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ORIGINAL ARTICLE *Andrology*

High-risk human papillomavirus in semen is associated with poor sperm progressive motility and a high sperm DNA fragmentation index in infertile men

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STUDY QUESTION: Does the presence of human papillomavirus (HPV) in semen impact seminal parameters and sperm DNA quality in white European men seeking medical help for primary couple's infertility?

SUMMARY ANSWER: HPV seminal infections involving high-risk (HR) genotypes are associated with impaired sperm progressive motility and sperm DNA fragmentation (SDF) values.

WHAT IS KNOWN ALREADY: HPV is commonly present in semen samples. However, whether the presence of HPV in semen is actually associated with impaired sperm parameters and SDF values have yet to be elucidated.

STUDY DESIGN, SIZE, DURATION: In this cross-sectional study, complete demographic, clinical and laboratory data from 729 infertile men were analysed.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Health-significant comorbidities were scored with the Charlson comorbidity index (CCI). Serum hormones and SDF index (measured by the sperm chromatin structure assay [SCSA]) were measured in every patient (SDF ≥30% was defined as pathological). Semen analysis was based on 2010 World Health Organisation reference criteria. Amplification by nested PCR was used to detect HPV-DNA sequences in semen samples. Descriptive statistics and linear regression models were used to test the association between the presence of HPV and clinical and seminal characteristics in the whole cohort.

MAIN RESULTS AND THE ROLE OF CHANCE: The overall rate of HPV positivity was 15.5% (113/729). Overall, 78/729 (10.7%) and 35/729 (4.8%) patients had HR HPV+ and low-risk HPV+, respectively. HPV16 was the most prevalent type (22.1%), followed by HPV43 (10.6%), HPV56 and HPV42 (both 8.8%). No differences were found in terms of clinical and hormonal characteristics between patients with or without seminal HPV. Sperm progressive motility was significantly lower ($P = 0.01$) while SDF values were higher ($P = 0.005$) in HPV+ men compared to those with no HPV. In particular, HR HPV+ men had lower sperm progressive motility ($P = 0.007$) and higher SDF values ($P = 0.003$) than those with a negative HPV test. Univariable analysis showed that HR HPV+ was associated with impaired sperm progressive motility ($P = 0.002$) and SDF values ($P = 0.003$). In the multivariable analysis, age, FSH levels and testicular volume were significantly associated with impaired sperm progressive motility (all $P \leq 0.04$). Conversely BMI, CCI, smoking habits and HPV status were not.

Only age ($P = 0.02$) and FSH ($P = 0.01$) were significantly associated with SDF, after accounting for BMI, CCI, testicular volume, smoking habits and HPV status.

LIMITATIONS, REASONS FOR CAUTION: Main limitations are the cross-sectional design of our study and the relatively small sample size of the subgroups. Additional limitations are the lack of a control group of normal fertile men and the lack of follow-up testing to check the clearance or the persistence of HPV in semen after a 6–12 months.

WIDER IMPLICATIONS OF THE FINDINGS: Overall, these observations point out the importance of an accurate investigation of seminal HPV presence in everyday clinical practice in the diagnostic work-up of infertile men.

STUDY FUNDING/COMPETING INTEREST(S): No external funding was used. There are no competing interests.

Key words: human papillomavirus / infertility / sperm DNA integrity / sperm progressive motility / risk factors

Introduction

Several conditions have been associated with male factor infertility (MFI), such as abnormalities of the urogenital tract, malignancies, endocrine disorders, immune factors and sexually transmitted infections (STI); however infertility is still idiopathic in nature in ~30–40% of men (Jungwirth *et al.*, 2017).

Human papillomavirus (HPV) is one of the most common sexually transmitted virus in both genders worldwide and skin represents the most commonly affected organ (Bezold *et al.*, 2007; Forman *et al.*, 2012; Ventimiglia *et al.*, 2016b). HPV low-risk (LR) genotypes can cause genital warts and respiratory papillomatosis, whereas persistent infection with high-risk types (HR) can promote the malignant transformation of cells in the cervix, but also in the vagina, vulva, anus, penis, mouth and throat (Dunne and Park, 2013; Ventimiglia *et al.*, 2016b). Beside the aetiological role in cancer, the association between HPV infection in females and adverse pregnancy outcomes has been extensively reported (Souho *et al.*, 2015). Conversely, the impact of HPV infection on male reproductive parameters is a matter of controversy (Foresta *et al.*, 2015; Ventimiglia *et al.*, 2016b).

