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RESEARCH ARTICLE

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Male and female human papilloma virus infection and assisted reproductive technology outcomes: A comprehensive assessment from prevalence in semen to obstetric outcomes

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Abstract

Infertility, affecting approximately 16% of the global population, has led to increased reliance on reproductive medicine. The impact of human papillomavirus (HPV) infection in one or both partners on outcomes of Assisted Reproduction Technologies (ART) remains unclear. This prospective cohort study aimed to evaluate prevalence and effects of HPV infection in subjects and couples candidates to ART. A total of *n* = 510 men and *n* = 246 women were included and *n* = 145 couples (*n* = 290 individuals) had both partners enrolled in the study. The HPV semen infection rate was 17% (95% CI: 14–20) with HPV-42, HPV-16, HPV-53 and HPV-51 as the most frequently detected genotypes. In women, 26% (95% CI: 21–32) tested HPV-positive in cervical swabs. In 6% (95% CI: 3–11) of the couples, both partners were positive but only three couples shared the same genotypes (HPV-16; HPV-39, HPV-51, and HPV-42; HPV-31). Follicular fluids were positive in 20% (95% CI: 11–33) of samples, showing genotype discrepancies with cervical tests. Semen treatment could not completely eliminate the virus in positive samples but reduced the positivity to one-third. No significant differences in semen and embryological variables, clinical pregnancy and live birth rates, neonatal and obstetrics outcomes were observed in subjects with positivity in semen or cervix compared to respective negative groups. Cumulative live birth rates per oocyte retrieval in couples where both partners were negative or both were positive did not differ, being 37% (95% CI: 28%–47%) and 44% (95% CI: 19–73), respectively. In conclusion, HPV testing should not be

Marco Reschini and Paola Viganò contributed equally to this study.

considered a prerequisite for accessing ART treatments. Robust inferences for natural fertility cannot be made using our findings, as the ART setting does not fully reflect natural conditions.

KEYWORDS
assisted reproductive techniques, human papilloma virus, infection, infertility, IVF outcomes

1 | INTRODUCTION

Infertility, defined as the inability to achieve pregnancy after 12 months of regular unprotected sexual intercourse, affects approximately 16% of the global population.[1] The importance of infertility treatments is growing worldwide, with an estimated half of the affected population seeking clinical assistance to conceive.[2] The number of children born after assisted reproduction technologies (ART) treatments has indeed increased steadily over the last four decades.

The umbrella term "assisted reproduction" encompasses various techniques involving in vitro manipulation of oocytes, sperm, and embryos, including cryopreservation procedures and preimplantation genetic testing.[3,4] The success of ART procedures hinges on the maintenance of precise environmental conditions due to the sensitivity of these cell types to external and microbiological factors. Consequently, meticulous management and continuous monitoring of conditions during fertility treatments are imperative to optimize patient outcomes.

Some sexually transmitted diseases (STDs) have the potential to disrupt several of these steps. Human papillomavirus (HPV) in particular, is one of the most common sexually transmitted infections. Despite the well-established scientific literature on HPV and its causality in cancers, the potential link between HPV infection and infertility remains controversial. Demonstrations of possible HPV presence on the sperm head, its binding to specific regions, the potential integration into the host genome and ability to affect the integrity of sperm DNA have raised concerns about its transmission during fertilization and persistence until the blastocyst stage.[5–10] However, results from the available literature exploring a possible association between HPV infections and semen concentration, motility, and morphology are contradictory. Reasons for such disagreement were attributed to limited sample sizes or the use of different genotyping techniques.[11] According to a meta-analysis published in 2020 on the effect of semen HPV infection on sperm variables, the presence of the virus can significantly reduce sperm concentration, motility, and morphology, but the studies included presented moderate to severe risk of bias, mostly due to selection bias, inappropriate management of confounding factors, or bias in the selection of the reported results.[12] The relationship between HPV and female infertility is also not clear. The presence of the virus has been detected in granulosa and endometrial cells.[10,13] However, no significant difference between HPV-infected and noninfected women in rates of in vitro fertilization (IVF) live birth rate was reported in a 2018 meta-analysis. Again, the overall quality of the evidence was very low.[14] Some studies, conversely, suggested potential risks during the first trimester of pregnancy, including an increased likelihood of miscarriage.[15] Quite surprisingly, while a few studies addressed the effect of semen infection on reproductive outcomes following IVF[12] and some investigated the impact of HPV female positivity on ART success rates,[10] the infertile couple was rarely investigated as a unit.[16]

Overall, the impact of HPV infection on the outcome of ART cannot be clearly disentangled from the literature. Robust information is needed to clarify whether HPV assessment in one or both partners could be of clinical interest. Thus, the objectives of this study were to: (i) determine the point prevalence of HPV infection in male and female populations undergoing IVF and establish the association between HPV presence in semen and risk factors for virus susceptibility as primary outcome; (ii) investigate the influence of HPV on semen variables and assess the efficacy of sperm treatment for IVF in eliminating the virus; (iii) analyze ART embryological variables, including fertilization, embryo cleavage, embryo morphology, and freezing, in the HPV-positive population versus the HPV-negative group; (iv) evaluate potential differences in pregnancy and live birth rates, as well as obstetric and neonatal outcomes, between HPV-positive and HPV-negative subjects and couples; and (v) confirm the possible presence of the virus in ovarian follicles.[10]

2 | MATERIALS AND METHODS

2.1 | Study design

This is a prospective cohort study conducted at the Infertility Unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan, Italy. Subjects undergoing autologous in vitro ART treatments were considered for inclusion in the study through a convenience sampling based on their accessibility to the Infertility Unit between February 2022 to November 2022. Couples undergoing non-in vitro procedures or donor cycles were excluded as well as women using frozen oocytes/embryos and those undergoing oocyte retrieval for fertility preservation. All recruited patients gave written informed consent for sample and data use. Ethical approval for the study was obtained from the local Ethical Committee (Comitato Etico Milano Area B, Protocol Number 613_2021).

2.2 | Study population

Women undergoing IVF treatments, with or without intracytoplasmic sperm injection (ICSI), were considered for inclusion in the study (Figure 1). Eligible women underwent HPV screening, and those testing positive for HPV underwent Pap smear tests. Colposcopy was conducted if deemed necessary to rule out cervical cancer in HPV-positive subjects. In a subgroup of HPV-positive women, follicular fluids collected on the day of oocyte retrieval were examined for the presence of the virus. Male participants also had to be undergoing IVF treatments. To be enrolled, they needed to provide a fresh semen sample, and their partner had to be undergoing IVF with at least one suitable oocyte for insemination or injection (Figure 1). Risk factors, including personal characteristics, lifestyle habits, and sexual behaviours, were investigated for all male participants. Demographic and clinical characteristics as well details of IVF procedures were extracted from patients' clinical and biological charts and from the locally used software Meditex (Critex GmbH). IVF and pregnancy outcome data were collected with a follow-up period of 1 year after oocyte retrieval. Clinical pregnancy was defined as the presence of an intrauterine gestational sac at first ultrasound (generally performed at 6–7 weeks' gestation). The cumulative pregnancy rate refers to the inclusion of all fresh and frozen embryo transfers from the same oocyte retrieval. Miscarriage was defined as the spontaneous loss of pregnancy before 20 weeks of gestation.[17]

2.3 | Semen analysis, treatment and sampling

Semen collection and analysis were accomplished in compliance with the guidelines set forth by the World Health Organization[18] as detailed in previous research.[19] Semen samples were collected after a sexual abstinence of 2–7 days and analysed within 60 min of ejaculation. For the purposes of this study, we considered semen volume, sperm concentration, progressive sperm motility and morphology. The laboratory responsible for semen analysis has implemented and maintained a continuous quality assurance program for several years. This program includes a quality manual with standardized operating procedures (SOP) and detailed instructions for the different processes. Internal quality control (IQC) is ensured by including IQC materials in the laboratory's regular workload, and their results are monitored using quality control charts. External quality control (EQA) is regularly performed through peer comparison.

