HPV infection and ART: the andrologist's point of view

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ABSTRACT

Human papilloma virus infection is a common sexually transmitted disease. In addition to its tropism for epithelial mucosae and skin, HPV can also infect the male urogenital tract and strongly bind the sperm head, causing a reduction of semen quality, with detrimental effects on both natural and assisted fertility. An association between HPV and assisted reproductive technology (ART) programs has been reported, and HPV infection of semen seems to have a negative influence on the outcome of these procedures. None of the standard procedures commonly used for sperm selection before ART showed efficacy in eliminating HPV from the sperm head. Several strategies have been suggested to improve viral clearance in semen of infertile patients, such as counseling and anti-HPV vaccination. Moreover, these strategies were able to increase the natural fertility of couples with HPV semen infection. Finally, a modified swim-up with the addition of an enzymatic treatment, despite reducing sperm motility, was reported to eliminate the virus from the sperm head. In this manuscript we propose a flow chart for the management of infertile couples with HPV semen infection.

KEYWORDS

HPV, HPV semen infection, assisted reproductive techniques, HPV vaccines.

Introduction

Human papilloma virus (HPV) is a term referring to a group of small non-enveloped epitheliotropic viruses with a double-stranded circular DNA genome made up of 8000 bp. The virion of these viruses has an icosaedric shape, with a diameter of 55 nm, and it is constructed of 52 capsomeres, each containing five molecules of the major capsid protein L1, and a smaller number of the minor capsid protein L2 [1]. There are more than 200 HPV genotypes, adapted to particular epithelial tissues, such as anogenital skin and mucosa [2]. On the basis of its oncogenic potential, HPV can be divided into two different groups: high-risk and low-risk viruses. The former, which include the well-known types 16 and 18, have been classified as oncogenic to humans according to the International Agency for Research on Cancer [3], and may cause neoplastic transformations in the following epithelial areas: cervical, vulvar, anogenital, penile and oropharyngeal [4]. The latter, such as types 6 and 11, are responsible for benign diseases such as genital warts [5]. The morbidity of cutaneous HPV lesions, particularly in immunosuppressed people [4], also deserves to be mentioned. HPV infections are primarily contracted by direct contact of skin or mucosa with an infected lesion. Genital HPV infection is largely transmitted through sexual intercourse, mostly penetrative, although non-penetrative genital, oral genital, and manual genital types of contact are also possible routes of transmission [6]. Epidemiologically, there is a clear difference in the prevalence of HPV infection between females and males: while in women the prevalence is high in the first years after they become sexually active and decreases thereafter, in men the prevalence remains high throughout life [7]. A similar difference between females and males can be observed in the prevalence of anti-HPV antibodies: in a 2011 study, Desai *et al*. observed that females had a higher prevalence of antibodies for all the HPV genotypes analyzed [8].