HPV is commonly present in semen samples, with a reported prevalence of 16% in infertile populations and 10% in men from the general population (Laprise *et al.*, 2014). However, whether the presence of HPV in semen is actually associated with impaired sperm parameters has yet to be elucidated. Previous evidence has suggested that HPV infection in semen could be implicated in male infertility through the reduction of sperm motility and alteration of semen viscosity, pH and leucocyte number (Foresta *et al.*, 2010; Garolla *et al.*, 2013; Yang *et al.*, 2013). However, this association was not confirmed in other recent studies (Golob *et al.*, 2014; Luttmer *et al.*, 2016).

Sperm DNA fragmentation (SDF) has progressively gained clinical importance in terms of assisted reproductive technology outcomes (Practice Committee of the American Society for Reproductive Medicine, 2015; Agarwal *et al.*, 2016; Esteves *et al.*, 2017). In this context, the current literature includes both studies that report a negative impact of HPV infection on SDF (Connelly *et al.*, 2001) and some studies that have failed to find any association between semen HPV and SDF values (Kaspersen *et al.*, 2013; Cortés-Gutiérrez *et al.*, 2017).

Overall, the impact of semen HPV infection on sperm parameters and SDF has been investigated by previous studies with relatively small cohorts, showing findings that were not unanimous (Foresta *et al.*, 2015). Therefore, we sought to cross-sectionally investigate the association between the presence of HPV in semen and sperm parameters and SDF rates in a homogeneous large cohort of white European men seeking medical help for the couple's primary infertility associated with male factors.

Materials and Methods

We analysed data from 729 white European men (age range 19–50 years) evaluated at a single academic centre for the couple's primary infertility between September 2014 and September 2017. According to the World Health Organisation (WHO) criteria, infertility was defined as not conceiving a pregnancy after at least 12 months of unprotected intercourse regardless of whether or not a pregnancy ultimately occurred (WHO web chapter on couple's infertility, 2017). Primary infertility was defined when a couple was never able to conceive (WHO web chapter on couple's infertility, 2017). Only MFI patients were included in the study; MFI was defined after a comprehensive gynaecological evaluation of the female partners. Baseline assessment included a detailed medical history and physical examination including the penile and anogenital region. Comorbidities were scored with the Charlson comorbidity index (CCI) (Charlson *et al.*, 1987). The CCI was categorized as 0 or ≥1. The BMI was calculated for every patient. Smoking habit was investigated according to the pack-year history and then categorized in three groups as follows: no smokers (never and former smokers), moderate smokers (0–1 pack-year history) and heavy smokers (>1 packs-year history). For the specific purposes of the analysis, smoking status was further categorized as: never/former smokers or current smokers (moderate + heavy smokers). All smokers had smoked for at least one year. Patients were considered active smokers if their quit date was within one year of the clinical evaluation. Similarly, alcohol assumption was categorized as abstainers (no alcohol consumption history), moderate drinkers (up to 2 drinks/day) and heavy drinkers (>2 drinks/day) (National Institute of Alcohol Abuse and Alcoholism, 1995). Testes volume was assessed through a Prader orchidometer. Cryptorchidism was excluded in every patient. None of the patients had skin lesion compatible with HPV infection.

Venous blood samples were drawn from every patient between 7 AM and 11 AM after an overnight fast. Follicle-stimulating hormone (FSH), luteinising hormone (LH), 17-β-estradiol (E2), inhibin B (InhB), total testosterone (tT) and sex hormone-binding globulin (SHBG) levels were measured for every patient. The same laboratory was used for the analysis of all parameters for all patients. Chromosomal analysis was performed in every patient (Ventimiglia *et al.*, 2016a); patients with abnormal karyotyping were excluded from the final analysis.

