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Autore/i: schillaci R

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Detection of oncogenic human papillomavirus genotypes on spermatozoa from male partners of infertile couples

Rosaria Schillaci, Ph.D.,[a] Giuseppina Capra, Ph.D.,[a] Carmela Bellavia, Ph.D.,[a] Giovanni Ruvolo, Ph.D.,[c] Concetta Scazzone, Ph.D.,[b] Renato Venezia, M.D.,[a] and Antonio Perino, M.D.[a]

[a] Dipartimento di Scienze per la Promozione della Salute e Materno Infantile "G. d'Alessandro" and [b] Dipartimento di Biopatologia e Biotecnologie Mediche e Forensi, University of Palermo; and [c] Centro di Biologia della Riproduzione, Palermo, Italy

Objective: To evaluate the prevalence of human papillomavirus (HPV) sperm infection and its correlation with sperm parameters in patients who attended a fertility clinic.
Design: Cross-sectional clinical study.
Setting: University-affiliated reproductive medicine clinic.
Patient(s): A total of 308 male partners of couples undergoing in vitro fertilization techniques.
Intervention(s): Specimens of semen were collected from all patients.
Main Outcome Measure(s): Sperm parameters were evaluated according to the World Health Organization manual. The presence of HPV DNA was researched by the combined use of two HPV assays and a highly sensitive nested polymerase chain reaction assay followed by HPV genotyping. To examine whether HPV was associated with the sperm, in situ hybridization (ISH) analysis was performed.
Result(s): Results of HPV investigation were compared with sperm parameters and ISH analysis. Twenty-four out of 308 semen samples (7.8%) were HPV DNA positive, but HPV infection did not seem to affect semen quality. Moreover, ISH revealed a clear HPV localization at the equatorial region of sperm head in infected samples.
Conclusion(s): Oncogenic HPV genotypes were detected on spermatozoa from asymptomatic subjects, but a role of the infection in male infertility was not demonstrated. (Fertil Steril® 2013;100:1236–40. ©2013 by American Society for Reproductive Medicine.)
Key Words: HPV infection, semen parameters, IVF, ICSI

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Human papillomaviruses (HPVs) are agents of the most common sexually transmitted disease. These small DNA viruses induce epithelial cell proliferation, and high risk HPV types cause almost all cases of cervical cancers and a variable proportion of noncervical malignancies, including vulvar, vaginal, anal, and oropharingeal, and cancer of the penis (1). Many studies have focused on HPV-related diseases in women, whereas fewer data are available on male infection. It is estimated that the prevalence of any HPV type in men ranged from 1.3% to 72.9%, with a prevalence of 65.4% in some age groups, especially among individuals 18–40 years of age (2, 3). Although HPV infection in men may be associated with low mortality and morbidity, investigation is still important owing to both its association with genital warts, penile cancer, anorectal cancer, and oropharyngeal cancer, and the role men play in HPV transmission to their female sexual partners (4, 5). In men, HPV DNA and RNA have been found not only in the penile shaft, glans, and urethra (3), but also in the ductus deferens, epididymis, and testis (6, 7).

Because the presence of the virus has also been demonstrated in semen (8), there is a growing interest in the impact of HPV infection on male fertility and reproductive function.

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Reprint requests: Rosaria Schillaci, Ph.D., Clinica Ostetrica e Ginecologica, Dipartimento di Scienze per la Promozione della Salute e Materno Infantile, Via Alfonso Giordano 3, 90127 Palermo, Italy (E-mail: rosaria.schillaci@unipa.it).

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Although data from recent studies suggest that placental HPV infection in early pregnancy can occur via infected sperm at fecundation (9) and reveal a possible role of HPV male infection as a cause of miscarriage in couples undergoing assisted reproduction technology cycles (10), the effect of infection on semen parameters is still a controversial topic. Some authors report that HPV infection is associated with reduced sperm motility (11–15), raising concerns about its possible role in idiopathic asthenozoospermia and thus in male infertility. Additionally, a very recent study by Garolla et al. (16) hypothesizes that HPV may impair sperm motility owing to higher antisperm antibody (ASA) production. On the other hand, other authors failed to observe any relation among sperm viral infection, ASA production, and sperm quality (17, 18).