Article history

Received 31 Aug 2023 – Accepted 14 Mar 2024

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HPV semen infection

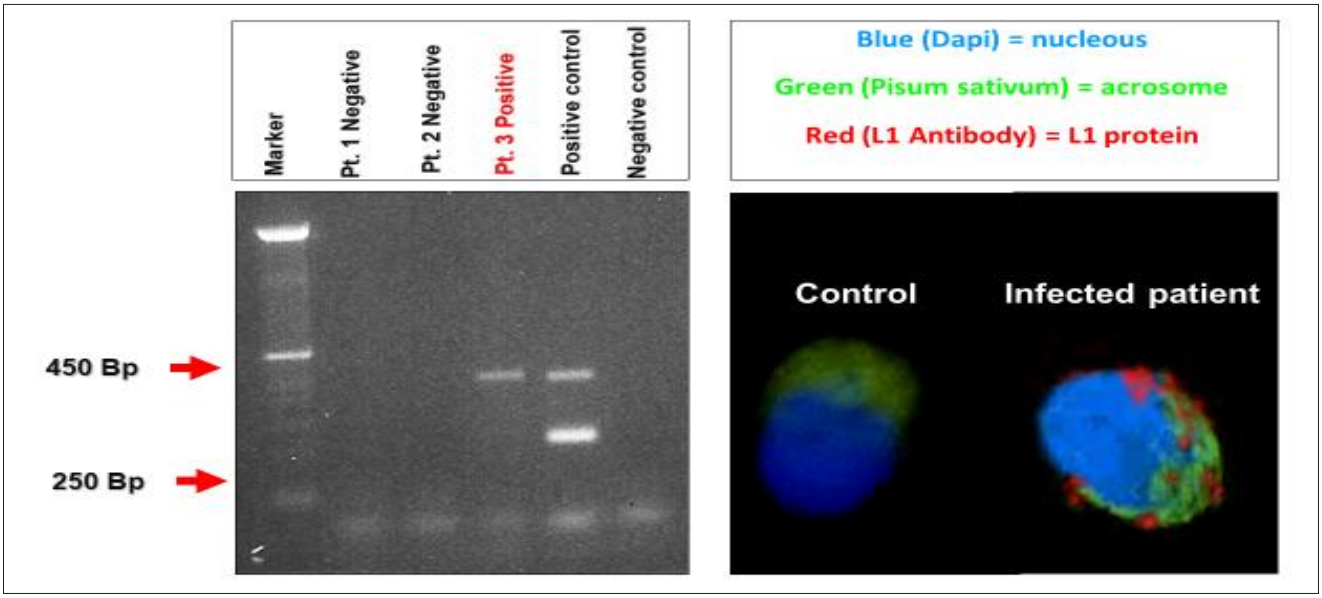
Semen infection is widely accepted, by clinicians and researchers, as a significant etiologic factor of male infertility and it is often associated with poor semen quality even in asymptomatic males. Chronic viral infections of the urogenital tract, especially human immune deficiency virus (HIV) infection, may be significantly associated with chronic low urethral tract inflammation and reduced fertility. Moreover, recent studies have shown that hepatitis B virus and hepatitis C virus semen infection can adversely modify semen parameters [9].

HPV virions can reside not only in the perianal region and external genitalia, including the foreskin, scrotum and glans penis, but also in the urethra, ductus deferens, epididymis, and testis [10]. Recently, a great deal of interest has been aroused

by the detection of HPV in semen, where it was found both in exfoliated cells and in spermatozoa [11]. In a 2019 study, Capra *et al*., through real-time polymerase chain reactions (PCRs), showed HPV DNA in every fraction of semen: spermatozoa, seminal cells, seminal fluid [12]. Figure 1 shows two examples of HPV infection in semen. Part A shows a PCR analysis of non-infected and infected patients. Part B shows the presence of HPV on the sperm surface in the acrosome region.

