Sperm human papillomavirus infection and risk of idiopathic recurrent pregnancy loss: insights from a multicenter case–control study

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Objective: To test the hypothesis claiming an association between human papilloma virus (HPV) sperm infection and idiopathic recurrent pregnancy loss (RPL).
Design: Multicenter retrospective case–control study.
Setting: Three university hospitals.
Patient(s): Cases included men belonging to couples affected by first trimester idiopathic RPL. Controls included men belonging to couples with proven fertility and no history of pregnancy loss; RPL was defined as the previous loss of 2 or more pregnancies. Couples were defined as "fertile" if they achieved a full-term pregnancy within the year before enrollment in the study. All participants conceived without assistance.
Main Outcome Measure(s): The association between HPV DNA sperm infection, as identified using polymerase chain reaction, and RPL.
Results: The HPV DNA sperm infection was detected in 23 of 117 cases (20%; 95% confidence interval [CI]: 13%, 28%) and in 3 of 84 controls (4%; 95% CI; 1%, 10%) ($P<.001$). A comparison across baseline characteristics and multiple regression analysis did not identify any potentially confounding factors. Multivariate regression models showed a significant association between HPV DNA sperm infection and RPL (adjusted odds ratio, 7.44; 95% CI: 2.08, 26.58; $P=.002$ [Model 1]; adjusted odds ratio, 8.96; 95% CI: 2.41, 33.44; $P=.001$ [Model 2]).
Conclusions: The prevalence of HPV sperm infection was significantly higher in couples affected by RPL than in their fertile counterparts. Notably, the semen sample was infected by HPV in approximately 1 out of 5 patients. (Fertil Steril® 2023;119:410-8. ©2022 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Human papilloma virus, recurrent pregnancy loss, seminal fluid

Recurrent pregnancy loss (RPL) is defined as the spontaneous failure of ≥2 clinical pregnancies before the fetus reaches viability (1–4). Large epidemiological studies conducted both in Europe and in the United States, have estimated that the average prevalence of RPL is between 1% and 4% of all women who achieve pregnancy (2, 5).

The etiology of RPL remains poorly understood and, despite extensive testing, the underlying cause of RPL is undetermined in approximately 40%–50% of cases (6, 7). Idiopathic RPL is associated with substantial adverse clinical and psychological consequences for affected couples; therefore, identifying the root causes of RPL is critical (6, 8). Recent studies in RPL suggest that sperm abnormalities may play a role in RPL pathophysiology (2). The association between alterations in conventional sperm parameters (i.e.,

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sperm viability, normal morphology, and total or progressive motility) and RPL is inconsistent (1). However, emerging data show higher sperm DNA fragmentation (SDF) in couples that experience RPL than in their fertile counterparts (1, 2, 8–10).

Surprisingly, although sexually transmittable infections are widely accepted as an etiologic factor of male infertility, they have not been thoroughly investigated as a possible cause of RPL (11). Early indirect evidence suggests that human papilloma virus (HPV) sperm infection negatively impacts fecundity, a measure that comprises the ability to both conceive and carry a fetus to viability (12–14). As such, HPV likely has a negative effect on sperm parameters. In fact, spermatozoa of infected patients exhibit a decrease in progressive motility, an impaired morphology, and a significantly high SDA compared with HPV-negative individuals (13). Accordingly, the prevalence of seminal HPV infection is considerably higher in men with infertility than among the general population (20.9% vs. 8.2%, respectively) (13). A recent meta-analysis demonstrated a significant association between seminal HPV infection and male infertility, even after adjusting for female infertility (adjusted odds ratio [aOR], 3.02; 95% confidence interval [CI]: 2.11, 4.33) (13). Furthermore, a large prospective multicenter study on male partners of women undergoing intrauterine insemination reported that the presence of HPV virions in sperm was associated with a four-fold reduction in the likelihood of achieving a clinical pregnancy (15). Finally, despite small sample sizes, studies conducted in an assisted reproductive technology (ART) setting showed a significantly high miscarriage risk in couples with HPV sperm infection (OR, 5.13; 95% CI: 2.40–10.94) (13).

