# Assisted Reproductive Technology and Obstetric Outcome in Couples when The Male Partner Has A Chronic Viral Disease

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Abstract-

**Background:** Assisted reproductive technology (ART) with washed semen can achieve pregnancy with minimal risk of horizontal and vertical transmission of chronic viral diseases (CVD) such as human immunodeficiency virus (HIV), hepatitis C virus (HCV) and hepatitis B virus (HBV) among serodiscordant couples. However, few studies have been made of the use made by these couples of ARTs or of the obstetric results achieved.

**Materials and Methods:** In this retrospective study, 93 men who were seropositive for HIV, HCV or HBV and who underwent assisted reproduction treatment at our centre (Hospital Universitario Virgen de las Nieves, Granada, Spain) were included. Washed semen was tested to detect viral particles. Non-infected women were tested before and after each treatment, as were the neonates at birth and after three months.

**Results:** A total of 62 sperm samples were washed, and none were positive for the detection of viral molecules. Semen samples from 34 HBV positive males were not washed since the female partner had immunity to hepatitis B. In total, 38 clinical pregnancies were achieved (22% per cycle and 40.9% per couple) out of 173 cycles initiated, and 28 births were achieved (16.2% per cycle and 30.1% per couple), producing 34 live births. The rate of multiple pregnancies was 21.4%. Obstetric and neonatal results were similar in the groups of couples studied. At follow-up, no seroconversion was detected in the women or neonates.

**Conclusion:** Sperm washing and intracytoplasmic sperm injection are shown to be a safe and effective option for reducing the risk of transmission or super infection in serodiscordant or concordant couples who wish to have a child. Pregnancies obtained by ART in couples when the male is CVD infected achieve good obstetric and neonatal results.

Keywords: HIV, HCV, HBV, Reproductive Techniques, Obstetric Labor Complications

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### Introduction

Assisted reproductive technology (ART) first came into use to address problems of infertility, and was subsequently applied to fertile couples with the aim of reducing the risk of transmission of genetic and infectious diseases. With this latter objective, ART has been applied to couples in which one or both partners are infected by human immunodeficiency virus (HIV), hepatitis C virus (HCV) or hepatitis B virus (HBV). Semprini et al. (1) recorded the first birth achieved after using washed semen from an HIV seropositive man. Today, the reproduction options for serodiscordant couples with a chronic viral disease (CVD) have been expanded. Among these possibilities are unprotected intercourse and timed intercourse with or without pre-exposure prophylaxis (PREP), ART using semen washed with or without testing for detectable viral load, donor sperm, the donation of embryos from seronegative couples, and adoption. However, many of these options are not accepted by the couple (2) or are not recommended by physicians, and so the option of washed semen is most often adopted (3).

To date, many studies have described the safety of the semen wash-intracytoplasmic sperm injection (ICSI) technique for couples that are serodiscordant for HIV (4). However, only a few of these studies (5-8), including the largest series to date, of over 3000 treatment cycles, published by CREATHE (9), have reported obstetric and neonatal results for the correct evaluation of ART results, as has been recommended by different groups (10).

A similar situation occurs with studies analysing the use of ART for couples in which the male partner is seropositive for HCV or HBV (11-18). In such cases, obstetric results are much more limited.

Since late 2005, the Human Reproduction Unit at Hospital Universitario Virgen de las Nieves, Granada, Spain has been the only one in the public health system of Andalucia (8.2 million inhabitants) providing fertility care for couples with an HIV, HCV or HBV infection. We follow the recommendations of the ethics taskforce of the european society of human reproduction and embryology (ESHRE) about suitable treatment compliance, avoidance of other risk factors such as drug abuse, treatment in reference centres with established protocols, a separate adapted laboratory, as well as separate tanks for storage of infected material, and appropriate multidisciplinary support (19).

The aim of this retrospective study was to deter-

mine, for couples in which the male was seropositive for HIV, HCV or HBV: i. the efficiency of sperm wash in terms of viral load; ii. the results of ART-ICSI; iii. the seroconversion rates after the treatment; and iv. the obstetric and neonatal outcome for such couples at a public hospital.

### Materials and Methods

A retrospective review was conducted of men who were seropositive for HIV, HCV or HBV and underwent assisted reproduction treatment between November 2005 and December 2009.

