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Correspondence:
Gianmartin Cito, Department of Urology, Careggi Hospital, University of Florence, Largo Brambilla, 3, Florence 50134, Italy.
E-mail: gianmartin.cito@gmail.com

*These authors contributed equally to this work.

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Influence of male human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infection on the reproductive outcomes in serodiscordant couples: a case–control study

[1]G. Cito, [2,*]M. E. Coccia, [2]R. Fucci, [2]R. Picone, [1]A. Cocci, [3]G. I. Russo, [2]F. Rizzello, [4]M. Trotta, [2]L. Badolato, [2]V. Basile, [2]L. Criscuoli, [1]S. Serni, [1]M. Carini and [1,*]A. Natali

[1]Department of Urology, Careggi Hospital, University of Florence, Florence, Italy, [2]Assisted Reproductive Technology Centre, Careggi Hospital, University of Florence, Florence, Italy, [3]Department of Urology, Vittorio Emanuele Hospital, University of Catania, Catania, Italy, [4]Department of Critical Care Medicine and Surgery, Infectious Disease Unit, Careggi Hospital, University of Florence, Florence, Italy

ABSTRACT

Background: Nowadays, serodiscordant couples (SDCs) with human immunodeficiency virus (HIV) or hepatitis C virus (HCV)-infected men have the chance to conceive safely, giving birth with a minimum risk of cross-infection.

Objective: To assess the impact of male HIV and HCV infection on the assisted reproductive technologies (ART) outcomes in SDCs, with HIV or HCV seropositive men and negative partners.

Materials and methods: Of 153 couples: 24 in Group 1 (HIV-seropositive men), 60 in Group 2 (HCV-seropositive men) and 69 in Group 3 (controls). Sperm-washing procedure was performed using a three-step system. Fresh ICSI cycles were carried out in HIV SDCs, HCV SDCs and controls. Seminal parameters, fertilization rate (FR), cleavage rate (CR), pregnancy rate per cycle (PR/C), miscarriage rate, implantation rate (IR) and live birth rate were evaluated.

Results: All the seropositive men have undetectable viral loads at the time of insemination, and both partners were free from comorbid infections. The median number of embryos transferred was 2.0 (IQR 1.0–3.0), with no differences among groups. FR was significantly reduced in HIV and HCV SDCs compared to the controls (66%, 61% and 75%, respectively; $p < 0.01$). CR was similar between groups ($p = 0.3$). IR was 12.1%, 11.1% and 14.1%, respectively, in the three groups ($p = 0.30$). PR/C was 21.7%, 17.6% and 20.2% in HIV, HCV and controls, respectively. Live birth rate per cycle was 17.4%, 15.7% and 15.9%, respectively. There were no significant differences in clinical pregnancies per cycle, as well as miscarriages and live births ($p = 0.30$; 0.30; 0.60, respectively).

Conclusions: The sperm-washing technique with ICSI may generate a promising way to improve pregnancy outcomes and to reduce the risk of viral transmission in these couples. In this setting, we can correctly counsel HIV- and HCV-infected men of SDCs with regard to the likelihood of father their own biological child.

INTRODUCTION

During the past years, the perspective on life of people suffering from human immunodeficiency virus (HIV) infection and its related disease, acquired immune deficiency syndrome (AIDS), has considerably changed. Indeed, the quality of life (QoL) of seropositive individuals greatly improved, thanks to the successful therapy with highly active anti-retroviral treatment (HAART) (Anderson, 1999; Englert *et al.*, 2001). Nowadays, HIV-serodiscordant couples (SDCs) who attempt to conceive naturally must take into account the risk of infecting each other. Actually, it has been expected 1/500–1000 acts of HIV transmission in unprotected intercourse (Anderson, 1999), with transfer from male to female and vice versa of 0.15 and 0.09%, respectively (Ajayi & Melie, 2003). The risk is affected by several aspects such as grade of virulence, viral load and phase of the disease (Maldelbrot *et al.*, 1997; Ethics Committees of ASRM, 2002). Indeed, it should be noted that in heterosexual HIV SDCs having sexual intercourse, when the man has undetectable viral loads (Quinn *et al.*,

2000) or has received HAART treatment, and both partners are free from genital infections, the risk of transmission is close to zero (de Vincenzi, 1994; Del Romero *et al.*, 2010).

