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Effect of human papillomavirus infection on semen quality and assisted reproductive technology outcomes: a prospective observational cohort study

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Abstract

Background Human papillomavirus (HPV) adversely affects human reproduction. We aimed to evaluate the prevalence of HPV infection in men and its correlation with semen parameters and reproductive outcomes.

Methods In this prospective observational cohort study, 384 semen samples were collected from 237 male partners of infertile couples. The presence of HPV DNA and genotyping were analyzed in semen by quantitative PCR. A total of 186 intrauterine inseminations (IUI) in 101 couples and 186 assisted reproduction techniques (ART) cycles in 155 couples were performed. Associations between HPV positivity and semen parameters and fertility outcomes were evaluated using a generalized linear mixed model.

Results The prevalence of HPV was 22.7%. Twenty-three HPV types were detected and 69.5% of positive samples presented at least one high risk (HR)-HPV genotype. HPV-18 (14%), HPV-53 (10%), and HPV-56 (10%) were the most prevalent HR-HPV genotypes followed by HPV-16, HPV-31, and HPV-51 (8%). HPV-42 was the most prevalent low risk (LR)-HPV genotype (25%). More than one HPV type was detected in 41% of HPV + samples. After capacitation, 30% of HPV + samples remained positive. We found no relationship between HPV infection and sperm volume, sperm concentration, and progressive motility both before and after semen capacitation. We observed a not significant different clinical pregnancy per cycle in the HPV − (6.8%) and HPV + (5.0%) IUI. We did not find any significant difference in fertilization, cleavage, quality of developed embryos, blastocyst formation nor in embryo utilization of ART cycles. Slightly lower cumulative pregnancy (33% *vs* 39%) and live-birth (25% *vs* 30%) rates and higher miscarriage rate (53% and 29%) were observed in HPV + with respect to HPV − cycles. Fifty-five neonatal outcomes from HPV − ($n = 45$) and HPV + ($n = 10$) cycles were available. No stillbirths as well as no malformations were recorded.

Conclusions This study confirmed previous findings that HPV DNA is present in semen of one quarter of infertile couples. No significant association of seminal HPV presence with semen parameters was found. We observed a trend of worst clinical outcomes in the HPV + group that is worth further investigation in a large population to draw definitive conclusions.

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Keywords Human papillomavirus (HPV), HPV semen infection, In vitro fertilization outcomes, Assisted reproductive technology (ART), Infertility

Background

Human papillomavirus (HPV) is the most prevalent sexually transmitted viral infections among men and women of reproductive age worldwide [1] and, while most infections are asymptomatic and are cleared 12–24 months post-infection [2], a small portion persist and progress to benign epithelial lesions and cancer [3]. There are almost 200 HPV genotypes [4] that, according to their oncogenic potential, can be divided into high risk (HR) and low risk (LR) groups [5]. The prevalence of HPV infection is about 40% of the general population (3.5–45% in men and 2–44% in women), with variations based on the HPV type and the anatomical site of infection [6].

The role of HPV in female diseases is well known and widely studied, due to many screening and research clinical programs. It is estimated that HPV is the cause of 99% of cervical cancers, 90% of anal cancer, 65% of vaginal cancers, 50% of vulvar cancers, and 45–90% oropharyngeal cancers [7]. In contrast, awareness in male HPV infection and related diseases is still insufficient [8]. HPV infection in men is associated with clinically symptomatic genital warts in the external genitals, or asymptomatic; the persistence of the infection in the latter cases may increase the risk of various cancers [9].

Recent studies suggest that HPV may affect fertility and perhaps even the effectiveness of assisted reproduction treatment (ART) [10]. Some studies suggest that female HPV infection may lower the success rate of ART [11, 12], increasing the risk of miscarriage and decreasing the live birth rate [13–15]. These findings, however, have not been confirmed by other reports [16–20], and the topic is still under discussion.

In male, HPV can be found not only along the entire male genital tract but frequently in semen (38%) [21]. Seminal HPV infection has often been associated with reduced sperm motility [22–24] and increased sperm DNA fragmentation [25, 26], although other studies have not found any effect on sperm quality [27–32]. The recent introduction of WHO 2021 evaluation criteria for the examination and processing of human semen [33] provided additional information regarding HPV impact on seminal parameters. Specifically, a significant correlation between HPV positivity, higher midpiece morphological defects, and diminished rapid progressive motility was found [34]. Regarding sperm DNA fragmentation index, only high-risk HPV infections affected DNA integrity [34].

HPV bound to the sperm head can likely be transmitted to oocytes [32] and in mouse model semen HPV infection has been shown to adversely affect embryo early development and implant [35–37]. The impact of HPV infection on ART success remains a topic of debate as some studies have shown that the presence of HPV at semen level reduces natural and assisted clinical pregnancy rates and increases the miscarriage rate [38–40], not confirmed by other authors [41, 42]. Jaworek et al. [41] found that the abortion rates in spontaneously pregnant women (5/46, 10.9%), couples treated with IVF (6/98, 6.1%), and couples treated with IUI (1/27, 3.7%) did not differ significantly ($P = 0.489$). Similarly, Carullo et al. observed no significant differences in embryological variables, clinical pregnancy and live birth rates, neonatal and obstetrics outcomes in subjects with HPV positivity [42].

Considering the inconclusive information available in the literature, we found interesting to expand the public data available on HPV in semen and the results of both inseminations and ART cycles (including conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). This prospective observational cohort study was aimed at evaluating both the prevalence of HPV in men undergoing IUI/ART and the impact of HPV infection on semen parameters, embryological, clinical, and neonatal outcomes.

