Human papillomavirus infection in couples undergoing in vitro fertilization procedures: impact on reproductive outcomes

A prospective study was performed to assess the relationship between human papillomavirus (HPV) infection in 199 infertile couples and outcome of assisted reproductive technologies (ARTs). A highly statistically significant correlation between pregnancy loss rate (proportion of pregnancies detected by β-hCG that did not progress beyond 20 weeks) and positive HPV DNA testing in the male partner of infertile couples, compared with HPV negatives, was observed (66.7% vs. 15%). (Fertil Steril® 2011;95:1845–8. ©2011 by American Society for Reproductive Medicine.)

Key Words: Abortion, ART, HPV infection, infertility, pregnancy loss

Human papillomaviruses (HPV) comprise a group of small DNA viruses that infect both cutaneous and mucous squamous epithelia. HPV infection has an estimated overall prevalence of 10% in the general female population during reproductive age (1, 2). Genital human papillomavirus infection is the most common sexually transmitted viral infection worldwide, and has been associated with precancer and cancer of the male and female anogenital mucosa (3, 4).

Although it is well known that sexually transmitted infections are the primary cause of infertility (5, 6), few studies have investigated the effect of HPV infection on human reproduction. In a previous study, a twofold increase in the rate of spontaneous abortion was reported in 66 women with current genital HPV infection (12%) compared with 900 HPV-negative women (6%), despite a lower anamnestic rate of pregnancies in HPV-positive women (7).

In addition, it has been shown that HPV infection was three times more prevalent in spontaneous abortion specimens compared with specimens from elective abortions (60% vs. 20%, respectively). The conclusions of this study suggested that HPV may be an etiologic agent of some miscarriages, and that these viruses may be closely linked to fetal pathology (8).

There is also speculation concerning HPV-related subfertility in men. Indeed, HPV has been isolated in seminal samples, and its presence has been correlated, with some controversy, with reduced sperm cell motility (9–12), sperm cell numbers (13), and semen pH (11). However, no clear correlation with male infertility has been demonstrated.

Given this scientific background, a prospective study was designed to investigate the role of HPV infection in infertile couples undergoing assisted reproductive technology (ART) cycles. The objectives of this study were [1] to assess the prevalence of HPV infection in infertile couples, and [2] to evaluate the correlation between HPV infection and ART outcome.

A total of 199 couples, after signing an informed consent, were enrolled in the program of medically assisted procreation at the Dipartimento Materno Infantile of the University of Palermo and the Centro di Biologia della Riproduzione, Palermo, Italy, from May 2008 to May 2009. Institutional Review Board approval was obtained before the start of the study.

The mean age of women and men were 34.7 $\pm$ 5.01 and 38.0 $\pm$ 6.36 years, respectively. Types of infertility were female (tubal occlusion, chronic anovulation, 24.1%), male (severe oligoasthenoteratozoospermia, 58.6%), couple (6.8%), and idiopathic (10.5%). Exclusion criteria were: cases of azoospermia, couples with $\geq$3 implantation failures, and cases of endometriosis. These two latter conditions are associated with a reduced implantation rate (14, 15).

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All women had undergone cervical cytologic screening (Papanicolaou test [PAP]) within the previous 12 months, with no cytologic abnormality reported. No patient tested positive for HIV, *Chlamydia trachomatis, Neisseria gonorrhoeae*, Herpes simplex, or *Treponema pallidum*.

Patients were treated with standard ovulation induction protocols and underwent cycles of ART. Of 199 couples, 33 (16.6%) underwent conventional oocyte insemination (IVF-ET), and 166 (83.4%), intracytoplasmic sperm injection (ICSI). A total of 339 embryos were transferred (average 2.3 ± 0.7). The following variables were evaluated: [1] mean age of women, [2] mean age of men, [3] number of oocytes, [4] cause of infertility, [5] clinical pregnancy rate (PR) (defined as the number of patients with fetal heart beat divided by the number or ART cycles), and [6] pregnancy loss rate (number of biochemical pregnancies and spontaneous miscarriages divided by the number of β-hCG positive measurements).

Cervical cells were obtained with the combined use of an Ayre's spatula and an endocervical cytobrush before oocyte recovery by transvaginal ultrasound-guided follicular puncture (14), and were placed in 20 mL of PreservCyt Solution (Cytyc Corp, Marlborough, MA). Total DNA was extracted with the QIAamp Mini Kit (Qiagen, Hilden, Germany).

All investigations, both for IVF and ICSI procedures, were carried out on spermatozoa prepared with the swim-up technique. After centrifugation at $300 \times g$ for 7 minutes, the supernatants were discarded and 0.3 mL of fertilization medium (Sage, Pasadena, California) were added to the pellet for ICSI procedure, and 1 mL of fertilization medium for IVF insemination.