Because HPV infection in males is usually considered to be transient and without detrimental clinical consequences, its presence in semen has not been adequately investigated. However, some studies, showing a reduction of sperm motility, have suggested that HPV may play a role in male infertility. One of the first clinical studies on this issue showed that in a cohort of 24 patients the incidence of asthenozoospermia (lower total velocity, straight-line velocity, or mean amplitude of lateral head displacement) was significantly higher among patients with HPV 16 and 18 sperm infection than among non-infected controls (75% vs. 8%) [13]. In 2010, assessing a cohort of male sperm donors, Foresta *et al*. observed significantly reduced sperm motility in patients with HPV DNA in the semen, independently of the genotype of HPV detected [14]. Recently, Moghimi *et al*. observed a significantly higher prevalence of high-risk HPV in infertile men, compared with fertile ones, with the percentage of normal sperm morphology and the sperm motility rate found to be significantly lower in men with HPV infections [15]. Furthermore, semen infection has been recognized as a risk factor for the presence of anti-sperm antibodies (ASAs) [16,17]. In fact, the prevalence of ASAs seems to be higher in infertile patients than in general population, and their presence is associated with reduced sperm motility [18,19]. From these data, HPV semen infection seems to be a significant risk factor for male infertility. In this regard, many studies, reviews and meta-analyses have reported that HPV semen infection is related to reduced fertility both in natural and assisted conceptions [20-29]. Although the role of ASAs in reproduction is controversial, various mechanisms have been proposed, suggesting how they may affect male fertility. These include sperm agglutination, impaired cervical mucus penetration, complement-mediated sperm injury through the female genital tract, and interference with male-female gamete interaction [30]. As several kinds of semen infection have been associated with the development of ASAs, different authors have tried to establish whether HPV infection is associated with their presence. In 2013, Garolla et al. studied the possible association between the presence of HPV and ASAs and their clearance time in the semen of infected and non-infected infertile patients and in fertile control subjects. They showed that more than 40% of the infected infertile patients had ASAs on the sperm surface. In contrast, sperm auto-immunity was significantly lower in non-infected infertile men and in fertile control subjects. Moreover, infected patients had a higher mean percentage of ASAs compared with non-infected ones. These findings suggested that the presence of HPV DNA on the sperm surface may promote an antigenic stimulus for ASA formation; the authors documented the presence of both viral proteins and immunoglobulins in the same sperm cells of samples with positive sperm mixed antiglobulin reaction test results, and thus confirmed that sperm autoimmunity was HPV dependent. Notably, when immunofluorescence for HPV 16-L1 was present on the sperm surface, they observed co-staining for IgA and IgG. This observation suggests that semen infection could represent a new clinical condition associated with the presence of ASAs. Finally, in infected males, significant viral clearance (approximately 85.3%) was obtained after 24 months of follow up. Interestingly the reduction in sperm infection paralleled the disappearance of ASAs and was significantly related to a progressive improvement in sperm motility [31]. Some authors also reported the presence of HPV sperm infection in cryovials from sperm cryobanks [25,32,33]. Because these sperm were cryopreserved for future use in ART treatments, the authors/these findings raised concerns about a possible detrimental effect of HPV infection on fertilization

Figure 1 **On the left:** PCR analysis for HPV DNA performed in semen samples from infertile patients. Patient 3 showed positivity for HPV DNA. **On the right:** Immunofluorescence at confocal microscope showing an infected sperm with HPV located at acrosome level and a negative control.



and embryo development. In a 2010 study, the prevalence of HPV semen infection was investigated in cryobank cryovials of sperm from two different groups: a group of 98 patients affected by testicular cancer and a group of 60 healthy controls [33]. The percentage of samples with HPV was not significantly different between the patients and controls but, in contrast, the percentage of infected sperm was significantly higher in the patients. Further studies demonstrated that use of infected sperm for ART techniques was associated with a higher rate of treatment failures. Perino et al. reported that, among couples who underwent ART, the abortion rate was higher in HPV-infected couples than in non-infected ones. Furthermore, the risk was higher when the male partner was infected rather than the female [34]. Recently, Depuydt *et al*., studying the infection in semen samples (sperm donor samples used for ART) from three different cryobanks, observed that no pregnancies were obtained from infected semen samples [25].

On the basis of these data, several authors have suggested that serious consideration should be given to the possibility of screening semen samples for HPV before sperm banking or before their use in ICSI procedures [25,33].

THE mechanism of HPV-sperm binding

From the first studies, it was clear that HPV, unlike what happens when it infects epithelial cells, does not enter sperm cells. No study has shown an increased number of aneuploidies in sperm cells of infected patients [31]. However, some authors have reported an increase in the sperm DNA alteration index [28], while others did not observe this alteration [35].