Methods

Study population and study design

This prospective observational cohort study was performed in a public tertiary level fertility center. We enrolled 237 infertile couples scheduled for intrauterine insemination (IUI) or in vitro fertilization at the Physiopathology of Human Reproduction, IRCCS Ospedale Policlinico San Martino, Genova, Italy, from January 2021 to October 2023. At recruitment, all patients were properly informed about the aim of the study and they gave written informed consent for the study and for the use of their data. The study was approved by the Ethical Committee of Regione Liguria (protocol code 168/2020). Exclusion criteria were azoospermia or severe hypoposia, cryopreserved semen. All samples were tested for infection of Chlamydia trachomatis, Mycoplasma, Ureoplasma, and common germs, and if positive were excluded. Men were routinely tested for human immunodeficiency virus type 1 or 2, hepatitis B or C virus, or

Treponema pallidum. Patients with genetic alterations, karyotype abnormalities, Y-chromosome microdeletions, or CFTR mutations were also excluded. A total of 260 semen samples were analyzed in order to detect the presence of HPV infection. We collected data of semen samples and IUI and/or ART cycles within 6 months of the HPV analysis. For 23 out of 237 patients, we collected two semen samples (this is why we have a total of 260 semen samples) because the next IUI or IVF/ICSI cycle was performed after more than 6 months from the first one. The median number of samples collected per subject was 1 (range 1–6).

Results of semen analysis of HPV negative (HPV −) and HPV positive (HPV +) patients were compared. We also evaluated the impact of HPV infection in semen on outcomes of IUI/ART cycles (IVF and ICSI) performed by the 237 infertile couples. Nineteen couples performed both IUI and IVF/ICSI cycles.

The study design is summarized in Fig. 1.

Semen processing

Semen samples were collected by masturbation into a sterile container after 2 to 5 days of abstinence. Sperm samples were liquefied at room temperature for 30–60 min. Semen volume, sperm concentration, and motility were evaluated following the World Health Organization (WHO) guidelines [33]. The semen analysis was performed by operators who were blinded for HPV test. We tested the presence of HPV DNA in two aliquots of semen from the same patient: one was untreated semen and one after two-layer density gradient (SpermGrad™, Vitrolife, Göteborg, Sweden) capacitation.

DNA isolation

Total DNA was purified from both native and capacitated aliquots by using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Briefly, 20 µL Proteinase K solution (> 600 mAU/mL) and 200 µL Buffer AL were added to 200 µL of semen sample in a 1.5-mL microcentrifuge tube. After mixing by pulse-vortexing for 15 s lysis was performed at 56 °C for 10 min. After the incubation, 200 µL ethanol (96–100%) were added to the sample and mixed again by pulse-vortexing for 15 s The mixture was applied to the QIAamp Mini spin column in a 2 mL collection tube and centrifuged at 6000 ×g for 1 min. Then, 500 µL Buffer AW1 were added to the QIAamp Mini spin column placed in a clean 2 mL collection tube. After centrifugation at 6000 ×g for 1 min, 500 µL Buffer AW2 were added to the column placed in a clean 2 mL collection tube, and centrifugation was performed at full speed (20,000 ×g) for 3 min. The column was placed in a clean 1.5 mL microcentrifuge tube, and 200 µL Buffer AE were added. After an incubation at room temperature for 1 min, the DNA was eluted by centrifuging at 6000 ×g for 1 min.

HPV DNA detection and genotyping

The molecular detection of HPV and its genotyping was performed using the Anyplex™ II HPV28 detection assay (Seegene®, Seoul, Korea) according to the manufacturer's instructions, and the CFX96 PCR Thermal Cycler (Bio-Rad, Hercules, CA, USA). This multiplex real-time polymerase chain reaction (PCR) allows for simultaneous detection and genotyping of 19 HR-HPV types, including HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82, and 9 LR-HPV types, including 6, 11, 40, 42, 43, 44, 54, 61, and 70, in a single reaction.

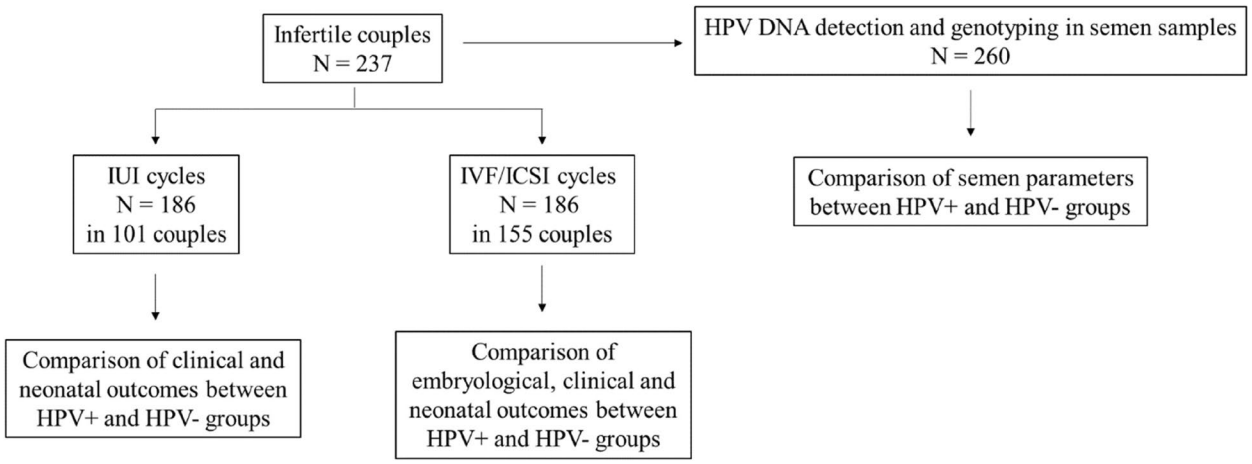

Fig. 1 Study design

All reactions included positive and negative controls provided in the kit. Analysis of the results was performed automatically using Seegene® Viewer software according to the manufacturer's instructions.

