Spontaneous fertility and in vitro fertilization outcome: new evidence of human papillomavirus sperm infection

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Objective: To evaluate the reproductive outcome of infertile couples undergoing assisted reproduction techniques (ART) with or without human papillomavirus (HPV) semen infection.
Design: Cross-sectional clinical study.
Setting: Units of andrology, reproductive medicine, and gynecology.
Patient(s): A total of 226 infertile couples.
Intervention(s): Male partners were evaluated by means of fluorescence in situ hybridization (FISH) for HPV on semen. After a diagnostic period, female partners underwent intrauterine insemination (IUI) or intracytoplasmic sperm injection (ICSI).
Main Outcome Measure(s): Seminal parameters and FISH analysis for HPV in sperm head. Spontaneous or assisted pregnancies, live births, and miscarriages were recorded. Statistical analysis included unpaired Student *t* test and chi-square test.
Result(s): Fifty-four male partners (23.9%) had HPV semen infection confined to sperm, confined to exfoliated cells, or in both cells. During the diagnostic period, noninfected couples showed spontaneous pregnancies. IUI and ICSI treatments were performed in, respectively, 60 and 98 noninfected and in 21 and 33 infected couples, with 38.4% and 14.2% cumulative pregnancy rates, respectively. The follow-up of pregnancies showed a higher miscarriage rate in infected couples (62.5% vs. 16.7%). Ongoing pregnancies of the latter group were characterized by HPV infection confined to exfoliated cells.
Conclusion(s): A reduction in natural and assisted cumulative pregnancy rate and an increase in miscarriage rate are related to the presence of HPV at sperm level. Although the exact mechanism by which sperm infection is able to impair fertility remains unclear, this aspect is worthy of further investigations. If confirmed, these results could change the clinical and diagnostic approach to infertile couples. (Fertil Steril® 2016;105:65–72. ©2016 by American Society for Reproductive Medicine.)
Key Words: HPV semen infection, IVF failure, male infertility, miscarriage, spontaneous fertility

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Human papillomavirus (HPV) currently represents an important factor responsible for both male and female cancer development and infertility (1, 2). Considering male HPV infection, this virus has been found not only along the whole male genital tract but even in semen and bound to sperm cells (3). Interestingly, the presence of HPV DNA at this site has been demonstrated to be associated with an impairment of sperm motility and the presence of antisperm antibodies (4, 5). Recently, new insights into human reproduction have suggested a role for HPV in infertile couples. In natural conception, the rate of spontaneous abortions and major birth defects appears to be controversial in HPV exposed couples, and well defined studies are needed to clarify this (6). A clinical study performed on women undergoing in vitro fertilization (IVF) reported a significant reduction of pregnancies in the

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presence of HPV cervical infection compared with no infection (7). Although the possible consequences of fetal exposure to HPV are not well defined, in vitro experiments have shown that HPV-transfected trophoblast cells have an increased rate of stage-specific maturation arrest and apoptosis and a reduced placental invasion into the uterine wall compared with control cells (8). We performed a study with the use of the hamster egg penetration test (HEPT) with human infected and noninfected sperm. HEPT showed that human HPV-infected sperm were able to penetrate hamster oocytes, even if the mean number of penetrated sperm per oocyte was lower compared with the control samples. Moreover, oocytes penetrated by transfected sperm expressed the viral genes, suggesting an active transcription of viral genes by the infected oocyte. Despite the high relevance of these data, it is unknown whether in vitro findings might apply to oocytes in vivo (9). Another study investigating the role of HPV infection in infertile couples undergoing assisted reproduction technology (ART) cycles, showed a reduced pregnancy rate and an increased spontaneous abortion rate in couples with HPV infection compared with those not infected. The risk was increased when HPV DNA testing was positive in the female partner, but it was even higher when sperm samples were infected (10). The aim of the present study was to evaluate the prevalence and localization of HPV semen infection in infertile couples attending a center for reproductive medicine. Moreover, we considered both natural and assisted reproductive outcome in couples with or without HPV semen infection.