The exact localization of HPV in sperm was clarified by *in vitro* immunofluorescence. To study the interaction between HPV and sperm cells, HPV type 16 capsids were used. It was shown that interaction of the virus with sperm could be reduced by two HPV infection inhibitors, heparin and carrageenan [36]. These findings were confirmed by others who performed fluorescent in situ hybridization (FISH) of infected semen samples from young adult men in order to study HPV DNA adhesion to the sperm cell surface [11]. To better understand the precise localization of HPV in the sperm head, immunofluorescence analysis was also performed using an antibody against the capsid protein L1 in intact sperm, which underwent an acrosome reaction. The signal for L1 was confirmed to be localized exclusively in the sperm head, especially in the equatorial region. After an acrosome reaction, the signal persisted in this region, thus excluding the presence of HPV in the acrosome and suggesting a preferential localization of HPV in the equatorial region. In the same study, a cytometry flow and an immunofluorescence analysis made it possible to demonstrate that binding between HPV and sperm cells involves the L1 capsid protein and the glycosamino-glycan Syndecan-I, expressed in the equatorial region of the sperm head. Moreover, PCR analysis of L1 gene expression confirmed infection of sperm by HPV DNA. Taken together, these data showed that sperm can be infected by HPV and that both HPV DNA and L1 capsid protein are bound to the sperm head, almost exclusively in the equatorial region [37].

In the literature, there is little evidence concerning the possibility that routine sperm washing techniques, used to select sperm before assisted reproduction, can eliminate HPV DNA from semen. On this basis, Foresta et al. evaluated viral clearance in infected sperm from infertile subjects treated with three different standard sperm selection procedures: sperm washing by centrifugation, discontinuous Ficoll-Paque Plus density gradients, and direct swim-up [38]. After sperm washing procedures, HPV detection, performed by in situ hybridization analysis, showed that the percentage of infected sperm remained unmodified. Using Ficoll gradients and swim-up procedures, there was a significant reduction in the percentage of infected sperm. However, despite the more efficient clearance obtained by swim-up, complete removal of infected sperm was recorded in only 19% of cases. These results suggest both the presence of very strong binding between HPV and sperm, and low efficacy of conventional sperm selection techniques in eliminating infected sperm.

Infected sperm and ART

While data are available on HIV, no precise data are available concerning the use of HPV-infected sperm in ART procedures. Moreover, little is known about whether infected cells used in ICSI procedures may interfere with the mechanisms of fertilization, implantation and embryo development. The literature is still insufficient to allow final conclusions to be drawn regarding the effect of HPV infection on the most important reproductive outcomes following natural and assisted fertility in women. However, it seems that when the infection is present in the male partner there is a negative effect on the ongoing pregnancy rate and live birth rate, as well as an increase in the rate of miscarriage [34]. Taken together, these findings should be further investigated in order to understand the real impact of HPV semen infection on fertility outcome. Interestingly, the presence of the virus has been associated with increased rates of wastage and miscarriage in both animals and humans, linked to transformation of cells, gene interruption and loss of chromosome heterozygosity or fragmentation of. host DNA fragmentation and apoptosis

In vitro studies

Many *in vitro* studies have shown a negative influence of HPV infection on several aspects of human fertility. For example, HPV transfected blastocyst and trophoblastic cells showed a reduction in decidua invasion capacity, potentially responsible for failure of the trophoblastic cell to invade the maternal uterine wall, subsequent placental dysfunction, and adverse pregnancy outcome [39]. Furthermore, several experimental studies have demonstrated that HPV contributes to pregnancy loss by transmitting viral genes to oocytes and determining DNA fragmentation and apoptosis of embryonic cells. The effects on embryo development seem to be stage related: from cellular instability at the two-cell level, through a decrease in blastocyst formation, to inhibition of the blastocyst hatching process [40,41]. However, there is little evidence to support the suggestion that HPV-infected sperm are able to interfere with embryo develop-

