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Presence of human papillomavirus in semen in relation to semen quality

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STUDY QUESTION: Is the presence of human papillomavirus (HPV) in semen associated with impairment of semen quality?

SUMMARY ANSWER: In a large cohort of males seeking fertility evaluation, no associations were observed between seminal HPV presence and semen parameters.

WHAT IS KNOWN ALREADY: HPV is commonly detected in semen samples. Whether the presence of HPV is related to impairment of semen quality, remains unclear.

STUDY DESIGN, SIZE, DURATION: This cross-sectional study included a cohort of 430 males.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Male partners in couples seeking fertility evaluation provided one semen sample per person. Semen samples were tested for HPV-DNA using GP5+/6+-PCR. Sperm concentration was counted and motility was assessed in a Makler counting chamber at a magnification of ×200. The presence of antisperm antibodies was assessed by a mixed agglutination reaction (MAR)-test.

MAIN RESULTS AND THE ROLE OF CHANCE: Overall HPV was detected in 14.9% (64/430) of semen samples, including 2.1% (9/430) that contained both high-risk (hr) HPV and low-risk (lr) HPV types, 8.8% (38/430) with exclusively hrHPV types and 4.0% (17/430) with exclusively lrHPV types. The presence of HPV in semen was not associated with the age of the participants, seminal pH, semen volume, total sperm count, sperm concentration, progressive motility or the presence of antisperm antibodies.

LIMITATIONS, REASONS FOR CAUTION: This study did not observe an association between HPV presence in semen and impairment of semen quality. However, we cannot exclude an effect of seminal HPV on early embryo development and clinical reproductive outcomes.

WIDER IMPLICATIONS OF THE FINDINGS: As HPV is frequently present in semen, screening of donor semen for HPV should be considered to prevent iatrogenic cervical HPV infections in the recipient. However our findings do not support standardized HPV testing of semen in the diagnostic work-up of subfertile couples.

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TRIAL REGISTRATION NUMBER: Not applicable.

Key words: human papillomavirus / HPV / male infertility / semen / semen quality

Introduction

Human papillomavirus (HPV) infection is one of the most common sexually transmitted infections worldwide (Bruni *et al.*, 2010). In both men and women, persistent infection with high-risk types of HPV (hrHPV) is associated with anogenital and oropharyngeal cancers, whereas low-risk HPV (lrHPV) types can cause genital warts (Forman *et al.*, 2012). The prevalence of genital HPV infections in men ranges widely (1.3–72.9%) (Dunne *et al.*, 2006; Smith *et al.*, 2011) depending on the population and sampled anatomical site (Giuliano *et al.*, 2007). A recent meta-analysis has revealed that HPV is commonly present in semen samples, with an estimated prevalence of 16% in infertile populations and 10% in men from the general population (Laprise *et al.*, 2014). Data on the underlying source of the presence of HPV in semen are very limited. We have recently found an association between HPV infection of the penile epithelium and the presence of HPV in semen, suggesting that HPV presence in semen might result from exfoliation of HPV-infected penile keratinocytes (Luttmer *et al.*, 2015). A remaining question to be answered is whether the presence of HPV in semen is associated with impairment of male fertility. This knowledge could be valuable for guidelines on the possible necessity of HPV testing in the diagnostic work-up for subfertile couples and in procedures of assisted reproduction. So far, conflicting results have been reported (Lai *et al.*, 1997; Rintala *et al.*, 2004; Foresta *et al.*, 2010a,b, 2015; Garolla *et al.*, 2012; Schillaci *et al.*, 2013; Yang *et al.*, 2013; Gizzo *et al.*, 2014; Golob *et al.*, 2014). Most previous studies on this topic have been performed on relatively small cohorts (Gizzo *et al.*, 2014; Foresta *et al.*, 2015). In the current study, we investigated the association between seminal HPV presence and semen parameters in a large cohort of 430 male partners of couples seeking fertility evaluation.

Materials and Methods

Study population

Study participants were recruited among male attendants of the fertility clinic of VU University Medical Center in Amsterdam, the Netherlands. According to national Dutch guidelines, couples who seek fertility evaluation because of an inability to conceive after more than one year of regular sexual intercourse are offered a diagnostic work-up which includes a basic semen analysis. Between September 2012 and January 2014, all male attendants providing a semen sample at VU University Medical Center for such primary fertility evaluation were asked to provide written consent for anonymous additional testing of their semen sample. Men with a history of vasectomy or testicular cancer were excluded from participation.

