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RESEARCH ARTICLE

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Impact of seminal low-risk human papillomavirus infection on sperm parameters of adult men

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ABSTRACT

Aim: We aimed to evaluate the impact of seminal low-risk human Papillomavirus (LR-HPV) infection on sperm conventional parameters.

Material and methods: This was a retrospective case–control study including patients attending to our center for infertility. Patients with evidence for high risk (HR)-HPV infection previously or at the time of enrollment, and/or with severe oligozoospermia (sperm concentration <5 mil/ml) were ruled out. Twenty selected patients positive for a LR-HPV and 20 control subjects with no evidence of HPV DNA and with available results of sperm analysis were consecutively enrolled.

Results: Patients positive for LR-HPV had a mean age of 31.0 + 11.0 years, while controls were 35.0 + 8.0-year-old ($p > .05$). Sperm concentration, total sperm count, sperm progressive motility, morphology, and leukocyte concentration did not differ between patients and controls. However, the prevalence of oligozoospermia was significantly higher in patients than controls (50% vs. 15%). No difference in the prevalence of astenozoospermia (30% vs. 40%) or teratozoospermia (15% vs. 15%) was found.

Conclusion: We found no difference in sperm conventional parameters in LR-HPV infected patients than in controls. These data might prompt to research the impact on LR-HPV genotype on male fertility. Particularly, evidence on sperm DNA fragmentation (SDF) and pregnancy outcome is needed.

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1. Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses worldwide, with about 6.2 million new cases annually [1]. The skin represents the most frequently affected tissue [2]. HPV indeed belongs to a family of small epitheliotropic viruses, with no envelope and with a double-stranded circular DNA genome, made of ~8000 bp. Its icosahedral structure contains the L1 and L2, that are the major and the minor virion's capsid proteins, respectively [3].

More than 200 HPV genotypes have been identified so far. Based on their oncological repercussions, HPV genotypes are divided into high-risk (HR) (e.g. HPV 16, 18, 31, and 45) and low-risk (LR) (e.g. HPV 6, 11, 30, 34, 40, 42, 43, 44, 54, 55, 61, 62, 64, 67, 69, 70, 72, 74, 81, 83, 84, and 91). So far, a great attention has been paid to HR-HPV genotypes, for their oncological repercussions. These are indeed associated with a higher risk of inducing cell metaplasia, by the expression of the E6 and E7 oncoproteins, which are required for induction and maintenance of malignancy in HPV-infected cells [4]. Accordingly, HR-HPV infection can promote malignant transformation of cells in the cervix, vagina, vulva, anus, penis, mouth, and throat [5]. Notably, HPV 16 infection has been associated with a significantly increased risk for developing prostate carcinoma [6].

In recent time, research has focused on the impact of HPV infection in human fertility. A significant association between HPV cervical infection and premature rupture of membranes, which may lead to miscarriage, has been reported [7]. Accordingly, some researches have shown a significantly higher prevalence of HPV infection in women experiencing spontaneous

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abortion [8,9]. In contrast, other data have shown opposite findings [10]. Concerning the impact on sperm parameters, the evidence supports the negative effects of HPV infection on sperm concentration, motility, and morphology, as well as on pregnancy rate and on miscarriage rate following *in vitro* fertilization (IVF) [11]. Particularly, HR-HPV infection may worsen sperm progressive motility and sperm DNA fragmentation (SDF), as derived from meta-analytic evidence [6]. E6/E7 proteins (to which HR-HPV infected spermatozoa are exposed) may indeed increase the apoptotic events, worsening the fertility potential [12,13]. For that reason, in infertile young couples with seminal HPV infection, a 6-month delay of fertility treatment can be suggested, in order to wait for a possible spontaneous viral clearance [14].

Remarkable, this counseling is suggested independently of the HPV genotype. Although a number of studies have already investigated the impact of seminal HR-HPV infection on sperm parameters, lesser data are currently available on LR-HPV. Due to the absence of E6/E7 proteins, LR-HPV may indeed have low or no influence on sperm parameters. A recent meta-analysis [15] highlighted that HR-HPV infection is more common vs. LR-HPV infection in infertile population (11.9% *vs.* 7.2%). The same meta-analysis claims that only few studies specifically reported the effects of HR- and LR-HPV on sperm parameters, which represents one of the main limitations on this topic.

With these premises, this retrospective case–control study was undertaken with the purpose of evaluating the impact of seminal LR-HPV infection on sperm conventional parameters. To accomplish this, we retrospectively screened the clinical charts of patients attending for infertility, that undergone to HPV DNA testing and sperm analysis. We included those with LR-HPV infection in the group of patients, and those with a negative HPV DNA test in the group of controls.