In the present study, we examined the role of HPV sperm infection in RPL. We specifically focused on pregnancies conceived without assistance to limit the contribution of several confounding factors associated with both difficulties in conceiving and ART.

MATERIALS AND METHODS

Aims

To test our hypothesis, we examined the relationship between the presence of sperm HPV infection (i.e., exposure factor) and occurrences of idiopathic RPL (i.e., outcome). We also conducted a secondary analysis to assess the association between high-risk HPV sperm infection and RPL. For exploratory purposes, the prevalence of sperm HPV infection was also compared between: couples with 2 and those with ≥ 3 previous pregnancy losses and couples with primary (i.e., couples who have never given birth to a live infant) and secondary (i.e., couples who have given birth to a live infant) idiopathic RPL. We compared the characteristics of participants and sperm parameters between subgroups (of both the full cohort and the case group alone) defined by sperm HPV infection status.

Design

This research comprised a retrospective multicenter case–control study conducted across 3 Italian University Hospitals (i.e., Humanitas S. Pio X Hospital, Humanitas University, Milan, Italy; Policlinico Gemelli Hospital, Catholic University of Scared Heart, Rome, Italy; Ospedale Università di Padova, Padova, Italy) between July 2020 and March 2022. The study protocol was approved by the institutional review board at the IRCCS Istituto Clinico Humanitas (determination nr. 3133/22). All included subjects signed an informed consent form before enrollment.

Study Population

Men were referred to the Obstetrics and Gynecology Departments of the 3 participating hospitals and were eligible for study participation if they met the following criteria: they were under the age of 50 and their female partner was between 25 and 42 years old; they exhibited no relevant comorbidities (i.e., tumors, cardiovascular diseases, metabolic diseases); and the couple was not affected by infertility (defined as the failure to conceive after 12 months of regular, unprotected sexual intercourse). Couples who achieved a previous pregnancy via ART were excluded from this study.

Study cases. Cases included men within couples affected by idiopathic RPL. This was defined as the previous loss of ≥ 2 pregnancies, according to the *European Society of Human Reproduction and Embryology* guidelines and the *American Society for Reproductive Medicine* committee opinion (1, 3, 16). Couples who conceived their previous pregnancy within 1 year and experienced spontaneous pregnancy losses within the first trimester of pregnancy were eligible for participation. Biochemical, ectopic, and molar pregnancies were not identified as RPL in this study. Women experiencing RPL underwent the following diagnostic work-up: genetic analysis of the pregnancy tissue; parental karyotyping; screening for antiphospholipid antibodies (lupus anticoagulant, and anticardiolipin antibodies [IgG and IgM]); screening for $\beta 2$ glycoprotein I antibodies (a$\beta 2$GPI); screening for antinuclear antibodies; thyroid screening (thyroid stimulating hormone and thyroid peroxidase antibodies); transvaginal 3 dimensional ultrasound; cervico-vaginal infections screening; and glucose metabolism assessment (1, 2, 16). If the full diagnostic work-up was negative, the couple was identified as experiencing "idiopathic RPL" and was eligible for the present study.

Study controls. Controls included men belonging to couples with proven fertility (i.e., full-term pregnancy with live birth achieved within the year preceding study enrollment) and no previous pregnancy losses. Female partners of these controls did not undergo the diagnostic work-up for RPL. Cases were selected first, followed by careful selection of appropriate controls. The age distribution of female partners was analyzed, and the proportion of female partners within age groups spanning 2 years each (e.g., 36–37 years; 38–39 years) was calculated. The control group was selected so that the proportion of female partners across age groups was identical to that of female partners belonging to couples affected by RPL.