Ethical approval

As semen samples were collected in routine diagnostics, handled anonymously and additional tests were performed on residual material only, no Institutional Review Board approval was required for this study, in concordance with Dutch legislation and the Dutch code of conduct on responsible use of human tissue for research (2011) (Federa, 2011).

Semen collection and analysis

Men were advised to have sexual abstinence three days prior to semen donation. The actual duration of sexual abstinence was self-reported by the participants. Following masturbation, semen samples were collected directly into a 100-ml polystyrene container (Sterilin, UK). Semen samples were analyzed within one hour after ejaculation. The basic semen analysis was based on WHO guidelines (World Health Organization, 2010) with the exceptions that semen volume was measured in a graded pipette with a 0.1-ml accuracy (after liquefaction), sperm concentration was counted and motility was assessed in a Makler counting chamber at a magnification of $\times 200$. The presence of antisperm antibodies was assessed by a mixed agglutination reaction (MAR)-test using IgG coated red blood cells (Checkcell, Immucor, USA) and N antiserum for human IgG (Siemens Healthcare Diagnostics, Germany). All assessments were performed by trained laboratory technicians who were blinded for the HPV test results.

HPV DNA detection and genotyping

After performance of basic semen analysis, the remaining semen sample was suspended in 10 ml PreservCyt medium (Hologic, USA) and stored at room temperature until further processing. Aliquots of 3 ml resuspended semen samples were used for DNA isolation. After automated DNA isolation using Macherey-Nagel magnetic beads and a Hamilton Star liquid handler (Hamilton Robotics, USA), DNA was subjected to GP5+/6+-PCR with enzyme immunoassay (EIA) read-out using cocktail probes for lrHPV and hrHPV types, respectively (Diassay, the Netherlands) (Jacobs *et al.*, 1997) and subsequent microsphere bead-based genotyping (Schmitt *et al.*, 2006). For DNA quality control, beta-globin PCR was performed on all samples. Specimens testing negative in both HPV and beta-globin PCR assays were considered invalid and therefore excluded from analyses. Samples were considered overall HPV-positive when they tested positive for either hrHPV or lrHPV type(s), and HPV-negative when they tested negative for both hrHPV and lrHPV types. The following 14 genotypes were classified as hrHPV types: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. Tested lrHPV types included: HPV 6, 11, 26, 30, 32, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 64, 67, 69, 70, 71, 72, 73, 81, 82 (variants mm4 and is39), 83, 84, 85, 86, 89 (formerly cp6108), and 90 (formerly jc9710). Samples that were EIA-positive but in which no genotypes could be detected were considered to contain uncharacterized types, referred to as HPV type X.

Statistical analysis

Estimating the HPV prevalence in semen in a cohort of men attending a fertility clinic to be around 16% (Laprise *et al.*, 2014), we calculated that we would be able to detect a difference in progressive sperm motility between HPV-positive and HPV-negative semen samples of at least 8% with a power of 80% by including a minimum of 354 men (two-sided $\alpha = 0.05$).

HPV positivity rates were calculated with 95% confidence intervals (CI) using the Wilson score method. In HPV-positive semen samples, the prevalence of separate HPV genotypes was determined (indicating frequencies in both single and multiple infections).The parameters seminal volume, total sperm count and concentration were log-transformed. First, an overall comparison of all mutually exclusive HPV-subgroups (both hrHPV and lrHPV-positive, exclusively hrHPV-positive, exclusively lrHPV-positive and HPV-negative samples respectively) was performed by one-way ANOVA with *post hoc* Fisher's least significant difference (LSD) testing. Secondly, HPV-positive subgroups (both hrHPV and lrHPV-positive, exclusively

hrHPV-positive and exclusively lrHPV-positive, respectively) were each compared with the HPV-negative group using a *post hoc* Dunnett's test. Linear regression was used for adjustment of the relation between HPV-positivity and semen volume for the duration of sexual abstinence. Semen parameters were compared between the overall HPV-positive and HPV-negative group using independent samples' t-tests. The percentages of semen samples with a positive MAR-test were compared between the groups using Fisher's exact test or Dunnett's test when appropriate. Two-sided *P*-values <0.05 were considered statistically significant.

Results

After written consent, a total of 431 participants provided one semen sample per person. One semen sample was of inadequate sample quality for HPV testing and was therefore excluded from analyses.