On the other hand, the global rise of hepatitis C virus (HCV) has largely generated a growth of HCV-seropositive sub-fertile couples (Piao *et al.*, 2014; Yip *et al.*, 2014). Currently, in the era of assisted reproductive technologies (ARTs) it is possible to avoid the virus transmission to the partner, conceiving safely and giving birth with a minimal risk of cross-infection. In this scenario, intrauterine insemination (IUI) or in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) is, however, necessary.

Nevertheless, there are different points to be discussed if the male or the female is infected separately. If the female is HIV- or HCV-positive and the male seronegative, the possibility of viral transmission to the partner and to the baby, the impact of pregnancy on the disease progression and the influence of the pathology on the pregnancy should be emphasized (Delvinge *et al.*, 2001). The male transmission can be eluded by inseminating spermatozoa by IUI. If seminal parameters are not fitting or after repeated unsuccessful efforts at IUI, IVF/ICSI should be considered mandatory.

If the male is seropositive and the female negative, the main challenge is to attain a sample of virus-free spermatozoa. HAART can reduce the plasma viral load to undetectable levels, decreasing the risk of sexual transmission (Gilling-Smith, 2000). Otherwise, sperm-washing process is a good option to eliminate HIV or HCV (Garrido *et al.*, 2005). The absence of detectable virus is verified using a polymerase chain reaction (PCR) nucleic acid-based sequence amplification assay. Successively, ICSI can be performed, in order to diminish the viral exposure to few motile sperm cells (Sauer & Chang, 2002).

So far, there is still a lot of confusion about the influence of male viral infection on the outcomes of an assisted reproductive programme, considering healthy uninfected women.

Thus, the aim of this work was to assess the impact of male HIV and HCV infection on the ART outcomes in a group of SDCs, in which the man is HIV- or HCV-seropositive and the woman negative.

MATERIALS AND METHODS

Study population

From February 2011 to August 2018, we carried out a single-centre retrospective analysis of 3375 infertile couples referred to our ARTs Centre for IVF treatment. Inclusion criteria were as follows: (i) female age range 18–40 years and male age range 18–45 years; (ii) couples HIV- or HCV-serodiscordant, when the man was HIV or HCV and the woman seronegative; (iii) couples interested to perform sperm-washing procedure. For HIV SDCs, we involved patients with a CD4 lymphocytes count > 200/mm and a stable viral load, evaluated <4 months before ART beginning. All patients were treated with HAART to reduce viremia below the threshold. All patients with anomalous liver function or chronic hepatitis were omitted. Moreover, we withdrew from the study all those men with azoospermia or severe criptozoospermia. Control group comprised seronegative males, partners of seronegative normo-ovulatory females with tubal-factor infertility and no evidence of other gynaecological pathologies (i.e. endometriosis).

A careful fertility evaluation was performed in both partners by a multidisciplinary specialized team. On day 2 of the cycle, all women were screened for hormonal assessment, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), anti-mullerian hormone (AMH), thyroid-stimulating hormone (TSH) and prolactin (PRL). Transvaginal ultrasounds for uterine and adnexal evaluation were performed, with particular focus on ovarian reserve on the day 3–5 of cycle. Moreover, uterine and tubal factor were evaluated by sonohysterosalpingography. Screening of cervical and breast cancer was completed through the Papanicolaou test and mammography. All men were tested for endocrinology profile (FSH, LH, total testosterone, TSH and PRL) and a detailed physical examination. Baseline characteristics, including male age and partner's age, were collected from our data records. Both male and female were screened by a dedicated infectious disease specialist for HIV 1/2, HCVab, HbsAg, HbsAb, HBcAb, Treponema Pallidum Hemagglutination and Venereal Disease Research Laboratories (TPHA-VDRL), Ab anti-Chlamidya Trachomatis and Ab anti-cytomegalovirus (CMV).