Semen Analysis

Semen samples were obtained via masturbation after 2–5 days of sexual abstinence and stored in sterile containers. Samples were allowed to liquefy for 30 minutes and were examined for seminal parameters according to the *World*

Health Organization guidelines (17). Furthermore, SDF was assessed in cases using a terminal deoxynucleotidyl transferase dUTP nick end labeling assay in a commercially available kit (Cell Death Detection Kit, Roche Diagnostics, Milan, Italy). Next, cells were analyzed using a Becton Dickinson *FACScan* System for measuring and analyzing flow cytometry in Cellquest software (Becton Dickinson, Oxford, UK). This measure was expressed as a percentage and labeled the "sperm DNA fragmentation index" (DFI) (i.e., the ratio of the number of spermatozoa with fragmented DNA to the total number of spermatozoa) (18). Seminal fluid analyses were performed by trained technicians in the participating hospital laboratories a few days after recruitment and sample collection to allow compliance with the period of sexual abstinence. We ensured a high-quality testing by implementing regular external quality assessment programs.

Sperm HPV-DNA Screening-Genotyping

Human papilloma virus-DNA screening-genotyping was performed through polymerase chain reaction amplification using the INNO-LiPA HPV Genotyping Extra assay (Innogenetics, Fujirebio Italia S.r.l., Pomezia, Italy) according to the manufacturers' documented protocols (19, 20). Glass slides containing at least 2×10^6 smeared sperm, fixed in a methanol-acetic acid solution, were used for fluorescence in-situ hybridization analysis to identify HPV (19–21). A total of 32 HPV genotypes may be detected using this technique, including 13 high-risk HPV genotypes (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, and HPV68), 6 possible high-risk (possible HR) HPV genotypes (HPV26, HPV53, HPV66, HPV70, HPV73, and HPV82), 9 low-risk HPV genotypes (HPV6, HPV11, HPV40, HPV42, HPV43, HPV44, HPV54, HPV61, and HPV81) plus 4 additional HPV genotypes (HPV62, HPV67, HPV83, and HPV89) (22).

Statistical Analysis

The target sample size was calculated based on the results of a recent meta-analysis that reported an increased risk of miscarriage (OR, 5.13; 95% CI: 2.40, 10.94), and a reduced chance of ongoing pregnancy (OR, 0.33; 95% CI: 0.13, 0.82) in patients with sperm HPV infection compared with their negative counterparts (13). By setting alpha and beta values at 0.05 and 0.20, respectively, the estimated sample size required in this study was at least 60 cases and 60 controls. The sample size was estimated using STATA 15.0 (StataCorp LP, College Station, TX, USA). Secondary analyses and sub analyses were considered exploratory because this study lacked sufficient power to assess the impact of additional exposures.

We compared baseline characteristics, the abuse of substances (i.e., tobacco and illicit drugs), socioeconomic status, the ethnic origin, prevalence of sexually transmitted infections, the severity of comorbidities, the *World Health Organization* sperm parameters, and the HPV sperm infection prevalence between cases and controls. The baseline characteristics of female partners and HPV cervical infection prevalence were also measured and compared between the study groups. Average family income was identified as the mean net household income of north-east, north-west, and central Italy (23). Comorbidities of participants and their partners were scored using the Charlson Comorbidity Index (24). A multiple logistic regression analysis including RPL as the dependent variable was performed to identify potential confounding factors. Two multivariate logistic regression models yielded adjusted measures of association between the exposure to sperm HPV DNA and RPL. Secondary analyses was conducted to assess the association between high-risk HPV sperm infection and RPL.

Comparisons between groups were performed using Chi-squared tests, Fisher's exact tests, and Mann-Whitney *U* (Wilcoxon) statistic, as appropriate.

The data were analyzed using IBM SPSS Statistics (Version 27). Statistical significance was set to an alpha level of 0.05. All statistical tests were two-tailed.

RESULTS

Included Subjects

During the study period, a total of 526 couples were evaluated for RPL, leading to 234 cases defined as "idiopathic," Among these couples, 117 agreed to participate in the study. A total of 1,860 men with proven fertility were deemed eligible for study participation as controls: 84 of these men agreed to participate in a semen analysis and were included in the study.