To prepare semen for conventional IVF or ICSI, a portion of the liquefied semen was layered onto a two-layered density gradient (80% and 40%, Sperm gradient, Fujifilm Irvine Scientific) in a sterile 14-mL conical tube and centrifuged at 160 x g for 15 min. The sperm pellet obtained was further centrifuged at 300 x g for 10 min with fresh washing medium, and the pellet resuspended in Sperm Medium (Multipurpose Handling Medium with Gentamicin (Fujifilm)) for samples undergoing ICSI. For those undergoing conventional IVF, after washing, 1 mL of HEPES medium was layered over the pellet and incubated for 30 min at 37°C at a 45-degree angle. Actively

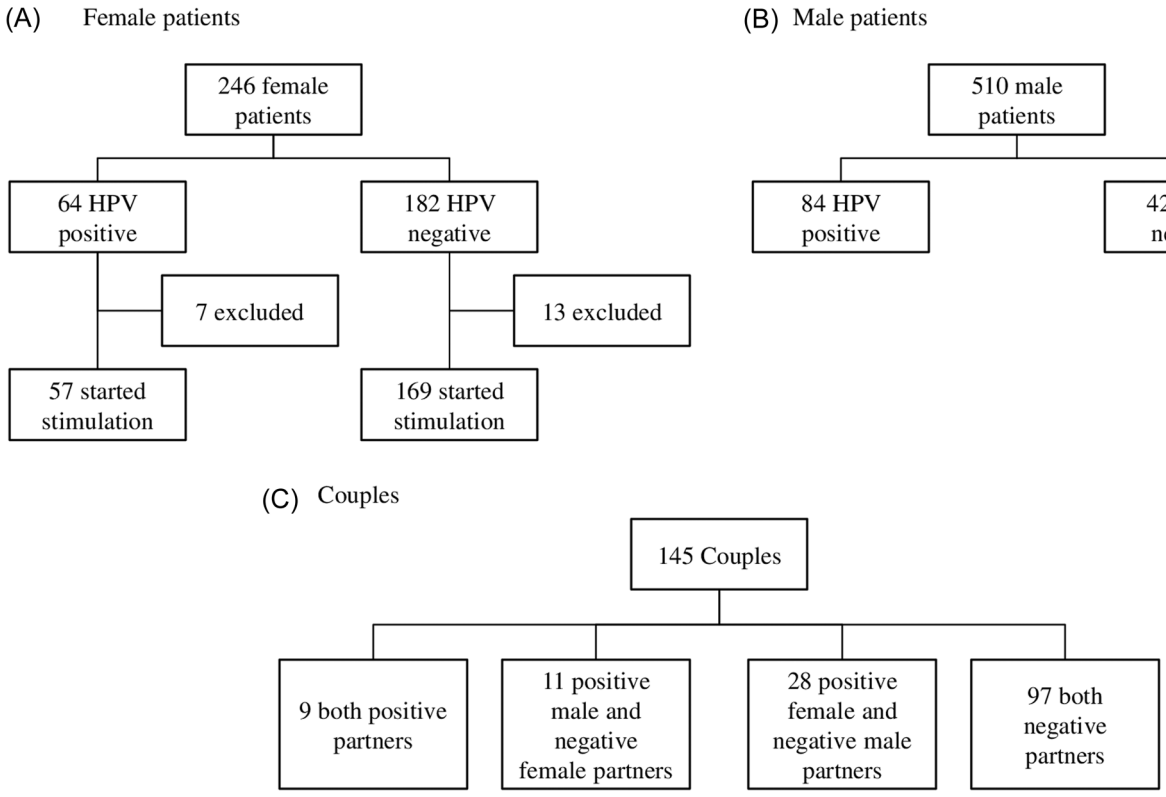

FIGURE 1 Flowchart showing the enrolled patients. HPV, human papillomavirus.

motile spermatozoa were collected with 0.2–0.3 mL of supernatant and transferred to a new tube. A 350 µL aliquot of the basal semen sample was sent to the Virology laboratory following liquefaction and another aliquot of the same volume was collected after treatment and stored at −20°C. In case where the initial sample tested positive for HPV, the aliquot of the post-treatment sample was subsequently analyzed for the presence of the virus. The ART laboratory operators were blinded for the virus presence. Lower reference limits for semen characteristics were set according to the guidelines of the World Health Organization[18] except for the severe male factor infertility for which we referred to the Guidelines on Sexual and Reproductive Health of the European Association of urology (number of spermatozoa <5 million/mL).[20] Consequently, the presence of a number of spermatozoa between 5 and 15 million/mL, was considered as a mild factor infertility.

2.4 | Cervical sampling

Female subjects were screened for HPV before starting the ovarian stimulation and the sample was sent to the laboratory on the same day or the day after, as per clinical procedure. Cervical samples collected by the endocervical brush scraping method were preserved and transported in ready-made vials eSwab® (COPAN) containing a specific solution for microbiological assays and stored at 2–8°C until the time of analysis within a couple of days. HPV test results were available in approximately 5–7 days. In case of a positive HPV test, if the previous Pap smear had been performed more than 6 months earlier, the Pap test was repeated within 10 days.

2.5 | Ovarian stimulation, oocyte retrieval, follicular fluid sample collection and embryo culture

The IVF procedure used in the authors' unit is detailed elsewhere.[21] Briefly, the regimen for ovarian stimulation and the initial dose of gonadotropins were determined based on age, Day 3 serum FSH levels, antimullerian hormone levels and antral follicle count. Human chorionic gonadotropin or a gonadotropin-releasing hormone agonist was administered subcutaneously once ≥3 leading follicles with a mean diameter greater than 18 mm were observed to induce final oocyte maturation. Oocyte retrieval was performed 36 h after the triggering of ovulation. Women previously diagnosed as HPV-positive in cervical sample tests had their follicular fluid collected to assess the presence of HPV in this biological fluid. After identifying and separating the oocyte for the IVF procedure, follicular fluid was centrifuged at 160 x g for 15 min, aliquoted, and stored at −20°C. Insemination was performed according to local protocols. Sixteen to 18 h after insemination/injection, fertilization was assessed using a standard procedure. Zygotes were cultured in a human Serum Albumin (hSA)-supplemented cleavage media culture under culture oil until Day 3 of development. After selecting embryos for transfer at the cleavage stage, the remaining embryos were cultured in hSA-supplemented blastulation media culture until blastocyst stage. The surplus expanded blastocysts obtained were cryopreserved. Patients at risk of ovarian hyperstimulation syndrome or with serum progesterone levels >1.5 ng/mL on the day of trigger underwent a freeze-all protocol, and no fresh embryos were transferred. Assessment of embryo/blastocyst scores followed the Istanbul Consensus.[22] Top-quality blastocysts were defined as those expanded with an inner cell mass and multicellular trophectoderm scored good or with only 1 of the 2 parameters scored fair and the other one scored good.

2.6 | DNA extraction, HPV-DNA detection, and genotyping

Nucleic acid extraction was performed with STARMag 96 × 4 Universal Cartridge Kit on platform Microlab NIMBUS IVD (Seegene NIMBUS). Detection of human papillomavirus including 19 high-risk HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82) and 9 low-risk HPV types (6, 11, 40, 42, 43, 44, 54, 61, 70) was performed using the Anyplex™ II HPV28 Detection Assay (Seegene) on CFX96™ Real-time PCR Detection System and CFX Manager™ Dx Software v3.1 (Bio-Rad). This multiplex real-time PCR assay simultaneously amplifies and detects 28 HPV genotypes targeting a fragment of 100 to 200 bp in length within the L1 region.[23] It incorporates a human constitutive gene as an endogenous internal control to monitor nucleic acid isolation efficiency, sample adequacy, and PCR inhibition. The internal control is co-amplified with the target nucleic acids in each clinical sample.

The Seegene Viewer programme was used for the automated reading and interpretation of the results. HPV positivity was graded as absent (−), low (+), intermediate (++), or high (+++), and samples were considered HPV-positive if the signal was + or greater, following the manufacturer's instructions. Patients were divided into two groups based on the presence of HPV (HPV-positive vs HPV-negative). The tests on semen were conducted once a week, to analyze a conspicuous amount of samples at the same time. In $n = 3$ semen samples, 50 uL of physiological solution was added to obtain the adequate volume requested by the instrument for DNA extraction. Before analysis, samples were stored at −20°C and transported to the virology lab using the same conditions.[24] In total the procedure took around 4 h.

2.7 | Sample size calculation and statistical analyses

The primary objective of the study was to evaluate HPV prevalence in the male and female populations candidates to ART, aiming to achieve a confidence interval (CI) width below ±5%. Assuming an HPV prevalence of 20%,[25–27] the estimated minimum number of patients required for the study was 240 (http://www.openepi.com/SampleSize/SSPropor.htm). For the male counterpart, we decided to recruit a two-fold higher number of subjects because we were also

interested in discerning risk factors for HPV infection. The calculated sample size allowed us to identify as risk factors, characteristics that are present in about 25% of men of the general population and considered as clinically relevant, showing an Odds Ratio (OR) for HPV infection >2 and setting type I and II errors to 0.05 and 0.20. These risk factors included smoking, alcohol and narcotic consumptions, and sexual habits.

Secondary outcomes included investigating the influence of HPV on semen variables, ART embryological variables, pregnancy and live birth rates, obstetric and neonatal outcomes, and prevalence of the virus in ovarian follicles.