2. Subjects and methods

2.1. Patient selection

This was a retrospective case–control study performed using the clinical charts of men who referred to the Division of Endocrinology, Metabolic Diseases, and Nutrition, University of Catania, for infertility. We consecutively screened the clinical charts of subjects attended from January 2018 to April 2021. Included patients were those positive for LR-HPV and with available sperm analysis at the time of infection. Exclusion criteria were the presence of HR-HPV infection previously or at the time of enrollment, the presence of not HPV-related male accessory gland infection (MAGI), and of severe oligozoospermia (sperm concentration <5 mil/ml), any endocrine comorbidity including any type of diabetes mellitus, cigarette smoking, overweight and/or obesity, mean testicular volume <12 ml (measured using Prader's orchidometer), any drug use. In particular, to exclude the presence of bacterial MAGI, symptomatic patients or those with signs at the physical examination (tenderness of epididymis or of vas deferens, abnormal rectal exploration) underwent sperm and urethral swab culture. Patients with positive culture or with signs/symptoms strongly suggesting a bacterial infection were excluded.

Subjects negative for HPV DNA and with available sperm analysis served as control. The retrospective screen of the clinical charts stopped when a number of 20 patients and 20 controls were reached.

2.2. Sperm analysis

The clinical charts screened contained information on the sperm parameters that were analyzed in the Unit of Endocrinology, Metabolic Diseases and Nutrition, University of Catania. For each patient and control, semen samples were collected by masturbation into a sterile container after 2–7 d of sexual abstinence and were analyzed immediately after liquefaction. According to the 2010 WHO guidelines, each sample was evaluated for seminal volume, pH, sperm count, progressive motility, morphology, and round cell concentration [16].

2.3. Screening of HPV DNA

Patients and controls from the screened clinical charts underwent to HPV DNA testing using the following procedure.

The sperm sample underwent to DNA extraction using PureLink® Genomic DNA Kits (Invitrogen, Carlsbad, CA, Catalog Numbers K1821-04). Real-time PCR (RT-PCT) was used for DNA amplification, using specific primers pair for the amplification of the L1 region of the viral genome, that is particularly preserved in the different HPV genotypes. As internal amplification control, the human gene thiosulfate sulfurtransferase that maps to the region 22q13.1 was simultaneously amplified.

To identify high (HPV 16, HPV 18, HPV 31, and HPV 45), intermediate (HPV 33, HPV 35, HPV 39, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 66, and HPV 68), or low (HPV 6 and HPV 11) risk HPV genotypes,

Fluorescent PCR Multiplex using fluorescent primer pairs for the target amplification region (E6 and E7), specific for each different HPV viral type searched, was used. A human polymorphic system short tandem repeats (STRs) served as internal control and it was amplified simultaneously with the viral genotypes. The visualization of the amplified products was performed by means of fluorescent capillary electrophoresis (Genetic Analyzer ABI Prism 3130 with Software Data Collection). The Standard Gene Scan 500 LIZ was used for the detection of the amplified products.

2.4. Statistical analysis

Results are reported as mean ± SD throughout the study. The normality of the variables was evaluated with the Shapiro–Wilk test. The Student *t*-test or the Mann–Whitney U-test was applied according to data normal or not-normal data distribution, respectively. Difference in the prevalence of abnormal sperm parameters between groups was assessed using the *chi*-squared test. Statistical analysis was performed using MedCalc Software Ltd. version 19.6 – 64 bit. A p value less than .05 was accepted as statistically significant.

As the majority of data support the concept that sperm motility is the parameter most likely affected by HPV infection, we choose this parameter for power calculation. A 32% control sperm progressive motility was assumed based on the WHO 2010 manual. A 25% absolute reduction was viewed to be of sufficient clinical importance to change practice. The study required a total of 34 participants (16 per group) to preserve a 90% power to detect a 5% absolute difference for a 5% two-sided test while accounting for a 5% drop-out rate.

2.5. Ethical approval

This study was conducted at the Division of Endocrinology, Metabolic Diseases and Nutrition of the teaching hospital "G. Rodolico – San Marco," University of Catania (Catania, Italy). The protocol was approved by the internal Institutional Review Board, and informed written consent was obtained from each participant after full explanation of the purpose and nature of all procedures used. The study has been carried out in accordance with the principles expressed in the Declaration of Helsinki.

Table 1. Range and baseline values of low-risk HPV-positive patients (LR HPV+) and controls.

Parameters	LR HPV+ ($n = 20$)	Controls ($n = 20$)
Sperm concentration (million/ml)	24.8 ± 13.0	25.7 ± 10.4
Total sperm count (million/ejaculate)	67.4 ± 63.7	73.0 ± 36.7
Sperm progressive motility (%)	32.6 ± 17.6	32.9 ± 17.6
Sperm total motility (%)	50.25 ± 17.82	58.8 ± 6.98
Sperm morphology (%)	18.5 ± 13.7	20.5 ± 14.3
Leukocytes (mil/ml)	1.6 ± 0.6	1.2 ± 0.6

Results are expressed as mean ± standard deviation.