Primary Analyses

In the primary analyses, HPV DNA sperm infection was detected in 23 of 117 cases (20%; 95% CI: 13%, 28%) and in 3 of 84 controls (4%; 95% CI: 1%, 10%) ($P<.001$). The baseline characteristics, abuse of substances (i.e., tobacco and illicit drugs), socioeconomic status, ethnic origin, the Charlson Comorbidity Index, and the semen parameters did not differ significantly between the study groups (Table 1). The multiple logistic regression analysis showed no significant association between the identified covariates and RPL (Table 2). Both adopted multivariate regression models yielded a significant association between HPV DNA sperm infection and RPL (aOR, 7.44; 95% CI: 2.08, 26.58; $P=.002$ [Model 1]; aOR, 8.96; 95% CI: 2.41, 33.44, $P=.001$) (Supplementary Table 1, available online). The primary analysis was repeated to include couples with a female partner aged $\geq$35 years: the prevalence of HPV DNA sperm infection was significantly higher in cases (20%; 95% CI: 12%, 30%) than in controls (2%; 95% CI: 0, 9%) ($P=.001$).

Secondary Analyses

The prevalence of high-risk and low-risk HPV genotypes in both study groups is reported in Table 3. In study cases, the high-risk HPV infection (15%; 95% CI: 9%–23%) was significantly more frequent than low-risk HPV infection (4%; 95% CI: 1%–10%; $P<.001$). We also observed a significant association between high-risk HPV genotypes sperm infection and RPL (OR, 4.91; 95% CI: 1.4, 17.26; $P=.01$). The multiple logistic regression analysis (considering high-risk HPV sperm infection as the exposure factor) showed no significant

TABLE 1

Baseline and sperm characteristics in cases and controls

Baseline and sperm characteristics	Cases (N = 117)	Controls (N = 84)	*P* value
Age (y)	38.7 ± 5.5	37.9 ± 4	.24
Body mass index (kg/m^2)	23.6 ± 3.3	22.8 ± 2.7	.08
Smoking habit, N (%)			.84
Yes	15 (13%)	12 (14%)	
No	102 (87%)	72 (86%)	
Use of illicit drugs[a], N (%)			.83
Current	2 (2%)	1 (1%)	
Previous	6 (5%)	3 (4%)	
Never	109 (93%)	80 (95%)	
Family income[b], N (%)			.50
Low (< € 25,000)	17 (14%)	11 (13%)	
Medium (€ 25,000-60,000)	78 (67%)	62 (74%)	
High (> € 60,000)	22 (19%)	11 (13%)	
Ethnicity, N (%)			.66
White	100 (85%)	72 (85%)	
Black	3 (3%)	0	
Asian	4 (3%)	3 (4%)	
Arab	7 (6%)	6 (7%)	
Hispanic	3 (3%)	3 (4%)	
History of sexually transmitted infections			.47
Yes	3 (3%)	1 (1%)	
No	114 (97%)	83 (99%)	
Charlson Comorbidity Index (value)	1 ± 1	0.9 ± 1.1	.42
Sperm characteristics			
Total sperm count (millions)	181 ± 123	217 ± 140	.06
Progressive motility (%)	36.0 ± 12.3	39.1 ± 11.5	.07
Sperm morphology (normal forms, %)	8.7 ± 5.7	9.9 ± 5.5	.35
Partner's age (y)	36 ± 3.9	35.3 ± 5.6	.35
Partner's age at last conception[c]	35.7 ± 4	35 ± 5.7	.29
Partner's body mass index (kg/m^2)	22.2 ± 3.2	22.6 ± 3.1	.36
Partner's smoking habit			.24
Yes	9 (8%)	11 (13%)	
No	108 (92%)	73 (87%)	
Partner's Charlson Comorbidity Index (value)	0.8 ± 1.1	1.1 ± 1.3	.11

Note: Data are expressed as mean ± standard deviation (SD) or as number (%). € = Euros.
[a] The current or previous use of the following illicit drugs was investigated: marijuana, hallucinogens, cocaine, heroin.
[b] The average family income (€ 34,265) was calculated as the mean net household income of north-east, north-west, and center of Italy (EU-SILC ITALIAN SURVEY - CROSS-SECTIONAL DATA: MICRODATA FOR RESEARCH PURPOSES, reference period: 2020) (23).
[c] Refers to the last conception that resulted in a clinical pregnancy.