Data Collected underwent analysis using the Statistical Package for Social Science (SPSS 27.0, IBM Corp). A binomial exact distribution model was used to estimate the 95% Confidence Interval (95% CI) of proportions. Differences between the groups were assessed using appropriate statistical tests, including the Fisher exact test, Chi-square test, Student *t*-test, Mann–Whitney, or McNemar test. The normality of data distribution was evaluated using the Shapiro–Wilk test, and non-normally distributed variables were compared using non-parametric statistics. Non-parametric tests were also applied to variables known in advance to be non-normally distributed regardless of the Shapiro-Wilk test result. Continuous variables were presented as mean ± standard deviation (SD) when normally distributed or median [interquartile range (IQR)] when non-normally distributed, while categorical variables were expressed as frequencies and percentages. Statistically significance was defined as P values less than 0.05. For live birth rate analysis, the OR and their respective 95% CI were calculated. A logistic multivariate regression model was employed to determine the adjusted OR, incorporating variables such as woman's age, total number of retrieved oocytes, and other factors identified in univariate baseline comparisons. Outcome data were reported in accordance with the guidelines recommended by the Core Outcome Measure for Infertility Trials (COMMIT) initiative.[28]

3 | RESULTS

As shown in Figure 1, a total of *n* = 510 men and *n* = 246 women were included in the study. Among these subjects, *n* = 145 couples (*n* = 290 individuals) had both partners enrolled in the study.

3.1 | Semen HPV prevalence, general characteristics and semen variables in men attending an IVF center

The overall HPV semen positivity rate was 17% (*n* = 84/510, 95% CI: 14%–20%), with a higher rate of high-risk genotypes (51%) compared to low-risk genotypes (29%). In 20% of cases (*n* = 17), both high- and low-risk genotypes were detected. The specific HPV genotypes detected in semen samples are described in Table S1. The most

TABLE 1A Baseline clinical characteristics of the male study groups.

Characteristics	HPV-positive (*n* = 84)	HPV-negative (*n* = 426)	*p*
Age	39 [36–42]	39 [31–42]	0.91
BMI (Kg/m^2)	24.7 [22.6–26.1]	25.2 [23.3–27.5]	0.06
Smoking status			0.39
Conventional cigarettes	27 (32%)	113 (27%)	
Electronic cigarettes	10 (12%)	41 (10%)	
Years of smoking	17 [10–20]	20 [10–20]	0.31
Alcohol consumption			0.27
Never	3 (4%)	42 (10%)	
Rarely	32 (38%)	164 (39%)	
1 or 2 times a week	37 (44%)	172 (40%)	
More than three times a week	12 (14%)	48 (11%)	
Narcotic consumption			0.10
Never	71 (85%)	384 (90%)	
Rarely	11 (13%)	35 (8%)	
1 or 2 times a week	0 (0%)	5 (1%)	
More than three times a week	2 (2%)	2 (1%)	
HPV vaccine	0 (0%)	5 (1%)	1.00
Age at first sexual intercourse	17 [16–18]	17 [16–19]	0.008
No. of previous partners	6 [4–15]	5 [2–10]	<0.001
Years of the current relationship	8 [4–10]	10 [7–14]	<0.001
No. of sexual intercourses per week	2 [1–3]	2 [1–3]	0.42
History of condylomas	6 (7%)	13 (3%)	0.11
Indication to IVF			0.49
Unexplained	37 (44%)	151 (36%)	
Endometriosis	11 (13%)	49 (12%)	
Tubal factor	3 (4%)	43 (10%)	
Disovulatory	3 (4%)	22 (5%)	
Genetic (PGT)	5 (6%)	22 (5%)	
Uterine factor	1 (1%)	6 (1%)	
Male factor	18 (21%)	86 (20%)	
Mixed	6 (7%)	47 (11%)	

Note: Data are reported as median [interquartile range] or number (percentage).

Abbreviation: HPV, human papillomavirus.

frequent genotypes detected in the seminal fluid were HPV-42 ($n = 14$ cases, 17%), HPV-16 and HPV-53 (both in $n = 11$ cases, 13%), and genotype HPV-51 ($n = 9$ cases, 11%).

There were no significant differences in age, BMI, smoking or alcohol habits, narcotic use, indication for IVF, or the number of sexual intercourses per week between the HPV-positive and HPV-negative groups (Table 1A). However, the HPV-positive group had a younger age at first sexual intercourse, a shorter duration of the current relationship, and a higher number of previous partners. Thirteen percent of HPV-positive males suffered of mild male factor infertility, compared to 15% in the HPV-negative population. Severe male factor infertility was present in 16% of cases in both groups.[18,20] Regarding fresh semen variables, no significant differences were observed in volume, concentration, progressive motility, normal morphology, or total motile sperm count between HPV-positive and HPV-negative groups (Table 1B). Only progressive motility showed a modest, though not statistically significant, increase in the HPV-positive group ($p = 0.05$). Similar nonsignificant findings in sperm variables were observed after sperm treatment.

Seventy-two samples initially found to be HPV-positive at baseline were also tested after treatment and $n = 21$ (29%) still showed positivity. Among these $n = 21$ persistently positive specimens, 57% ($n = 12$) maintained high-risk genotypes, 33% ($n = 7$) low-risk genotypes, and 10% ($n = 2$) had both high and low-risk genotypes. The specific HPV genotypes detected in semen samples pre- and post-treatment are described in Table S1. Given that, at our institution, the semen treatment for conventional IVF involved more rounds of sperm selection compared to ICSI, we sought to investigate whether persistent positivity differed between the two methods. Despite conventional IVF samples having a notably higher concentration of post-treatment motile sperm (median and [interquartile range] 1.0 [0.5–3.0]) than ICSI samples (median and [interquartile range] 0.6 [0.5–1.7]), no statistical difference was found in the percentage of persistently positive specimens between the two strategies (34% for conventional IVF vs. 24% for ICSI).

3.2 | Impact of HPV on IVF success rates in men

Conventional IVF was more frequently performed than ICSI with semen samples from HPV-positive men ($p = 0.04$), indicating that HPV did not impair sperm motility. No differences were observed in fertilization rates, fertilization failure, embryo cleavage rate, "top-quality" embryos and "top-quality" blastocysts, or the number of cryopreserved embryos between HPV-positive and HPV-negative groups, although the partners of HPV-positive men tended to be older and to have recruited a lower number of oocytes (Table 2A).

The cumulative live birth rate per oocyte retrieval was 30% (95% CI: 21%–40%) in the HPV-positive group and 33% (95% CI: 28%–37%) in the negative group. The OR for live birth in affected men was 0.88 (95% CI: 0.53–1.46, $p = 0.61$), and the adjusted OR considering partner's age and number of oocytes retrieved was 1.03 (95% CI: 0.61–1.75, $p = 0.92$). In the subgroup analysis of patients who underwent conventional IVF, the cumulative live birth rate per oocyte retrieval was significantly lower in couples where the man was HPV-positive (OR = 0.41, 95% CI: 0.18–0.94, $p = 0.03$). However, after adjusting for partner's age and number of retrieved oocytes, the OR attenuated to 0.60 (95% CI: 0.24–1.49, $p = 0.27$). In the subgroup where ICSI was performed, the cumulative live birth rate per oocyte

TABLE 1B Semen quality of the male study groups.

Characteristics	HPV-positive ($n = 84$)	HPV-negative ($n = 426$)	p
Male factor			0.88
Mild	11 (13%)	63 (15%)	
Severe	13 (16%)	70 (16%)	
Pre-treated semen			
Volume (mL)	3.0 [1.9–3.5]	2.8 [2.0–3.8]	0.71
Concentration ($\times 10^6$/mL)	51.5 [18.3–78.0]	41.5 [17.0–78.0]	0.40
Progressive motility (%)	52 [37–64]	48 [32–49]	0.05
Normal morphology (%)	4 [3–6]	4 [3–5]	0.67
Total motile sperm count ($\times 10^6$)	69.7 [19.5–120.5]	52.0 [12.2–111.7]	0.24
Post-treated semen			
Concentration ($\times 10^6$/mL)	4.1 [2.6–9.5]	4.1 [2.5–11.0]	0.79
Progressive motility (%)	90 [70–95]	88 [70–95]	0.43
Normal morphology (%)	5 [4–7]	5 [3–6]	0.07
Total motile sperm count ($\times 10^6$)	0.60 [0.39–1.82]	0.67 [0.36–1.75]	0.80

Note: Data are reported as median [interquartile range] or number (percentage).
Abbreviation: HPV, human papillomavirus.

retrieval in HPV-positive patients showed an increase, though not statistically significant (OR = 1.55, 95% CI: 0.79–3.02, $p = 0.20$). Even after adjusting for partner's age and number of retrieved oocytes, the OR remained nonsignificant (1.63, 95% CI: 0.82–3.21, $p = 0.16$). Lastly, no significant differences were observed regarding obstetrical and neonatal outcomes (Table 2B). These findings remained

TABLE 2A Outcomes in the two studied groups of men who started the IVF cycle.