All the men carried out semen analysis in two determinations, evaluated according to the 2010 World Health Organization (WHO) guidelines. On the day of fresh cycle, we assessed seminal parameters, as follows: volume, pH, total sperm concentration, total sperm count/mL, viability, progressive motility (PR), non-progressive motility (NP), immobility (IM) and morphology. Thereafter, the sperm-washing procedure was performed, according to Semprini protocol, in order to eliminate the leucocytes from HIV-infected semen. After the sperm wash, we analysed sperm concentration/mL and motility.

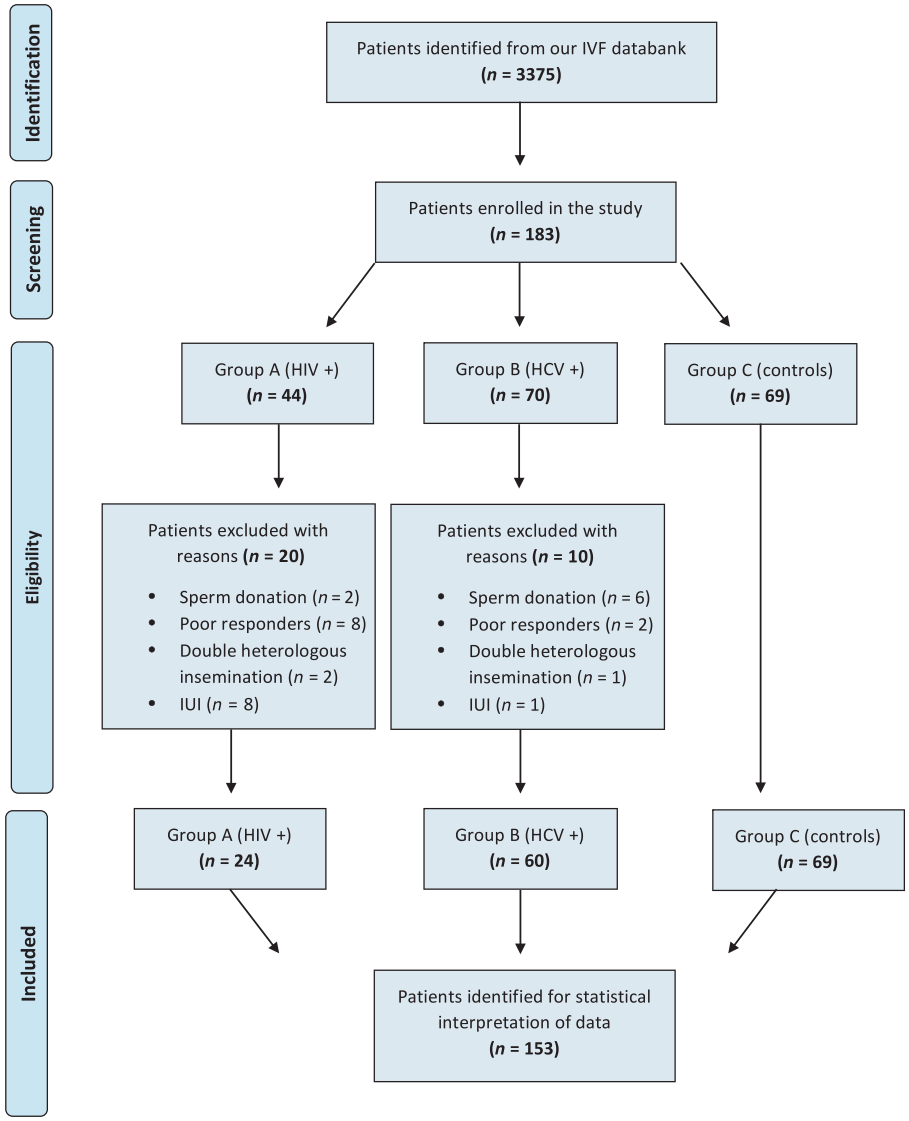
Study schedule

Three groups were detected (Fig. 1): couples with HIV-seropositive men and negative partners (Group 1), couples with HCV-seropositive men and negative women (Group 2), and controls with healthy seronegative male and seronegative female suffering from tubal infertility (Group 3).