The mean age of the remaining 430 men was 36.2 years (CI 35.6–36.8). HPV positivity rates in semen are summarized in Table I. Overall HPV positivity in the total study population was 14.9% (64/430; CI 11.8–18.6%). In 2.1% (9/430; CI 1.1–3.9%) of samples, both hrHPV and lrHPV types were detected. In 8.8% (38/430; CI 6.5–11.9%) of samples, exclusively hrHPV types were present, and 4.0% (17/430; 2.5–6.2%) of samples contained exclusively lrHPV types. The presence of overall HPV in semen was not related with the age of the participants; nor was the presence of hrHPV or lrHPV (separately or in combination).

In the majority of HPV-positive semen samples, a single HPV-type was detected (47/64; 73.4%; CI 61.5–82.7%). The genotype distribution is shown in Table II. HPV16 was the most prevalent type (15/64; 23.4%; CI 14.7–35.1%), followed by HPV31, HPV51 and HPV56 (each type detected in 7/64; 10.9%; CI 5.4–20.9%), and HPV42 (6/64; 9.4%; CI 4.4–19.0%).

Table III shows participant characteristics and semen parameters in the total study population and in strata of seminal HPV presence, including an overall comparison between all HPV-subgroups. Comparisons of different HPV-positive subgroups with HPV-negative samples (as a control group) are shown in Table IV. The duration of sexual abstinence differed significantly between all HPV-subgroups ($P = 0.025$; Table III) and between the overall HPV-positive group and HPV-negative group ($P < 0.001$; Table IV). Men with exclusively hrHPV-positive semen had kept a mean period of 3.0 days (CI 2.8–3.2) of sexual abstinence, whereas men with HPV-negative semen had a mean abstinence period of 4.0 days (CI 3.8–4.3; *post hoc* LSD $P = 0.007$; *post hoc* Dunnett $P = 0.022$, Table IV). Secondly, the semen volume differed significantly between all HPV-subgroups ($P = 0.039$; Table III). Semen samples that tested exclusively hrHPV-positive had a mean volume of 2.9 ml, which was significantly lower than that of HPV-negative samples (3.4 ml; *post hoc* LSD $P = 0.033$). However, after adjustment for multiple testing (*post hoc* Dunnett $P = 0.096$) or adjustment for duration of sexual abstinence (volume β −0.152, CI −0.323–0.019, $P = 0.081$), the association between volume and HPV-status lost statistical significance. No statistically significant associations were found between the presence of overall HPV in semen and seminal parameters (i.e. pH, volume, total sperm count, sperm concentration, progressive motility, or the presence of antisperm antibodies; Table IV).

Table I Presence of HPV in semen samples.

HPV presence in semen	*n*	Percentage (95% CI)
Overall HPV	64/430	14.9% (11.8–18.6%)
High-risk HPV *and* low-risk HPV type(s)	9/430	2.1% (1.1–3.9%)
Exclusively high-risk HPV type(s)	38/430	8.8% (6.5–11.9%)
Exclusively low-risk HPV type(s)	17/430	4.0% (2.5–6.2%)

Percentages do not sum to total due to rounding.

Discussion

In this large cohort, comprising male partners of couples seeking fertility evaluation, we confirmed that HPV-DNA is commonly present in semen. We could not demonstrate any association between the presence of HPV in semen and semen parameters.

To our knowledge, this is the second largest study assessing seminal HPV presence in relation to semen parameters (Gizzo *et al.*, 2014; Foresta *et al.*, 2015). We detected an overall HPV prevalence of 14.9%, which is in accordance with a recent meta-analysis (Laprise *et al.*, 2014) describing a pooled prevalence of 16% in fertility clinic attendees. We recently showed that healthy Dutch volunteers had a markedly higher seminal HPV prevalence of 27%, which might be explained by the age difference between populations (36 years in the present cohort in contrast to 22 years in the healthy volunteers). Other studies in asymptomatic men (students, fathers-to-be) have reported prevalences of HPV in semen ranging from 2 to 31% (Rintala *et al.*, 2004; Foresta *et al.*, 2010a; Yang *et al.*, 2013), and estimates in semen donors were 16 to 26% (Olatunbosun *et al.*, 2001; Kaspersen *et al.*, 2011).

Table II Detected HPV genotypes in semen (both single and multiple infections).