Infertility treatments

Women included in the IUI cohort received a daily dose of 50 mg Clomiphene citrate started on the third day after menstruation. When at least one follicle >16 mm in diameter was found by transvaginal sonography, we administrated 5.000 IU human chorionic gonadotropin (hCG) for ovulation induction. IUI took place 36 h after hCG administration.

In ART cycles, controlled ovarian hyperstimulation, oocyte recovery, embryo culture, and morphological evaluation of embryos were performed as described previously [43]. Oocytes were fertilized with IVF or ICSI. Embryo transfer (ET) was generally performed 72 h after oocyte collection. However, based on the number of embryos available, the ET could be performed on day 2 (if the patient had only one to two fertilized oocytes) or day 5 (if the patient had at least four good quality cleavage-stage embryos). Surplus blastocysts were cryopreserved.

The serum beta hCG test was performed 14 days after IUI or oocyte recovery. The presence of a gestational sac at the 6rd–7 th gestational week was defined as a clinical pregnancy. Miscarriage was defined as a loss of pregnancy after ultrasonographic detection of a gestational sac.

Sample size calculation and data analysis

The primary objective of this study was to estimate the prevalence of HPV infection among males undergoing assisted reproductive technology. Assuming an expected prevalence of 20% [44] and a desired 95% confidence level, a sample size of 246 individuals would be required to achieve an error margin of +5%. We enrolled 237 men, which provide a precision of approximately +5.1%, considered acceptable for prevalence estimation purposes.

We assessed the impact of semen HPV infection on primary endpoints (semen volume, sperm concentration and motility) and retrieved information for infertility treatments performed. The reported outcomes of cycles were fertilization rate (defined as the number of fertilized oocytes per number of MII oocytes inseminated), cleavage rate (defined as the number of cleaved embryos per number of fertilized oocytes), quality of embryos, blastulation rate (defined as the total number of blastocysts formed per number of embryos cultured up to day 5–7), embryo utilization rate (defined as the number of embryos utilized (transferred or cryopreserved) per number of fertilized oocytes in the same cycle), implantation rate (defined as the number of fetal cardiac activities at 12 weeks of gestation per number of transferred embryos), pregnancy rate (defined as the pregnancies with at least one gestational sac and fetal cardiac activities per number of embryo transfers), miscarriage rate (defined as the number of abortions per number of pregnancies), live birth rate (defined as live-born babies divided by number of ET), and birthweights (expressed as percentile and standard deviation score (SDS) for gestational age, according the Italian reference curves) [45]. Descriptive statistics were reported as means ± standard deviation (SD) or median (range) for continuous variables, and as absolute frequencies and percentages for categorical variables.

Comparisons of semen parameters in HPV –and HPV +groups were performed using a Generalized Estimating Equation (GEE) model, to consider the correlation between observations originated from the same subject (different semen samples from subsequent collections from the same man). For this purpose, *p*-values were adjusted for age at sample collection, smoking habits, and body mass index (BMI).

Comparisons of demographic data and embryological outcomes between groups (HPV −*vs.* HPV +) were performed using a GEE model, to take into account the correlation between observations originated from the same subject (subsequent cycles in the same couple). For this purpose, *p*-values were adjusted for male and female age at sample collection, infertility cause, and type of treatment. In the GEE model, an unstructured correlation matrix was used as the correlation structure. Comparisons of clinical outcomes and perinatal characteristics were performed using the chi-square test or the *T*-test or *U*-Mann–Whitney test, as appropriate. Analyses were carried out by SAS version 9.4 (SAS Institute, Cary, NC) and MedCalc® software (Ostend, Belgium) by using the paired Student's *t*-test and chi-square, as appropriate. A *p*-value < 0.05 was considered statistically significant.

Results

HPV prevalence

A total of 260 samples from 237 male subjects were analyzed. At baseline (the first visit at Centre) the mean age of men was 38.5 ± 5.6 years.

The analysis for HPV performed on semen samples revealed a positivity rate of 22.7% (59/260 samples) or 23.6% considering the prevalence in our populations (56/237 patients) since more samples belong to the same patient. Table 1 shows the characteristics of the whole cohort of men enrolled in the study and in the HPV −and HPV +groups.

A total of 28 HPV genotypes were analyzed and 23 HPV types were detected. Among the 59 HPV +semen samples, 69.5% (41/59) presented at least one high-risk

Table 1 Baseline characteristics of the enrolled population and of HPV − and HPV + groups

	All patients	HPV − patients	HPV + patients	*p*-value*
Number	237	181	56	-
Age, mean ± SD	38.5 ± 5.6	38.7 ± 5.6	38.0 ± 5.7	0.324
Number of samples collected, median (range)	1 (1–6)	1 (1–6)	1 (1–4)	0.709
BMI, mean ± SD	25.6 ± 3.7	25.5 ± 3.6	26.1 ± 4.0	0.330
Smoke habit, *N* (%)				
No	131/237 (55.3)	100/181 (55.2)	31/56 (55.4)	0. 899
Former	66/237 (15.6)	32/181 (17.7)	5/56 (8.9)	0.169
Yes	69/237 (29.1)	49/181 (27.1)	20/56 (35.7)	0.169

p-values were obtained from GEE model. GEE analysis was performed at patient-level

N, number; *SD*, standard deviation; *BMI*, body mass index

* Comparison between HPV − and HPV + groups

HPV type. HPV-18, HPV-53, and HPV-56 were the most prevalent HR-HPV genotypes (HPV-18: 10% (6/59); HPV-53: 10% (6/59); HPV-56: 14% (8/59)) followed by HPV-16 (8%, 5/59), HPV-31 (8%, 5/59), and HPV-51 (8%, 5/59). HPV-42 was the most prevalent LR-HPV genotype (25%, 25/59). In 41% (24/59) of the positive samples, more than one HPV type was detected, and 29% (7/24) multiple infections included only high-risk viral types.