ment when injected into the oocyte cytoplasm (such as during *in vitro* fertilization). To better understand this process, Foresta *et al*. performed an *in vitro* study evaluating the ability of a virus-infected sperm to transfer HPV DNA and capsid proteins to the oocyte during fertilization. After transfecting a human sperm with a plasmidic episome containing HPV E6 and E7 proteins, they used the hamster egg-human sperm penetration test to show the ability of infected sperm to transfer the capsid protein L1 to the oocyte, and the expression of E6 and E7 viral protein in the fertilized oocyte [37]. Their findings demonstrated that both sperm transfected with E6 and E7 genes and sperm exposed to HPV L1 capsid protein are able to penetrate the oocyte. This laboratory data, combined with the observation that HPV DNA is detected in a larger proportion of spontaneous abortions than voluntary terminations of pregnancy [34], may suggest an active role for HPV (which is carried to the egg by the sperm) in preterm gestation. This phenomenon could lead to an increase in the fragmentation of embryonic DNA resulting in alteration and apoptosis of the embryo [37]. It should be remembered, however, that the situations described above refer to *in vitro* models that may not reflect the *in vivo* situation, where the entry of viral DNA into the egg has never been proven [31].

In vivo studies

In experimental murine models it was found that HPV genomes are expressed in fertilized oocyte, blastocyst and trophoblastic cells. The viral genome was frequently associated with cellular changes such as inhibition of the blastocyst hatching process, and DNA fragmentation and apoptosis, often with lethal effects for early embryo development [42]. These data have been confirmed by further studies, in which authors highlighted that the detrimental effects of HPV on embryo development are stage specific: early exposure of embryos to HPV was linked to death of the two-cell embryo, while delaying exposure, to the late embryo stages, seemed to permit embryo development [40]. In addition, as reported before, Depuydt *et al*., studying the infection in semen samples (provided by sperm donors, for use in ART procedures) from three different cryobanks, observed that no pregnancies were obtained from infected semen samples [25].

Treatment of infertile infected patients

It is well known that HPV infections are frequently long lasting, and it has been suggested that certain anatomical sites could act as viral reservoirs able to support persistence of the infection. Moreover, since there is still no effective and resolutive treatment for HPV infection and its related problems, including the consequences of HPV infection on fertility, it is mandatory, pending the development of effective prevention and therapy solutions, to educate and counsel infected couples, especially when they are seeking fertility treatments and ART procedures.

Counseling

It is important to educate and provide careful counseling to couples where at least one partner is infected with HPV. A 2014 study clearly showed the effectiveness of this strategy. In fact, couples in this situation who had carefully followed advice given to them during the first counseling session showed a significant reduction in the persistence of the infection. More specifically, partners were advised to: pay particular attention to hygiene of both of their reproductive tract and their hands; use only their own underwear and towels; avoid oral and anal sex; reduce or quit smoking; have only protected intercourse; and, in the case of HPV-related lesions, treat them and monitor the genital area over time. Couples who complied strictly with the multiple suggestions made during the first counseling session, when monitored at 6, 12, 18 and 24 months, showed significantly reduced viral persistence [43].

Vaccination

HPV vaccines are produced using recombinant DNA technology. They are prepared from purified L1 structural proteins that self-assemble to form HPV-like empty shells, termed virus-like particles, which mimic the native HPV external coat. Since they are hollow and do not contain viral DNA, they have the potential to elicit an immune response without the ability to cause infection. This makes them completely safe even for immunosuppressed people. Vaccination is recommended before the start of sexual activity, and national immunization programs often target 11-12-year-olds.

Recent evidence suggests that vaccination is a valid tool even in patients who have already contracted the infection [44]. In particular, adjuvant vaccination in adult males with persistent HPV semen infection is able to reduce the average elimination time of the virus, thus accelerating the healing time. Moreover, it has been shown that patients with semen infection receiving vaccination have a faster rate of seroconversion and greater viral clearance, identified as the percentage of HPV DNA detectable in seminal fluid [45]. In the study in question, the authors evaluated three groups of patients with HPV semen infection: patients without anti-HPV antibodies (seronegative) at recruitment, who were candidates for vaccination (vaccine arm), patients seronegative at recruitment but not candidates for vaccination (seronegative control arm), and patients who were seroconverted at recruitment (seroconverted control arm). During the two-year follow up, vaccinated patients showed significantly faster healing of disease paralleled by a reduction in the percentage of HPV-positive sperm in the semen and massive development of high-titer seroconversion. Interestingly, a group of 30 patients displaying seroconversion at the start of the study, despite having a much lower serum antibody titer, also showed some improvement of humoral immunity in the anti-HPV response. In view of the aforementioned effects of HPV on oocyte fertilization and embryo development, these data were highly suggestive of a possible impact of male vaccination on both natural and assisted fertility. This issue was addressed in a more recent study in 2018, where 151 infertile couples were retrospectively selected according to detection of HPV DNA in the male partner's semen. In this study, 79 males received the quadrivalent vaccine as off-label vaccine. The remaining male partners from 71 couples refused to receive vaccination and served as controls. Over a one-year follow up, variations in sperm parameters and data on natural