HPV genotypes		*n*	Percentage (95% CI)
High-risk	16	15	23.4% (14.7–35.1%)
	18	3	4.7% (1.6–12.9%)
	31	7	10.9% (5.4–20.9%)
	33	1	1.6% (0.3–8.3%)
	35	2	3.1% (0.9–10.7%)
	45	3	4.7% (1.6–12.9%)
	51	7	10.9% (5.4–20.9%)
	52	3	4.7% (1.6–12.9%)
	56	7	10.9% (5.4–20.9%)
	58	1	1.6% (0.3–8.3%)
	59	2	3.1% (0.9–10.7%)
	66	5	7.8% (3.4–17.0%)
Low-risk	6	5	7.8% (3.4–17.0%)
	26	1	1.6% (0.3–8.3%)
	32	1	1.6% (0.3–8.3%)
	42	6	9.4% (4.4–19.0%)
	43	4	6.3% (2.5–15.0%)
	67	2	3.1% (0.9–10.7%)
	70	1	1.6% (0.3–8.3%)
	81	1	1.6% (0.3–8.3%)
	83	1	1.6% (0.3–8.3%)
	90	5	7.8% (3.4–17.0%)
	HPV type X	3	4.7% (1.6–12.9%)

Total HPV-positive $n = 64$; indicated frequencies include presence of types both in single infections ($n = 47$) and multiple infections ($n = 17$).

Table III Participant characteristics and semen parameters in the total study population and in strata of HPV presence.

	Total group (n = 430)	Overall HPV positive (hrHPV and/or lrHPV) (n = 64; 15%)	Both hrHPV and lrHPV positive (n = 9; 2%)	Exclusively hrHPV positive (n = 38; 9%)	Exclusively lrHPV positive (n = 17; 4%)	HPV negative (n = 366; 85%)	P[b]
Age of participant (years)	36.2 (35.6–36.7)	36.1 (34.5–37.6)	35.6 (32.6–38.6)	35.3 (33.1–37.5)	37.9 (35.0–40.8)	36.2 (35.6–36.8)	0.524
Duration of sexual abstinence (days)	3.9 (3.7–4.1)	3.2 (2.9–3.4)	4.1 (2.8–5.4)	3.0 (2.8–3.2)	3.1 (2.9–3.4)	4.0 (3.8–4.3)	0.025
pH of semen	8.1 (8.1–8.1)	8.1 (8.0–8.1)	8.1 (7.9–8.2)	8.1 (8.0–8.2)	8.1 (8.0–8.2)	8.1 (8.1–8.1)	0.776
Volume of semen (ml/ejaculate)	3.4 (3.2–3.5)	3.1 (2.7–3.5)	2.5 (1.9–3.1)	2.9 (2.4–3.4)	4.0 (3.0–4.9)	3.4 (3.2–3.6)	0.039
Sperm count ($\times 10^6$/ejaculate)	184.6 (169.9–199.2)	157.5 (126.1–188.8)	142.3 (72.4–212.2)	130.4 (95.0–165.7)	226.0 (153.5–298.6)	189.3 (173.0–205.6)	0.073
Sperm concentration ($\times 10^6$/ml)	56.7 (52.9–60.5)	52.1 (42.8–61.5)	54.7 (29.4–80.0)	46.8 (35.0–58.6)	62.8 (43.8–81.8)	57.5 (53.4–61.7)	0.280
Progressive sperm motility (PR, %)	58.2 (56.4–60.5)	60.2 (55.5–64.9)	65.3 (50.0–80.7)	56.8 (50.2–63.4)	65.0 (60.2–69.8)	57.9 (55.8–59.9)	0.320
Positive MAR-test result[a] (n)	119/408 (29.2%)	15/61 (24.6%)	1/7 (14.2%)	8/37 (21.6%)	6/17 (35.3%)	104/347 (30.0%)	0.578[c]

Presented data are means (95% confidence intervals).
[a]n = 408; samples in which MAR-test was not performed or not to determine were excluded, presented data are positive n/total n (percentage).
[b]P-value obtained by one-way ANOVA, comparing the four mutually exclusive groups (both hrHPV and lrHPV positive; exclusively hrHPV positive; exclusively lrHPV positive; HPV negative) unless otherwise specified.
[c]P-value obtained by Fisher's exact test, comparing the four mutually exclusive groups (both hrHPV and lrHPV positive; exclusively hrHPV positive; exclusively lrHPV positive; HPV negative).

Table IV Comparison of semen parameters in different subgroups of HPV-positive versus HPV-negative semen samples.