Characteristics	HPV-positive ($n = 84$)	HPV-negative ($n = 426$)	p
Partner's age	38 [36–40]	37 [34–40]	0.09
No. of oocytes retrieved	6 [3–8]	6 [4–10]	0.05
Technique			0.04
Conventional IVF (cIVF)	42 (50%)	158 (37%)	
ICSI	42 (50%)	265 (63%)	
Fertilization rate (%)—total	78 [51–100]	75 [50–100]	0.41
Fertilization rate (%)—cIVF	76 [50–100]	79 [50–100]	0.50
Fertilization rate (%)—ICSI	79 [54–100]	75 [50–100]	0.63
Embryo Cleavage rate (%)	100 [100–100]	100 [100–100]	0.52
Top quality embryos (%)	50 [0–100]	50 [0–75]	0.37
Top quality blastocysts (%)	50 [0–100]	50 [0–100]	0.71
Fresh embryo transfer performed	58 (69%)	271 (64%)	0.38
Stage of embryo transferred at[a]			0.14
Cleavage stage	56 (97%)	269 (99%)	
Blastocyst stage	2 (3%)	2 (1%)	
Number of embryo transferred[a]			0.71
1	55 (95%)	261 (96%)	
2	3 (5%)	10 (4%)	
Reasons not to perform fresh embryo transfer			0.71
High risk of OHSS	10 (39%)	67 (43%)	
No suitable oocytes	0 (0%)	3 (2%)	
Total fertilisation failure	5 (19%)	31 (20%)	
No viable embryos	5 (19%)	15 (10%)	
PGT	3 (12%)	25 (16%)	
Others	3 (12%)	14 (9%)	
No. of cryopreserved embryos	1 [0–3]	1 [0–2]	0.70
No. of frozen embryo transfers[b]			0.52
0	50 (60%)	224 (53%)	
1	21 (25%)	117 (28%)	
2	6 (7%)	51 (12%)	
≥3	7 (8%)	34 (8%)	

Note: Data are reported as median [interquartile range] or number (percentage).

Embryo cleavage rate was calculated as the total number of embryo at cleavage stage divided by the number of fertilized oocytes.

Top quality embryo was defined as type 1 embryo.

Top-quality blastocyst was defined as those expanded with an inner cell mass and multicellular trophectoderm scored good or with only 1 of the 2 parameters scored fair and the other one scored good.

Abbreviations: HPV, human papillomavirus; OHSS, Ovarian hyperstimulation syndrome; PGT, Preimplantation Genetic Testing.

[a]Data refer to women performed fresh embryo transfer ($n = 58$ and $n = 271$ for positive and negative women, respectively).

[b]Only single embryo transfers were performed.

TABLE 2B Pregnancy outcomes in the male studied groups.

Characteristics	HPV-positive ($n = 84$)	HPV-negative ($n = 426$)	p
Cumulative clinical pregnancies	35 (42%)	174 (41%)	0.90
Adverse outcome of clinical pregnancies			0.49
Ectopic pregnancy	1/35 (3%)	3/174 (1%)	
Miscarriage	8/35 (23%)	31/174 (18%)	
Pregnancy termination[a]	1/35 (3%)	1/174 (1%)	
Cumulative live births	25 (30%)	139 (33%)	0.61
Preterm deliveries (<37 weeks)	1/25 (4%)	10/139 (7%)	1.00
Neonatal weight <2500 g	1/25 (4%)	13/139 (9%)	0.70
Obstetrical complications[b]	1/25 (4%)	12/139 (9%)	0.69
Neonatal complications[c]	0/25 (0%)	2/139 (1%)	1.00
Live births per ET	25/115 (22%)	139/601 (23%)	0.75

Note: Data are reported as number (percentage).

Abbreviations: HPV, human papillomavirus.

[a]Pregnancy termination was decided because of multiple malformations (HPV-positive group) and chromosome 21 aneuploidy (HPV-negative group).

[b]One pregnancy in the HPV-positive group was complicated by gestational diabetes. In the HPV-negative group, there were four cases of gestational diabetes, five cases of preterm premature rupture of membranes, one case of single umbilical artery, one case of placenta previa, and one case of monochorionic pregnancy with induced abortion of the second twin.

[c]Neonatal complications include 1 child with congenital malformations of cardiac septa and a premature baby with bronchial dysplasia and retinopathy.

consistent when analyzed based on semen HPV positivity before and after semen treatment (Table 3), HPV genotypes (Table S2), and the presence of single or multiple HPV genotype infections (Table S3).

3.3 | General characteristics and HPV prevalence in women attending an IVF center

Among the $n = 246$ recruited women, 26% ($n = 64$) (95% CI: 21%–32%) tested positive for HPV in the cervical swab. High-risk genotypes were more prevalent than low-risk genotypes (59% vs 19%, respectively). The specific HPV genotypes detected in cervical samples are described in Table S1. The most frequent HPV genotypes detected were HPV-16 and HPV-54 ($n = 9$ cases, 14%), HPV-31, HPV-59, and HPV-42 ($n = 8$ cases, 13%), and HPV-53 and HPV-68 ($n = 7$ cases, 11%). Of the examined samples, 22% ($n = 14$) were infected with both high- and low-risk genotypes. No significant difference was found in age, BMI, previous pregnancies and deliveries, indication to IVF, duration of infertility, antral follicle count or AMH levels between HPV-positive and HPV-negative groups (Table 4). In the group of HPV-positive women, the Pap smear yielded positive results in five cases ($n = 3$ ASCUS, $n = 1$ high grade squamous intraepithelial lesion and $n = 1$ low grade squamous intraepithelial lesion), prompting further examination through colposcopy, which subsequently returned negative findings.

In a subgroup of positive patients, follicular fluids aspirated during the oocyte retrievals were analyzed for the presence of the virus. Out of $n = 46$ samples tested, 20% were positive ($n = 9$) (95% CI: 11%–33%). In one case, the genotype detected in the follicular fluid (HPV-31) differed from the one detected in the cervical test (HPV-59), while in $n = 5$ cases, the genotypes detected in the follicular fluid were fewer than those identified in the cervical test. Finally, in one case, the presence of the virus was tested not only in

TABLE 3 Outcomes in the studied groups of men undergoing IVF cycle according to the semen pre- and post-treatment HPV-positivity.

Characteristics	Pre- and post-treatment HPV-positive semen samples ($n = 21$)	Pre-treatment HPV-positive and post-treatment HPV-negative semen samples ($n = 51$)	Pre-treatment HPV-negative semen samples ($n = 426$)	p
Pre-treated semen				
Volume (mL)	2.6 [1.8–4.2]	3.0 [2.0–3.5]	2.8 [2.0–3.8]	0.71
Concentration ($\times 10^6$/mL)	55.0 [35.5–80.0]	52.0 [14.0–83.0]	41.5 [17.0–78.0]	0.29
Progressive motility (%)	57 [38–66]	52 [40–62]	48 [32–49]	0.40
Normal morphology (%)	4 [3–6]	4 [3–6]	4 [3–5]	0.78
Total motile sperm count ($\times 10^6$)	78.8 [35.1–120.3]	76.4 [12.7–120.9]	52.0 [12.2–111.7]	0.73
Post-treated semen				
Concentration ($\times 10^6$/mL)	6.0 [2.8–9.0]	4.0 [2.8–15.0]	4.1 [2.5–11.0]	0.49
Progressive motility (%)	92 [77–95]	90 [69–95]	88 [70–95]	0.34
Normal morphology (%)	5 [4–7]	5 [4–7]	5 [3–6]	0.09
Total motile sperm count ($\times 10^6$)	0.78 [0.46–1.64]	0.61 [0.32–2.55]	0.67 [0.36–1.75]	0.63
Fertilization rate (%)—total	85 [67–100]	75 [50–100]	75 [50–100]	0.27
Embryo Cleavage rate (%)	100 [88–100]	100 [85–100]	100 [100–100]	0.38
Top quality embryos (%)	50 [0–80]	50 [5–100]	50 [0–75]	0.52
Top quality blastocysts (%)	50 [37–83]	50 [0–100]	50 [0–100]	0.99
Fresh embryo transfer performed	16 (76%)	32 (63%)	271 (64%)	0.49
No. of cryopreserved embryos	1 [0–3]	1 [0–2]	1 [0–2]	0.30
Cumulative clinical pregnancies	11 (52%)	19 (37%)	174 (41%)	0.49
Cumulative live births	7 (33%)	15 (29%)	139 (33%)	0.89

Note: Data are reported as median [interquartile range] or number (percentage).

Embryo cleavage rate was calculated as the total number of embryo at cleavage stage divided by the number of fertilized oocytes.

Top quality embryo was defined as type 1 embryo.

Top-quality blastocyst was defined as those expanded with an inner cell mass and multicellular trophectoderm scored good or with only 1 of the 2 parameters scored fair and the other one scored good.

Abbreviation: HPV, human papillomavirus.

TABLE 4 Baseline clinical characteristics of the female study groups.