fertility were recorded in both vaccinated and control patients. Compared with the latter, the vaccinated patients showed faster viral clearance paralleled by a progressive improvement of sperm motility and a reduction in the percentage of anti-sperm antibodies. Furthermore, the couples where the male partner received vaccination recorded, compared with controls during the one-year follow-up, a higher number of pregnancies and deliveries associated with a lower miscarriage rate [46].

To assess and clarify any prognostic factor able to predict the effectiveness of the HPV vaccine in promoting viral clearance from the male genital tract, De Toni et al. performed a retrospective cohort study, collecting clinical records from 379 male patients with persistent seminal HPV detection who received therapeutic HPV vaccination. They recorded i) genital HPV-DNA assessment by INNO-LiPA genotyping; ii) semen HPV-DNA analysis using the FISH method; iii) serum antibody titer. All the data were collected before vaccine administration (T0) and 6 months after the vaccination cycle ended (T1). The authors observed complete clearance of genital HPV DNA in 326 (86%) patients and assessed that serum HPV-antibody titer at T1 was the most important prognostic factor associated with HPV DNA clearance, suggesting that anti-HPV antibodies represent a suitable marker of adequate immune response to HPV vaccination in patients with genital infection, and, therefore, of healing [47].

Taken together, these results represent a rationale for a significant improvement in the management of infertile men with risk factors for semen infection by HPV.

Modified swim-up

Recently, studies investigating the role of HPV infection in infertile couples undergoing ART procedures showed a correlation between HPV sperm infection and increased risk of pregnancy loss after ART [23,34]. Considering the high prevalence of HPV semen infection in infertile patients and the absent or poor effect of standard semen selection procedures (centrifugation, discontinuous density gradient and direct swim-up) in eliminating HPV from sperm, it is of paramount importance to find new techniques to achieve sperm negativization from HPV infection. In an attempt at eliminating HPV from infected sperm samples, a recent study tested a modified swim-up with addition of the enzyme heparinase-III [48]. The rationale for this treatment was to cleave the binding between HPV and its putative ligand, Syndecan-I, on the sperm surface. Compared with the normal swim-up technique, the modified version was able to abolish binding between HPV and sperm in 100% of cases of infected sperm (as shown in Figure 2). This was confirmed by FISH, a technique allowing detection of the HPV virion bound to the sperm head surface [11].

Nonetheless, compared with the state before the treatment, reductions in viability and motility were shown in the sperm of patients who underwent modified swim-up procedures. Even though this reduction was not associated with impaired DNA integrity, potentially allowing the use of selected sperm in ART, the use of heparinase III is not currently approved for use in ART procedures.

Another study used the same swim-up technique modified with another enzyme, hyaluronidase, which is already approved for some ARTs. Given its mechanism, able to cleave the linkages between N-acetylglucosamine and glucuronate, the authors hypothesized that the enzyme might be able to break the linkage between HPV virions and syndecan-like GAG moieties on the sperm surface. In this study the authors collected semen samples from, respectively, five normo-zoospermic fertile volunteers (control subjects), five infertile oligo-astheno-teratozoospemic patients (both these two groups with negative detection of HPV-DNA in semen), and 12 infertile patients with HPV DNA detected in semen. Semen samples from the first two groups were incubated with HPV VLPs from the quadrivalent HPV vaccine. The authors investigated the effect of the enzyme at different concentrations both on sperm incubated with HPV VLPs and on real samples from infertile patients with positive

Figure 2 HPV infected semen sample before and after treatment with Heparinase III.