	Overall HPV positive (hrHPV and/or lrHPV; n = 64)		Both hrHPV and lrHPV positive (n = 9)		Exclusively hrHPV positive (n = 38)		Exclusively lrHPV positive (n = 17)	
	Mean difference[a] or ratio[b] of overall HPV-positive to HPV-negative samples (95% CI)	P[d]	Mean difference[a] or ratio[b] of double hrHPV- and lrHPV-positive to HPV-negative samples (95% CI)	P[f]	Mean difference[a] or ratio[b] of exclusively hrHPV-positive to HPV-negative samples (95% CI)	P[f]	Mean difference[a] or ratio[b] of exclusively lrHPV-positive to HPV-negative samples (95% CI)	P[f]
Age of participant[a] (years)	−0.15 (−1.76 to 1.46)	0.853	−0.62 (−4.57 to 3.32)	0.986	−0.87 (−2.91 to 1.16)	0.780	1.7 (−1.20 to 4.63)	0.584
Duration of sexual abstinence (days)	0.84 (0.49 to 1.19)	0.000	−0.08 (−1.66 to 1.50)	0.999	1.03 (0.68 to 1.38)	0.022	−0.9 (0.57 to 1.25)	0.276
pH of semen[a]	−0.03 (−0.10 to 0.04)	0.445	−0.04 (−0.22 to 0.13)	0.947	−0.04 (−0.13 to 0.05)	0.738	0.0 (−0.12 to 0.14)	0.997
Volume of semen[b] (ml/ejaculate)	0.91 (0.79 to 1.04)	0.155	0.79 (0.57 to 1.10)	0.429	0.83 (0.70 to 0.99)	0.096	1.18 (0.92 to 1.51)	0.460
Sperm count[b] ($\times 10^6$/ejaculate)	0.77 (0.51 to 1.17)	0.226	0.59 (0.21 to 1.68)	0.692	0.58 (0.34 to 0.99)	0.127	1.66 (0.79 to 3.52)	0.474
Sperm concentration[b] ($\times 10^6$/ml)	0.84 (0.58 to 1.23)	0.364	0.74 (0.29 to 1.90)	0.900	0.69 (0.43 to 1.11)	0.323	1.40 (0.71 to 2.74)	0.712
Progressive sperm motility[a] (PR, %)	2.32 (−2.91 to 7.56)	0.384	7.47 (−5.70 to 20.64)	0.595	−1.05 (−7.70 to 5.61)	0.985	7.1 (1.64 to 12.64)	0.372
Positive MAR-test result[b,c] (n)	0.76 (0.41 to 1.43)	0.447[e]	0.39 (0.50 to 3.28)	0.746	0.65 (0.29 to 1.46)	0.641	1.274 (0.46 to 3.54)	0.952

[a]Presented data are mean differences of parameter in HPV-positive and HPV-negative samples.
[b]Presented data are mean ratios of parameter in HPV-positive to HPV-negative samples.
[c]n = 408; samples in which MAR-test was not performed or not to determine were excluded, presented data are positive n/total n (percentage)
[d]P-value obtained by independent samples' t-test unless otherwise specified.
[e]P-value obtained by Fisher's exact test.
[f]P-value obtained by Dunnet's test using the HPV-negative group as a control.

As recently outlined in two systematic reviews (Gizzo et al., 2014; Foresta et al., 2015), previous studies on semen parameters in relation to seminal HPV presence have shown conflicting results. The majority of published studies (which were performed in different types of populations and had a total sample size of 1944 participants (Foresta et al., 2015)) described a reduced sperm motility in HPV-positive samples. This association could however not be confirmed in three studies (of which one was performed in fathers-to-be, and two in men seeking fertility evaluation) (Rintala et al., 2004; Schillaci et al., 2013; Golob et al., 2014) and in the present study, all together representing 1143 participants.

In the present study, the semen analysis (which is in part a subjective analysis, such as the ranking of sperm cell motility) was performed by technicians who were blinded for HPV-test results.

Limitations of our study include the absence of data on sperm morphology, which was not tested in each primary semen analysis, but performed only on the physician's request. Secondly, MAR-testing was performed for IgG only. Although binding sites on sperm for IgG and IgA have been shown to be highly concordant (Ford et al., 1996), we might have underestimated the number of samples with antisperm antibodies (both in HPV-positive and HPV-negative samples).

In a previous study, we have found that the presence of HPV in semen is closely related to HPV infections of the penile epithelium, even on a genotype-specific level (Luttmer et al., 2015). The current findings, demonstrating that HPV presence in semen is not associated with an impairment of semen function, are in line with the suggestion that HPV presence in semen does not necessarily affect spermatozoa but is rather a side-effect of desquamation of HPV-infected penile keratinocytes (Luttmer et al., 2015).