Characteristics	HPV-positive ($n = 64$)	HPV-negative ($n = 182$)	p
Age	36 [34–40]	36 [33–39]	0.35
BMI (Kg/m^2)	22.2 [20.0–24.7]	22.6 [20.2–26.6]	0.23
Indication to IVF			0.84
Unexplained	21 (33%)	54 (30%)	
Endometriosis	7 (11%)	17 (9%)	
Tubal factor	7 (11%)	27 (15%)	
Disovulatory	2 (3%)	9 (5%)	
Recurrent miscarriages	0 (0%)	1 (0.5%)	
Genetic (PGT)	0 (0%)	1 (0.5%)	
Male Factor	17 (27%)	55 (30%)	
Mixed	10 (15%)	18 (10%)	
Previous IVF cycles	23 (36%)	87 (48%)	0.11
Previous gynaecological surgery	17 (27%)	37 (20%)	0.30
Duration of infertility (years)	2 [2–4]	2 [2–4]	0.80
Previous pregnancies	22 (34%)	61 (34%)	1.00
Previous deliveries	8 (13%)	35 (19%)	0.26
AMH (ng/mL)	1.8 [0.8–3.6]	2.1 [1.0–3.9]	0.62
AFC	9 [6–15]	10 [7–17]	0.50

Note: Data are reported as median [interquartile range] or number (percentage).

Abbreviations: AFC, Antral Follicle Count; AMH, Anti-mullerian hormone; HPV, human papillomavirus; PGT, Preimplantation Genetic Testing.

the follicle pool but also in the follicular fluid of the first and last follicle. In the follicular fluid of the first follicle aspirated from the left ovary, the test detected the presence of HPV genotypes 18, 31, and 51, while in the last follicle, only HPV-18 was identified. The first follicle of the right ovary was tested again and showed the same types: 18, 31, and 51.

3.4 | Impact of HPV on IVF success rates in women

No differences in duration of stimulation, total dose of gonadotropins, number of retrieved and suitable oocytes, fertilization rates, rate of insemination techniques, number of cryopreserved embryos, and clinical pregnancy rates were observed between HPV-positive and HPV-negative women (Tables 5A and 5B). Seven women in the HPV-positive group and $n = 13$ in the HPV-negative group did not start the ovarian stimulation for the IVF cycle within 6 months after the HPV test and were excluded from subsequent analysis. The cumulative live birth rate per oocyte retrieval showed no variation between women with and without HPV (28% [95% CI: 18%–41%] vs. 33% [95% CI: 26%–40%], respectively; $p = 0.62$). The OR for live birth in affected women was 0.81 (95% CI: 0.42–1.57, $p = 0.53$) and the OR adjusted for age and number of oocytes retrieved was 0.76 (95% CI: 0.37–1.57, $p = 0.46$). Ultimately, no significant differences were observed regarding obstetrical and neonatal outcomes (Table 5B). These findings remained consistent when analyzed based on HPV genotypes (Table S4), and the presence of single or multiple HPV genotype infections (Table S5).

3.5 | General characteristics and HPV prevalence in couples attending an IVF center

In $n = 145$ couples, both partners underwent HPV test. The prevalence of the virus in this subgroup was consistent with previous reports,[16] at 26% in women and 14% in men. In $n = 39$ couples (27%), only one partner was HPV-positive. In 6% of couples ($n = 9$) (95% CI: 3%–11%), both partners were positive. Statistical analysis confirmed that the two infectious events were not independent. Overall, in 33% of couples undergoing an IVF procedure, at least one partner tested positive for the virus. Among the $n = 9$ couples in which both partners tested positive, only three exhibited the same genotypes. In one couple, both partners were positive for genotype HPV-16; in another, both had genotypes HPV-39, HPV-51, and HPV-42; while in a third couple, both partners were positive for genotype HPV-31. In $n = 3$ couples, partial genotype overlap was observed between the male and female partners. Specifically, in one couple, the male partner tested positive for genotypes HPV-16, HPV-68, HPV-73, and HPV-43, while the female partner was positive for genotypes HPV-56 and HPV-68. In a second couple, the male partner had genotypes HPV-16 and HPV-51, while the female partner had genotypes HPV-31 and HPV-51. In the remaining couple, the male partner tested positive for genotypes HPV-53 and HPV-40, while the female partner had genotypes HPV-31, HPV-53, and HPV-54. In the other $n = 3$ couples, where both partners tested positive, the genotypes were different. In the first of these couples, the male partner was positive for genotypes HPV-59 and HPV-66, while the female partner had genotypes HPV-16 and HPV-68. In the second couple, the man was positive for genotype HPV-61, while the woman had genotypes HPV-51 and HPV-6. In the last couple, the man was positive for genotype HPV-26, while the woman had genotypes HPV-66, HPV-42, and HPV-54 (Table S6). No significant differences were found in age, BMI, previous IVF cycles, pregnancies, and surgical interventions, indication to IVF, duration of infertility, antral follicle count, or AMH levels among the four groups (both partners positive, both partners negative, HPV-positive men and HPV-negative women and HPV-positive men and HPV-negative women) (Table 6).

TABLE 5A Outcomes in the two studied groups of women who started the IVF cycle.

Characteristics	HPV-positive (*n* = 57)	HPV-negative (*n* = 169)	*p*
Duration of stimulation (days)	9 [7–10]	9 [7–10]	0.92
Total dose of gonadotropins (IU)	1600 [1350–2025]	1675 [1350–2025]	0.95
Interrupted stimulation	2 (4%)	13 (8%)	0.37
Oocyte retrievals	55 (96%)	156 (92%)	0.37
No. of oocytes retrieved[a]	5 [3–8]	5 [3–8]	0.84
No suitable oocyte[a]	1/55 (2%)	4/156 (3%)	1.00
Semen not available[a]	1/55 (2%)	1/156 (1%)	0.45
Technique[b]			0.63
Conventional IVF (cIVF)	23/53 (43%)	73/151 (48%)	
ICSI	30/53 (57%)	78/151 (52%)	
Fertilization rate (%)—total	80 [50–100]	67 [50–100]	0.34
Fertilization rate (%)—cIVF	80 [40–100]	75 [50–100]	0.85
Fertilization rate (%)—ICSI	79 [50–100]	67 [50–83]	0.20
Fresh embryo transfer not performed[b]	18/53 (34%)	47/151 (31%)	0.73
Reason for fresh embryo transfer not performed			0.88
High risk of OHSS	9/18 (50%)	25/47 (53%)	
Total failed fertilisation	5/18 (28%)	9/47 (19%)	
No viable cleavage embryos	3/18 (17%)	9/47 (19%)	
Others	1/18 (5%)	4/47 (9%)	
No. of cryopreserved embryos	1 [0–3]	1 [0–2]	0.34
No. of frozen embryo transfers[c]			0.98
0	30 (55%)	90 (58%)	
1	15 (27%)	41 (26%)	
2	7 (13%)	18 (12%)	
≥3	3 (5%)	7 (4%)	

Note: Data are reported as median [interquartile range] or number (percentage).

Abbreviations: HPV, human papillomavirus; ICSI, Intracytoplasmatic Sperm Injection; IU, International Units; IVF, In vitro fertilization; OHSS, Ovarian hyperstimulation syndrome; PGT, Preimplantation Genetic Testing.

[a]Data refer to women undergoing oocyte retrieval (*n* = 55 and 156 for positive and negative women, respectively).

[b]Data refer to subjects with at least one suitable oocyte and semen available (*n* = 53 and *n* = 151 for positive and negative women, respectively).

[c]Only single embryo transfers were performed.

3.6 | Impact of HPV on IVF success rates in couples

No differences in the duration of stimulation, total dose of gonadotropins, number of retrieved oocytes, fertilization rates, rate of insemination techniques, number of cryopreserved embryos, and clinical pregnancy rates were observed among the four groups considered (both partners positive, both partners negative, HPV-positive men and HPV-negative women and HPV-positive men and HPV-negative women) (Tables 7A and 7B). The live birth rates were 37% (95% CI: 28%–47%), 36% (95% CI: 15%–65%), 25% (95% CI: 13%–43%), and 44% (95% CI: 19%–73%) in couples where both partners were negative, only the male partner was positive, only the female partner was positive, and both partners were positive, respectively, with no significant differences observed ($p = 0.62$). Regarding obstetrical and neonatal outcomes, no significant differences were found (Table 7B).

4 | DISCUSSION

This prospective study involving *n* = 756 subjects undergoing IVF, aimed to assess the prevalence of HPV and its impact on IVF outcomes. To the best of our knowledge, this is the only study that has

TABLE 5B Pregnancy outcomes in the female studied groups.