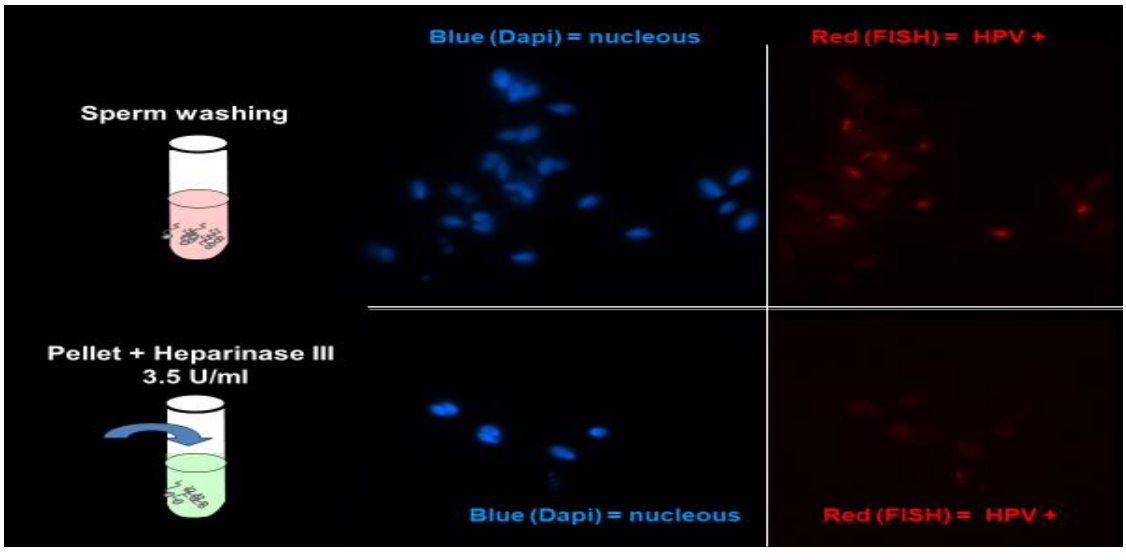
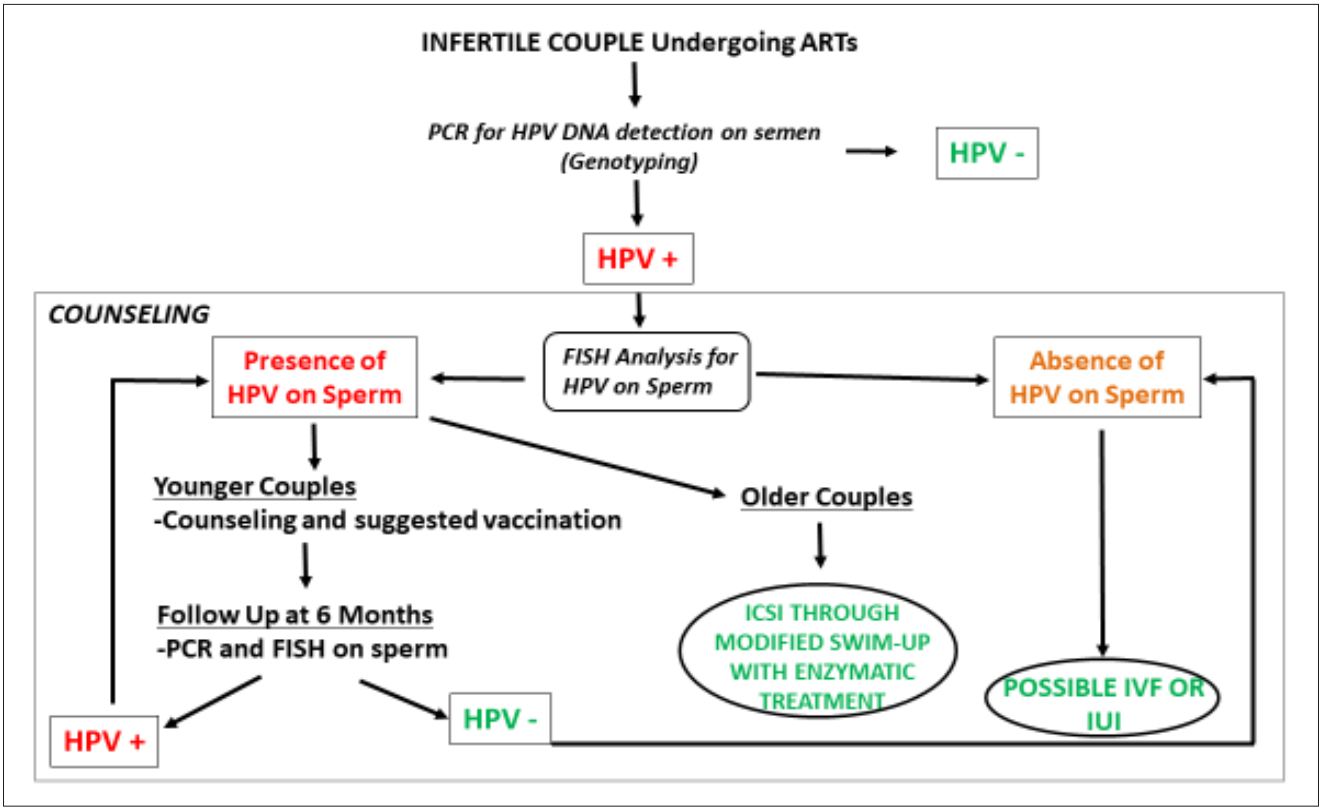