Patients underwent at least two consecutive semen analyses, both showing below standard values for normal semen parameters according to the WHO criteria (Cooper *et al.*, 2010). For the specific purposes of this analysis, we considered semen volume, sperm concentration, sperm

progressive motility, morphology, number of leucocytes and SDF index, as measured by sperm chromatin structure assay (SCSA). SDF was considered pathological if ≥30%.

Amplification by nested PCR was used to detect HPV-DNA sequences in all semen samples; PCR was performed at the same laboratory for every patient. Samples were considered overall HPV positive (HPV+) when they tested positive for either HR HPV or LR HPV types; conversely, HPV negative (HPV−) was defined when they tested negative for both HR HPV and LR HPV types. Tested HR HPV types were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. LR HPV were HPV 6, 11, 26, 30, 32, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, 85, 86, 89 and 90.

Colour-Duplex ultrasound was used to detect spermatic vein reflux and to classify the grade of varicocele in every patient along with the clinical examination (Baazeem *et al.*, 2011). Additional exclusion criteria were symptoms of genitourinary infections, a history of vasectomy, infertility treatment in the preceding year and a positive semen culture (including Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis, Mycoplasma genitalium, Mycoplasma parvum and common uropathogens).

Data collection followed the principles outlined in the Declaration of Helsinki. All patients signed an informed consent agreeing to share their own anonymous information for future studies. The study was approved by the IRCCS San Raffaele Hospital Ethical Committee (Prot. 2014—Pazienti Ambulatoriali).

Statistical analyses were performed using SPSS v.20 (IBM Corp., Armonk, NY, USA). Distribution of data was tested with the Shapiro–Wilk test. Data are presented as medians (interquartile range; IQR) or frequencies (proportions). A 95% CI was estimated for the association of categorical parameters. First, demographics characteristics, hormonal values and semen parameters were compared between HPV+ and HPV− groups with the Mann–Whitney test and the Chi-square test. Second, an overall comparison of all the mutually exclusive HPV subgroups (HR HPV+ that includes patients with exclusively HR HPV and concomitant HR and LR HPV+, exclusively LR HPV+ and HPV−) was performed by Kruskal–Wallis test with multiple comparisons. Finally, univariable (UVA) and multivariable (MVA) linear regression analyses tested the associations between clinical variables (e.g. age, BMI, CCI, FSH, testicular volume, smoking habits and HPV status) and sperm progressive motility. Similarly, UVA and MVA linear regression analyses were used to identify variables associated with the SDF score. All tests were two sided, and statistical significance level was determined at $P < 0.05$.

Results

Table I lists patients characteristics, also segregated as for HPV+ and HPV− individuals. Of the 729 patients, an overall HPV positivity was found in 113 (15.5%) individuals. Median (IQR) patient's age at presentation was 37 (34–40) years. Overall, HPV+ and HPV− patients did not differ in terms of age, BMI, CCI, recreational habits, testis volume and hormonal profile (Table I). Table I also details semen parameters; semen volume, sperm concentration, normal sperm morphology and semen leucocyte count were comparable between HPV+ and HPV− patients. Conversely, sperm progressive motility was significantly lower in HPV+ than in HPV− patients ($P = 0.01$). Furthermore, SDF values were higher in HPV+ than in HPV− men ($P = 0.005$). Higher rates of impaired progressive motility (78.7 vs. 67.2%; $P = 0.03$) and greater SDF values (59.3 vs. 48.6%; $P = 0.002$) were more frequently observed in HPV+ than in HPV− individuals (Table I).

Of the 113 HPV-positive men, 78 (69.0%) and 35 (31.0%) patients had HR HPV+ and LR HPV+, respectively. In 84 (74.3%) cases, a single HPV type was detected. In terms of genotype distribution, HPV16 was the most prevalent type (22.1%), followed by HPV43 (10.6%), HPV56 and HPV42 (both 8.8%) (Table II).

Table III shows patients' characteristics and semen parameters as segregated according to HPV presence. Clinical and hormonal characteristics were similar among groups (all $P > 0.05$). Conversely, sperm progressive motility ($P = 0.01$) and SDF ($P = 0.005$) differed significantly among the groups. Multiple comparison analysis showed that HR HPV+ men had lower sperm progressive motility ($P = 0.007$) and higher SDF values ($P = 0.003$) compared to HPV− patients. No further differences were seen among groups.