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association between covariates and RPL (Supplementary Table 2). Both adopted multivariate regression models yielded a significant association between high-risk HPV DNA sperm infection and RPL (aOR, 5.68; 95% CI: 1.55, 20.84; P=.009 [Model 1]; aOR, 6.72; 95% CI: 1.71, 26.56; P=.007) (Supplementary Table 3). The HPV cervical infection prevalence and the distribution of high-risk HPV and low-risk HPV genotypes did not differ between women in couples affected by idiopathic RPL and fertile controls (Table 3).

Sub Analyses

We did not observe significant differences in baseline characteristics, abuse of substances, socioeconomic status, Charlson Comorbidity Index, ethnic origin, seminal parameters, and HPV sperm infection prevalence between men belonging to couples with primary (N = 94) and secondary (N = 23) idiopathic RPL. Characteristics of female partners and rates of HPV cervical infection did not differ between groups (Supplementary Table 4). Additionally, no significant differences were observed by comparing the prevalence of HPV DNA sperm infection between cases belonging to couples with 2 previous pregnancy losses and those with ≥3 previous pregnancy losses (12%; 95% CI: 4%–24% and 26%; 95% CI: 16%–38%, respectively; P=.07) (Fig. 1). The prevalence of HPV DNA sperm infection in cases with a female partner aged between 29 and 34 years (N = 35) was compared with that in cases with a female partner aged ≥35 years (N = 82), and showed no significant differences (20%; 95% CI: 8%–37% and 20%; 95% CI 12%–30%, respectively; P=1.0).

The baseline characteristics, abuse of substances (i.e., tobacco and illicit drugs), socioeconomic status, ethnic origin, the Charlson Comorbidity Index, and the semen parameters were compared between HPV-positive and HPV-negative participants across the full cohort and no significant differences were observed (Supplementary Table 5). Similar results were found after restricting the analyses to men with HR-HPV sperm infection (Supplementary Table 6). The mean number of previous pregnancy losses was significantly higher in participants with HPV sperm infection than among those without infection (2.9 ± 1.5 and 1.6 ± 1.7, respectively; P<.001). The DFI did not significantly differ between HPV-positive and

TABLE 2

Multiple logistic regression analysis

Covariates	Wald test	*P* value	Odds ratio (95% confidence interval)
Age at conception (female partner)	3.72	.05	0.15 (0.02, 1.03)
Age at conception (male partner)	0.02	.96	1.06 (0.09, 12.17)
Body mass index (female partner)	1.73	.19	0.71 (0.43, 1.18)
Body mass index (male partner)	1.64	.20	1.18 (0.92, 1.51)
Current smoker (female partner)	1.41	.24	1.91 (0.66–5.52)
Current smoker (male partner)	0.16	.69	1.21 (0.47–3.1)
Non-White ethnicity	0.61	.44	1.74 (0.44–6.92)
Family income	1.41	.23	1.89 (0.66, 5.4)
Charlson Comorbidity Index (female partner)	1.8	.18	2.44 (0.66, 8.94)
Charlson Comorbidity Index (male partner)	2.44	.12	0.06 (0, 2.1)
Positive history of sexually transmitted infections (male partner)	0.14	.71	1.76 (0.09–33.1)
Current or previous use of illicit drugs (male partner)	0.52	.47	0.60 (0.15–2.40)
Positive human papilloma virus cervical infection	0.05	.82	1.1 (0.53–2.23)
Semen parameters			
Total sperm count	3.02	.08	0.39 (0.14–1.13)
Progressive motility	2.85	.09	0.53 (0.26–1.11)
Normal sperm morphology	0.13	.72	0.87 (0.41–1.85)

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HPV-negative cases (Supplementary Table 7). This finding was also observed after restricting the analysis to cases with high-risk HPV sperm infection.