Sperm preparation

Semen specimens were collected by masturbation after 2 to 7 days of sexual abstinence. After liquefaction in the incubator set at 37 °C for 30 min, ejaculates were examined and categorized. Seminal samples were treated under sterile conditions, using the density gradient centrifugation method with 95% and 50% gradient layers (PureSperm ®100, Nidacon Mölndal, Sweden). Each sample was distributed into 1 mL aliquots, which were relocated into 15-mL conical tubes (Nunc) containing 1 mL of the 50% fraction and 1 mL of the 95% fraction. Oligospermic specimens were managed using smaller volumes of the gradient layers. The semen samples, layered on top of the double density gradient, were centrifuged at 300 g for 20 min. Supernatants were prudently dismissed using a sterile Pasteur pipette for each tube. The pellets of the same sample were subsequently placed into a new conical tube, re-suspended in 2.5 mL of sperm medium (Flushing, Origio, Malov, Denmark) and centrifuged at 250 g for 10 min. The tube was placed at a 45° angle and incubated for 1 h in the incubator set at 37 °C. After incubation, the upper 1 mL of medium was recovered and divided into two parts. One part was utilized for post-wash HIV/HCV testing (at the Serology Section of the Careggi Hospital-

Figure 1 Study schedule.



University), and the other part was used for ICSI treatment, once PCR results were available. One drop of medium was used to assess microscopically sperm concentration/mL and motility.

ICSI and embryo transfer

The individualized predictive factors of ovarian response, including anti-Müllerian hormone (AMH) and antral follicle count (AFC), are used to guide the treatment protocol. The standard starting dose of recombinant FSH was 225–375 IU (Gonal-F; Serono, Italy), relying on the woman's age and baseline serum FSH level. The follicular growth was checked with sequential ultrasound images, and the dose of FSH was regulated according to the follicular response. In the presence of ≥14 follicles, a short protocol with gonadotropin-releasing hormone (GnRH) antagonist was used. Serial ultrasound scans were performed to control the follicular advance and to programme the appropriate time of oocytes retrieval. When at least two follicles had reached a maximal diameter of 17–18 mm, 250 mcg recombinant human chorionic gonadotropin (HCG; Ovitrelle; Merk Serono, Milan, Italy) was subcutaneously administered. Transvaginal oocyte retrieval was performed approximately 36 h later.

All oocytes retrieved by HIV and HCV SDCs were submitted to ICSI procedure. The incubation of injected eggs was performed in 20 μL drops. All the zygotes were assessed 16–18 h by ICSI to evaluate the presence of two distinct pronuclei. Consequently, all the embryos were valued on days 2, 3 and 5 of the development with an inverted microscope. We considered high-quality cleavage-stage embryos those with all of this qualities: four cells on day 2 or 8–10 cells on day 3, <15% fragmentation, regular blastomeres, non-existence of multinucleation, colourless cytoplasm with moderate granulation and no inclusions, lack of perivitelline space granularity, no imperfections of zona pellucida (classified with the score A). The absence of these measures were evaluated of low quality (score B-C). Thereafter, ICSI outcomes, as well as fertilization rate (FR), cleavage rate (CR) and pregnancy rate per cycle (PR/C), were evaluated. The total and normal oocyte FR was calculated by total number of fertilized oocytes and 'two pronuclear' (2PN) fertilized oocytes by the number of injected oocytes,

Table 1 Baseline characteristics and reproductive outcomes of the study population (n = 153)