To be enrolled, all the couples were required to sign informed consent, and to attest to safe sex practices since four months before beginning the treatment and not to have sex from one month before until one month after the end of the treatment. Male and seropositive female partners were requested to be under the care of an infectious disease specialist and to provide a full report of their disease including current serological study, CD4 counts in blood (only for HIVseropositive), blood viral load with a maximum age of 4 months and treatment received and evolution of the disease for the past 12 months. To enrol an HIV positive female, both undetectable viral load and CD4 counts >200 cells/mm<sup>3</sup> were required. Seronegative female partners were required to provide a current serological study and a blood viral load. HBV negative females with HBV positive male partners were vaccinated, and if immunity was not achieved, the partner's semen was washed and viral load was determined. The couples had to wait an average of two years for ICSI treatment, as our hospital is a public one and it has a waiting list. Every couple was allowed a maximum of two attempts (two cycles with embryo transfer) to achieve a pregnancy.

A standard evaluation was performed on both partners, consisting of anamnesis and physical examination. Female fertility was also assessed by gynaecological examination, a vaginal ultrasound examination of the uterus and ovaries, a vaginal sample for bacteriological testing and a smear test. Basal hormonal study was determined from blood samples on day 3 of the menstrual cycle. The men were also subjected to seminal analysis, following world health organization (WHO) criteria (20). The ART laboratory used for all procedures was separated from the laboratory facilities used for couples negative for HIV, HBV and HCV.

Semen samples were obtained after sexual abstinence of 3 days, and subsequently kept and manip-

ulated in a class II biological safety cabinet. After liquefaction, semen parameters were evaluated as outlined by the WHO (20) and samples were processed by centrifuging (20 minutes at 300 g) through a discontinuous density gradient 80-40% (PureSperm 100 and PureSperm Buffer, Nidacon International AB, Mölndal, Sweden) with 1 ml per layer, with the semen pippetted directly on top of the upper layer. Pellets were washed 1:2 (vol: vol) with PureSperm Wash (Nidacon, Mölndal, Sweden) for 8 minutes at 300 g, then the supernatant was removed and the pellet was resuspended in 3 ml of Pure Sperm Wash. The concentration was assessed and the sample was divided in two, and one half was then used to test for viral presence. The other half was frozen with Sperm Freezing Medium (Irvine Scientific, Santa Ana, CA), and stored until needed for use, after the PCR test for HIV resulted negative.

Quantification of HIV RNA, HCV RNA and HBV DNA in final processed semen was performed by real-time PCR COBAS TaqMan<sup>TM</sup> 96 instrument (COBAS Ampliprep<sup>TM</sup> analyser; Roche Diagnostics GmbH, Manheim, Germany).

Ovarian stimulation protocols were selected according to clinical data, patient's age and hormonal profile and the result of any previous stimulation. Normally, the long protocol was adopted for the first cycle, and then, for the second one, the long or the short protocol was chosen depending on the results of the first cycle. The cycles were monitored by serial transvaginal ultrasound examination and serum estradiol  $(E_2)$  levels. When the follicles had reached a mean diameter of 18-22 mm, human chorionic gonadotropin (hCG) was administered, and 34-36 hours later, oocyte retrieval was performed with ultrasound guidance. The oocytes collected were incubated in G-IVF Plus supplemented with human serum albumin (HSA, Vitrolife, Göteborg, Sweden) and the mature oocytes with extrusion of the first polar body were then microinjected with sperm selected from the motile fraction. The procedure was performed as previously described (21). The embryos were incubated in G-1 Plus supplemented with HSA (Vitrolife, Göteborg, Sweden) in incubators separated from those of seronegative infertile couples and transferred at day 2-3 into Embryoglue (Vitrolife, Göteborg, Sweden). Progesterone (400 mg/day) and folic acid (0.4 mg/ day) were prescribed on the day of oocyte retrieval and maintained until the patient was instructed to suspend this medication. The remaining embryos were vitrified for possible future transfers.

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Complications such as ovarian hyperstimulation syndrome (OHSS) were noted. Clinical pregnancy was assessed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy as described by the International Committee for Monitoring Assisted Reproductive Technology. Delivery was defined as the expulsion or extraction of one or more foetuses from the mother after 20 completed weeks of gestational age, and live birth was considered to be the complete expulsion or extraction from its mother of a product of fertilization, irrespective of the duration of the pregnancy, which, after such separation, breathes or shows any other evidence of life such as heart beat, umbilical cord pulsation, or definite movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta remains attached (22).

Comparisons of rates between groups were performed using chi-squared tests. All the tests were twosided, with a p value of 5% considered as significant.