Table IV depicts linear regression models testing the associations between clinical variables (age, BMI, CCI, FSH, testicular volume, smoking habits and HPV status) and either sperm progressive motility or SDF values. In the UVA, age ($P = 0.04$), FSH values ($P < 0.001$), testicular volume ($P < 0.001$) and having HR HPV+ ($P = 0.002$) were associated with impaired sperm progressive motility. Conversely BMI, CCI and smoking status were not. Similar findings were found in UVA testing the association between clinical variables and SDF. In the MVA, age, FSH levels and testicular volume (all $P \leq 0.04$) were significantly associated with impaired sperm progressive motility. Conversely BMI, CCI, smoking habits and HPV status were not. Only age ($P = 0.02$) and FSH ($P = 0.01$) were significantly associated with SDF.

Discussion

The aim of this cross-section real-life study was to investigate the prevalence of and the association between the presence of HPV in semen and semen parameters and sperm DNA integrity in a cohort of white European men seeking medical help for the couple's primary infertility.

This study was prompted by the extensive controversies throughout the recent literature regarding the impact of seminal HPV infection on sperm parameters (Bezold *et al.*, 2007; Golob *et al.*, 2014; Foresta *et al.*, 2015; Garolla *et al.* 2016; Luttmer *et al.*, 2016) and, more specifically, the substantial lack of research exploring the association of different genotypes of HPV infection (namely HR and LR HPV) with the same parameters or sperm DNA alterations in primary infertile men. Recent meta-analyses have shown that HPV semen infection is a risk factor for male fertility (Lyu *et al.*, 2017; Xiong *et al.*, 2018). However, the specific mechanism underlying this association has yet to be elucidated.

We found an overall HPV prevalence of 15.5% in primary infertile men, which is in line with that reported in a recent study showing a pooled HPV prevalence of 16% in infertile population (Laprise *et al.*, 2014). Moreover, we also confirmed that HR HPV are more frequent than LR HPV in infertile men and that HPV16 is one of the most common genotypes detected (Damke *et al.*, 2017; Lyu *et al.*, 2017).

Several pathogenic mechanisms have been proposed to explain the effect of seminal HPV infection on male infertility. One of the most commonly proposed mechanisms is the impairment of semen parameters. However, previous studies on semen parameters in relation to seminal HPV presence have shown conflicting results. Lai *et al.* (1997), for instance, first reported that the incidence of asthenozoospermia (lower velocity, straight-line velocity, mean amplitude of lateral head displacement) was higher among patients with HPV16 and 18 sperm infection than non-infected controls. These findings were subsequently confirmed by several clinical studies (Foresta *et al.*, 2010;

Table I Characteristics and descriptive statistics of the whole cohort segregated according to HPV test (*N* = 729)