Parameter	Group 1 (HIV+)	Group 2 (HCV+)	Group 3 (controls)	p value*	1 vs. 2[a]	2 vs. 3[a]	1 vs. 3[a]
Male age (years)	40.0 (36.0–43.0)	44.0 (39.0–46.0)	39.0 (35.0–42.0)	<0.01	0.02	<0.01	NS
Female age (years)	36.0 (33.0–40.0)	38.0 (36.0–40.0)	36.0 (34.0–40.0)	<0.05	NS	NS	NS
Oocytes retrieved (n)	6.0 (4.0–8.0)	7.0 (5.0–9.0)	7.0 (4.0–9.0)	0.09	NS	NS	NS
Oocytes inseminated (n)	5.0 (4.0–6.0)	6.0 (4.0–7.0)	5.0 (3.0–7.0)	0.02	0.03	NS	0.02
Oocytes 2PN fertilized (n)	3.0 (2.0–3.0)	3.0 (1.0–4.0)	3.0 (2.0–5.0)	0.20	NS	NS	NS
Oocytes total 1-2-3PN fertilized (n)	3.0 (2.0–4.0)	3.0 (2.0–4.0)	4.0 (2.0–6.0)	0.12	NS	NS	NS
Fertilization rate 2PN (%)	66.0 (50.0–75.0)	55.0 (40.0–72.0)	66.0 (50.0–85.0)	<0.01	NS	NS	<0.01
Fertilization rate 1-2-3PN(%)	66.0 (50.0–75.0)	61.0 (50.0–75.0)	75.0 (60.0–100.0)	<0.01	NS	NS	<0.01
Embryos obtained (n)	3.0 (2.0–3.0)	3.0 (2.0–3.0)	3.0 (2.0–5.0)	0.87	NS	NS	NS
Embryos transferred (n)	2.0 (1.0–3.0)	2.0 (2.0–3.0)	2.0 (1.0–2.0)	<0.01	NS	NS	<0.01
Cleavage rate (%)	100.0 (75.0–100.)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	0.23	NS	NS	NS

Values are median (IQR). NS, not significant; PN, pronuclear. *ANOVA test. [a]Bonferroni test.

Table 2 Pregnancy outcomes

Parameter	Group 1 (HIV+)	Group 2 (HCV+)	Group 3 (Controls)	p value*	1 vs. 2[a]	2 vs. 3[a]	1 vs. 3[a]
Implantation rate, n (%)[a]	10/82 (12.1)	24/216 (11.1)	16/113 (14.1)	0.30	NS	NS	NS
Pregnancy rate per cycle, n (%)[a]	10/46 (21.7)	18/102 (17.6)	14/69 (20.2)	0.30	NS	NS	NS
Miscarriage rate per cycle (%), n (%)[a]	2/10 (20.0)	2/18 (11.1)	4/14 (28.5)	0.38	NS	NS	NS
Live birth rate per cycle (%), mean (SD)[a]	8/46 (17.4)	16/102 (15.7)	11/69 (15.9)	0.60	NS	NS	NS

Variables not normally distributed. NS, not significant; SD, standard deviation. *ANOVA test. [a]Bonferroni test.

respectively. The CR was calculated by the number of embryos obtained by the number of normal fertilized oocytes. The luteal phase was supported with a daily administration of 50 mg intramuscularly of natural progesterone in oil (Prontogest; Amsa; Italy) starting the day after the oocyte retrieval. Embryos were transferred into the uterine cavity 72 to 120 h after ICSI procedure. At our centre, embryo transfers are performed under transabdominal ultrasound guidance using a Wallace catheter (© COOK Medical Incorporated Bloomington, IN, USA). Our laboratory policy on transfer of donor oocytes embryos is to transfer two embryos, either on day 3 or day 5, based on morphology. Supernumerary embryos are frozen. After 14 days, HCG test was performed. The implantation rate was defined as the number of gestational sacs per embryo transferred. Clinical pregnancy was defined by HCG levels above 50 mU/L and documented by transvaginal ultrasound visualization of an intrauterine gestational sac with a heartbeat at around 5–6 weeks of gestation. Pregnancy loss before 20 weeks of gestation and all biochemical pregnancies were considered as miscarriages. Live birth rate was defined as the percentage of all cycles that lead to live birth.

Ethical considerations and statistical analysis

The couples have been adequately counselled on potential risks of the procedure. In HIV and HCV SDCs, the safety of the ART was guaranteed by a local quality manual, according to the international guidelines. All participants were fully informed of the issues involved and signed a detailed consent form, agreeing to have their data anonymously utilized. All procedures were performed in accordance with the ethical standards of the institutional (CS/1158/05; CS/1158/06) and national research committee and with the 1975 Helsinki Declaration. Continuous variables are presented as median and interquartile range (IQR). ANOVA test has been used for comparing three or more group means for statistical significance and post hoc analyses using the Bonferroni test. Categorical variables are presented as rate and percentage, and were tested with the chi-square test. A p-value < 0.05 was set as statistically significant.