Characteristics	HPV-positive ($n = 57$)	HPV-negative ($n = 169$)	p
Cumulative clinical pregnancies	19 (33%)	66 (39%)	0.53
Twin pregnancy	1/19 (5%)	1/66 (2%)	0.40
Adverse outcome of clinical pregnancies			0.45
Ectopic pregnancy	1/19 (5%)	1/66 (2%)	
Miscarriage	1/19 (5%)	9/66 (14%)	
Pregnancy termination[a]	1/19 (5%)	1/66 (2%)	
Cumulative live births	16 (28%)	55 (33%)	0.53
Preterm deliveries (<37 weeks)	1/16 (6%)	3/55 (6%)	1.00
Neonatal weight <2500 g	1/16 (6%)	4/55 (7%)	1.00
Obstetrical complications[b]	2/16 (13%)	7/55 (13%)	1.00
Neonatal complications	0/16 (0%)	0/55 (0%)	1.00
Live births per ET	16/74 (22%)	55/203 (27%)	0.36

Note: Data are reported as number (percentage).

Abbreviation: HPV, human papillomavirus.

[a]Pregnancy termination was decided because of multiple malformations (HPV-positive group) and chromosome 21 aneuploidy (HPV-negative group).

[b]One pregnancy in the HPV-positive group was complicated by preterm premature rupture of membranes and one by gestational hypothyroidism and gestational hypertension. In the HPV-negative group, there were five cases of gestational diabetes, one case of monochorionic pregnancy with induced abortion of the second twin and one case of pre-eclampsia.

addressed HPV effects separately in male and female populations and comprehensively in infertile couples, considering all aspects of IVF and pregnancy outcomes.

The prevalence of HPV in semen of men undergoing IVF observed in this study (17%) aligns with existing literature confirming the accuracy of our estimates.[37] A 17% positivity rate was found by both Luttmer et al.[38] and Yang et al. in the infertile male population.[39] We also confirmed that 1) in semen, high-risk HPV genotypes are more common[40] and that 2) the semen treatment procedures can significantly reduce but not completely eliminate the virus.[36] Factors like the number of previous partners, age at first sexual intercourse, and years in the current relationship were shown to be significantly associated with HPV positivity, although the clinical relevance is uncertain due to potential recall bias.

In the present study, HPV prevalence in women was slightly higher than in men and in Southern Europe.[29] However, in Italy specifically, reproductive-age women have been shown to have a high prevalence of high-risk genotypes.[30] Interestingly, only 6% of couples had both partners coinfected, and only 2% of them shared the same genotypes. These prevalence data in couples are similar to those described by Perino et al. in another Italian population undergoing IVF, where only 4.5% of couples had both partners HPV-positive in these specific sites.[16] A much higher rate of co-infection has been reported in the literature when the virus is localized at the penile sulcus.[31] The low co-infection rate in our couples may be attributed to the well-known transient nature of the virus in 80% of the female subjects.[32] In infected male patients, a significant viral clearance (approximately 85%) is known to occur after 24 months.[33,34] While prevalence data can be considered consolidated, explaining the differences among various studies in relation to the adverse effects of the infection on seminal fluid parameters and IVF outcomes is much more difficult. Despite some literature suggesting detrimental effects on sperm and reproductive outcomes,[29] our study found no significant differences in semen variables, fertilization rate, clinical pregnancy rate, and live birth rate between the HPV-positive and negative groups. Data on women are also controversial. A recent Italian study did not support a negative impact of the cervical infection on IVF success rates, despite the HPV-positivity in 61% of granulosa cells and 48% of endometrial tissues of women with HPV-positive cervical swabs.[10] Conversely, a recent report in a Chinese cohort found an inverse association between the presence of HPV in the uterine cavity and the rates of implantation, biochemical, clinical, and ongoing pregnancy. However, the prevalence of the virus in the uterine cavity of infertile women undergoing IVF was estimated at only 2.2%, raising serious doubts about the origin of the infection and its relevance to fertility.[13]

The reproductive function seems to be primarily affected by HPV infection in semen. The meta-analysis by Siristatidis et al. has shown significantly lower live birth/ongoing pregnancy rates and higher miscarriage rates when the virus was present in the semen. However, caution was recommended in interpreting the findings as the overall quality of the evidence was considered very low.[14] Importantly, the variability in sperm treatment procedures across studies may contribute to these inconsistencies.[16,35] Although the semen treatment cannot completely eliminate the virus from all samples, Ficoll gradients and swim-up techniques were shown to be able to significantly reduce the rate of infected cells from 24.7% ± 8.9% to 8.5% ± 5.7% and 3.7% ± 2.3%, respectively.[36] This reduction is expected to greatly decrease the probability of an oocyte being fertilized by an infected sperm. Moreover, this change may be even more marked if both the procedures, the gradient and the swim-up, are performed, as for our conventional IVF samples. In any case, from a clinical point of view, we agree with the ESHRE guidelines (2021) that *HPV-positive males should be informed that no current semen preparation technique can eliminate the virus from the infected semen sample*.[41]

Explaining the controversy regarding the impact of the virus on semen quality is more challenging. Meta-analyses on this topic underlined the limitations to consider when interpreting the results. Most of the studies were not designed prospectively. High heterogeneity across the included studies was observed for seminal variables. For instance, both a significant worsening of sperm

TABLE 6 Baseline clinical characteristics of the couples who started the IVF cycle.

Characteristics	Both HPV-positive ($n = 9$)	Male HPV-positive and female HPV-negative ($n = 11$)	Female HPV-positive and male HPV-negative ($n = 28$)	Both HPV-negative ($n = 97$)	p
Female age	36 [35–39]	37 [32–40]	36 [33–40]	36 [33–39]	0.91
Male age	38 [34–41]	39 [35–44]	40 [35–44]	38 [35–42]	0.38
Female BMI (Kg/m^2)	22.9 [21.4–24.6]	23.9 [20.2–26.6]	22.2 [19.8–25.0]	21.8 [19.7–26.4]	0.67
Indication to IVF					0.70
Unexplained	5 (56%)	3 (27%)	7 (25%)	30 (31%)	
Endometriosis	0 (0%)	1 (9%)	6 (21%)	9 (9%)	
Tubal factor	1 (11%)	2 (18%)	2 (7%)	14 (14%)	
Disovulatory	0 (0%)	0 (0%)	1 (4%)	7 (7%)	
Male factor	2 (22%)	4 (36%)	6 (21%)	26 (27%)	
Mixed	1 (11%)	1 (9%)	6 (21%)	11 (11%)	
Previous IVF cycles	3 (33%)	4 (36%)	8 (29%)	48 (50%)	0.21
Previous gynaecological surgery	2 (22%)	1 (9%)	28 (29%)	21 (22%)	0.62
Duration of infertility (years)	2 [2–4]	2 [2–4]	2 [2–4]	2 [2–5]	0.75
Previous pregnancies	4 (44%)	2 (18%)	6 (21%)	35 (36%)	0.29
Previous deliveries	2 (22%)	0 (0%)	0 (0%)	21 (22%)	0.02
AMH (ng/mL)	1.20 [0.50–2.00]	2.13 [1.17–2.67]	3.37 [0.86–4.65]	2.03 [1.06–4.42]	0.32
AFC	9 [7–15]	9 [6–26]	8 [5–15]	11 [7–18]	0.16

Note: Data are reported as median [interquartile range] or number (percentage).

Abbreviations: AFC, Antral Follicle Count; AMH, Anti-mullerian hormone; HPV, human papillomavirus; PGT, Preimplantation Genetic Testing.

morphology and no differences in the percentage of normal spermatozoa were reported in relation to HPV infection by the same group in different studies.[33,35,42] Furthermore, many of the studies did not adequately adjust for important confounding variables, including lifestyle habits, such as smoking and alcohol consumption.[12,26]

This study has limitations. First, for some secondary outcomes, we cannot exclude some type II errors. Pregnancy rate was one of our secondary outcomes and the sample size calculation was not set up to detect a small significant difference between groups. A particular comment should be dedicated to the miscarriage rate. We, as well as others, could not identify differences in miscarriage rate following IVF in association with HPV-positivity[43] but some meta-analyses showed evidence, albeit of low quality, of a higher miscarriage rate in pregnancies derived from infected semen.[12,14] The prevalence of HPV sperm infection was shown to be higher in couples affected by unexplained recurrent pregnancy loss than in their fertile counterparts.[44] Whether the semen treatment for IVF can reduce the miscarriage rate by reducing the rate of positive spermatozoa from the samples remains to be demonstrated.

Second, we have confirmed the virus presence at follicular level[10] but we were unable to assess its origin. The detection of the virus in the endometrium might suggest its arrival in the tubes and at the ovarian site, but iatrogenic contamination cannot be excluded (the needle used during the oocyte retrieval may be contaminated in the vagina and then could carry the virus into the ovarian milieu). In several patients, not all cervical genotypes were detected in follicular fluids indicating a possible contamination. In the single case when we collected follicular fluids separately for the first and last aspirated follicles, HPV-positivity persisted. If the latest follicle had been negative, contamination would have been more plausible but, since it was also positive, no conclusion could be drawn. Additionally, we could not determine the clinical relevance of the virus presence in follicular fluid; the sample size of this corollary evaluation was insufficient for meaningful conclusions on IVF outcome, and we did not investigate whether any long-term health problem might develop in women HPV-positive in follicular fluid. Third, we did not evaluate HPV binding to the sperm surface. However, this mechanistic approach was beyond the scope of this study and has been adequately addressed previously.[5,45,46] Finally, semen variables were not evaluated by an automatic technology and variability across operators could not be excluded, despite blinding for the virus presence.