DISCUSSION

Main Findings

In the present study, the prevalence of HPV sperm infection was significantly higher in couples affected by RPL than in their fertile counterparts. Notably, we detected approximately 1 in 5 participants with HPV DNA in the semen sample. Moreover, high-risk HPV was more commonly observed than low-risk HPV in men belonging to couples affected by RPL. An association between HPV sperm infection and RPL was also identified after including high-risk HPV infection as an exposure factor.

Interpretation of Results

Our findings support claims stating a detrimental impact of HPV sperm infection on the risk of pregnancy loss (13). Furthermore, the present study's design may shed light on the pathophysiological mechanisms linking sperm HPV infection with spontaneous abortion (25, 26). The exclusion of couples who experienced repeated losses with abnormal karyotypes leads us to speculate that HPV does not have a detrimental effect on the structure of embryonic chromosomes. We observed no difference in DFI between cases with and without HPV DNA in seminal fluid, which contradicts the notion that the infection may be associated with a high risk of miscarriage by increasing SDA. Importantly, we failed to observe an impact of HPV sperm infection on seminal parameters (13, 27–33). This lack of association between the infection and semen quality was also found after restricting the analysis to high-risk HPV, which are considered genotypes with the highest risk of damaged sperm (8, 13). Our findings differ from most previous studies conducted on men with infertility that demonstrate a significant connection between HPV in sperm and decreased sperm concentration, motility, and morphology (34, 35). To reconcile these seemingly contradictory results, we may hypothesize that, although both circumstances lead to a failed viable pregnancy, the detrimental mechanisms through which HPV acts in the 2 populations (i.e., infertile men and men belonging to couples with RPL) differ. Although the underlying reason remains unknown; when infection occurs, the virus may not have an "all-or-nothing" effect on the seminal fluid. Instead, it may exert a detrimental impact with differing levels of severity. If HPV considerably alters seminal parameters, this may directly impair the ability to conceive. Conversely, if sperm alterations are not particularly severe, conception may occur. However, in the latter case the virus may still compromise other stages of the reproductive process or pregnancy development (27–33). A recent study conducted on a mouse model reported that spermatozoa carrying exogenous HPV DNA can act as vectors, transmitting HPV to the embryo through fertilized oocytes (36). Interestingly, Bober et al. (37) demonstrated an association between high-risk HPV trophoblast infection and miscarriage in humans. These

TABLE 3

High and low-risk HPV infection status in cases and controls.

	Cases (n = 117)	Controls (n = 84)	*P* value
HPV sperm infection			.03
Absent	94 (80%)	81 (96%)	
High-risk HPV infection	18 (16%)	3 (4%)	
Low-risk HPV infection	5 (4%)	0	
Partner's HPV cervical infection			.23
Absent	86 (73%)	70 (83%)	
High-risk HPV infection	15 (13%)	8 (10%)	
Low-risk HPV infection	16 (14%)	6 (7%)	

Note: Data are expressed as number (%). HPV = Human papilloma virus.