Of the 59 samples tested positive, 30% (18/59) remained positive even after capacitation: 72% (13/18) of semen samples presented high-risk HPV, and in 3 of them, two HPV types were detected. The most prevalent HR-HPV genotypes after capacitation were HPV-51 (17%, 3/18), HPV-53 (11%, 2/18)), HPV-56 (11%, 2/18), and HPV-73 (11%, 2/18). HPV-42 remained the most prevalent LR-HPV genotype (28%, 5/18) also in capacitated semen. Figure 2 shows the prevalence of the HPV types detected in the HPV + semen samples before and after capacitation.

HPV and semen characteristics

A total of 384 semen samples from the 237 male partners of the infertile couples were analyzed because 99 couples performed subsequent cycles; specifically, the range number of both IUI treatments and ART cycles per couple was 1–3. Therefore, several semen samples from subsequent collections from the same man were included in the semen parameters analysis.

The results are summarized in Table 2. No statistically significant difference was found in sperm parameters between HPV-infected and noninfected semen samples both before and after semen capacitation. We found no relationship between HPV infection and sperm volume or sperm concentration. HPV − and HPV + semen samples had similar progressive motility (mean 44.9% *vs.* 42.9%, $p = 0.673$) and nonprogressive motility (mean 9.2% *vs.* 9.5%, $p = 0.257$). Neither oligo- nor asthenozoospermia was associated with the detection of HPV DNA in semen.

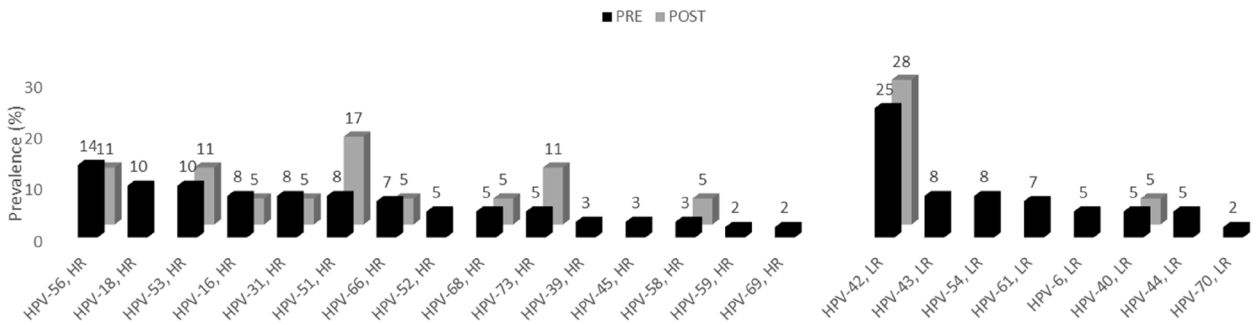

Fig. 2 Prevalence of the different genotypes detected in the HPV + semen samples before (PRE, calculated on the 59 HPV + samples) and after (POST, calculated on the 18 samples that remained positive) capacitation. HR, high-risk; LR, low-risk

Table 2 Seminal parameters in the total samples and HPV − and HPV + groups

	All samples	HPV − samples	HPV + samples	*p*-value*
PRE-capacitation				
Number of semen samples	384	294	90	-
Semen volume, ml	2.5 ± 1.2	2.5 ± 1.2	2.5 ± 1.1	0.666
Sperm concentration (million/ml)	52.6 ± 40.1	52.6 ± 40.5	52.2 ± 39.1	0.445
Motility type a (%)	12.6 ± 10.3	11.96 ± 9.8	14.70 ± 11.7	0.398
Motility type b (%)	30.8 ± 9.9	30.9 ± 9.7	30.3 ± 10.9	0.351
Motility type c (%)	9.5 ± 5.9	9.5 ± 5.9	9.2 ± 6.1	0.257
Progressive motility, a + b (%)	43.4 ± 15.7	42.9 ± 15.9	44.9 ± 14.9	0.673
POST-capacitation				
Sperm concentration (million/ml)	27.0 ± 24.0	27.4 ± 24.4	25.6 ± 22.9	0.226
Motility type a (%)	34.4 ± 17.5	34.2 ± 17.7	35.1 ± 17.2	0.949
Motility type b (%)	47.3 ± 15.2	47.3 ± 14.9	47.2 ± 16.1	0.620
Motility type c (%)	8.4 ± 8.2	8.7 ± 8.3	7.3 ± 7.5	0.858
Progressive motility, a + b (%)	81.7 ± 18.6	81.5 ± 18.8	82.3 ± 17.9	0.703

Values are mean ± standard deviation

* Comparison between HPV − and HPV + groups. *p*-value was derived from GEE model, which was performed at patient level (adjusted for age at sample collection, smoking habits, and BMI)

HPV and intrauterine insemination outcomes

One hundred and one couples were enrolled from January 2021 to October 2023, resulting in 186 IUI treatments analyzed. The mean age at enrollment was 38.1 ± 5.2 years for men and 35.0 ± 3.6 years for women. The median number of cycles per couple was 1 (range 1–3).

The analysis for HPV conducted on semen samples showed a positivity rate of 19.8% (20/101). Among the 20 positive male patients, 3 (15.0%) remained positive after semen capacitation.

Clinical characteristics of patients are shown in Table 3.

Ten singleton clinical pregnancies were obtained in IUI cycles performed with HPV − semen, with a clinical pregnancy rate per cycle of 6.8% (10/146). One of them hesitated in a spontaneous miscarriage and one was a late miscarriage due to severe restriction of growth of the fetus. Eight healthy children were born with a live birth per couple of 9.9%.