	Overall	−HPV	+HPV	*P*-value*
Number of individuals	729 (100%)	616 (84.5%)	113 (15.5%)	
Age (years)				0.31
Median (IQR)	37.0 (34–40)	37.0 (33.5–40)	37 (34–41)	
Range	19–50	19–50	19–50	
Partner's age (years)				0.82
Median (IQR)	34.0 (32–37)	34 (32–37)	34 (32–38)	
Range	19–53	19–53	20–45	
BMI (kg/m^2)				0.22
Median (IQR)	25.3 (23.4–27.5)	25.3 (23.4–27.5)	24.9 (23.3–27.2)	
Range	17.7–41.6	18.5–41.6	17.7–37.1	
CCI (score)				0.70
Median (IQR)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	
Median (SD)	0.1 (0.5)	0.1 (0.6)	0.12 (0.5)	
Range	0–8	0–8	0–3	
Total testis volume (Prader estimation)				0.95
Median (IQR)	15.0 (12–20)	15.0 (12–20)	15.0 (12–20)	
Range	3–25	3–25	3–25	
Smoking status, *n* (%)				0.96
No smokers/former smokers	509 (69.8)	430 (69.8)	79 (69.9)	
Active smokers	220 (30.2)	186 (30.2)	34 (30.1)	
Alcoholic status, *n* (%)				0.86
Abstainers	395 (54.2)	335 (54.5)	60 (53.1)	
Moderate drinkers	265 (36.3)	222 (36.0)	43 (38.1)	
Heavy drinkers	69 (9.5)	59 (9.5)	10 (8.8)	
Varicocele, *n* (%)	391 (53.6)	338 (54.9)	53 (46.9)	0.12
FSH (mUI/ml)				0.92
Median (IQR)	5.5 (3.1–10.5)	5.5 (3.0–10.9)	6.0 (3.5–9.0)	
Range	0.6–48.8	0.6–48.8	1.3–55.0	
LH (mUI/ml)				0.56
Median (IQR)	4.1 (2.9–5.7)	4.0 (2.8–5.6)	4.1 (3.3–5.3)	
Range	0.7–77.0	0.7–77.0	0.8–44.0	
InhB (pg/ml)				0.09
Median (IQR)	113.2 (57.8–173.2)	110.4 (53.2–172.4)	129.0 (95.6–185.1)	
Range	0.1–439.4	0.1–439.4	6.0–311.1	
tT (ng/ml)				0.11
Median (IQR)	4.5 (3.3–5.7)	4.5 (3.3–5.6)	4.7 (3.5–6.4)	
Range	0.8–11.8	0.8–11.8	0.1–15.5	
E_2 (pg/ml)				0.33
Median (IQR)	27.0 (23–37)	26.0 (23–36)	29.0 (24–38)	
Range	5.0–100.0	5.0–100.0	5.0–100.0	
SHBG (nmol/l)				0.05
Median (IQR)	32.0 (24–41)	31.0 (23–40)	32 (27–44.9)	
Range	2.4–88.0	2.4–88.0	12–73	
Semen volume (ml)				0.82
Median (IQR)	3.0 (2.0–4.0)	3.0 (2.0–4.0)	3.0 (2–4)	
Range	0.1–9.0	0.1–9.0	0.2–9.0	

Continued

Table I *Continued*

	Overall	−HPV	+HPV	*P*-value*
Sperm concentration				0.91
Median (IQR)	12.2 (3.6–34.9)	12.4 (3.1–35)	10.9 (5.0–32.8)	
Range	0.5–200.5	0.5–200.5	0.1–122.0	
Concentration ≤15 × 10^6/ml, *n* (%)	404 (55.4)	339 (55.0)	65 (57.5)	0.57
Progressive motility				0.01
Median (IQR)	21.0 (8.0–37.5)	22.0 (10–39)	16.0 (5.0–31.0)	
Range	0.0–90.0	0.0–90.0	0.0–72.0	
Progressive motility ≤32%, *n* (%)	503 (68.9)	414 (67.2)	89 (78.7)	0.03
Normal morphology				0.21
Median (IQR)	2.0 (0–4)	2.0 (0–4)	1.0 (0–4)	
Range	0.0–91.0	0.0–91.0	0.0–66.0	
Normal morphology ≤4%, *n* (%)	503 (68.9)	422 (68.5)	81 (71.6)	0.11
Leucocytes (×10^6/mL)				0.21
Median (IQR)	0.33 (0.21–0.43)	0.30 (0.21–0.39)	0.35 (0.25–0.43)	
Range	0.0–0.65	0.0–0.54	0.0–0.65	
SDF (%)				0.005
Median (IQR)	31.4 (22.3–50.6)	28.3 (21.3–48.4)	35.9 (25.6–55.0)	
Range	0.4–99.8	0.4–99.8	1.4–85.7	
SDF ≥30%, *n* (%)	366 (50.2)	299 (48.6)	67 (59.3)	0.002

Keys: CCI = Charlson comorbidity index; tT = total testosterone; SDF = sperm DNA fragmentation index.
*P-value according to the Mann–Whitney test and Chi-square test, as indicated.