RESULTS

As shown in Fig. 1, from our IVF databank, we extracted 228 couples for this case-control retrospective study: 44 couples had men HIV-seropositive, 70 had men HCV-seropositive, and 69 were controls. From HIV and HCV group, 30 couples were excluded from the study. Overall, 153 couples were considered for statistical interpretation of data: 24 couples were included in Group 1 (HIV-seropositive men), 60 in Group 2 (HCV-seropositive male) and 69 in Group 3 (controls). Overall, 217 fresh ICSI cycles were performed: 46 cycles in Group 1, 102 cycles in Group 2 and 69 cycles in Group 3.

The demographic data and the reproductive outcomes of the cases and controls are presented in Table 1. The cause of HIV and HCV infection in the man was: drug addiction (27.2% and 33.3%, respectively), sexual intercourse (68.2% and 58.3% respectively) and unknown causes (4.5% and 8.3, respectively). Except for control group, all of the females were healthy and had no determined pathology, such as diminished ovarian reserve, low response to ovarian stimulation protocol or chromosomal abnormalities. All men who underwent sperm-washing process have undetectable (<50 copies/mL) viral loads at the time of insemination. After sperm-washing, no seroconversion of partners or offspring was recorded.

Among couples including HIV- and HCV-positive men, we found a significantly reduced total FR compared to the controls (66%, 61% and 75%, respectively; $p < 0.01$). CR was similar between groups ($p = 0.3$). As shown in Table 2, implantation, clinical pregnancy per cycle, miscarriage and live birth rates were comparable between groups, not showing significant differences. The live births, summing singleton and twin pregnancies, for couples with an HIV-positive man, HCV-positive man and controls were respectively 12, 22 and 23.

Table 3 showed the results of semen parameters. The median sperm concentration/mL was comparable between groups, suggesting that sperm production was not affected by the infection. The median total sperm count was significantly higher in HIV-infected males, although no relevance is supposed on the overall

Table 3 Comparison between seminal parameters in groups, before and after washing (AW)

Parameter	Group 1 (HIV+)	Group 2 (HCV+)	Group 3 (Controls)	*p* value*	1 vs. 2[a]	2. vs 3[a]	1 vs. 3[a]
Abstinence time (days)	3.5 (2.0–5.0)	3.5 (2.0–5.0)	3.5 (3.0–5.0)	0.83	NS	NS	NS
Volume (mL)	3.2 (2.3–4.5)	2.5 (2.0–3.1)	2.8 (2.0–3.8)	<0.01	<0.01	NS	NS
pH	7.6 (7.4–7.8)	7.8 (7.6–7.8)	7.6 (7.6–7.8)	0.035	0.02	NS	NS
Viability (%)	78.0 (67.0–82.0)	70.0 (61.0–75.0)	73.5 (70.0–80.)	<0.01	0.04	NS	0.01
Concentration (sperm/mL)	36.0 (8.0–74.0)	39.0 (18.0–77.0)	38.0 (18.0–62.0)	0.38	NS	NS	NS
Total sperm count	158.4 (30.0–218.5)	84.0 (40.8–167.4)	122.5 (42.0–202.4)	<0.01	0.01	NS	NS
Progressive motility (PR), (%)	50.0 (30.0–70.0)	40.0 (30.0–50.0)	55.0 (40.0–65.0)	<0.01	<0.01	NS	<0.01
Non-progressive motility (NP), (%)	10.0 (10.0–10.0)	10.0 (10.0–15.0)	10.0 (5.0–10.0)	0.90	NS	NS	0.03
Immobility (IM), (%)	30.0 (20.0–50.0)	45.0 (40.0–60.0)	40.0 (30.0–45.0)	<0.01	0.01	NS	<0.01
Morphology (%)	5.0 (4.0–6.0)	5.0 (4.0–7.0)	5.0 (4.0–6.0)	0.52	NS	NS	NS
Concentration (sperm/mL) AW	6.0 (0.6–16.0)	3.0 (1.3–10.0)	4.0 (1.5–12.0)	0.03	NS	NS	NS
Motility AW (%)	95.0 (85.0–95.0)	95.0 (70.0–95.0)	90.0 (85.0–95.0)	0.45	NS	NS	NS