The blood viral load of the female partner was tested using RNA polymerase chain reaction (PCR) at 3 weeks, 3 months and 6 months after the ART. If the woman became pregnant, the blood viral load was tested every 2 months, and the serological study performed every 3 months. The infants were also tested at birth and at age 3 months.

### Results

After considering a total of 105 couples, 93 couples were included in this study and twelve were rejected as they did not meet the inclusion criteria; two men had active drug consumption, three men did not present adequate adherence to highly active antiretroviral therapy (HAART), another two men had active opportunist infection and five women were aged older than 40 years.

In 33 of the couples treated, the male was HIV seropositive (23 men were also HCV positive, one was HBV positive and another was both HCV and HBV positive). In another 23 couples, the male was HCV seropositive (one man was also HBV positive), and in the remaining 37 couples, the male was HBV seropositive. The men were infected with different genotypes of HCV: 43.5% (20 men) with genotype 1, 6.5% (n=3) with genotype 2, 26.1% (n=12) with genotype 3 and 23.9% (n=11) with genotype 4. The characteristic profile of the couples who underwent ICSI is shown in table 1.

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Reference	ble 1: Characteristic profile of HIV (n=33)		HCV (n=2	HBV (n=37)		
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Female partner						
Age (Y)	$34.6\pm4.1$	25-40	$34.2 \pm 2.8$	29-39	$34.0\pm3.8$	26-40
HIV, n (%)	2 (6.1)					
Hepatitis C, n (%)	1 (3)		1 (4.4)			
Hepatitis B, n (%)	0(0)		0 (0)			
Known HIV/HCV/HBV diagnosis (Y)	$6.5 \pm 2.1$	5-8	$5\pm0$			
On HAART therapy, n (%)	2 (100)					
HCV treatment, n (%)	0(0)		0 (0)			
Viral load (IU/mL)			376000			
Undetectable viral load HIV, n (%)	2 (100)					
Undetectable viral load HCV, n (%)	0(0)		0 (0)	•		
Undetectable viral load HBV, n (%)	0					
CD <sup>4+</sup> T-cell count (mm <sup>3</sup> )	$666 \pm 5.7$	662-670				
HBV immunity, n (%)		SK			34 (91.9)	
Male partner						
Age (Y)	40.1 ± 5.7	28-55	39.4 ± 6.1	32-50	$36.4 \pm 3.8$	26-44
Hepatitis C, n (%)	23 (69.7)					
Hepatitis B, n (%)	2 (6.1)		1 (4.4)			
Known HIV/HCV/HBV diagnosis (Y)	11 ± 4.8	2-19	$17 \pm 6.1$	10-21	$10.3\pm8.5$	4-20
On HAART therapy, n (%)	28 (84.9)					
HCV treatment, n (%)	3 (9.1)		3 (13)			
Viral load (IU/mL)	31631.5 ± 47110.4*	68-123000	3367025.6±4075433.2	1071-15937448	1837.5 ± 2577.3	17-10481.1
Undetectable viral load HIV, n (%)	27 (81.8)					
Undetectable viral load HCV, n (%)	7 (30.4)		3 (13)			
Undetectable viral load HBV, n (%)	1 (50)		1 (100)		7 (18.9)	
CD4+ T-cell count (mm <sup>3</sup> )	569.3 ± 296.6	109-1654				

Table 1: Characteristic profile of couples undergoing IVF-ICSI

\*; Viral load HIV in copies/ml

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A total of 62 sperm washes from 59 couples were performed, and none were positive for the detection of viral molecules. The semen samples from the 34 HBV-positive males were not washed since the female partner had immunity to hepatitis B.

The results of our ICSI programme, with respect to viral infection, are summarized in table 2. A total

of 173 cycles were performed for 93 couples, whereas 25 cycles (14.5%) were cancelled before oocyte retrieval: 2 cycles (8%) due to the presence of an ovarian cyst, 19 cycles (76%) because of low response, 2 cycles (8%) due to hyper-response of the woman, one cycle (4%) due to failure of down-regulation, and one couple failed to attend for oocyte retrieval.