Current guidelines for assisted reproductive technologies (Association of Biomedical Andrologists; Association of Clinical Embryologists; British Andrology Society; British Fertility Society; Royal College of Obstetricians and Gynaecologists, 2008; Practice Committee of American Society for Reproductive Medicine, 2013) do not include the screening of semen for presence of HPV-DNA. As proposed previously (Luttmer et al., 2015), screening of donor semen for HPV may be considered to prevent unnecessary viral transmission to the female genital tract (and thereby increasing the risk of 'iatrogenic' development of cervical premalignant lesions). Donors with HPV-positive semen could be followed-up and re-invited to donate after viral clearance (within approximately seven months (Giuliano et al., 2011)).

Recently, researchers have also suggested standardized HPV testing on semen for diagnostic purposes in unexplained infertility (Foresta et al., 2015) and prior to procedures of assisted reproduction (Gizzo et al., 2014; Foresta et al., 2015). However, so far only two studies have addressed whether men with HPV-positive semen have altered clinical reproductive outcomes when compared with men with HPV-negative semen (Perino et al., 2011; Garolla et al., 2015). Perino et al. observed that seminal HPV positivity was not associated with pregnancy rates but might be related to the occurrence of spontaneous abortions (Perino et al., 2011). Recently, using fluorescence in-situ hybridization (FISH) for HPV detection, Garolla et al. described a decreased conception rate and an increased miscarriage rate in couples with HPV-positive semen (Garolla et al., 2015). The presence of HPV-DNA was described in products of conception in both spontaneous and voluntary abortions (Hermonat et al., 1997; Matovina et al., 2004), as well as in placentas at term (Skoczyński et al., 2011). In-vitro and animal studies have shown negative effects of semen-transferred HPV on embryonic development (Calinisan et al., 2002) and hatching (Henneberg et al., 2006); *in vitro*, HPV16-transfected human spermatozoa have been demonstrated to penetrate oocytes, enabling viral gene transcription (Foresta et al., 2011). In our opinion, it seems ethical to introduce HPV-screening in the work-up of subfertile couples only if sufficiently powered studies have demonstrated that seminal HPV-positivity affects clinical reproductive outcomes and, secondly, if an evidence-based consensus is reached on the preferred management in case of seminal HPV-positivity. A specific semen washing technique using a modified swim-up with enzymatic treatment (Garolla et al., 2012) has shown promising results for the elimination of HPV from semen, but should be more extensively studied.

In conclusion, this study confirmed earlier findings that HPV-DNA is commonly present in semen of men seeking fertility evaluation. However, no association of seminal HPV presence with semen parameters was observed. Therefore, screening for HPV in semen should currently only be considered for donor semen to prevent unnecessary viral transmission to the female genital tract, but not for diagnostic settings in assisted reproduction. Further large studies are needed to elucidate the clinical relevance of HPV presence in semen by focusing on conception, early embryo development and clinical reproductive outcomes.

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Authors' roles

C.J.L.M.M., M.G.D., P.G.A.H., P.J.F.S., and R.L. designed the study. R.L. coordinated the sample collection, managed the database, and performed statistical analyses, interpretation of data, and drafting and final writing of the manuscript. M.G.D., P.J.F.S., P.G.A.H., D.A.M.H., D.T.M.P. and C.J.L.M.M. contributed to the HPV testing, interpretation of data and writing of the manuscript. I.H. contributed to the semen analyses, interpretation of data and writing of the manuscript. J.B. contributed to the statistical analyses, interpretation of data and writing of the manuscript.

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Conflict of interest

P.J.F.S. has been on the speakers bureau of Roche, Gen-Probe, Abbott, Qiagen and Seegene and has been a consultant for Crucell B.V. J.B. has

been on the speakers bureau of Qiagen and has been a consultant for Roche, DDL Diagnostic Laboratory, GlaxoSmithKline and Merck. D.A.M.H. has been member of the scientific advisory boards of Amgen and Pfizer, and has been on the speakers bureau of Hologic/Gen-Probe. C.J.L.M.M. has been on the speakers bureau of GlaxoSmithKline, Qiagen, Merck, Roche, Menarini and Seegene, has served occasionally on the scientific advisory board of GlaxoSmithKline, Qiagen, Merck, Roche and Genticel, and has occasionally been a consultant for Qiagen. Formerly, C.J.L.M.M. was a minority shareholder of Delphi Biosciences, which bankrupted in 2014. C.J.L.M.M. is a minority shareholder of Diassay B.V. P.J.F.S., D.A.M.H. and C.J.L.M.M. have minority stake in Self-Screen B.V., a spin-off company of VU University Medical Center. R.L., M.G.D., P.G.A.H., D.T.M.P., and I.H. do not have any conflicts of interest to disclose.