The strengths of the study include its prospective design, the complete blindness of HPV test results by the IVF staff, its large

TABLE 7A Outcome in couples who started the IVF cycle.

Characteristics	Both HPV-positive (*n* = 9)	Male HPV-positive and female HPV-negative (*n* = 11)	Female HPV-positive and male HPV-negative (*n* = 28)	Both HPV-negative (*n* = 97)	*p*
Duration of stimulation (days)	8 [7–11]	8 [7–11]	9 [8–10]	9 [7–10]	0.96
Total dose of gonadotropins (IU)	1600 [1350–2140]	1650 [1200–1800]	1800 [1350–2065]	1715 [1350–2025]	0.65
Interrupted stimulation	0 (0%)	0 (0%)	2 (7%)	4 (4%)	0.68
No. of oocyte retrievals	9 (100%)	11 (100%)	26 (93%)	93 (96%)	0.68
No. of oocytes retrieved[a]	4 [2–7]	3 [2–8]	5 [3–9]	5 [4–9]	0.51
Technique[b]					0.83
Classical IVF	4 (44%)	4 (36%)	13 (50%)	47 (50%)	
ICSI	5 (56%)	7 (64%)	13 (50%)	46 (50%)	
Fertilization rate (%)	100 [80–100]	100 [60–100]	79 [33–100]	67 [50–95]	0.08
Fresh embryo transfer not performed[b]	1 (11%)	4 (36%)	12 (46%)	26 (28%)	0.17
Reason for fresh embryo transfer not performed					0.34
High risk of OHSS	1 (100%)	1 (25%)	6 (50%)	15 (57%)	
Total failed fertilisation	0 (0%)	0 (0%)	4 (33%)	7 (27%)	
No viable cleavage embryos	0 (0%)	2 (50%)	2 (17%)	2 (8%)	
Others	0 (0%)	1 (25%)	0 (0%)	2 (8%)	
No. of cryopreserved embryos	1 [0–3]	1 [0–2]	2 [1–5]	1 [0–2]	0.34
No. of frozen embryo transfers[c]					0.62
0	5 (56%)	8 (73%)	14 (54%)	48 (52%)	
1	3 (33%)	3 (27%)	5 (19%)	30 (32%)	
2	0 (0%)	0 (0%)	5 (19%)	10 (11%)	
≥3	1 (11%)	0 (0%)	2 (8%)	5 (5%)	

Note: Data are reported as median [interquartile range] or number (percentage).

Abbreviaitons: HPV, human papillomavirus; ICSI, Intracytoplasmatic Sperm Injection; IU, International Units; IVF, In vitro fertilization; OHSS, Ovarian hyperstimulation syndrome; PGT, Preimplantation Genetic Testing.

[a]Data refer to women undergoing oocyte retrieval.

[b]Data refer to subjects with at least one suitable oocyte and semen available.

[c]Only single embryo transfers were performed.

sample size, the assessment of HPV's impact on infertility at the couples' level, and the evaluation of multiple outcomes. CORE outcomes were not different in infertile couples when semen, the cervix or both were HPV-positive compared to negative controls.

Clarifying the impact of semen HPV infection on natural or IVF conceptions represents an important health issue. Our findings suggest that the infection is unremarkable for ART outcomes; therefore, screening for HPV via standard DNA detection procedures in couples scheduled for ART cannot be recommended. Since HPV infection was not found to impact on semen quality and conventional IVF results (this may be viewed as a surrogate possibility to evaluate natural fertilization), it is unlikely to significantly affect natural fertility as well. It is however important to note that ART conditions differ in several aspects from natural conditions and the impact of HPV infection on natural fertility must be explored with specific studies using different designs.

In conclusion, based on our findings it is reasonable not to require an HPV test as a prerequisite for accessing ART treatments. Despite the widespread prevalence of HPV, this study found no significant impact of HPV on semen variables, embryo development, cumulative pregnancy rate, or cumulative live birth rate per oocyte retrieval in infertility couples undergoing IVF. Whether this lack of impact is due to IVF laboratory procedures mitigating the virus's effects on in vitro outcomes or because HPV does not affect natural reproductive outcomes remains unknown.

TABLE 7B Pregnancies outcome in couples.

Characteristics	Both HPV-positive (*n* = 9)	Male HPV-positive and female HPV-negative (*n* = 11)	Female HPV-positive and male HPV-negative (*n* = 28)	Both HPV-negative (*n* = 97)	p
Cumulative clinical pregnancies	5 (56%)	4 (36%)	8 (29%)	45 (46%)	0.31
Twin pregnancy	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0.94
Adverse outcome of clinical pregnancies					0.33
Ectopic pregnancy	0 (0%)	0 (0%)	0 (0%)	1 (2%)	
Miscarriage	0 (0%)	0 (0%)	1 (12%)	7 (16%)	
Pregnancy termination[a]	1 (20%)	0 (0%)	0 (0%)	1 (2%)	
Cumulative live births	4 (44%)	4 (36%)	7 (25%)	36 (37%)	0.62
Preterm deliveries (<37 weeks)	0 (0%)	0 (0%)	0 (0%)	3 (8%)	0.72
Neonatal weight <2500 g	0 (0%)	0 (0%)	0 (0%)	3 (8%)	0.72
Obstetrical complications[b]	0 (0%)	0 (0%)	0 (0%)	5 (14%)	0.51
Neonatal complications	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.00
Live births per ET	4/15 (27%)	4/10 (40%)	7/45 (16%)	36/133 (27%)	0.30

Note: Data are reported as number (percentage).

Abbreviation: HPV, human papillomavirus.

[a]Pregnancy termination was decided because of multiple malformations (HPV-both positive group) and chromosome 21 aneuploidy (HPV-both negative group).

[b]In the HPV-both negative group, there were three cases of gestational diabetes, one case of monochorionic pregnancy with induced abortion of the second twin and one case of pre-eclampsia.

AUTHOR CONTRIBUTIONS

Edgardo Somigliana and Paola Vigano' designed the study and were in charge of the study conduct. Giorgia Carullo, Ludovica Basili, Davide Marinello, Giorgia Di Stefano, Irene Mondini and Mattia Volpi recruited the patients, collected the samples and obtained the clinical baseline and outcomes data. Sara Uceda determined the virus's presence. Giorgia Carullo, Davide Marinello, Irene Mondini and Mattia Volpi performed the laboratory procedures in semen. Marco Reschini did the statistical analysis and prepared the figure/tables. Giorgia Carullo, Marco Reschini and Paola Viganò drafted the manuscript. Maira Casalechi, Stefania Noli, Edgardo Somigliana, Antonia Valzano, Annapaola Callegaro and Paolo Vercellini revised the manuscript. All authors approved the final version.

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CONFLICT OF INTEREST STATEMENT

Edgardo Somigliana has received honoraria for lectures from Ibsa and Gedeon Richter, handles grants research from Ferring and Ibsa, and is the Editor-in-chief of Human Reproduction Open. Paola Vigano' is the coeditor-in-Chief of the Journal of Endometriosis and Uterine Disorders. The other authors declare that they have no conflict of interest. Paolo Vercellini serves as Associate Editor for Human Reproduction; has received royalties from Wolters Kluwer for chapters on endometriosis management in the clinical decision support resource UpToDate. The remaining authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Ethical approval for the study was obtained from the local Ethical Committee (Comitato Etico Milano Area B, Protocol Number 613_2021).