3. Results

Among 100 clinical charts of patients tested for HPV DNA consecutively screened, the overall rate of HPV positivity was 38% (38/100). In detail, 20 were positive for a LR-HPV (20%), 18 for a HR-HPV (18%). The remaining 62 were negative for HPV.

Patients positive for LR-HPV had a mean age of 31.0 ± 11.0 years, while controls were 35.0 ± 8.0 year old ($p > .05$). The distribution of LR genotypes was as follows: HPV 6 in 8/20 patients (40%), HPV 11 in 4/20 patients (20%), HPV 55, 61,62 in 4/20 patients (20%), HPV 72,83,84 in 4/20 patients (20%).

The mean values of conventional sperm parameters in patients and controls are resumed in Table 1. Sperm concentration, total sperm count, sperm progressive motility, morphology, and leukocyte concentration did not differ between patients and controls (Figure 1). Notably, the prevalence of oligozoospermia was significantly higher in LR-HPV + that LR-HPV – patients (50%, 10/20 *vs.* 15%, 3/20; $p = .04$). No difference in the prevalence of astenozoospermia (30%, 6/20 *vs.* 40%, 8/20; $p = .5$) or teratozoospermia (15%, 3/20 *vs.* 15%, 3/20; $p = 1.0$) was found. Finally, LR-HPV + patients had a significantly higher prevalence of leukocytospermia compared to the LR-HPV- ones (100%, 20/20 *vs.* 70%, 14/20; $p = .02$).

4. Discussion

HPV infection represents one of the major causes of sexually transmitted viral diseases worldwide [1]. Mounting evidence points to HPV infection as a novel target in male idiopathic infertility [14]. However, the majority of data refer to HR-HPV genotypes. Scanty evidence is available on LR-HPV. At the best of our knowledge, this is the first study specifically designed to understand the impact of seminal LR-HPV infection on conventional sperm parameters. We found no difference in sperm concentration, total count, motility, and morphology between patients and controls.

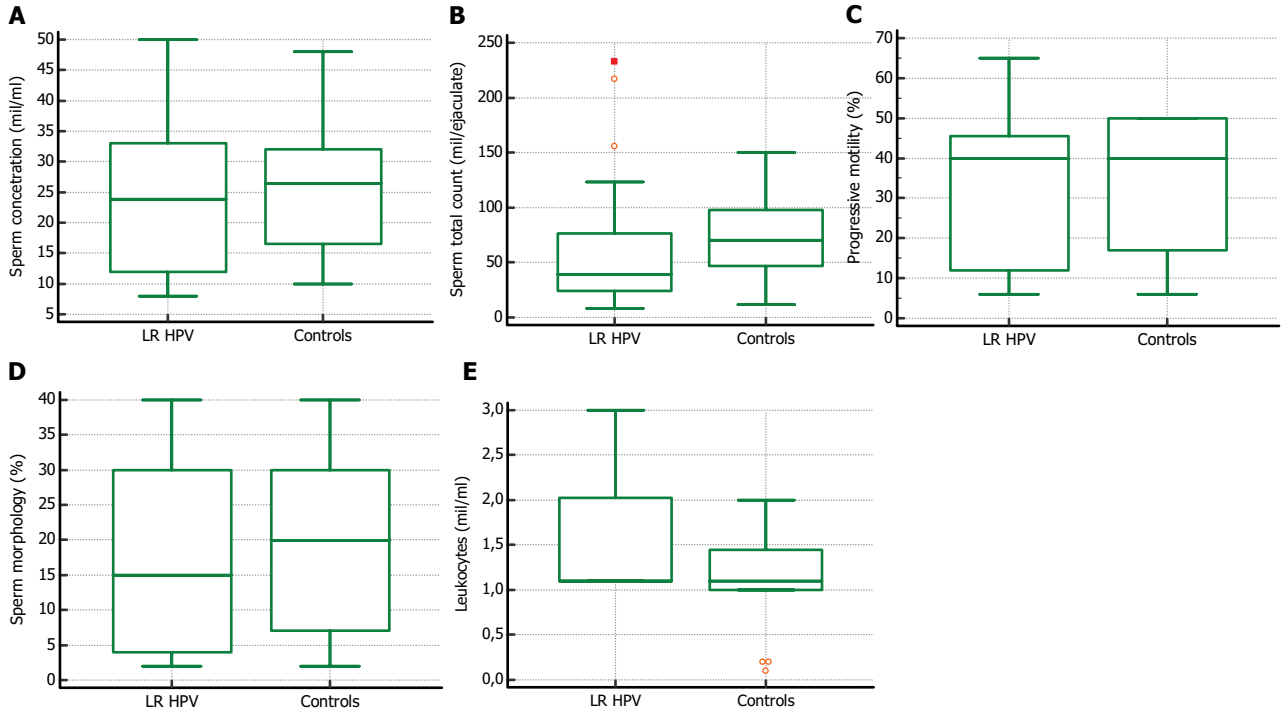

Figure 1. Conventional sperm parameters in HPV positive patients and controls. Sperm concentration (A), total sperm count (B), sperm progressive motility (C), morphology (D) and semen leukocytes concentration (E) do not differ in patients and controls. Six graphics representing the box plots of sperm concentration, total sperm count, progressive motility, sperm morphology, and leukocytes in patients with low-risk HPV and controls.