Table	Table 2: IVF performance and outcome of IVF-ICSI cycles									
	HIV (n=33)		HCV (n=23)		HBV (n	=37)				
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range				
Number of cycles initiated, n	61		48		64					
Total FSH	$2409.6 \pm 1153.8$	1013-5550	$2305\pm964.3$	1275-4725	2315.5 ± 845.8	825-4875				
Days of stimulation	$11.2 \pm 2.7$	7-22	$10.7 \pm 2.2$	7-16	$10.6 \pm 1.9$	8-18				
Follicles >17 mm	$5.3 \pm 4.1$	0-15	5.7 ± 3.4 0-13		5.6 ± 3.1	1-14				
Peak estradiol level (pg/ml)	$2760.6 \pm 1408.2$	672-6816	2536,4 ± 1417.1 759.8-6230		$2214.6 \pm 1302.6$	263-5240				
LH (peak estradiol)	$2.4 \pm 1.7$	0.30-8	2.8 ± 1.9	0.21-9	$2.4 \pm 1.6$	0.1-6.1				
Coasting, n (%)	5 (8.2)		1 (2.1)							
Days of coasting	$1.8 \pm 1.3$	1-4	$5\pm0$		$0\pm 0$					
Number of retrievals	48		43		56					
Cycle cancellation rate, n (%)	13 (21.3)		4 (8.3)		8 (12.5)					
Number ofoocytes retrieved per retrieval	$10.6 \pm 6.1$	1-23	$9.4\pm4.6$	0-20	$9.5 \pm 5.2$	0-19				
Number of mature oocytes for ICSI per retrieval	8.5 ± 5.4	1-20	$7.6 \pm 4.1$	0-15	$7.5 \pm 4.4$	1-18				
Number of normally fertilized oocytes per retrieval	4.6 ± 3.6	0-17	4.1 ± 2.9	0-12	4.5 ± 3.4	0-15				
Rate of normally fertilized oocytes per retrieval, (%)	54.6 ± 29.1	0-100	52.3 ± 21.1	0-100	$60.5 \pm 28.4$	0-100				
Embryo transfers										
Retrievals with not enough embryos for fresh transfer, n (%)	6 (18.2)		5 (11.6)		3 (5.4)					
Number of embryos transferred per embryo transfer	$1.9 \pm 0.4$	1-3	2.1 ± 0.5	1-3	$2.0 \pm 0.5$	1-3				
Implantation rate fresh (%)	13.2		16.9		16.8					
Implantation rate thaw (%)	22.2		0		45.5					
Cycles with cryopreservation										
Retrievals with enough embryos for fresh transfer and cryopreservation, n (%)	7 (14.6)		5 (11.6)		12 (21.4)					
Retrievals with cryopreservation (no fresh transfer), n (%)	2 (4.2)		0 (0)		0 (0)					
Number of embryos cryopreserved per cryopreservation	$1.9 \pm 0.4$	1-3	$1.4 \pm 0.9$	1-3	2.8 ± 2	1-8				
Number of embryos thawed, n (%)	19 (59.4)		5 (71.4)		16 (48.5)					
Number of embryos survived and transferred after thawing (cryosurvival), n (%)	9 (47.4)		2 (40)		11 (68.8)					
OHSS, n (%)	1 (1.6)		0 (0)		0 (0)					

## Table 2: IVF performance and outcome of IVF-ICSI cycle

With respect to the different infection diseases, there were no significant differences in the number of oocytes retrieved, the number of mature oocytes, the fertilization rate, the number of embryos transferred or the number of embryos cryopreserved per retrieval. In the case of two women, no transfer was performed, due to ovarian hyper stimulation syndrome (OHSS) and cystitis.

The mean fertilization rate achieved was 56.2%, with a mean implantation rate of 15.7% for fresh transfers and 31.8% for thawed embryos. In total, 38 clinical pregnancies (22% per cycle and 40.9% per couple) took place, with 28 live births delivered (16.2% per cycle and 30.1% per couple). The miscarriage rate was 26.3% and that of multiple pregnancies, 21.4% (Table 3). As a result, 34 newborns (22 singles and 6 twins) were delivered (Table 4). Preterm deliveries (<37 weeks) occurred in 8 neonates, mostly arising from multiple gestation. Extreme prematurity (<32 weeks) was reported in one neonate, 6 babies were born with low birth weight (<2500 g) and one with very low birth weight (<1500 g). No significant differences were recorded in fertilization, pregnancy rates, obstetric or neonatal results for the different groups of CVD.

No seroconversion was detected in any of the 34 newborns (tested at birth and at age 3 months) or in the 62 women treated with washed sperm during assisted reproduction programmes.