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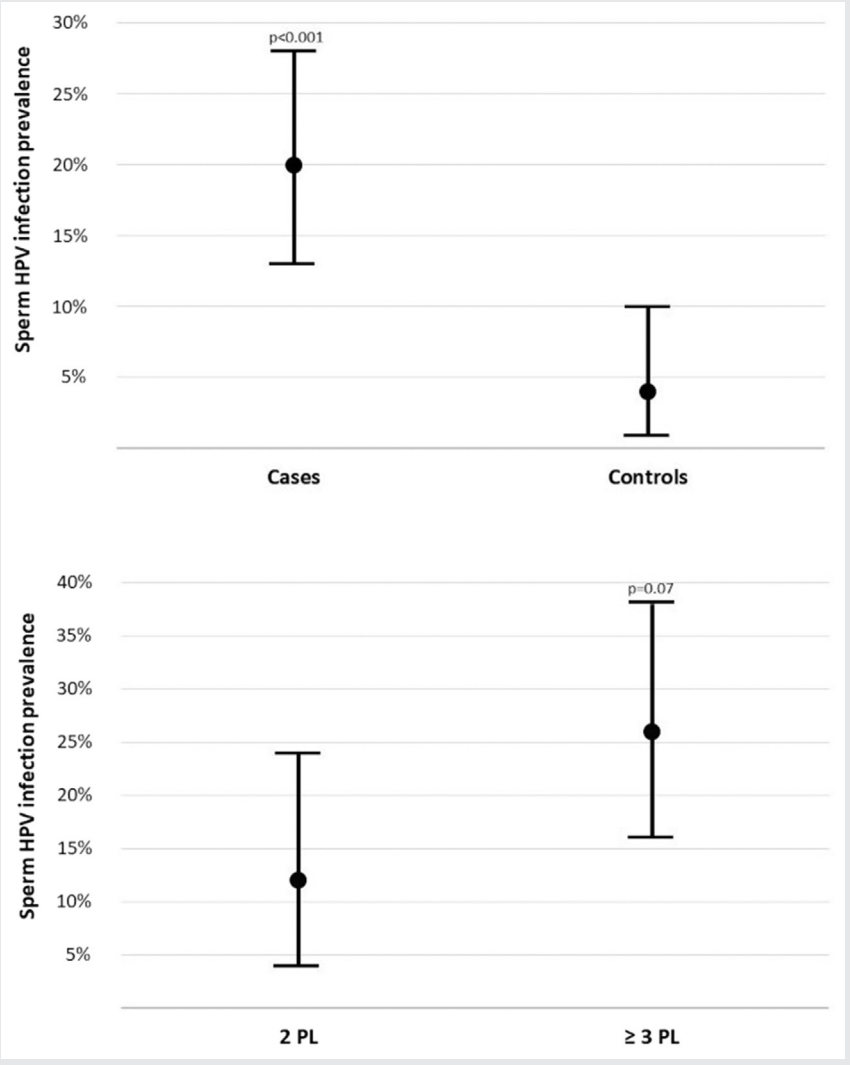
FIGURE 1



Sperm HPV infection prevalence
Prevalence of sperm HPV infection in men belonging to couples affected by recurrent pregnancy loss (cases) and in fertile controls (20%; 95% confidence interval [CI]: 13%, 28% and 4%; 95% CI: 1%, 10%, respectively; *P*<.001) (*upper panel*). Prevalence of sperm HPV infection in men belonging to couples with 2 and ≥3 previous pregnancy losses (12%; 95% CI: 4%–24% and 26%; 95% CI: 16%–38%, respectively; *P*=.07 (*lower panel*). HPV = human papilloma virus, CI = confidence interval.

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findings lead us to hypothesize that HPV infection may not only interfere with embryonic gene expression but also with the placentation process (13). Albeit intriguing, both these theories require additional, adequately powered studies.

Study Strengths

To our knowledge, this is the first study to explicitly investigate and report an association between sperm HPV infection and RPL. In recent years, evidence supporting a negative impact of HPV seminal fluid infection on fertility has been growing steadily (13, 21, 33–35). The present study adds substantially to this literature by including fertile couples who conceived without assistance. This allows us to circumvent several possible confounding factors associated with both infertility and ART. Finally, the extensive and meticulous diagnostic work-up included in screening participants allowed us to identify couples affected by idiopathic RPL with a high degree of certainty.