In the IUI groups performed with HPV + semen, a singleton and a twin clinical pregnancy were obtained (clinical pregnancy rate: 5.0%, 2/40). The singleton ended with a voluntary termination of pregnancy due to trisomy 21 of the fetus in a 39-year-old woman. Two healthy girls were born from the twin pregnancy, with a live-birth per couple of 10.0%.

We performed a subgroup analysis on the effects on reproductive outcomes of the most prevalent HPV genotypes (namely, HPV-18 (15%, 6/40 HPV + cycles),

Table 3 Baseline characteristics of the couples which performed IUI and of HPV − and HPV + groups

	All couples	HPV − couples	HPV + couples	*p*-value*
Number of couples	101	81	20	-
Number of IUI cycles	186	146	40	-
Female age, years, mean ± SD	35.0 ± 3.6	34.9 ± 3.4	35.5 ± 4.6	0.490
Male age, years, mean ± SD	38.1 ± 5.2	38.2 ± 5.4	37.5 ± 4.6	0.582
Infertility cause, *N* (%)				
Diminished ovarian reserve	14/101 (13.9)	9/81 (11.1)	5/20 (25.0)	0.211
Ovulatory endocrine factor	19/101 (18.8)	15/81 (18.5)	4/20 (20.0)	0.868
Endometriosis	1/101 (1.0)	1/81 (1.2)	0/20 (0)	0.431
Other	2/101 (2.0)	2/81 (2.5)	0/20 (0)	0.860
Idiopathic	65/101 (63.3)	54/81 (66.7)	11/20 (55.0)	0.473

* Comparison between HPV − and HPV + groups. *p*-values were obtained from GEE model. GEE analysis was performed at couple-level. *N*, number; *SD*, standard deviation

HPV-42 (17%, 7/40 HPV + cycles), HPV-54 (22%, 9/40 HPV + cycles)) and multiple infections that were detected in 37% of HPV + cycles (15/40). The outcomes of these subgroups are shown in the Additional file 1: Table S1. Unfortunately, the subgroups are not large enough to provide realistic or definitive information.

In only 3 HPV + couples (3/20, 15%) who underwent IUI treatment, the semen remained positive after sperm capacitation, so we could not carry out an informative analysis on the effects on reproductive outcomes (just to note, none of these couples have achieved pregnancy).

HPV and ART cycles outcomes

From January 2021 to October 2023, 155 couples were enrolled, resulting in 186 cycles analyzed. The mean age at enrollment was 38.4 ± 5.9 years for men and 35.7 ± 4.2 years for women. The median number of cycles per couple was 1 (range 1–3).

The analysis for HPV conducted on semen samples showed a positivity rate of 23.2% (36 out of 155). Among the 36 positive male patients, 10 (27.8%) remained positive when the test was repeated after semen capacitation.

Table 4 indicates that HPV − and HPV + cycles did not differ in woman's and man's mean age during the cycle ($p = 0.674$ and $p = 0.558$, respectively), number of cycles per couple ($p = 0.243$), type of treatment (ICSI: $p = 0.866$; IVF: $p = 0.876$), and cause of infertility.

Characteristics of the ART cycles are reported in Table 5. We did not find any significant difference in fertilization rate ($p = 0.926$), cleavage rate ($p = 0.944$), quality of developed embryos ($p = 0.590$), blastocyst formation rate ($p = 0.699$) nor in the embryo utilization rate ($p = 0.507$).

The percentage of women experiencing embryo transfer cancelation was equal in both groups (Table 5) either due to delayed embryo transfer (freeze-all strategy, $p = 0.813$), failure of fertilization ($p = 0.941$) or failure of embryo cleavage ($p = 0.762$). We performed embryo transfers on day 2–3 ($p = 0.965$) or day 5 ($p = 0.651$) without any difference between HPV − and HPV + cycles. In the study period, there were 69 vitrified-warmed embryo transfers, which included 14 embryos from HPV + cycles. Clinical pregnancy, implantation, live-birth, and miscarriage rates were not significantly different between the two groups; a not significative, slightly lower cumulative pregnancy rate and live-birth rate were observed in HPV + couples in comparison to HPV − group (33% *vs* 39% and 25% *vs* 30%, respectively). Accordingly, a trend of a higher miscarriage rate was found in HPV + with respect to HPV − cycles (53% and 29%, respectively).

Table 4 Baseline characteristics of the couples which performed IVF/ICSI and of HPV − and HPV + groups

	All couples	HPV − couples	HPV + couples	*p*-value*
Number of couples	155	119	36	-
Number of cycles	186	141	45	-
Number of cycles per couple, median (range)	1 (1–3)	1 (1–3)	1 (1–2)	0.243
Female age, mean ± SD	35.7 ± 4.2	35.9 ± 4.1	35.3 ± 4.6	0.674
Male age, mean ± SD	38.4 ± 5.9	38.4 ± 5.6	38.4 ± 6.9	0.558
Type of treatment, *N* (%)				
ICSI	99/186 (53.2)	76/141 (53.9)	23/45 (51.1)	0.866
IVF	87/186 (46.8)	65/141 (46.1)	22/45 (48.9)	0.876
Infertility cause, *N* (%)				
Female infertility cause				
Diminished ovarian reserve	34/155 (21.9)	23/119 (19.3)	11/36 (30.6)	0.228
Ovulatory endocrine factor	13/155 (8.4)	13/119 (10.9)	0/36 (0)	0.084
Endometriosis	14/155 (9.0)	11/119 (9.2)	3/36 (8.3)	0.867
Tubaric factor	16/155 (10.3)	13/119 (10.9)	3/36 (8.3)	0.891
Other	4/155 (2.6)	3/119 (2.5)	1/36 (2.8)	0.616
Male infertility cause	38/155 (24.5)	31/119 (26.0)	7/36 (19.4)	0.558
Oligozoospermia	16/155 (10.3)	13/119 (10.9)	3/36 (8.3)	0.8912
Asthenozoospermia	10/155 (6.4)	7/119 (5.9)	3/36 (8.3)	0.8994
Oligo-asthenozoospermia	12/155 (7.7)	11/119 (9.2)	1/36 (2.8)	0.3657
Idiopathic	36/155 (23.2)	25/119 (21.0)	11/36 (30.6)	0.332