Although a higher prevalence of oligozospermia was observed, no difference in the prevalence of asthenozoospermia or teratozoospermia was described.

Few studies have compared the impact of LR- *vs.* HR-HPV seminal infection on sperm parameters. A study carried out in 229 patients reported a prevalence of exclusively HR-HPV in 5.7% of cases, and of exclusively LR-HPV in 6.1% of cases. By comparing the conventional sperm parameters of these two groups, a significantly worse semen viscosity was reported in the HR- compared to the LR-HPV group. No difference in the other semen parameters was found [17]. A cross-sectional study on 430 infertile patients reported a prevalence of exclusively HR- or LR-HPV genotypes equal to 8.8% and 4%, respectively. Interestingly, no difference was found between HR- and LR-HPV infected patients in sperm conventional parameters, including volume and viscosity [18]. Finally, among 729 patients with infertility, HR-HPV genotype has been isolated in the semen of the 10.7% of patients, and LR-HPV in the 4.8%. Sperm progressive motility and SDF were significantly worse in the group with HR-HPV compared to that with LR-HPV [5]. Particularly, the median value of sperm progressive motility was 12.5% in the HR group, and 22.0% both in the LR and in control group. SDF resulted 40.3% in HR-HPV patients, 31.5% and 28.3% in LR-HPV and control group. No difference in the other conventional sperm parameters was found [5]. To the best of our knowledge, no other studies have compared sperm parameters in patients with HR- and LR-HPV seminal infection. The results from these studies may support the worse impact of HR-HPV on semen viscosity, sperm progressive motility and SDF compared to LR-HPV, although other research is still needed.

These data are in line with those reported in the present analysis. Accordingly, no significant difference of sperm conventional parameters, including motility and morphology, was found between LR-HPV infected patients and controls. Although the prevalence of oligozoospermia was significantly higher in the group of patients, no direct cause-effect relationship between LR-HPV infection and the reduction of sperm count can be inferred. The majority of the available data indeed suggest that HPV infection may mostly impact on sperm motility or morphology, as well as on SDF [5,14]. Therefore, the role of chance cannot be excluded and a wider sample size is needed to solve this issue. The exclusion criteria adopted in this study justify, in our opinion, the limited series analyzed.

Taken together, these findings suggest no negative repercussions of LR-HPV infection on sperm parameters. From a physical-pathological point of view, the lack of a negative impact of LR-HPV genotypes on

sperm parameters might be ascribed to the absence of the E6/E7 proteins in the infected sperm. Accordingly, *in vitro* exposure of washed sperm to E6/E7 deoxyribonucleic acid fragments from HPV for 24 h resulted in a significant reduction of velocity and of amplitude of lateral head displacement, as well as of SDF, in some HPV genotypes [12]. Another study confirmed the reduction of sperm motility in the presence of HPV E6/E7 fragments from all the HPV types tested after 24h of incubation [13].

The practical value of these findings concerns the counseling of infertile patients. Currently, in the presence of seminal HPV infection, when fatherhood is searched, some authors suggest to postpone infertility treatment to six months, due to the likelihood of spontaneous viral clearance, in case of young couples. In contrast, IVF using treated sperm should be considered in older ones [14,19]. Apparently, this algorithm does not differ according to the HPV genotype. However, the lack of E6/E7 proteins in LR-HPV may suggest no influence of these genotypes on fertility.

Finally, this study is limited by the limited sample size, and the retrospective design. Prospective, well-sized studies are needed to confirm our findings.

In conclusion, we found no difference in sperm conventional parameters in LR-HPV infected patients than in controls. The data of this study might prompt to further research the impact on LR-HPV genotype on male fertility. Particularly, evidence on SDF and pregnancy outcome is needed.

Disclosure statement

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. R.C. drafted and wrote the manuscript. A.A. and R.A.C. selected the subjects, critically analyzed the data. S.L.V. and R.A.C. performed the clinical analysis, A.G. performed the HPV analysis in the semen, E.G. and S.D.C. contributed to interpretation of data and to the writing of manuscript. A.E.C. and S.L.V. drafted and supervised the project and revised the manuscript critically.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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