MATERIALS AND METHODS

Patients

We conducted an observational prospective cohort study of 250 infertile couples seeking a child for ≥ 2 years and scheduled for intrauterine insemination (IUI) or IVF at the Human Reproductive Medicine and Gamete Cryopreservation Unit of the Gynecology and Obstetrics Clinic in Bruneck Hospital from January 2013 to December 2014. At recruitment, all patients were properly informed about the aim of the study and were enrolled only if they gave written informed consent for the study and for the use of their data according to Italian privacy law. The study was approved by the Institutional Review Board of our hospital (protocol no. 2336P). We considered eligibility for the study to include normo-ovulatory women with the following characteristics at recruitment: normal responder according to the Bologna criteria (11), idiopathic/unexplained infertility, age 25–35 years, body mass index (BMI) 18–30 kg/m^2, and negative Pap smear and genital swab for the presence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and bacterial vaginosis. We excluded patients with a history of ovarian/tubal surgery, cervical dysplasia, endometriosis, pelvic inflammatory disease, tubal occlusion, or polycystic ovarian syndrome. In addition, we excluded patients treated for benign endouterine disease (such as endometrial polyps, submucous myomas, intrauterine synechiae, and uterine septus) in the 6 months before the IUI/IVF cycle. Patients with a history of smoking, karyotype abnormalities, mutations of the cystic fibrosis gene, major systemic disease (such as diabetes, multiple sclerosis, adrenal diseases, thyroid dysfunction, alteration in basal serum prolactin value, hypogonadotropic or hypergonadotropic hypogonadism, acquired or inherited thrombophilia, and immunologic disorders), previous neoplasia, previous chemo- or radiotherapy, or untreated uterine diseases (polyps, myomas, synechiae, septus) also were excluded.

Considering male partner, we included subjects aged 25–40 years with normal or altered sperm parameters according to World Health Organization guidelines 2010 (12), and we excluded azoospermic patients. We also excluded subjects with current infection of *Chlamydia trachomatis*, ureoplasma, *Neisseria gonorrhoeae*, or other sperm infections, seropositivity toward human immunodeficiency virus type 1 or 2, human T-cell lymphotropic virus type 1 or 2, hepatitis B or C virus, or *Treponema pallidum*. Patients with genetic alterations, karyotype abnormalities, Y-chromosome microdeletions, or CFTR mutations were also excluded. Each male partner underwent fluorescent in situ hybridization (FISH) of the ejaculated semen for the detection of HPV-DNA sequences in the spermatozoa and exfoliated cells both at recruitment and on the day of IUI/IVF.

Semen Processing

Semen samples were obtained by means of masturbation after 3 days of sexual abstinence. After liquefaction at room temperature, semen volume, pH, sperm concentration, viability, motility, and normal morphology were determined according to World Health Organization guidelines for semen analysis (12). In each sample we also performed the spermMar test to detect sperm antibodies.

Antisperm Antibody Detection

Sperm antibodies were detected using the spermMar Test kit for IgG and IgA (FertiPro). Semen samples were treated according to the kit protocol. The test was considered to be positive when spermatozoa were partially or totally covered by latex particles. The reactivity of the test was confirmed by the next formation of growing agglutinates of latex particles themselves. Alternatively, freely moving spermatozoa uncovered by latex particles were considered to be negative.

FISH for HPV

This analysis was performed at diagnosis and repeated on the day of ART. Glass slides containing $\geq 2 \times 10^6$ adhered sperm were fixed in a methanol-acetic acid solution for ≥ 1 hour at -20°C. To permeabilize, samples were digested with pepsin diluted 1:25,000 in prewarmed 0.01 mol/L HCl for 10 minutes at 37°C. Permeabilization of the specimens was stopped with 3- to 5-minute washes in phosphate-buffered saline solution (PBS), then samples were dehydrated in 70%, 80%, and absolute ethanol for 2 minutes and finally air dried. Samples were then overlaid with 20 mL hybridization solution (Pan Path) containing biotin-labeled HPV DNA probe (a mix of total genomes containing the conserved HPV region). Each slide was covered with a glass coverslip, and the edges were sealed with nail polish to prevent loss of the mixture during denaturation and hybridization. After denaturation of cellular target DNA and HPV DNA probe on a heating block for 5 minutes at