* comparison between HPV − and HPV + groups. *p*-values were obtained from GEE model. GEE analysis was performed at couple-level. *N*, number; *SD*, standard deviation

Table 5 Embryological and clinical outcomes of all analyzed cycles and of HPV –and HPV + cycles

	All cycles	HPV − cycles	HPV + cycles	*p*-value
Number of cycles	186	141	45	-
Fertilization (%)	61.6 ± 30.2	61.5 ± 30.1	61.9 ± 30.7	0.926*
Cleavage (%)	97.2 ± 13.1	97.5 ± 12.0	96.4 ± 16.1	0.944*
Top quality embryo (%)	66.7 ± 31.3	66.8 ± 30.7	66.4 ± 33.5	0.590*
Blastulation (%)	45.4 ± 30.7	46.4 ± 31.4	42.5 ± 29.1	0.699*
Embryo utilization (%)	61.8 ± 31.4	62.0 ± 31.3	61.3 ± 32.1	0.507*
Canceled ET, *N* (%)	48/186 (25.8)	35/141 (24.8)	13/45 (28.9)	0.725
Delayed ET, *N* (%)	29/186 (15.6)	22/141(15.6)	7/45 (15.6)	0.813
Absence of fertilized oocytes, *N* (%)	14/186 (7.5)	10/141 (7.1)	4/45 (8.9)	0.941
Absence of viable embryos, *N* (%)	5/186 (2.7)	3/141 (2.1)	2/45 (4.4)	0.762
Cycles with day 2–3 ET, *N* (%)	77/186 (41.4)	58/141 (41.1)	19/45 (42.2)	0.965
Cycles with day 5 ET, *N* (%)	61/186 (32.8)	48/141 (34.0)	13/45 (28.9)	0.651
Implantation (%)	29.3 ± 49.7	29.2 ± 49.2	29.7 ± 52.1	0.789*
Pregnancy per ET in fresh cycles, *N* (%)	44/138 (32)	35/106 (33)	9/32 (28)	0.752
Miscarriage, *N* (%)	10/44 (23)	8/35 (23)	2/9 (22)	0.703
Live-birth rate, *N* (%)	40/138 (29)	32/106 (30)	8/32 (25)	0.745
ET in freeze–thaw cycles, *N* (%)	64	49	14	-
Cumulative pregnancy per ET, *N* (%)	76/202 (38)	61/155 (39)	15/46 (33)	0.572
Cumulative miscarriage, *N* (%)	24/76 (32)	18/61 (29)	8/15 (53)	0.1453
Cumulative live-birth, *N* (%)	55/202 (27)	45/155 (29)	10/46 (22)	0.455
Cumulative live-birth per couple, *N* (%)	55/155 (35)	45/119 (38)	10/36 (28)	0.369

Values are mean ± SD unless otherwise stated

* *p*-values were obtained from GEE model, which was adjusted for male and female age at sample collection, infertility cause, and type of treatment

Among ART cycles, the most prevalent HPV genotypes were HPV-42 (27%, 12/45 HPV + cycles), HPV-53 (13%, 6/45 HPV + cycles), and HPV-56 (18%, 8/45 HPV + cycles), and multiple infections were detected in 40% of HPV + cycles (18/45). The outcomes of these subgroups are shown in the Additional file 2: Table S2. Unfortunately, the subgroups are not large enough to provide realistic or definitive information.

Semen samples of 10 couples who underwent a total of 14 ART cycles remained positive after sperm capacitation. The outcomes of this subgroup are shown in the Additional file 3: Table S3. Unfortunately, this subgroup is not large enough to provide realistic or definitive information.

Perinatal characteristics of babies

A total of 55 neonatal outcomes from HPV − (n = 45, of which 10 were from 5 twin gestations) and HPV + (n = 10, of which 2 were from a twin gestation) cycles were available. Fifteen newborns derived from transfers of a cryopreserved blastocysts; 40 were from fresh cycles. Two pregnancies from frozen embryos are still ongoing. The perinatal characteristics of the newborns are detailed in Table 6. All the newborns weighed appropriately for the gestational age (mean: 47° centile, with a mean SDS of 0.16 ± 1.53), with the exception of eight pregnancies from HPV − cycles (1 fetus from one singleton pregnancy and 7 from 4 twin pregnancies). We did not observe any difference in birthweights between babies born from HPV − and HPV + cycles. No stillbirths as well as no malformations were recorded among the newborns of HPV + cycles.