Values are median (IQR). AW, after washing; NS, not significant. *ANOVA test. [a]Bonferroni test.

embryo quality results because severe male factor was excluded. Progressive motility (PR) resulted significantly lower in HIV and HCV group, compared to the controls (50%, 40%, 55%, respectively). After sperm washing, seminal characteristics are comparable between groups independently of the infection. Indeed, we did not find a significant correlation between groups in terms of sperm concentration/mL and motility, after sperm washing processing ($p = 0.03$; $p = 0.45$).

DISCUSSION

Nowadays, the advances of ARTs in assisting HIV and HCV patients in Europe have produced successful pregnancies, free of mother's or child's seroconversion (Wu & Ho, 2015). In 1992, Semprini was the first one to describe the sperm-washing method, consisting of three steps: (i) purifying the liquefied semen through a gradient; (ii) washing the retrieved spermatozoa to eliminate seminal plasma; and (iii) swim-up to retrieve highly motile spermatozoa (Semprini *et al.*, 1992). The analysis of the final specimen by using a PCR assay would guarantee the removal of HIV-1 virus throughout the wash practise. There are several studies that stated the effectiveness of semen washing to prevent HIV transmission and assist pregnancy in HIV-discordant couples (Zafer *et al.*, 2016). All of the previous studies that measured HIV transmission to infants reported no cases of vertical transmission, demonstrating the safety of the procedure (Kim *et al.*, 1999; Sunderam *et al.*, 2008). Overall, each of them agrees with recommending 'sperm washing' as a safer alternative to natural conception for HIV SDCs wishing to have children. Even now, in our country there are few centres that accept SDCs for ART purpose. On these bases, we proposed to all of our HIV-seropositive patients the sperm-washing protocol, in order to decrease the risk of vertical and horizontal viral transmission. Equally, we treated couples with HCV-seropositive males by the same procedure, since it was shown that HCV can be detected in semen, although with low prevalence (Levy *et al.*, 2000). Overall, in our case series we did not report partner HIV/HCV seroconversion or transmission of the newborn.

The reason why in our protocol we opted for ICSI in HIV- and HCV-seropositive patients is supported by previous studies. Normally, IVF with ICSI offers 2–5 times higher pregnancy rates compared to IUI, meaning less recurrent exposure to HIV-1 (Pena *et al.*, 2003).

Nevertheless, the choice of ICSI vs. IVF in the absence of sperm alterations is discussed in literature and a lack of uniformity in the decision of the best clinical practise emerges (van Leeuwen *et al.*, 2009). Indeed, some authors even looked at the violation of the zona pellucida as a mode of introducing HIV directly into the oocyte, maybe leading to viral genome integration.

Based on the previous literature (Sauer & Chang, 2002), according to the multidisciplinary team, our protocol provides that ICSI procedure represents the gold standard for couples involving HIV- or HCV-positive men.

In our study design, we decided to exclude all couples with HIV- or HCV-seropositive women, in order to focus on the male influence and to reduce all possible bias related to the partner. Indeed, it is widely known that the female infection could compromise fertility in different ways. First of all, menstrual abnormalities occurred up to 20% of cases of infection (Kimani *et al.*, 1996). Moreover, tubal pathologies may also happen at a greater occurrence because of a higher frequency of pelvic inflammatory disease (Gray *et al.*, 1998), further to their major vulnerability to infection. On the other hand, we included in the control group all couples with fertile healthy seronegative men and females suffering from tubal infertility factor, without other pathologies. In this way, we assumed the oocyte quality to be optimal, in order to isolate the male factor.