	Table 3: Pregnancy data							
		HIV		HCV		HBV		
	n	(%)	n	(%)	n	(%)		
Intrauterine death	0		0		0			
Clinical pregnancy rate fresh	8		10		14			
Per IVF cycle		(13.1)		(20.8)		(21.9)		
Per retrieval		(16.7)		(23.3)		(25)		
Per embryo transfer fresh		(20)		(27.0)		(27.5)		
Per couple		(24.2)		(43.5)		(37.8)		
Clinical pregnancy rate thaw	2		0		4			
Per thaw cycle		(33.3)		(0)		(44.4)		
Per embryo transfer thaw		(40)		(0)		(66.7)		
Clinical pregnancy rate total	10		10		18			
Per IVF cycle		(16.4)		(20.8)		(28.1)		
Per retrieval		(20.8)		(23.3)		(32.1)		
Per embryo transfer thaw		(22.2)		(25.6)		(31.6)		
Per couple		(30.3)		(43.5)		(48.6)		
Miscarriage rate	2	(20)	2	(20)	6	(33.3)		
Ectopic	0	(0)	0	(0)	0	(0)		
Live birth delivery rate fresh	7		8		9			
Per IVF cycle		(11.5)		(16.7)		(14.1)		
Per retrieval		(14.6)		(18.6)		(16.1)		
Per embryo transfer fresh		(17.5)		(21.6)		(17.6)		
Per couple		(21.2)		(34.8)		(24.3)		
Live birth delivery rate thaw	1		0		3			
Per thaw cycle		(16.7)		(0)		(33.3)		
Per embryo transfer thaw		(20)		(0)		(50)		
Live birth delivery rate total	8		8		12			
Per IVF cycle		(13.1)		(16.7)		(18.8)		
Per retrieval		(16.7)		(18.6)		(21.4)		
Per embryo transfer		(17.8)		(20.5)		(21.1)		
Per couple		(24.2)		(34.8)		(32.4)		

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	Table	4: Delivery d	lata			
	HIV		HCV		HBV	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Total number couples delivered	8		8		12	
Total number of infants delivered	10		10		14	
Total number of deliveries						
Singletons, n (%)	6 (75)		6 (75)		10 (83.3)	
Twins, n (%)	2 (25)		2 (25)		2 (16.7)	
Triplets, n (%)	0 (0)		0 (0)		0 (0)	
Multiple gestation rate, n (%)	2 (25)		2 (25)		2 (16.7)	
Vaginal birth, n (%)	6 (75)		3 (37.5)		9 (75)	
Cesarean section, n (%)	2 (25)		5 (62.5)		3 (25)	
Full-Term delivery (≥ 37 weeks), n	6		7	Ď	9	
Number of infants, n (%)	8 (80)		8 (80)		9 (64.3)	
Gestational age (weeks)	$39 \pm 1.5$	38-42	39 ± 1.5	37-41	$38 \pm 1$	37-40
Birth weight (g)	$2925.6\pm2630$	2630-3315	$2809\pm470.2$	2150-3700	$3376\pm353.7$	2760-3890
Preterm delivery (<37 weeks gestation), n	1		1		3	
Number of infants, n (%)	1 (10)		2 (20)		5 (35.7)	
Gestational age (weeks)	36 ± 0	36-36	$34\pm0$		$36\pm0.6$	35-36
Birth weight (g)	$2880 \pm 0$	2880-2880	$2342.5\pm343$	2100-2585	$2286\pm531.6$	1840-3200
Fetal death, n (%)	0 (0)		0 (0)		0 (0)	
Maternal seroconversion, n (%)	0 (0)		0 (0)		0 (0)	
Delivered offspring seroconversion, n (%)	0 (0)		0 (0)		0 (0)	
Extreme prematurity (<32 weeks)						
Number of infants, n (%)	1 (10)		0 (0)		0 (0)	
Low birth weight (<2500 g)						
Number of infants, n (%)	0 (0)		2 (20)		4 (28.6)	
Birth weight	-		$2125\pm35.4$	2100-2150	$2057.5 \pm 169.2$	1840-2215
Very low birth weight (<1500 g)						
Number of infants, n (%)	1 (10)		0 (0)		0 (0)	
Birth weight	$750\pm0$					

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### Discussion

Our data show that sperm wash testing for detectable viral load of final processed semen and ICSI seems to be a safe and effective option for serodiscordant couples to conceive and to avoid transmitting the virus to the mother and child.