Limitations

The main limitation in this study includes the possible confounding effect of covariates. We aimed to control for confounding variables through a detailed comparison of baseline characteristics across the study groups and analyzing

these variables using a multiple regression logistic analysis. Both methods did not yield significant associations between the identified covariates and the outcome of interest. Furthermore, to examine the effect of advanced maternal age, we also restricted the main analysis to include patients aged ≥35 years. Unfortunately, true age matching was not possible owing to the difficulty in recruiting the control group. However, we opted to retain the number of cases included in the analysis to avoid selection bias and increase statistical power. Despite compelling results reported in this study, we cannot make causal inferences detailing the role of HPV sperm infection in the pathophysiology of RPL. A randomized controlled design would rectify this challenge and would almost nullify the impact of uncontrolled bias. We also recognize that conducting a prospective study is particularly challenging for several reasons: the need to recruit couples in the preconception period; difficulty in selecting men willing to undergo semen sample collection in the absence of fertility problems (which represents an obstacle in any study because of the social and cultural implications of this exam modality); the low incidence of idiopathic RPL; and the length of follow-up required. Another important limitation of this study is the absence of data regarding SDF in the controls. Finally, this study is underpowered; therefore, it is difficult to draw firm conclusions from the results of secondary and subgroup analyses.

Future Perspectives

Despite limitations of this work, our results mark a critical starting point for additional research. This work motivates 2 important next steps for investigators to pursue. Firstly, prospective studies could establish the reproductive prognosis of men with sperm HPV infection. Second, the pathophysiological basis behind the association reported in the present study requires further investigation. A clear causal link between seminal HPV and RPL could open new therapeutic solutions for couples struggling with RPL. In this regard, recent evidence suggests that vaccination against HPV may have both a preventive and a therapeutic role by hastening recovery from an HPV sperm infection (19, 38). This finding may be particularly critical because there are currently no sperm preparation techniques capable of eliminating the virus from an infected semen sample (39).

CONCLUSIONS

The present case–control study shows an association between sperm HPV infection and idiopathic RPL. To our knowledge, this is the first reported link between HPV infection and idiopathic RPL; however, future research is required to gain a clearer understanding of this connection and its potential implications for clinical practice. Considering the limited knowledge that exists surrounding the etiology of RPL and prospective therapies for HPV sperm infection, we believe that the data presented here motivate new research initiatives on this topic.

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Infección espermática por el virus del papiloma humano y riesgo de aborto recurrente idiopático: hallazgos de un estudio multicéntrico caso-control.

Objetivo: Evaluar la hipótesis que relaciona la infección espermática por virus del papiloma humano (HPV) y el aborto de repetición (RPL).

Diseño: Estudio multicéntrico retrospectivo caso-control.

Marco: Tres hospitales universitarios.

Paciente(s): Los casos incluyeron varones pertenecientes a parejas afectas de aborto recurrente idiopático de primer trimestre. Los controles incluyeron varones pertenecientes a parejas con fertilidad probada y sin historia de aborto; RPL se definió como la pérdida previa de 2 o más embarazos. Las parejas se definieron como "fértiles" si habían conseguido un embarazo a término dentro del año anterior a su inclusión en el estudio. Todos los participantes concibieron sin reproducción asistida.

Medida del resultado(s) principal(es): Asociación entre infección espermática por DNA de HPV, identificado utilizando reacción en cadena de la polimerasa, y RPL.

Resultado(s): La infección espermática por DNA de HPV se detectó en 23 de 117 casos (20%; intervalo de confianza del 95% [CI]:13%, 28%). La comparación de las características basales y el análisis de regresión múltiple no identificaron ningún potencial factor de confusión. Los modelos de regresión multivariable demostraron una asociación significativa entre la infección espermática por DNA de HPV y RPL (odds ratio ajustado 7.44; CI 95%:2.08, 26,58; P= .002 [Modelo 1]; odds ratio ajustado 8,96; CI 95%: 2.41, 33.44; P< .001 [Modelo 2].

Conclusiones: La prevalencia de infección espermática por HPV fue significativamente más elevada en las parejas afectas de RPL que en sus homólogas fértiles. Es destacable el hecho de que el semen estuviera infectado por HPV en aproximadamente 1 de cada 5 pacientes.