However, there is still a grey area about the impact of viral male infection on the reproductive outcomes. Several studies are reported in literature, investigating the clinical outcomes of HIV- or HCV-seropositive men, but very few with a case–control study design. Some authors believed that male HIV infection has no effect on fertilization, implantation, pregnancy and live birth rates (Melo *et al.*, 2008; Yang *et al.*, 2015; Vankerkem *et al.*, 2017). Equally, two out of the three matched control studies in literature did not find differences in clinical pregnancy rates (Santulli *et al.*, 2011; Prisant *et al.*, 2010), whereas the third one detected higher rates of FR and PR/C for cases involving HIV-positive men (Kashima *et al.*, 2009). However, in most studies a retrospective non-controlled design was involved with clinical pregnancy rates per cycle and per embryo transfer varying from 17.7 to 42.0 and 26.6 to 46%, respectively (Veiga *et al.*, 1999; Marina *et al.*, 2003; Mencaglia *et al.*, 2005; Savasi *et al.*, 2007; Sauer *et al.*, 2009). Moreover, Prisant *et al.* detected fertilization and cleavage rates significantly lower in HCV males SDCs than in controls. However, as a strong limitation, they considered only cycles post-thaw sperm recovery (Prisant *et al.*, 2010). In our study, we considered all fresh cycles, in order to remove all possible negative side effect of the sperm cryopreservation.

In our study cohort, we observed a significant reduction in fertilization rates for HIV- and HCV-positive men in comparison

with controls. This probably could occur because antioxidant defences, including superoxide dismutase and glutathione peroxidase, decline during HCV or HIV chronic infection (Levent *et al.*, 2006).

Nevertheless, in our experience the overall pregnancy rates and live birth rates per cycle in HIV- and HCV-seropositive men were comparable to those of the matched control seronegative group.

Regarding the sperm quality in HIV-positive males, controversial reports are currently discovered in literature. Several studies detected an important injury of the gonads in seropositive males, as well as worse sperm production and quality (Diehl *et al.*, 2003; Mital *et al.*, 2004). Otherwise, the specific immunologic circumstance in HIV-positive males and the therapies received could meddle with sperm quality and epigenetics.

In this setting, all viruses have a potentially negative effect on male reproductive function. The HIV itself has profound negative effects on semen quality, as does infection with HCV, so treatment with anti-virals generally improves semen quality in men with longer duration of infection and/or greater symptoms. Currently, men with HIV infections take combination anti-retroviral therapy (cART), involving three or more medications, making it difficult to assess the toxicity of individual medications. For example, zidovudine alone or cART have minimal negative effects on semen quality. This effect could possibly be attributed to the mitochondrial toxicity of nucleoside inhibitors of reverse transcriptase (Sergerie *et al.*, 2004). However, the studies that investigate the influence of HIV medications on semen quality are controversial.

In general, HIV infection itself has been associated with hypogonadism and semen abnormalities, and cART increases the CD4 cell count and general health of patients, which are related to better semen quality (Drobnis & Nangia, 2017).

In this respect, our study population comprised fertile men with normal testicular volume and hormonal levels in the normal range.

Moreover, in our study, although total sperm count was significantly higher in HIV patients, the extensive sperm processing and testing lead to poor final sperm concentration. Overall, after sperm washing no significant differences were found in sperm concentration and motility.

According to previous studies, HIV-positive men seem to produce fewer motile spermatozoa (Umapathy, 2005). This relationship might be mediated by the activation of leucocytes, which caused oxidative stress on the spermatozoa (Umapathy *et al.*, 2001).

This study is troubled by some limitations. The study design was retrospective, and some data were missing: the duration of infection and information about HIV treatment (type and duration). However, all the SDCs access to ART until after the infectious disease specialist has evaluated the clinical assessment. Sample size of study population was limited for HIV- and HCV-positive couples, as a result of stringent recruitment criteria, which led us to exclude several couples from the statistical interpretation of data, due to our specific primary outcome.

CONCLUSION

Infertility treatment advances have helped HIV and HCV SDCs to conceive successfully and safely. The sperm-washing technique and the appropriate ovarian stimulation protocol with ICSI may generate an encouraging way to improve pregnancy outcomes and to minimize the risk of viral transmission in these couples. In this setting, we can correctly counsel HIV- and HCV-infected males of SDCs with regard to the likelihood of fathering their own biological child.

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