Discussion

The prevalence of seminal HPV infection differs greatly between different groups of men, and in general it is higher in men affected by idiopathic infertility compared with fertile controls [46]. In this large cohort, comprising male partners of couples undergoing infertility treatments, seminal HPV infection was detected in 23.6% of men. This data agrees with that established in similar studies in which infertile couples were enrolled [28, 47] and a recent meta-analysis of the current literature which reported that the prevalence of HPV infection is significantly higher in infertile men compared to the general population (20.9% versus 8.2%) [44]. As expected, more than one HPV genotype was found in the same sample [48] and the HR genotypes were detected in more than half of the HPV + samples. The most common genotypes

Table 6 Neonatal characteristics of live births in all cycles and in HPV − and HPV + cycles

Parameter	All cycles	HPV − cycles	HPV + cycles	*p*-value*
N. newborns	55	45	10	-
N. lost follow-up	0	0	0	-
N. ongoing pregnancies	2	2	0	-
Birthweight (grams)				
Total	2925.7 ± 788.4	2919.2 ± 866.9	2955.1 ± 232.8	0.898
Singletons	3184.0 ± 509.3	3227.1 ± 545.8	2995.7 ± 244.2	0.251
Multiples	2000.0 ± 920.1	1841.5 ± 943.3	2792.5 ± 10.6	0.200
N. birthweight < 2500 g				
Total	8	8	0	-
Singletons	1	1	0	-
Multiples	7	7	0	-
Gestational age (weeks)				
Total	37.1 ± 3.8	37.3 ± 3.7	38.0 ± 1.6	0.562
Singletons	38.5 ± 1.3	38.6 ± 1.2	38.3 ± 1.7	0.559
Multiples	32.2 ± 5.4	32.6 ± 5.6	37	-
N. prematurity < 37 weeks				
Total	11	9	0	-
Singletons	3	1	0	-
Twins	8	8	0	-
Birthweight centiles				
Total	46.7 ± 29.2	43.3 ± 29.8	38.4 ± 14.9	0.617
Singletons	44.9 ± 27.9	46.3 ± 29.9	38.6 ± 16.6	0.488
Multiples	53.3 ± 33.9	37.5 ± 29.8	31	-
SDS score				
Total	0.16 ± 1.53	− 0.03 ± 1.4	− 0.33 ± 0.42	0.508
Singletons	0.06 ± 1.41	0.09 ± 1.5	− 0.33 ± 0.47	0.442
Multiples	0.53 ± 1.92	− 0.46 ± 0.9	− 0.33 ± 0.25	0.842

Values are mean ± SD unless otherwise stated

* Comparison between HPV − and HPV + groups

found in our cohort have been already identified as the most prevalent HPV types in general population (HPV-16) and associated with infertility (HPV-31, HPV-56, and HPV-42) [49].

The standard two-layer density gradient method used in semen preparation for infertility treatments has been shown to be effective in eliminating HPV DNA in 70% of our positive samples. This confirms the need to standardize new strategies of sperm washing [50, 51]. Modified swim-up with heparinase-III [23] and hyaluronidase [53] are promising methods to remove HPV virions from human semen samples efficiently. In particular, the enzyme hyaluronidase is approved for its use in ART, and it is able to break the linkage between HPV and syndecan-like glycosaminoglycan components on the sperm surfaces. Recently, two pregnancies were reported after the application of the hyaluronidase-based sperm washing in infertile couples with HPV semen infection [53].

Although conventional semen treatments could not completely eliminate the virus from all samples and in about 30% of cases the viral positivity was detected even after sperm capacitation, we found no evident effect on the semen parameters and reproductive treatments. We suppose that the presence of HPV could not be associated with viral activity. In support of this hypothesis, Faja et al. recently showed that HPV-DNA was not transcriptionally active in semen samples [54].

In the present study, we were unable to detect any significant difference in semen volume, sperm concentration, and motility between men having seminal HPV infection and uninfected men. The results of our study imply that HPV infection does not seem to affect sperm quality in terms of causing clinically significant alterations of sperm parameters. Our results agree with previous reports [27–30], but others correlated HPV semen infection with asthenozoospermia, reduced semen volume and count, increased DNA sperm fragmentation

index, altered pH and viscosity of semen, and production of anti-sperm antibodies [46]. A possible explanation for these conflicting results may be heterogeneous populations (i.e., donors, infertile patients, men seeking fertility evaluation), different HPV DNA detection protocols, or coinfection with other urogenital infections. Moreover, HPV has been detected both in exfoliated and sperm cells [55], and all the semen components (spermatozoa, somatic cells and/or plasma) may contain viral DNA [48]. Therefore, the discordant relationship between semen quality and HPV DNA presence may be due to the different sample types analyzed (native ejaculate, plasma or spermatozoa), thus not considering which cells of the semen are infected.

Based on our results, the presence of HPV in seminal fluid does not appear to significantly alter the embryological, clinical, and neonatal outcomes. So far, only four studies have addressed the impact of HPV + semen in ART techniques. Garolla et al. [38] have reported a significant reduction in natural and assisted cumulative pregnancy rates in both IUI (60 HPV − *vs.* 21 HPV +) and ICSI (98 HPV − *vs.* 33 HPV +) in infertile couples. Similarly, the large study of Depuydt et al. [39] showed that women inseminated with HPV + sperm had 4 times fewer clinical pregnancies compared with women who had HPV − partners (732 infertile couples undergoing 1753 IUI cycles). Moreover, an increase in miscarriage rates related to the presence of HPV at the sperm level was reported [14, 38]. Conversely, more recently, no association between HPV positivity in semen and fertility outcomes, including abortion rates, was found in 260 infertile couples who underwent IVF ($n = 161$) and IUI ($n = 53$) treatments or became pregnant spontaneously ($n = 46$) [41]. In line with Jaworek et al., we found no significant association between the presence of HPV DNA in semen and reduced ART success rates. Nevertheless, data obtained in IVF/ICSI treatments suggest that HPV infection may negatively affect male reproductive competence, resulting in a tendency to reduce cumulative pregnancy rates (39% in HPV − *versus* 33% in HPV + cycles) and live-birth rates (29% in HPV − *versus* 22% in HPV + couples), and increase miscarriages (29% in HPV − *versus* 53% in HPV + cycles). An in vitro study indicated that sperm might function as a vector for HPV transfer into the oocyte, and HPV could negatively influence early embryo development [32]. The presence of HPV DNA was described in products of conception in both spontaneous and voluntary abortions [13, 56], as well as in placentas at term [57]. In vitro and animal studies have shown negative effects of semen-transferred HPV on embryonic development by initiating apoptosis of embryonic cells through DNA fragmentation [35] and by inhibiting the blastocyst hatching process [36]. As a consequence, abortion rates could be increased in ART-treated couples where the male partner is HPV positive. This conclusion was supported by clinical studies in which infertile couples with an HPV positive male partner had significantly higher abortion rates than those with an HPV negative male partner [14, 38].