95°C, hybridization was performed by incubating the samples at 37°C overnight in a humidified chamber. Thereafter the coverslips were carefully removed, and the slides were washed in PBS for 10 minutes. After 15 minutes' incubation at 37°C with the differentiation reagent (Pan Path), the slides were washed three times in PBS. The biotin-labeled HPV probe was detected by means of incubation with 1:200 streptavidin Texas Red (Vector Laboratories) for 40 minutes at room temperature. After detection, the slides were washed twice in PBS/0.01% Triton and then twice in PBS and mounted with a solution containing DAPI and antifade (Bioblue; Bioview). Samples were analyzed with the use of a fluorescence microscope (Nikon Vico video confocal microscope) equipped with a triple band-pass filter set (FITC, TRITC, DAPI). For each slide, ≥ 200 spermatozoa and ≥ 200 exfoliated cells were analyzed. Evaluation of nuclear hybridization signals was performed by three investigators. When nuclei were completely and homogeneously stained and multiple small spots or single large signals were present, the sperm cells were classified as positive. The method was tested on control slides containing CaSki cells, a human cervical carcinoma cell line with stably integrated and transcriptionally active HPV genomes, that served as a control for the specific probe. Cells smeared on salinated glass slides were fixed with 4% paraformaldehyde in PBS for 10 minutes. After fixation, cells were subjected to 3- to 5-minute washes in PBS and then dehydrated with 5-minute ethanol washes (30%, 60%, and 95%). Cell smears were then air-dried and stored at 4°C until use.

Design of the Study

At recruitment, couples were divided into two groups based on the presence or absence of HPV semen infection, and outcomes were recorded after 2 consecutive phases. The first phase was a 6-month period (diagnosis period) ranging from enrollment to the beginning of ART cycles. A further period of 12 months (ART period), in which couples underwent IUI/ICSI, was considered to be the second phase. Primary outcomes were the prevalence of HPV on semen according to FISH analysis at diagnosis and on the day of ART, and clinical pregnancy rates during both periods. During the study we also recorded ongoing pregnancy rates, miscarriages, and healthy born babies. Figure 1 shows the flow diagram of the whole study.

ART Treatments

Six months after the first visit, all couples who had not already achieved spontaneous pregnancy were enrolled for IUI for three cycles or ICSI for one cycle. If the number of total motile spermatozoa was not suitable for IUI ($\leq 5 \times 10^6$/mL), couples were enrolled only for IVF.

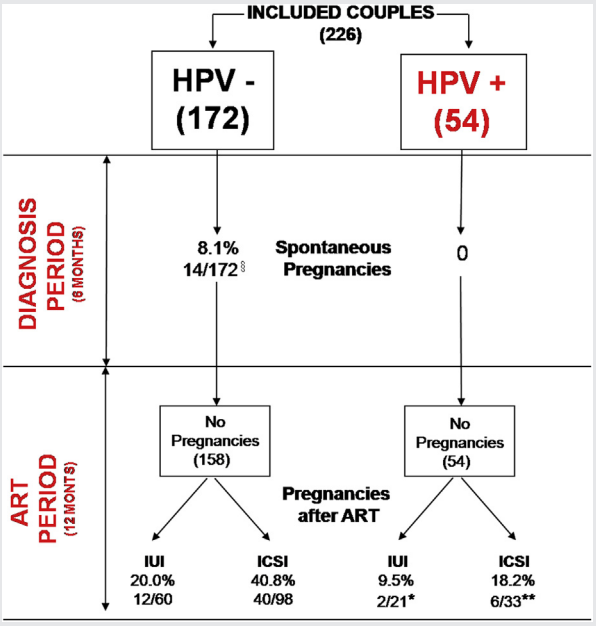
Women included in the IUI cohort received a daily dose of 50 UI recombinant FSH (Puregon; Organon) started on the third day after menstruation. When at least one follicle >16 mm in diameter was found by means of transvaginal sonography (TVS) we administrated 10,000 UI hCG for ovulation induction. IUI took place 36 hours after hCG administration.

Women included in the ICSI cohort received a controlled ovarian hyperstimulation (COH) cycle with the use of a flexible-scheme short-antagonist protocol with a daily dose of GnRH-antagonist (0.25 mg/0.5 mL) cetrorelix (Cetrotide; Merck-Serono) started from the TVS detection of at least one follicle >14 mm in diameter and continued until hCG administration (flexible protocol). All women were also treated with the use of recombinant fFSH (Gonal-F; Merck-Serono) with a starting dose (maintained for the first 5 days) of 150 IU administered on the 3rd day after menstruation (pending basal E_2 <0.3 nmol/L). The clinicians decided the subsequent dose adjustment during the cycle according to the biochemical and TVS features of the ovarian response.