Table II HPV genotypes in semen samples (*N* = 113)

	Type	Number	%		Type	Number	%
High risk	16	25	22.1	Low risk	6	7	6.2
	18	9	7.9		26	1	0.8
	31	9	7.9		32	1	0.8
	33	4	3.5		42	10	8.8
	35	5	4.4		43	12	10.6
	45	2	1.7		62	2	1.7
	51	4	3.5		67	2	1.7
	52	1	0.8		70	1	0.8
	56	10	8.8		81	3	2.6
	58	5	4.4		83	1	0.8
	59	7	6.2		90	8	7.1
	66	5	4.4				

Indicated frequencies include the presence of types both in single infection (*n* = 84) and multiple infections (*n* = 29)

Garolla *et al.*, 2013; Yang *et al.*, 2013). However, other recent studies failed to find any association between HPV detection and sperm parameters (Schillaci *et al.*, 2013; Golob *et al.*, 2014; Luttmer *et al.*, 2016). Moreover, only two small cross-sectional studies have analysed the specific impact of HR and LR HPV on semen parameters and their findings did not eventually show any significant association (Luttmer *et al.*, 2016, Damke *et al.*, 2017). To the best of our knowledge, we presented one of the largest studies assessing seminal HPV presence in relation to sperm parameters, with a specific focus on the effect of different groups of HPV genotype (HR and LR HPV genotypes). Our observations confirmed the potentially detrimental impact of HPV seminal detection on sperm progressive motility. Of clinical importance, in the UVA this negative effect emerged to be pronounced in patients with HR HPV genotypes than those with negative HPV, but a similar association was not confirmed in the MVA.

Other potential explanations for male subfertility in patients with semen HPV+ have been suggested to be (i) the presence of antisperm antibodies, which may reduce male fertility by interfering with sperm motility and sperm–oocyte binding (Garolla *et al.*, 2013); (ii) a glandular dysfunction related to prostate disturbance that may change the proportion of fluids secreted from the prostate and the seminal vesicles (Damke *et al.*, 2017) and (iii) a reduced rate of sperm DNA integrity.

In particular, the effect of semen HPV infection on sperm DNA integrity has been a debated topic in the recent literature. Connelly *et al.* (2001), in an *in vitro* study, found that sperm cells transfected with exogenous HPV E6/E7 DNA had higher percentages of breakages characteristic of apoptosis compared to the uninfected controls. Similarly, Lee *et al.* (2002) performed an *in vitro* study incubating sperm cells with HPV-DNA encoding for E6 and E7 proteins and found an increased SDF, including exon 5 and exon 8 of the p53 gene. Conversely, other *in vivo* studies failed to find any association between HPV infection and sperm DNA integrity (Kaspersen *et al.*, 2013; Cortés-Gutiérrez *et al.*, 2017). Our cross-sectional, real-life study showed that semen HPV+ patients, in particular those with HR HPV

Table III Descriptive statistics of the whole cohort segregated according to HPV test (*N* = 729)

	HR HPV+ (*n* = 78; 10.7%)	LR HPV+ (*n* = 35; 4.8%)	HPV− (*n* = 616; 84.5%)	*P*-value*
Age (years)	37.5 (33–41) [19–50]	36.0 (35–41) [21–50]	37.0 (33.5–40) [19–50]	0.602
BMI (kg/m^2)	24.7 (23.3–26.9) [18.1–33.6]	25.4 (23.0–27.7) [17.7–37.1]	25.3 (23.4–27.5) [18.5–41.6]	0.412
CCI (score)	0.0 (0.0-0-0) [0.0–3.0]	0.0 (0.0-0-0) [0.0–3.0]	0.0 (0.0-0-0) [0.0–6.0]	0.927
Testis volume (Prader estimation)	15.0 (12–20) [3.0–25.0]	15.0 (12–20) [4.0–25.0]	15.0 (12–20) [3.0–25.0]	0.812
FSH (mUI/ml)	5.0 (3.6–10.5) [1.3–55.0]	6.1 (3.2–7.7) [1.3–47.3]	5.5 (3.0–10.9) [0.6–48.8]	0.865
LH (mUI/ml)	4.3 (3.3–6.3) [1.6–44.0]	4.9 (3.2–6.5) [0.8–22.6]	4.0 (2.8–5.6) [0.7–77.0]	0.516
tT (ng/ml)	4.5 (3.4–6.3) [0.1–15.5]	5.6 (4.1–6.8) [0.8–8.8]	4.5 (3.3–5.6) [0.8–11.8]	0.097
Semen volume (ml)	3.0 (2.0–4.0) [0.2–9.0]	3.0 (2.0–3.2) [1.0–6.0]	3.0 (2.0–4.0) [0.1–9.0]	0.633
Sperm concentration (×10^6/ml)	10.0 (5.0–29.9) [0.1–122.0]	14.5 (3.7–43.9) [0.3–84.7]	12.4 (3.1–35) [0.5–200.5]	0.866
Progressive motility (%)	12.5 (5.0–30.0)§ [0.0–60.0]	22.0 (2.0–38.7) [0.0–72.0]	22.0 (10–39) [0.0–90.0]	0.010
Normal morphology (%)	1.0 (0.0–4.0) [0.0–66.0]	2.0 (0.0–3.0) [0.0–34.0]	2.0 (0.0–4.0) [0.0–91.0]	0.407
Leucocytes (×10^6/ml)	0.35 (0.23–0.46) [0.0–0.65]	0.32 (0.21–0.41) [0.0–0.55]	0.30 (0.21–0.39) [0.0–0.54]	0.113
SDF (%)	40.3 (26.5–59.5)§ [10.1–85.7]	31.5 (24.2–52.7) [1.5–85.7]	28.3 (21.4–48.4) [0.4–99.8]	0.005