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REFERENCES

1. Chou D, Tunçalp Ö, Hotamisligil S, Norman J, Say L. Steps through the revision process of reproductive health sections of ICD-11. *Gynecol Obstet Invest*. 2012;74(3):228-232.
2. Chambers GM, Harrison C, Raymer J, et al. Infertility management in women and men attending primary care—patient characteristics, management actions and referrals. *Hum Reprod*. 2019;34(11):2173-2183.
3. Huang JYJ, Rosenwaks Z. Assisted reproductive techniques. *Methods Mol Biol*. 2014;1154:171-231.
4. Sciorio R, Aiello R, Janssens R. Considerations on staffing levels for a modern assisted reproductive laboratory. *JBRA Assist Reprod*. 2022;27:(1):120-130.
5. Pérez-Andino J, Buck CB, Ribbeck K. Adsorption of human papillomavirus 16 to live human sperm. *PLoS One*. 2009;4(6):e5847-e5847.
6. Kaspersen MD, Larsen PB, Ingerslev HJ, et al. Identification of multiple HPV types on spermatozoa from human sperm donors. *PLoS One*. 2011;6(3):e18095.
7. Foresta C, Patassini C, Bertoldo A, et al. Mechanism of human papillomavirus binding to human spermatozoa and fertilizing ability of infected spermatozoa. *PLoS One*. 2011;6(3):e15036.
8. Capra G, Schillaci R, Bosco L, Roccheri MC, Perino A, Ragusa MA. HPV infection in semen: results from a new molecular approach. *Epidemiol Infect*. 2019;147:e177.
9. Capra G, Notari T, Buttà M, Serra N, Rizzo G, Bosco L. Human papillomavirus (HPV) infection and its impact on male infertility. *Life*. 2022;12(11):1919.
10. Zullo F, Fiano V, Gillio-Tos A, et al. Human papillomavirus infection in women undergoing in-vitro fertilization: effects on embryo development kinetics and live birth rate. *Reprod Biol Endocrinol*. 2023;21(1):39.
11. Sucato A, Buttà M, Bosco L, Di Gregorio L, Perino A, Capra G. Human papillomavirus and male infertility: what do we know? *Int J Mol Sci*. 2023;24(24):1756217562.
12. Weinberg M, Sar-Shalom Nahshon C, Feferkorn I, Bornstein J. Evaluation of human papilloma virus in semen as a risk factor for low sperm quality and poor in vitro fertilization outcomes: a systematic review and meta-analysis. *Fertil Steril*. 2020;113(5):955-969.e4.
13. Bai M, Sun D, Shu J, et al. Assisted reproductive technology treatment failure and the detection of intrauterine HPV through spent embryo transfer media sample. *J Med Virol*. 2024;96(3):e29468.
14. Siristatidis C, Vaidakis D, Sertedaki E, Martins WP. Effect of human papilloma virus infection onin-vitrofertilization outcome: systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2018;51(1):87-93.
15. Bober L, Guzowski G, Moczulska H, Sieroszewski P. Influence of human papilloma virus (hPV) infection on early pregnancy. *Ginekol Pol*. 2019;90(2):72-75.
16. Perino A, Giovannelli L, Schillaci R, et al. Human papillomavirus infection in couples undergoing in vitro fertilization procedures: impact on reproductive outcomes. *Fertil Steril*. 2011;95(5):1845-1848.
17. Allison JL, Schust DJ. Recurrent first trimester pregnancy loss: revised definitions and novel causes. *Curr Opin Endocrinol Diabetes Obes*. 2009;16(6):446-450. doi:10.1097/med.0b013e3283327fc5
18. World Health Organization*WHO laboratory manual for the examination and Processing of human semen*. 6th ed. World Health Organization; 2021.
19. Boeri L, Pozzi E, Capogrosso P, et al. Infertile men with semen parameters above WHO reference limits at first assessment may deserve a second semen analysis: challenging the guidelines in the real-life scenario. *PLoS One*. 2023;18(1):e0280519.
20. EAU Guidelines *Edn. presented at the EAU Annual Congress, Paris*. EAU Guidelines; 2024. https://uroweb.org/guidelines/sexual-and-reproductive-health/chapter/male-infertility
21. Somigliana E, Sarais V, Reschini M, et al. Single oral dose of vitamin D3 supplementation prior to in vitro fertilization and embryo transfer in normal weight women: the SUNDRO randomized controlled trial. *Am J Obstet Gynecol*. 2021;225(3):283.e1-283.e10.
22. Balaban B, Brison D, Calderon G, et al. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Hum Reprod*. 2011;26(6):1270-1283.
23. Rollo F, Donà MG, Pichi B, Pellini R, Covello R, Benevolo M. Evaluation of the anyplex II HPV28 assay in the detection of human papillomavirus in archival samples of oropharyngeal carcinomas. *Arch Pathol Lab Med*. 2020;144(5):620-625.
24. Afrakhteh H, Joharinia N, Momen A, et al. Relative frequency of hepatitis B virus, human papilloma virus, Epstein-Barr virus, and herpes simplex viruses in the semen of fertile and infertile men in Shiraz, Iran: a cross-sectional study. *Int J Reprod Biomed* 2021;19:699-706.
25. Jeršovienė V, Gudlevičienė Ž, Rimienė J, Butkauskas D. Human papillomavirus and infertility. *Medicina*. 2019;55(7):377.
26. Moreno-Sepulveda J, Rajmil O. Seminal human papillomavirus infection and reproduction: a systematic review and meta-analysis. *Andrology*. 2020;9(2):478-502.
27. La Vignera S, Vicari E, Condorelli RA, et al. Prevalence of human papilloma virus infection in patients with male accessory gland infection. *Reprod Biomed Online*. 2015;30(4):385-391.
28. Duffy JMN, Bhattacharya S, Bhattacharya S, et al. Standardizing definitions and reporting guidelines for the infertility core outcome set: an international consensus development study. *Fertil Steril*. 2021;115(1):201-212.
29. Tramontano L, Sciorio R, Bellaminutti S, Esteves SC, Petignat P. Exploring the potential impact of human papillomavirus on infertility and assisted reproductive technology outcomes. *Reprod Biol*. 2023;23(2):100753.
30. Agarossi A, Ferrazzi E, Parazzini F, Perno CF, Ghisoni L. Prevalence and type distribution of high-risk human papillomavirus infection in women undergoing voluntary cervical cancer screening in Italy. *J Med Virol*. 2009;81(3):529-535.
31. Garolla A, Pizzol D, Vasoin F, Barzon L, Bertoldo A, Foresta C. Counseling reduces HPV persistence in coinfected couples. *J Sex Med*. 2014;11(1):127-135.
32. Brouwer AF, Campredon LP, Walline HM, et al. Incidence and clearance of oral and cervicogenital HPV infection: longitudinal analysis of the MHOC cohort study. *BMJ Open*. 2022;12(1):e056502.
33. Garolla A, Pizzol D, Bertoldo A, De Toni L, Barzon L, Foresta C. Association, prevalence, and clearance of human papillomavirus and antisperm antibodies in infected semen samples from infertile patients. *Fertil Steril*. 2013;99(1):125-131.e2.
34. Doorbar J. The human papillomavirus twilight zone—latency, immune control and subclinical infection. *Tumour Virus Res*. 2023;16:200268.
35. Garolla A, Engl B, Pizzol D, et al. Spontaneous fertility and in vitro fertilization outcome: new evidence of human papillomavirus sperm infection. *Fertil Steril*. 2016;105(1):65-72.e1.
36. Foresta C, Pizzol D, Bertoldo A, Menegazzo M, Barzon L, Garolla A. Semen washing procedures do not eliminate human papilloma virus sperm infection in infertile patients. *Fertil Steril*. 2011;96(5):1077-1082.
37. Boeri L, Capogrosso P, Ventimiglia E, et al. High-risk human papillomavirus in semen is associated with poor sperm progressive motility and a high sperm DNA fragmentation index in infertile men. *Hum Reprod*. 2019;34(2):209-217.
38. Luttmer R, Dijkstra MG, Snijders PJF, et al. Presence of human papillomavirus in semen in relation to semen quality. *Hum Reprod*. 2016;31(2):dev317.
39. Yang Y, Jia CW, Ma YM, Zhou LY, Wang SY. Correlation between HPV sperm infection and male infertility. *Asian J Androl*. 2013;15(4):529-532.

40. Muscianisi F, De Toni L, Giorato G, Carosso A, Foresta C, Garolla A. Is HPV the novel target in Male idiopathic infertility? A systematic review of the literature. *Front Endocrinol*. 2021;12:643539.
41. Mocanu E, Drakeley A, Kupka MS, et al. ESHRE guideline: medically assisted reproduction in patients with a viral infection/disease. *Hum Reprod Open*. 2021;2021(4):hoab037.
42. Foresta C, Garolla A, Parisi S, et al. HPV prophylactic vaccination in males improves the clearance of semen infection. *EBioMedicine*. 2015;2(10):1487-1493.
43. Jaworek H, Koudelakova V, Oborna I, et al. Impact of human papillomavirus infection on semen parameters and reproductive outcomes. *Reprod Biol Endocrinol*. 2021;19(1):156.
44. Busnelli A, Garolla A, Tersigni C, et al. Sperm human papillomavirus infection and risk of idiopathic recurrent pregnancy loss: insights from a multicenter case–control study. *Fertil Steril*. 2023;119(3):410-418.
45. Brossfield JE, Chan PJ, Patton WC, King A. Tenacity of exogenous human papillomavirus DNA in sperm washing. *J Assist Reprod Genet*. 1999;16:325-328.
46. Olatunbosun O, Deneer H, Pierson R. Human papillomavirus DNA detection in sperm using polymerase chain reaction. *Obstet Gynecol*. 2001;97:357-360.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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