On all of the women, we performed a subcutaneous injection of 10,000 IU hCG for ovulation induction. Oocyte retrieval took place 36 hours after hCG administration, and all of the oocytes were fertilized by means of ICSI technique. When obtained, one blastocyst was transferred 5 days after pick-up (after selection for quality).

All patients received high-dose P supplementation (90 mg vaginally) for luteal phase support until β-hCG assay, which was performed 14 days after embryo transfer. Clinical pregnancy was confirmed by positive serum β-hCG test 2 weeks after embryo transfer/IUI, and ongoing pregnancy as an uncomplicated pregnancy over 12 gestational weeks.

FIGURE 1



Flow diagram of the study. A group of 226 infertile couples eligible for assisted reproduction (172 with no human papillomavirus [HPV] semen infection and 54 with HPV semen infection) were followed for spontaneous and assisted pregnancies during a diagnosis period of 6 months and during a further 12-month period in which they underwent to intrauterine insemination (IUI) or intracytoplasmic sperm injection (ICSI). HPV− = noninfected group; HPV+ = infected group. *HPV semen infection confined to exfoliated cells (two cases). **HPV semen infection in sperm and exfoliated cells (two cases), confined to sperm (three cases), confined to exfoliated cells (one case). §P=.04 vs. infected.

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All women were scheduled with combined oral contraceptive (COC; Gestodiol 20: 20 μg ethinylestradiol and 75 μg gestodene) the previous month before starting COH. Moreover, all women received dietary supplementation with inositol and folic acid (Dikirogen; Pizeta Pharma) during COH.

