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# 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® 2015;■:■–■. ©2015 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 presence of HPV cervical infection compared with no infection (7).

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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 $\geq 2$ 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 $\geq 1$ 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