Data presented as median (IQR) [range]
Keys: HR = High-risk; LR = Low-risk; CCI = Charlson Comorbidity Index; tT = total Testosterone; SDF = Sperm DNA fragmentation index
*p value according to the Kruskal-Wallis test, as indicated; § $P < 0.01$ vs. HPV−

genotypes, had higher SDF values than those with HPV−. It has been speculated that the chromosome breakage in HPV infected cells may increase the cellular susceptibility to DNA damage or the defective repair of DNA damage as a consequence of reduced p53 or pRB function (Buitrago-Pérez *et al.*, 2009). However, this mechanism has not been demonstrated in sperm cells yet. Once again, our results underline the importance of HPV testing in the work-up of infertile couples. Further studies are needed to assess if these recently found genetic abnormalities are associated with the presence of HPV in the nucleus and not only in its typical location on the cell surface (Foresta *et al.*, 2015).

The first strength of the current study is that we have investigated a relatively large homogenous cohort of patients comprehensively studied with a thorough hormonal evaluation, with an accurate assessment of possible confounders for impaired semen parameters, such as smoking and alcoholic consumption and health comorbidities. Of primary importance, most of the previous studies that aimed to test the relationship between HPV infection and alterations in semen parameters have not considered hormonal values and recreational habits, thus limiting the validity of their findings. In fact, as reported by our linear regression models, we showed that HR HPV positivity was univariably associated with lower sperm motility and higher SDF values. However, in the MVA, which adjusted current findings for the effects of clinical and hormonal characteristics, HPV status was no longer associated with sperm motility and SDF. Indeed, in MVA analyses, HPV status may have lower magnitude of effects when compared to standard prognosticators (such as age, FSH and testicular volume and smoking status). Further, an important point of strength of the study is that only primary infertile men seeking medical help for the couple's infertility in an outpatient setting were considered, thus providing our results with a strong characterization in the real-life setting.

Likewise, the study is not devoid of limitations. Although the methodologies used in our study are comparable to those reported in most of the studies already published on the same topic, it is necessary to stress the point that we were not able to clearly distinguish the simple presence of HPV in semen samples from a HPV infection accompanied by a host response. The study reports the results of a retrospective cross-sectional analysis of data prospectively collected in a relatively

Table IV Linear regression models predicting sperm progressive motility and SDF (beta; *P*-value [95% CI]) in the whole cohort (*N* = 729)