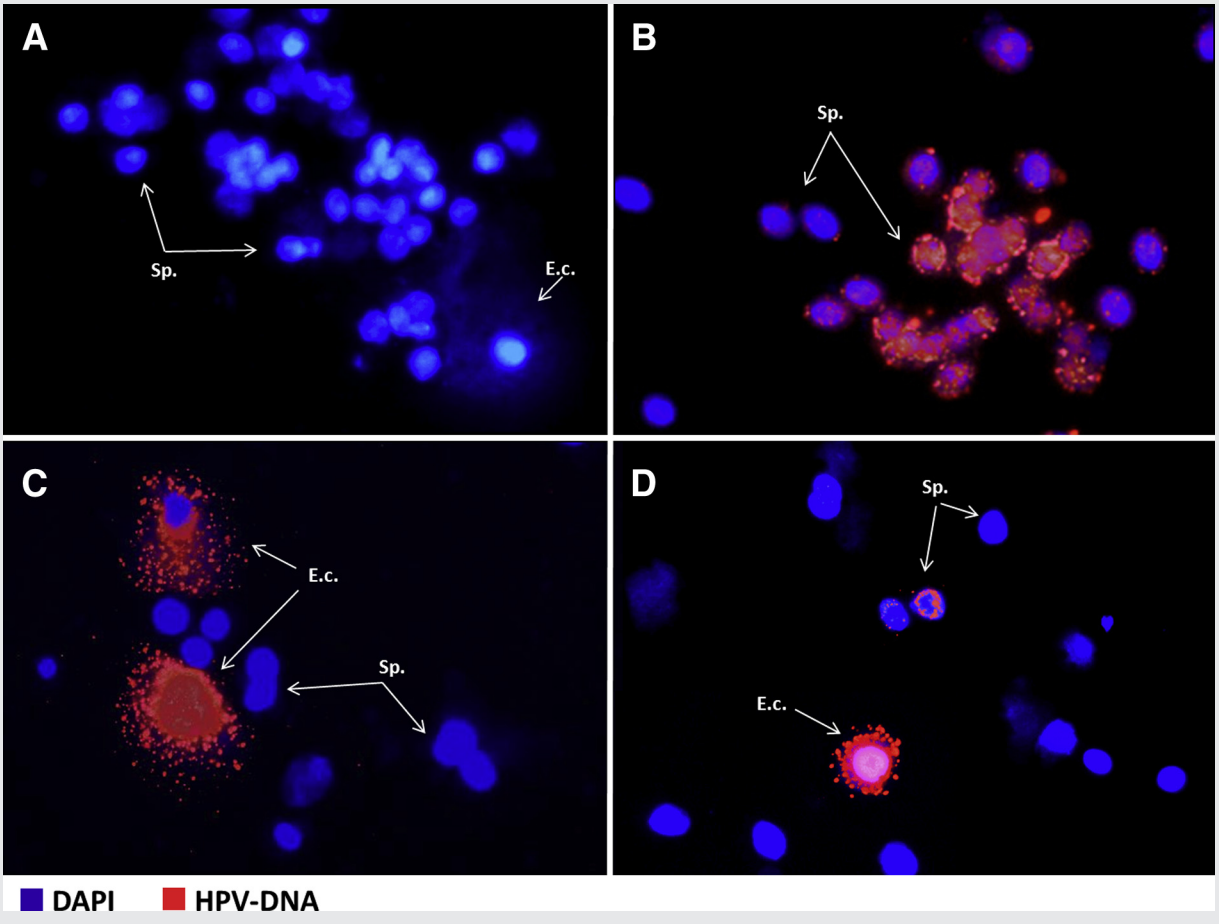
Statistical Analysis

The results are expressed as mean ± SD, and categoric variables are expressed as percentages. Comparisons between groups were performed with the use of unpaired Student *t* test after acceptance of normality according to the Kolmogorov-Smirnov test and the chi-square test for categoric data. Comparisons of proportion were performed with a one-sided nonparametric resampling test. Probability (*P*) values of $< .05$ were considered to be statistically significant.

RESULTS

Among 250 observed couples, 226 completed the whole study period and were included in these results. The mean age at enrolment was 34.2 ± 4.1 years for men and 31.3 ± 3.2 years for women. The FISH analysis for HPV performed in semen samples of male partners showed a 23.9% positivity (54 out of 226 patients). The results of FISH analysis was confirmed in the semen samples used for ART. Mean ages of male and female partners of infected and noninfected groups were not significantly different. Men showed the presence of the virus in sperm and/or exfoliated cells. In particular, 28 patients (51.9%) had infection confined to sperm, 8 (22.2%) confined to exfoliated cells, and 14 (25.9%) in both sperm and exfoliated cells. Figure 2 shows examples of FISH analysis for HPV in semen samples of infertile patients. During the diagnosis period (6 months) 14 out of 172 couples without HPV semen infection (8.1%) had spontaneous pregnancy while there were none in the infected group ($P = .04$). During the ART period (12 months), 12 out of 60 couples (20%) had successful IUI and 40 out of 98 (40.8%) had successful ICSI treatment. In the infected group, 2 out of 21 (9.5%) and 6 out of 33 (18.2%) had successful IUI and ICSI treatments, respectively. Among infected patients with successful ART,

FIGURE 2



Examples of fluorescence in situ hybridization analysis for HPV on semen samples of infertile patients: (**A**) negative sample; (**B**) infection confined to sperm; (**C**) infection confined to exfoliated cells; (**D**) infection in both sperm and exfoliated cells. *Arrows* indicate sperm (Sp.) and exfoliated cells (E.c.).

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both IUI cases showed HPV infection confined to exfoliated cells and ICSI cases showed the following pattern: three cases confined to sperm, one confined to exfoliated cells, and two in both sperm and exfoliated cells. Fertilization rates obtained with the use of ICSI were not different between noninfected (86.7%) and infected subjects (84.8%). In contrast, blastocyst formation rate was significantly reduced in the latter group (54.1% in noninfected vs. 27.3% in infected patients; $P<.05$ [data not shown]).

Table 1 shows sperm parameters and fertility outcome of subjects with noninfected sperm compared with those of HPV-infected semen. Considering all patients of the latter group, the percentage of motile sperm was significantly reduced (25.9 ± 16.2%) and the presence of antisperm antibodies significantly higher (40.7%) compared with noninfected subjects (34.3 ± 14.9% and 10.5%, respectively). The same trend was observed considering subjects from couples who had no pregnancy (sperm motility and antisperm antibodies 25.1 ± 17.7% and 43.4% in infected and 32.7 ± 15.1% and 12.2% in noninfected). No significant difference in sperm parameters was found comparing subjects with different fertility outcomes of noninfected and infected groups. Figure 3 reports the cumulative pregnancy rate and the pregnancy outcome observed in noninfected and infected couples. Cumulative pregnancy rates recorded in noninfected and infected couples were, respectively, 38.4% and 14.2% ($P<.05$). At the time of writing, among the 66 pregnancies recorded in noninfected cases, 36 were still ongoing, 19 had given birth to healthy babies, and 11 resulted in miscarriage. Among the eight pregnancies recorded in the infected group, three were still ongoing and no babies had been born. In this group, we observed a significantly higher miscarriage rate (62.5% vs. 16.7% of noninfected; $P<.05$). In particular, all pregnancy losses of the infected group took place very early (three at 5th and two at 6th gestational week). The localization of HPV semen infection was different in cases with ongoing pregnancies or miscarriage. The three cases with ongoing pregnancies showed infection confined to exfoliated cells, and the five with miscarriage always showed the presence of infected sperm (two cases in both sperm and exfoliated cells and three cases confined to sperm). Supplemental Table 1 (available online at www.fertstert.org) presents sperm parameters of subjects with HPV-infected and -noninfected semen distinguished on the basis of pregnancy outcome. No sperm parameter reached statistically significant difference when comparing noninfected and infected patients with different pregnancy outcome.