Once the pregnancy was successfully completed, no negative consequences have been observed in newborns of cycles with HPV + semen. Only one singleton in a 39-year-old woman after IUI with HPV + semen ended with a voluntary termination of pregnancy due to trisomy 21 of the fetus. No stillbirths as well as no malformations were recorded among the newborns of HPV + cycles. We did not observe any difference in birthweights between babies born from HPV − and HPV + cycles. This is the first report about the perinatal characteristic of babies from HPV-infected semen, and it represents an undoubtedly comforting information.

In summary, this study found no significant impact of HPV on semen variables and reproductive outcomes. Whether this lack of impact is due to the presence of not transcriptionally active virus in semen samples or semen processing decreasing the probability of an oocyte being fertilized by an infected sperm or the limited sample size within the HPV + group reducing the statistical power to detect small significant differences remains unknown. However, since ART treatments differ in several aspects from natural conditions, clarifying the impact of semen HPV infection on natural conceptions is worth exploring. In this context, with the introduction of HPV vaccination on both female and male adolescents, this important health issue will be hopefully reduced in the near future.

In our opinion, it seems appropriate to introduce HPV screening for donor semen in order to avoid possible viral transmission to the recipient, but routinely clinical application of HPV screening in the diagnostic workup of infertile couples and before ART cycles should be postponed until an evidence-based consensus is eventually reached on the impact of seminal HPV-positivity on clinical reproductive outcomes. According to the latest ESHRE guidelines, the possibility of HPV testing could be discussed with couples, HPV-positive women should be informed that ART does not eliminate the risk of vertical transmission, HPV-positive men should be informed that no current semen preparation technique can ensure complete removal of the virus from the infected semen sample, and couples with a known positive HPV test should be informed that HPV is a transient infection, and postponing the ART treatment could be an option depending on individual circumstances [58]. Secondly, there is no standardized management in case of seminal HPV positivity: there is not a gold-standard protocol for the detection of HPV in semen samples, no

information was provided on the role of different HPV genotypes in reproductive outcomes or on threshold values of the amount of virus in semen to be considered critical. The specific semen washing technique using a modified swim-up with enzymatic treatment [52] has shown promising results for the elimination of HPV from semen, but should be studied more extensively.

We are aware that this study has some limitations. First, female partners underwent cervical cytologic screening (Papanicolaou test, PAP test) within the previous 12 months, with no cytologic abnormality reported, but were not routinely tested for HPV. Secondly, the number of cases that remained positive after semen capacitation and underwent infertility treatments was limited; therefore, it was not possible to investigate the influence of persistent HPV infection on semen quality and reproductive outcomes. Third, we did not have a mechanistic approach, so we did not assess the HPV localization and possible binding to the sperm surface. Moreover, we did not assess sperm morphology and DNA fragmentation index because at our Center they are not routinely analyzed in samples that will be used for IUI and ART treatments. Finally, although our overall cohort size was relatively large, we acknowledge that the limited sample size within the HPV + group may have reduced the statistical power to detect a small significant difference between groups.

Conclusions

This study confirms previous findings that HPV DNA is present in semen of one quarter of infertile couples. No significant association of seminal HPV presence with semen parameters was found. We observed a trend of worst clinical outcomes in the HPV + group (lower pregnancy rate and live birth rate and higher miscarriage rate) that is worth further investigation in a large population to draw definitive conclusions.

Abbreviations

ART	Assisted reproduction techniques
BMI	Body mass index
ET	Embryo transfer
GEE	Generalized Estimating Equation
HR	High risk
HPV −	HPV negative
HPV +	HPV positive
hCG	Human chorionic gonadotropin
HPV	Human papillomavirus
IVF	In vitro fertilization
IU	International Units
ICSI	Intracytoplasmic sperm injection
IUI	Intrauterine inseminations
LR	Low risk
N	Number
PCR	Polymerase chain reaction
SD	Standard deviation
SDS	Standard deviation score
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-04152-5.

Additional file 1: Table S1. Baseline characteristics of the couples and clinical outcomes of HPV- IUI cycles, the most prevalent HPV genotypes (HPV-18, HPV-42, HPV-54) and multiple HPV+ cycles.

Additional file 2: Table S2. Baseline characteristics of the couples and clinical outcomes of HPV- ART cycles, the most prevalent HPV genotypes (HPV-18, HPV-42, HPV-54) and multiple HPV+ cycles

Additional file 3: Table S3. Baseline characteristics of the couples and clinical outcomes of HPV- ART cycles, HPV+ PRE (detected before capacitation) and HPV+ POST (detected positive also after capacitation) cycles

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Authors' contributions

S.S. designed the study, performed DNA isolation and contributed to critical discussion; E.C. and M.B. performed HPV DNA detection and genotyping; E.M. processed semen samples and collected biological data; F.B. performed statistical analyses; I.C. performed cycles as embryologist; C.M. and F.S. performed patient recruitment and treatments, collected clinical data; A.M. contributed to critical discussion; P.S. performed cycles as embryologist, interpreted the data, drafted the manuscript, and contributed to critical discussion; P.A. designed the study and contributed to critical discussion. All authors read and approved the final article.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. Data are located in controlled access data storage at our Institute.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of Regione Liguria (protocol code 168/2020). Written informed consent was obtained from all patients, and participation was voluntary.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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