References

Association of Biomedical Andrologists; Association of Clinical Embryologists; British Andrology Society; British Fertility Society; Royal College of Obstetricians and Gynaecologists. UK guidelines for the medical and laboratory screening of sperm, egg and embryo donors (2008). *Hum Fertil (Camb)* 2008;**11**:201–210.

Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, De Sanjose S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis* 2010;**202**:1789–1799.

Calinisan JH, Chan SR, King A, Chan PJ. Human papillomavirus and blastocyst apoptosis. *J Assist Reprod Genet* 2002;**19**:132–136.

Dunne EF, Nielson CM, Stone KM, Markowitz LE, Giuliano AR. Prevalence of HPV infection among men: A systematic review of the literature. *J Infect Dis* 2006;**194**:1044–1057.

Federa. Human Tissue and Medical Research: Code of conduct for responsible use (2011). 2011;1–36. Available from: http://www.federa.org/sites/default/files/digital_version_first_part_code_of_conduct_in_uk_2011_12092012.pdf.

Ford WC, Williams KM, McLaughlin EA, Harrison S, Ray B, Hull MG. The indirect immunobead test for seminal antisperm antibodies and fertilization rates at in-vitro fertilization. *Hum Reprod* 1996;**11**:1418–1422.

Foresta C, Garolla A, Zuccarello D, Pizzol D, Moretti A, Barzon L, Palù G. Human papillomavirus found in sperm head of young adult males affects the progressive motility. *Fertil Steril* 2010a;**93**:802–806.

Foresta C, Pizzol D, Moretti A, Barzon L, Pal G, Garolla A. Clinical and prognostic significance of human papillomavirus DNA in the sperm or exfoliated cells of infertile patients and subjects with risk factors. *Fertil Steril* 2010b;**94**:1723–1727.

Foresta C, Patassini C, Bertoldo A, Menegazzo M, Francavilla F, Barzon L, Ferlin A. Mechanism of human papillomavirus binding to human spermatozoa and fertilizing ability of infected spermatozoa. *PLoS One* 2011;**6**:e15036.

Foresta C, Noventa M, De TL, Gizzo S, Garolla A. HPV-DNA sperm infection and infertility: from a systematic literature review to a possible clinical management proposal. *Andrology* 2015;**3**:163–173.

Forman D, de Martel C, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, Vignat J, Ferlay J, Bray F, Plummer M *et al.* Global burden of human papillomavirus and related diseases. *Vaccine* 2012;**30**(Suppl 5):F12–F23.

Garolla A, Lenzi A, Pal G, Pizzol D, Bertoldo A, De Toni L, Foresta C. Human papillomavirus sperm infection and assisted reproduction: a dangerous hazard with a possible safe solution. *Hum Reprod* 2012;**27**:967–973.

Garolla A, Engl B, Pizzol D, Ghezzi M, Bertoldo A, Bottacin A, Noventa M, Foresta C. Spontaneous fertility and in vitro fertilization outcome: new evidence of human papillomavirus sperm infection. *Fertil Steril* 2015. doi:10.1016/j.fertnstert.2015.09.018. [Epub ahead of print].

Giuliano AR, Nielson CM, Flores R, Dunne EF, Abrahamsen M, Papenfuss MR, Markowitz LE, Smith D, Harris RB. The optimal anatomic sites for sampling heterosexual men for human papillomavirus (HPV) detection: the HPV detection in men study. *J Infect Dis* 2007;**196**:1146–1152.

Giuliano AR, Lee J-H, Fulp W, Villa LL, Lazcano E, Papenfuss MR, Abrahamsen M, Salmeron J, Anic GM, Rollison DE *et al.* Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. *Lancet* 2011;**377**:932–940.

Gizzo S, Ferrari B, Noventa M, Ferrari E, Patrelli TS, Gangemi M, Nardelli GB. Male and couple fertility impairment due to HPV-DNA sperm infection: update on molecular mechanism and clinical impact—Systematic review. *Biomed Res Int* 2014;**2014**:230263.