DISCUSSION

The possible cause-effect relationship existing between HPV infection and fertility impairment represents one of the most fascinating and debated themes of human reproduction in recent years (10, 13, 14). Starting from male sperm infection, several authors have detected, in different studies, a higher prevalence of HPV semen infection in infertile men compared with healthy control subjects (14, 15). Moreover (4, 16), a recent literature review which analyzed all strengths and possible bias of studies on this topic (13), in

TABLE 1

Sperm parameters and fertility outcome of subjects with human papillomavirus (HPV)–infected semen compared with noninfected sperm.

Variable	Fertility outcome	Semen volume, mL	Sperm concentration, 10^6/mL	Sperm count, $\times 10^6$	Motility (a+b), %	Normal morphology, %	Sperm viability, %	Sperm antibodies, n (%)
Noninfected patients (n = 172)	No pregnancy (n = 106)	2.7 ± 1.4	50.1 ± 50.6	129.9 ± 126.3	32.7 ± 15.1	15.0 ± 14.6	67.6 ± 24.8	13 (12.2)
	Spontaneous pregnancy (n = 14)	2.3 ± 1.3	54.6 ± 48.5	138.4 ± 99.6	35.1 ± 16.9	13.7 ± 12.1	71.2 ± 29.4	0
	IUI pregnancy (n = 12)	2.7 ± 1.1	61.3 ± 57.9	145.5 ± 131.3	33.9 ± 12.6	14.3 ± 13.2	68.4 ± 31.6	0
	IVF pregnancy (n = 40)	2.8 ± 1.9	48.8 ± 52.4	126.6 ± 135.4	28.7 ± 14.3	10.18 ± 9.6	59.3 ± 26.6	5 (12.5)
	All (n = 172)	2.7 ± 1.5	52.2 ± 50.3	131.9 ± 128.4	34.3 ± 14.9	14.8 ± 13.7	66.1 ± 27.3	18 (10.5)
Infected patients (n = 54)	No pregnancy (n = 46)	2.3 ± 1.7	67.2 ± 48.0	146.6 ± 125.6	25.1 ± 17.7[a]	16.1 ± 14.0	65.1 ± 34.7	20 (43.4)[a]
	Spontaneous pregnancy (n = 0)	–	–	–	–	–	–	–
	IUI pregnancy (n = 2)	2.5 ± 1.3	61.5 ± 54.6	150.7 ± 140.9	29.8 ± 14.9	17.7 ± 13.1	70.6 ± 36.4	0
	IVF pregnancy (n = 6)	2.4 ± 1.5	51.7 ± 43.9	123.8 ± 128.2	24.6 ± 15.3	14.9 ± 15.4	60.6 ± 38.2	2 (33.3)
	All (n = 54)	2.3 ± 1.6	58.9 ± 48.8	145.6 ± 131.5	25.9 ± 16.2[b]	16.2 ± 14.1	65.2 ± 35.8	22 (40.7)[b]

[a] $P<.01$ vs. noninfected patients.
[b] $P<.001$ vs. noninfected patients.