It is currently estimated that following recent advances in antiretroviral treatment, when total suppression of the viral load is achieved, the risk of sexual transmission is 1:100000 (23). When ICSI is combined with routine viral detection testing of the final processed semen before using the sample, the risk of transmission is significantly reduced (24-26). In the case of HCV, sexual transmission had been considered to occur only rarely (27, 28). However, recent data indicate that sexual transmission of HCV can occur, especially among HIV-infected persons. Centers for Disease Control and Prevention surveillance data show that 10% of persons with acute HCV infection report contact with a known HCV-infected sex partner as their only risk of infection (29). For the case of HBV, it has been reported that around 25% of stable sexual contacts between patients with chronic HBV infection experience seroconversion (30), although vaccination against HBV drastically reduces the risk of sexually transmitted infection (31).

To date, no seroconversions have been reported following ICSI treatment in which semen samples are processed by density gradient centrifugation of sperm, with or without routine testing of final semen processes (4). In our study, no seroconversions were observed, either among the 34 neonates (tested at birth and at age 3 months), or among the 62 women treated with washed sperm during assisted reproduction programmes.

Some assisted reproduction centres perform ICSI for all couples, rather than Intrauterine insemination (IUI), justifying this on the greater success rate, and therefore, less exposure to possibly contaminated sperm, or on the theory that ICSI requires only *in vitro* contact between a single sperm and egg, which should dramatically reduce the risk of transferring the viral particles that are often present in the semen or seminal cellular compartment (5, 32).

Other authors consider this theory to be unproven, and believe the process could involve the introduction of viral particles directly into the oocyte, with as yet unknown effects (33-35), as well as imposing higher costs and greater demands on healthcare staff, and being associated with a higher number of obstetric complications. At our hospital, ICSI is routinely applied to all couples, because as is the case at other centres (18, 36), and we do not also possess analytical results with which to discount the presence of viral particles on the same day as the sperm washing is performed. Therefore, the semen must be frozen, and in consequence, we do not obtain sufficient motile sperm for IUI to be performed.

Considerable differences have been reported in the percentage of positive results for RNA-HIV of final processed semen, ranging from 0% (37) to 40% (38). Similar discrepancies have been reported for HCV, with detection rates ranging from 0 (39, 40) to 57% (41). These differences may be due to the heterogeneity of the patients treated, to the sensitivity of the PCR kits used or to the elimination of PCR inhibitors (42). For this reason, it is essential that all concerned, like ourselves and other researchers, should participate in external quality control programmes (43, 44).

Our results for clinical and ongoing pregnancies achieved for couples in which the male partner is HIV positive are similar to those described by other authors (18, 45), although lower than those reported by some (7, 8). This could be because the mean number of embryos transferred by the latter (3.54 and 3.54 and 3.55 andrespectively) is significantly higher than our value of 2 embryos. However, our multiple pregnancy rates (21.4%) is lower than that obtained by these authors (57.1 and 41%, respectively), and is similar to that obtained in another study, in which fewer embryos were transferred (46). It should be taken into account that many of these patients are fertile couples, with no reproductive problems, and therefore, the number of embryos transferred should be minimised in order to reduce the risk of multiple pregnancy.

With respect to clinical and ongoing pregnancies for couples with HCV and HBV-positive male partners, our results are similar to those published by other authors (13, 15, 18).

Although we found no statistically significant differences in the pregnancy rates among the three types of couples studied, the trend for rates to be lower among the HIV-infected couples could be related to reduce fertility rate among HIV-infected men as a result of treatment or infection (47, 48). With respect to the difference in implantation rates observed among all groups, between fresh and

between the embryo and the endometrium in cycles with thawed embryos (49). In turn, this factor could result from the fact that endometrial development in frozen-thawed cycles can be controlled more precisely than in cycles of controlled ovarian hyperstimulation with gonadotropins (50).

Although not expressly described, we found no significant differences between embryology laboratory results for males who were only HIV seropositive, and those who were positive for both HIV and HCV. This has also been reported by other authors, in studies of these two groups of patients, or comparing them with the results obtained from healthy couples (14, 51).

Although the rate of caesarean delivery was higher among the group in which the male partner was HCV infected, these differences were not statistically significant. Moreover, the overall rate of caesareans in our study (35.7%) was lower than that described by other authors for this type of couple (6-8). The obstetric and neonatal complications recorded were similar to those published in the literature for non-serodiscordant couples (52, 53).

### Conclusion

We showed sperm washing and ICSI is a safe and effective option for reducing the risk of transmission or super infection in serodiscordant or concordant couples who wish to have a child. The pregnancies obtained by ART among couples in which the male partner has CVD produce good obstetric and neonatal results.

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