However, precise data about the presence and significance of HPV in sperm are not available. In particular, the exact localization and mechanism of infection by HPV in sperm, as well as the role of infected sperm cells as a transmission vector for the virus are still unclear. A study by Perez-Andino et al. (19) revealed that HPV-16 capsids bind efficiently to two distinct sites at the equatorial region of the sperm head surface, suggesting that these sites may mediate HPV binding. Other observations of HPV-6, -16, -18, and -31 association to or near the sperm head equatorial segment were documented in sperm donors (20). Recently, Foresta et al. (21) revealed a possible mechanism of infection by HPV in human sperm based on the interaction between the capsid-protein L1 and the primary attachment receptor syndecan-1 localized at the equatorial region of the sperm head. Sperm transfected with HPV E6/E7 genes and sperm exposed to HPV L1 capsid protein are capable of penetrating the oocyte and transferring the virus into hamster eggs (21).

The aim of the present study was to detect HPV in semen and investigate its correlation with sperm parameters in patients who attended our fertility clinic.

MATERIALS AND METHODS

Patients

A cohort of 308 male partners of couples undergoing conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), after signing an informed consent, were enrolled in the study at the Reproductive Medicine Unit, University of Palermo, Italy, and the Centro di Biologia della Riproduzione, Palermo, Italy, from October 2010 to October 2012. Institutional Review Board approval was obtained before starting the study. No patient tested positive for HIV, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, Herpes simplex, or *Treponema pallidum*. All subjects, aged 28 to 57 years (mean 38.7 ± 5.9 years), collected semen for standard sperm analyses and examination for HPV DNA.

Semen Processing

Semen samples were obtained by masturbation after 3–5 days of sexual abstinence. After liquefaction at room temperature, semen volume, pH, sperm concentration, motility, and normal morphology were evaluated according to World Health Organization guidelines for semen analyses (22). Sperm cells were then separated by swim-up technique. After centrifugation at 300*g* for 7 minutes, supernatants were discarded and 0.3 mL of fertilization medium (Origio; Medicult) was added to the pellet for ICSI procedure or 1 mL of the same medium for IVF insemination. Samples were then incubated at 37°C in 5% CO_2 for 45 minutes.

Detection of HPV DNA

After ICSI/IVF insemination, the supernatant was centrifuged at 300*g* for 7 minutes and the pellet fixed in Preservcyt Solution (Cytyc Corp.). HPV testing was then performed directly on sperm cells. All semen samples were processed for DNA extraction (23). Amplifications were carried out in a Mastercycler (Eppendorf) and polymerase chain reaction (PCR) products were analyzed in 8% polyacrylamide gel. HPV detection and typing were performed with the combined use of the HPV Inno-Lipa Genotyping System (Innogenetics) (24) and a nested PCR assay with PGMY09/11 and GP05+/06+ primer pairs, followed by direct cycle sequencing of PCR products for HPV genotyping. HPV genotypes were considered to be low risk or high risk according to two recently published epidemiologic classifications of HPV types (25).

Semen In Situ Hybridization

Sperm in situ hybridization (ISH) for HPV was a modification of the procedure described by Sarrate et al. (26). Briefly, after separation by swim-up procedure, samples containing $\geq 1 \times 10^6$ sperm were permeabilized with 0.075 mol/L KCl and incubated for 30 minutes at 37°C in a water bath. After centrifugation for 5 minutes at 1,000*g*, the supernatant was removed, the pellet fixed in a methanol–acetic acid solution for ≥24 hours at −20°C, and 10 μL sperm sample smeared on clean grease-free slides. After dehydration, samples were incubated in a solution of 5 mmol/L [1,4-dithiothreitol, 1% Triton X-100, and 50 mmol/L 2-amino-2-(hydroxymethyl)-1,3-propanediol] for 8 minutes at 37°C to induce chromatin decondensation. Denaturation and hybridization were performed with a commercial kit (Zytofast HPV- Screening Plus CISH; Zytovision).

Samples were overlaid with 10 μL hybridization solution containing digoxigenin (DIG)–labeled HPV DNA probe (Zytofast HPV 6/11/16/18/31/33/35 probe). Each slide was then covered with a glass coverslip, denaturated at 75°C for 5 minutes, and hybridized at 37°C for 60 minutes in a Thermobrite System (Iris Sample Processing).

Thereafter, slides were washed twice in 1× Tris-buffered solution (TBS) and incubated with three to four drops of mouse anti-DIG per slide for 30 minutes at 37°C. After three washes in 1× TBS, three to four drops of anti–mouse horseradish polymerase per slide were added to the samples, which were then incubated for 30 minutes at 37°C. Incubation was followed by three washes in 1× TBS.