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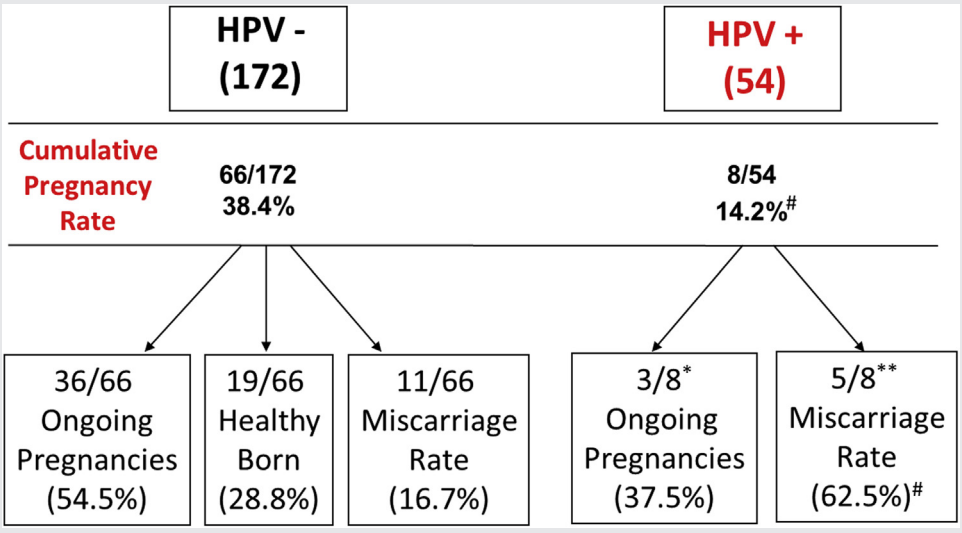
FIGURE 3



Comparison of cumulative pregnancy rates, ongoing pregnancies, live births, and miscarriages observed in noninfected and infected couples. Abbreviations as in Figure 1. *HPV semen infection confined to exfoliated cells (three cases). **HPV semen infection in sperm and exfoliated cells (two cases) and confined to sperm (three cases). #$P<.05$ vs. noninfected.

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an overall sample size of 1,920 patients showed a strong association between HPV semen infection and asthenozoospermia. Another important finding which certainly deserves further elucidation is the high prevalence of antisperm antibodies in the seminal fluid of HPV-infected men, which leads to speculation that the reduced motility may be related to the presence of antibodies on the sperm surface (17). In the present study we certainly confirmed this important result, reporting a significant reduction of sperm motility in patients with FISH analysis positive for HPV, and observed a high prevalence of HPV semen infection (~24%) in infertile couples who underwent ART. Our findings are similar to those of a recent meta-analysis by Laprise et al., which reported a total prevalence of HPV sperm infection in men attempting fertility treatments of ~16% compared with 10% in the general population (18). Combining this evidence, our data show that during the diagnosis period, only couples without HPV semen infection had spontaneous pregnancy (8.1%), which is not surprising. Moreover, this is the first study that clearly demonstrates a significant reduction of spontaneous pregnancy rate in couples without any known infertility factor if not the presence of HPV in semen. Increasingly strong evidence even seems to suggest that the infected spermatozoa may act as a carrier of viral genome into the oocyte during conception (19).

Consequences of this infection could range from the active viral replication in fertilized oocyte and trophoblastic cells to very early miscarriage events, even those clinically undetectable (20). This represents a serious concern for both spontaneous fertility and ART treatment. In our sample, we reported a significant reduction of success rate by ART in both IUI (from 20% in HPV-negative to 9.5% in HPV-positive sperm) and ICSI (from 40.8% in HPV-negative to 18.2% in HPV-positive sperm) cohorts, with a significant reduction of cumulative pregnancy rates (both spontaneous and assisted) in all HPV-positive couples. In ICSI cycles, the reduced pregnancy rate of infected couples was related to impaired blastocyst formation. The main factor that could explain this phenomenon is, in our opinion, the HPV infection, because the noninfected and the infected groups were similar and homogeneous for all confounding factors. It is also very important to emphasize that all patients with successful IUI/ICSI treatment and positive semen infection showed the HPV localization in exfoliated cells and none of the five patients with sperm infection had ongoing pregnancies. This last speculation is confirmed by a recent paper that demonstrated the ability of HPV-infected spermatozoa to fertilize the oocyte, but with a greatly reduced penetration rate compared with HPV-negative sperm (9).