Golob B, Poljak M, Verdenik I, Kolbezen Simoniti M, Vrtačnik Bokal E, Zorn B. High HPV infection prevalence in men from infertile couples and lack of relationship between seminal HPV infection and sperm quality. *Biomed Res Int* 2014;**2014**:956901.

Henneberg AA, Patton WC, Jacobson JD, Chan PJ. Human papilloma virus DNA exposure and embryo survival is stage-specific. *J Assist Reprod Genet* 2006;**23**:255–259.

Hermonat PL, Han L, Wendel PJ, Quirk JG, Stern S, Lowery CL, Rechtin TM. Human papillomavirus is more prevalent in first trimester spontaneously aborted products of conception compared to elective specimens. *Virus Genes* 1997;**14**:13–17.

Jacobs MV, Snijders PJF, Van den Brule AJC, Helmerhorst TJM, Meijer CJLM, Walboomers JMM. A general primer GP5+/GP6+-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. *J Clin Microbiol* 1997;**35**:791–795.

Kaspersen MD, Larsen PB, Ingerslev HJ, Fedder J, Petersen GB, Bonde J, Höllsberg P. Identification of multiple HPV types on spermatozoa from human sperm donors. *PLoS One* 2011;**6**:e18095.

Lai YM, Lee JF, Huang HY, Soong YK, Yang FP, Pao CC. The effect of human papillomavirus infection on sperm cell motility. *Fertil Steril* 1997;**67**:1152–1155.

Laprise C, Trottier H, Monnier P, Coutlée F, Mayrand M-H. Prevalence of human papillomaviruses in semen: a systematic review and meta-analysis. *Hum Reprod* 2014;**29**:640–651.

Luttmer R, Dijkstra MG, Snijders PJFF, Jordanova ES, King AJ, Pronk DTMM, Foresta C, Garolla A, Hompes PGA, Berkhof J *et al.* Presence of human papillomavirus in semen of healthy men is firmly associated with HPV infections of the penile epithelium. *Fertil Steril* 2015;**104**:838–844.e8.

Matovina M, Husnjak K, Milutin N, Ciglar S, Grce M. Possible role of bacterial and viral infections in miscarriages. *Fertil Steril* 2004;**81**:662–669.

Olatunbosun O, Deneer H, Pierson R. Human papillomavirus DNA detection in sperm using polymerase chain reaction. *Obstet Gynecol* 2001;**97**:357–360.

Perino A, Giovannelli L, Schillaci R, Ruvolo G, Fiorentino FP, Alimondi P, Cefal E, Ammatuna P. Human papillomavirus infection in couples undergoing in vitro fertilization procedures: impact on reproductive outcomes. *Fertil Steril* 2011;**95**:1845–1848.

Practice Committee of American Society for Reproductive Medicine. Recommendations for reducing the risk of viral transmission during fertility treatment with the use of autologous gametes: A committee opinion. *Fertil Steril* 2013;**99**:340–346.

Rintala MAM, Grénman SE, Pöllänen PP, Suominen JJO, Syrjänen SM. Detection of high-risk HPV DNA in semen and its association with the quality of semen. *Int J STD AIDS* 2004;**15**:740–743.

Schillaci R, Capra G, Bellavia C, Ruvolo G, Scazzone C, Venezia R, Perino A. Detection of oncogenic human papillomavirus genotypes on spermatozoa from male partners of infertile couples. *Fertil Steril* 2013;**100**:1236–1240.

Schmitt M, Bravo IG, Snijders PJF, Gissmann L, Pawlita M, Waterboer T. Bead-based multiplex genotyping of human papillomaviruses. *J Clin Microbiol* 2006;**44**:504–512.

Skoczyński M, Goździcka-Józefiak A, Kwaśniewska A. Prevalence of human papillomavirus in spontaneously aborted products of conception. *Acta Obstet Gynecol Scand* 2011;**90**:1402–1405.

Smith JS, Gilbert PA, Melendy A, Rana RK, Pimenta JM. Age-specific prevalence of human papillomavirus infection in males: A global review. *J Adolesc Health* 2011;**48**:540–552.

World Health Organization. *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 5th edn. 2010;1–286. Available from: http://whqlibdoc.who.int/publications/2010/9789241547789_eng.pdf.

Yang Y, Jia C-W, Ma Y-M, Zhou L-Y, Wang S-Y. Correlation between HPV sperm infection and male infertility. *Asian J Androl* 2013; **15**:529–532.