	Sperm progressive motility		SDF	
	UVA model	**MVA model**	**UVA model**	**MVA model**
Age	−0.12; 0.04	−0.36; 0.035	0.66; 0.006	0.40; 0.02
	[−0.81–−0.03]	[−0.74–−0.08]	[0.21–1.31]	[0.21–1.63]
BMI	−0.38; 0.12	−0.24; 0.62	−0.21; 0.51	0.14; 0.72
	[−0.85–0.09]	[−0.81–0.33]	[−0.85–0.42]	[−0.64–0.93]
CCI	−1.1; 0.46	−0.41; 0.66	0.41; 0.89	1.79; 0.71
	[−4.15–1.97]	[−4.39–2.34]	[−5.61–6.44]	[−3.14–11.44]
FSH	−0.73; <0.001	−0.51; 0.015	1.78; 0.001	1.38; 0.01
	[−1.01–−0.46]	[−0.85–−0.09]	[1.37–8.34]	[1.10–6.65]
Testis volume	0.77; <0.001	0.66; 0.003	−0.37; 0.11	−0.47; 0.10
	[0.44–1.11]	[0.22–1.03]	[−0.83–0.85]	[−1.03–1.09]
Smoking status				
Yes vs. No	1.82; 0.16	−0.81; 0.61	2.92; 0.06	1.61; 0.52
	[0.80–4.22]	[−5.14–3.47]	[0.91–8.95]	[−4.34–7.85]
HPV status				
Negative	Ref.	Ref.	Ref.	Ref.
High risk	−7.37; 0.002	−3.05; 0.33	8.59; 0.003	1.54; 0.082
	[−12.1–−2.64]	[−9.14–3.13]	[2.86–14.31]	[0.21–4.34]
Low risk	−0.32; 0.923	2.36; 0.59	0.85; 0.831	−0.28; 0.55
	[−6.80–6.17]	[−6.34–11.07]	[−6.93–8.63]	[−3.13–1.13]

Keys: UVA = univariate model; MVA = multivariate model, CCI = Charlson comorbidity index; SDF = sperm DNA fragmentation index.

large, homogenous cohort of white European men seeking medical help for the couple's primary infertility, but deserves external validation with an independent, larger and more diverse sample. Second, we were unable to check for the clearance or the persistence of HPV in semen by repeating the search for viral DNA and genotyping after a 6–12 months period of time. Likewise, our analyses did not allow us to perform a viral load quantification by real-time PCR in the samples, which could have been of clinical relevance in terms of final HR HPV clearance rates (Trevisan *et al.*, 2013). Third, our data did not allow us to analyses semen samples in terms of MAR test findings and the level of some inflammatory markers that may be associated with impaired sperm parameters (La Vignera *et al.*, 2013). Fourth, by definition our analyses did not consider patients with normozoospermia who, in turn, may be infertile because a pure male factor as a consequence of a number of sperm function impairments at the molecular level. Finally, our study did not include a control group of normal fertile men.

Nevertheless, our results indicate that an accurate investigation of the presence of seminal HPV may be important in the diagnostic work-up of infertile men.

Conclusions

This cross-sectional real-life study confirms previous findings that HPV DNA is common in semen of primary infertile men. Clinically relevant, we showed that HPV seminal presence, in particular that involving exclusively HR genotypes, was associated with impaired sperm progressive motility and SDF values. Overall, these observations point out the importance of an accurate investigation of seminal HPV presence over the everyday clinical practice diagnostic work-up of primary infertile men not only because of its potential pathophysiologic negative impact towards male fertility potential but also in terms of overall men's health. Given the conflicting evidence in the literature in terms of the association between HPV and semen parameters and sperm DNA integrity, further large cohort studies are needed to corroborate our results.

Authors' roles

L.B.: study concept and design, data collection, statistical analyses and data interpretation and drafting of the manuscript. P.C.: study concept and design, data collection and statistical analyses and data interpretation. E.V.: study concept and design, data collection and statistical analyses and data interpretation. W.C.: data collection. F.P.: data collection. E.P.: data collection. F.C.: data collection. M.C.: data interpretation, critical revision of the manuscript and final approval of the version to be published. P.V.: data interpretation, critical revision of the manuscript and final approval of the version to be published. E.M.: data interpretation, critical revision of the manuscript and final approval of the version to be published. F.M.: data interpretation, critical revision of the manuscript and final approval of the version to be published. A.S.: study concept and design, data collection, data

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Conflict of interest

None declared.

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