Regarding the consequences of oocyte fertilization by infected spermatozoa there is still much to understand. A recent study demonstrated that in the HPV-infected oocyte there is an active viral genome expression (9). Moreover, in different in vitro studies, the peculiar negative effects of HPV presence in blastocyst and trophoblast cells has been documented (20). Indeed, the rate of apoptotic processes in HPV-transfected trophoblast cells was significantly higher compared with negative control cells, and this phenomenon was associated with a progressive decrease in the invasive capacity of trophoblast (21–23). Finally, it has been shown that HPV embryo infection may be associated with a significant reduction of blastocyst formation, particularly at the 2-cell embryo stage. This observation suggests that the negative effect of HPV strikes the early embryo development (8). This evidence could explain our finding of a significantly higher miscarriage rate observed in ART couples with HPV sperm

infection (62.5% vs. 16.7%). Our data are in accordance with the only prospective study performed in this field, which reported a similar miscarriage rate (66.7%) in couples undergoing ART with HPV semen infection (10). Certainly our results, though in accordance with Perino et al., would seem, in a superficial view, to be in disagreement with various published studies that failed to find a cause-effect relationship between HPV and miscarriage (24–26). However, all of those works are affected by some bias (27) and did not consider which cells of the semen are infected and the negative effect of infected sperm on blastocysts.

CONCLUSION

We think that greater attention should be paid to assess the HPV clinical status, particularly in men with idiopathic infertility in couples undergoing fertility treatments. In these cases, it would be useful to perform an HPV test and, in the case of infection, a FISH analysis in semen to detect HPV at the sperm level (13, 17, 18). We are conscious that HPV testing would increase the costs for couples and that there is no treatment for HPV infection. However, this behavior could be supported by the following considerations: 1) Despite there being no treatment for HPV infection, the estimated clearance for the virus is >60% at 6 months and in the case of young couples there is the chance to wait for spontaneous healing to restore normal sperm parameters and the chance of spontaneous pregnancy or to improve the ART outcome; 2) for aged couples who can not wait for spontaneous clearance, we have previously demonstrated that there is the chance to eliminate HPV from sperm through a specific washing procedure of semen with heparinase III (17); 3) the cost of testing for HPV in semen is very little compared with a failed ART procedure.

To our knowledge, this is the first prospective study that demonstrates both a significant reduction of spontaneous and assisted pregnancy rate and a significant increase in miscarriage rate in couples with HPV sperm infection undergoing ART. The immediate relapse in daily clinical practice is the identification of a new important factor of both male and couple infertility that should not be underestimated during the evaluation of idiopathic infertile couples, especially when all other known causes of infertility are ruled out. Our results are supported by a concise study design but with a small sample size. However, our sample is homogeneous and our findings clearly show a significant impairment of pregnancy outcome in the case of HPV sperm infection. Despite the possible limitations of the present study, if our results are confirmed they could significantly change the clinical approach to HPV-infected couples. In light of these insights, it is mandatory to plan new clinical and laboratory studies aimed to definitively confirm the present data and to deepen the knowledge of biologic mechanisms at the basis of HPV-related infertility.

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SUPPLEMENTAL TABLE 1

Sperm parameters of subjects with human papillomavirus (HPV)–infected and –noninfected semen according to pregnancy outcome.

Variable	Pregnancy outcome	Semen volume, mL	Sperm concentration, 10^6/mL	Sperm count, $\times 10^6$	Motility (a+b), %	Normal morphology, %	Sperm viability, %	Sperm antibodies, n (%)
Noninfected patients (n = 66)	Ongoing pregnancy (n = 36)	2.7 ± 1.6	54.3 ± 56.6	136.1 ± 110.7	33.7 ± 14.5	12.5 ± 12.3	70.3 ± 30.1	3 (8.3)
	Healthy born (n = 19)	2.8 ± 1.5	58.4 ± 49.8	144.7 ± 128.4	34.2 ± 15.2	15.7 ± 13.1	70.5 ± 30.4	1 (5.2)
	Miscarriage (n = 11)	2.7 ± 1.8	50.2 ± 43.3	129.6 ± 139.1	29.3 ± 13.6	10.7 ± 10.2	58.8 ± 26.7	1 (9.1)
Infected patients (n = 8)	Ongoing pregnancy (n = 3)	2.4 ± 1.7	57.5 ± 51.7	138.7 ± 137.7	27.1 ± 15.6	16.8 ± 13.9	68.4 ± 36.0	1 (33.0)
	Healthy born (n = 0)	–	–	–	–	–	–	–
	Miscarriage (n = 5)	2.5 ± 1.1	55.4 ± 46.8	130.1 ± 129.6	26.3 ± 14.4	15.2 ± 14.8	63.2 ± 35.7	1 (20.0)

Note: All *P* values were not significant.

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