viral load therefore continues. No seroconversions were observed in a more retrospective study of 77 HIV-1-serodiscordant couples who attempted conception through timed unprotected intercourse and in which the HIV-positive male partner had an undetectable blood viral load (< 500 copies/ml) through HAART [29]. This study did not, however, look for seroconversions in those couples who failed to conceive and the population size was too small to allow conclusions on the safety of this approach. Vernazza *et al.* have recently proposed additional measures to improve the safety of timed unprotected intercourse [30].

One limitation of our retrospective study was the loss to follow-up in 7.1% of couples. This loss to follow-up was due to geographical issues in the early days of the assisted reproduction programmes in the Italian centre. As these assisted reproduction programmes were offered to reduce the contamination risk, it is reasonable to presume that any female seroconversion following treatment would have been reported to the centre concerned.

The present study is the first multicentre study of the use of sperm washing in HIV-1-serodiscordant couples, in which the male partner was infected, who wished to conceive. It is the largest series published to date and the first with sufficient case numbers to confirm the safety and efficacy of assisted reproduction, where sperm washing was used as the primary means of avoiding HIV infection in the female partner. We recommend that public health authorities sustain and promote networks in order to analyse the results of assisted reproduction programmes, tailored to the needs of HIV-serodiscordant couples, and ensure a comprehensive database for the outcome of treatment and follow-up of all female patients treated is maintained, thereby facilitating epidemiologic studies.

According to our present data and other studies that have demonstrated the feasibility of such approach (see review by Gilling-Smith *et al.* [31]) it is neither ethically nor legally justifiable to exclude individuals from infertility services on the basis of male HIV-infection. For many countries in the world the first priority of the policy against HIV is to improve education, to allow access to HIV screening, to encourage condom use and to offer antiretroviral therapy where appropriate. In countries where these approaches are now in place we recommended that assisted reproductive programmes, such as IUI with sperm washing, should be integrated into a global public health initiative against HIV.

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