The samples were incubated at 37°C in 5% CO_2 for 45 minutes. After ICSI/IVF insemination the supernatant was centrifuged at $300 \times g$ for 7 minutes and the pellet was fixed in PreservCyt Solution (Cytyc Corp). Therefore HPV testing was performed directly on sperm cells.

All cervical and semen samples were processed for DNA extraction (16). Amplifications were carried out in a Mastercycler (Eppendorf, Hamburg, Germany) and the polymerase chain reaction (PCR) products were analyzed in 8% polyacrylamide gel (15). The HPV detection and typing were performed with the combined use of the HPV INNO-LiPA Genotyping system (Innogenetics N.V., Ghent, Belgium) (17, 18) and a nested PCR assay with PGMY09/11 and GP05+/06+ primer pairs, followed by direct cycle sequencing. The HPV genotypes were considered low risk or high risk according to two recently published epidemiological classifications of HPV types (19, 20).

The association between pregnancy and miscarriage for demographic and clinical variables was assessed using the χ^2 test or Fisher's exact test, as appropriate. A P value $\leq .05$ was considered statistically significant. To measure the association level, crude odds ratio (OR) and the 95% corresponding test-based confidence interval (CI) were calculated. Multivariate logistic regression was applied to obtain the adjusted OR with respective 95% CI. Variables tested for inclusion in the multivariate model were those significantly associated with miscarriage at $P \leq .05$ in the univariate analyses.

The 199 infertile couples who underwent ART were tested for HPV infection. The male partner was HPV positive in 9.5% of couples (19/199), whereas the female partner had a positive HPV DNA test in 17.5% of couples (35/199). Both partners were HPV positive in 4.5% (9/199) of couples. The most frequent genotypes in women were HPV-16 and HPV-66, whereas HPV-51 and HPV-52 were most frequently identified in the male partner of infertile couples.

Univariate analysis showed no statistically significant differences in the rate of pregnancy in terms of HPV status. When considering the HPV infection of couples, the PR was 33.3% and 31.6%, respectively, in HPV negative and HPV positive men, and 31.1% and 42.9%, respectively, in HPV negative and HPV positive women. Conversely, miscarriage rates showed statistically significant differences. Couples who underwent ART cycles experienced an increased risk of pregnancy loss when HPV DNA testing was positive in the male partner, compared with noninfected patients (66.7%–15%, $P<.01$). It is worth noting that all pregnancies in HPV-positive couples resulted in miscarriage, whereas there was a 15.9% overall miscarriage rate in HPV-negative couples ($P<.001$).

Finally, multivariate analysis showed a statistically significant risk of miscarriage correlated with male age and to the presence of male HPV infection (Table 1). Previous experimental studies have shown that infected spermatozoa may play a role as carriers of HPV DNA both in the reproductive tract and within the oocyte, with the possibility of detrimental effects during fertilization (21, 22).

A recent clinical study reported a significant reduction in pregnancies in women with papillomavirus cervical infection who had undergone IVF compared with women who were HPV negative (23% vs. 57%). However, no significant difference in the rate of miscarriage was found (23). It should be underscored that this study did not investigate HPV infection in the male partners.

In our prospective study we investigated the prevalence of HPV infection in infertile couples undergoing IVF procedures, and its impact on the reproductive outcome. The results of our study showed, for the first time, a significant increase in the risk of pregnancy loss when HPV infection was diagnosed in sperm cells of the male partner.

Noteworthy was the fact that all pregnancies achieved when both partners were HPV infected resulted in miscarriages. However, even if the risk of sexually transmitted diseases from infected seminal liquid has been widely studied, especially for some viral diseases (hepatitis B and HIV), little attention has been paid to the diagnosis of papillomavirus infection in ART programs.

Some studies have shown how HPV DNA is present in sperm cells both in infected and healthy individuals, and that sperm washing in preparing and purification of seminal liquid used in ART is not able to remove the risk of transmission of viral infection (24, 25). Although the precise mechanism through which the virus infects sperm cells has yet to be completely identified, a recent study has shown that HPV capsid binds to two distinct sites at the equatorial region of the sperm head surface, suggesting that sperm cells promote virus dispersal and mucosal penetration within the female genital tract (26). Several experimental studies have shown that the role of papillomavirus in causing pregnancy loss is probably associated with the possibility of transmitting virus-destabilized genes to oocytes during fertilization, thus determining apoptosis of embryonic cells through DNA fragmentation (22, 27, 28). Furthermore, our results may