After the addition of 3,3-diaminobenzidine tetrahydrochloride and nuclear blue solutions, samples were washed in water, dehydrated in ethanol (70%–85%–95%–100%), and then incubated in xylene for 2 minutes. The negative control sample was processed in the same manner.

After drying, evaluation of sample material was carried out by light microscopy with ×100 magnification.

Statistical Analysis

Data were analyzed with SPSS version 13.0. Significant differences between groups with or without HPV were determined by the Mann-Whitney test at $P<.05$.

RESULTS

A total of 308 semen samples were analyzed from patients who attended our fertility clinic, with an average age of 38.7 ± 5.9 years: 7.8% (24/308) tested positive for HPV and 41.6% of these (10/24) had an infected female partner. As reported in Table 1, most genotypes were of high risk, and a prevalence of HPV-52 (5/24; 21%) was found. Among the 24 patients, 83.3% (20/24) presented high-risk HPV and 16.7% (4/24) low-risk HPV. In 16.7% (4/24) of the samples, more than one HPV type was detected, and all multiple infections included high-risk viral types. Among the ten infected couples, 40% (4/10) showed concordance of all viral types and 50% (5/10) concordance of at least one. In particular, 90% (9/10) of semen samples presented high-risk HPV and 10% (1/10) low-risk HPV. Moreover, in 40% (4/10) of the infected couples, a multiple infection by high-risk HPV was detected.

No statistically significant difference was found in sperm parameters between HPV-infected and noninfected semen samples (Table 2). Median value of sperm concentration was 10 × 10^6/mL and 15 × 10^6/mL in HPV-positive and HPV-negative men, respectively ($P=.19$). HPV-infected and noninfected semen samples had similar rapid/slow progressive motility (medians 30% vs. 20%; $P=.63$) and nonprogressive motility (medians 20% vs. 20%; $P=.06$). We found no relationship between HPV infection and sperm volume (medians 3.5 vs. 3; $P=.56$), pH (medians 7.6 vs. 7.4; $P=.40$) or normal morphology (medians 60% vs. 55%; $P=.70$) evaluated in HPV-positive patients and HPV-negative patients, respectively.

Regarding the transmission of HPV infection, ISH performed on HPV-infected semen samples clearly showed attachment of HPV to the equatorial region of the sperm head, supporting the observation of other authors regarding mechanism of entry of the virus into the sperm (19–21) (Fig. 1).

TABLE 1

Type of human papillomavirus (HPV) infection in 24 infected patients undergoing IVF treatment and concordance of viral types between sexual partners.

ID	HPV, female	HPV, male
28	59, 66	59, 61, 66, 84
31		59,73
37		59
78		16
84		83
156	87	87
159	16, 31	16,31
176	66	16
203		11, 18, 44, 59, 52
210		52
213		53
216	59, 66	51
221		31
224		66
228		51
235		51
237	70	70
241	39	39
247	18	33
271		54
280		52
282	18, 31	52
289		52
306	66	44

Schillaci. Oncogenic HPV genotypes in sperm. Fertil Steril 2013.

TABLE 2

Human papillomavirus (HPV) infection and sperm parameters in patients undergoing IVF treatment.

	Infected (n = 24)	Noninfected (n = 284)	*P* value
Age, y	38.9 ± 6.7	38.7 ± 5.9	.88[a]
Sperm volume, mL	3.5 (2)	3 (2)	.56[b]
pH	7.5 (0.2)	7.45 (0.23)	.40[b]
Sperm number, 10^6/mL	10 (39.2)	15 (35)	.19[b]
Motility a+b, %	30 (45)	20 (40)	.63[b]
Motility c, %	20 (15)	20 (20)	.06[b]
Normal morphology, %	60 (35)	55 (25)	.70[b]

Note: Values are presented as mean ± SD or median (interquartile range).
[a] Student *t* test.
[b] Mann-Whitney test.

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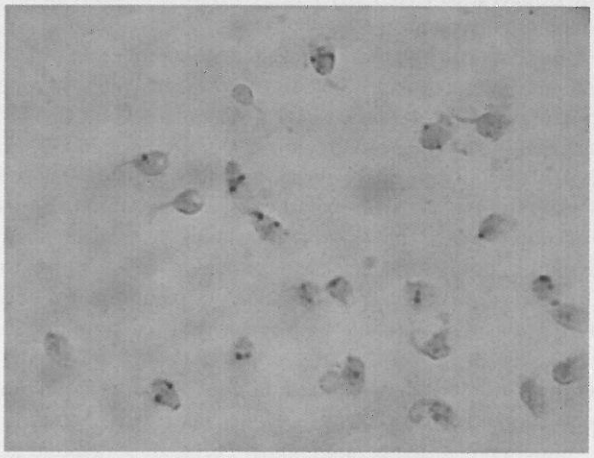
DISCUSSION

According to study results, HPV infection was prevalent in 7.8% of the male partners of infertile couples with a detection

FIGURE 1



Detection and localization of human papillomavirus (HPV) in human sperm. In situ hybridization for HPV DNA on sperm from a patient with HPV-16 in semen.

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of high-risk HPV in 83.3% of positive seminal samples; HPV-52 was the most prevalent viral type. Earlier data from a Mexican study (27) reported a higher prevalence of HPV infection (59.73%) in semen samples from patients in an assisted reproduction program. That study also revealed that infection with HPV-16 was more frequent in both oligozoospermic and normozoospermic patients. In the latter, a minor presence of abnormal spermatic cells and leukocytes were found compared with oligozoospermic patients. A possible explanation for the evident discrepancy in the HPV prevalence and distribution identified in these two studies includes different geographic areas, sociodemographic characteristics, behavioral/lifestyle factors, sexual behavior between male samples studied, and detection techniques performed (2, 28–31).

Seminal HPV infection was frequently associated with reduced sperm motility (12–15), although other studies did not find any effects on sperm quality (8). The striking differences in literature data could be related to the relatively small sample size of cohorts evaluated in the different studies. For this reason, a multicenter study would be needed. Recently, Garolla et al. (16) reported that HPV sperm infection can be frequently associated with ASAs and lower sperm motility leading to a reduction of male fertility. This finding is in contrast with other observations that excluded the existence of a relationship between sperm viral infection, ASAs production, and sperm quality alteration (17, 18). However, in a previous study (10) we demonstrated that pregnancy and implantation rates were not affected by HPV infection in the men of couples undergoing IVF, but HPV positivity of semen from male partners increased the pregnancy loss rate compared with noninfected patients (66% vs. 15%).

Thus, in accordance with data from Rintala et al. (8), our results suggest that HPV infection is not associated with an impairment of sperm parameters. Moreover, the recent detection of HPV in 16% of semen samples from 188 healthy Danish donors (20) and the observation of an early placental HPV infection occurring in noninfected women (9) suggest a role of sperm as HPV carrier rather than its involvement in semen quality alteration.

Our data confirm that the mechanism of infection by HPV in human sperm probably consists, as previously described, of an interaction between the HPV capsid and a specific receptor localized in the equatorial region of the sperm head (19–21). The HPV ability to infect sperm, together with the absence of alterations of sperm parameters, entails the risk that procedures such as intracytoplasmic sperm injection, involving no natural selection of sperm cells, could use HPV-carrying sperm. These data suggest caution in the use of HPV-positive seminal samples for assisted reproduction techniques or sperm banking and emphasize the need to perform the HPV screening in the general infertility population.

Moreover, in the present study, 10/24 HPV-infected patients (41.6%) had a female partner who was HPV positive, with 9/10 (90%) presenting high-risk HPV. None of the male partners presented any visible lesions, although it has been reported that penile lesions are more frequent in partners of high-risk HPV carriers (32). Concordance of at least one viral type was found in 50% of the infected couples (5/10), suggesting that HPV-infected men could have an important role in transmission to women and maintenance of infection; as a consequence, there is a higher risk of developing cervical cancer. Several lines of evidence have suggested that the sexual behavior of men can contribute to the risk of cervical cancer in their sexual partners (33–35). Several cancers of the anogenital tract and upper aerodigestive tract and their precursor lesions in men are now understood to be caused by infection with sexually transmitted HPV, and more than one-fourth of HPV-associated cancers in the United States occur in men (36). In Europe, the overall estimated epidemiologic burden of HPV-related cancers and nonmalignant diseases is high in men. Approximately 30% of all new cancer cases attributable to high-risk HPV types that occur yearly in Europe were estimated to occur in men (37, 38). According to an Italian study , the economic burden attributable to noncervical HPV-6, -11, -16, and -18–related diseases was higher among men than among women (60.6% vs. 39.4% of the total, respectively). The economic costs among men, in fact, represented more than one-third (38.8%) of the total direct costs of HPV-6, -11, -16, and -18–related diseases, including cervical conditions (cervical cancer, dysplasia, and CIN1/2/3 lesions), i.e., 291 million euros. This observation could inform Italian national and regional health policy evaluations of the economic value of extending the anti-HPV immunization programs to cohorts of boys (39).

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