Figure 3 Flow chart for the management of infertile couples undergoing ART based on the presence or absence of HPV semen infection (**HPV**: Human Papillomavirus; **PCR**: Polymerase chain reaction; **ICSI**: Intra cytoplasmic sperm injection; **FISH**: Fluorescent in situ hybridization; **ARTs**: Assisted reproductive technologies



semen detection of HPV DNA. Total removal of virions was observed at the highest concentration of the enzyme (80 UI/ml). Although this treatment, too, led to significant alteration of sperm motility, this effect was not sufficient to compromise use of the spermatozoa in ART. In support of this consideration, no alterations of sperm morphology, DNA integrity or markers of capacitation and acrosome reaction were observed.

New experiments, aimed at testing other enzymes capable of removing HPV from the sperm surface without detrimental effects on sperm quality, are in the trial phase in our lab [49].

Conclusions

Because HPV sperm infection may be a major cause of male infertility and seems to play an important role in spontaneous abortions, semen samples from patients undergoing ART should be always tested for the presence of HPV. Figure 3 shows a flow chart summarizing the main steps in the management and treatment of infertile couples undergoing ART in the presence of HPV semen infection.

References

1. Doorbar J, Quint W, Banks L, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30 Suppl 5:F55-70.
2. Bouvard V, Baan R, Straif K, et al; WHO International Agency for Research on Cancer Monograph Working Group. A review of human carcinogens--Part B: biological agents. Lancet Oncol. 2009;10(4):321-2.
3. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Human Papillomaviruses. Lyon (FR): International Agency for Research on Cancer; 2007. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 90.) Available from: https://www.ncbi.nlm.nih.gov/books/NBK321760/.
4. Cubie HA. Diseases associated with human papillomavirus infection. Virology. 2013;445(1-2):21-34.
5. de Sanjosé S, Brotons M, Pavón MA. The natural history of human papillomavirus infection. Best Pract Res Clin Obstet Gynaecol. 2018;47:2-13.
6. Dunne EF, Park IU. HPV and HPV-associated diseases. Infect Dis Clin North Am. 2013;27(4):765-78.
7. De Vuyst H, Clifford G, Li N, Franceschi S. HPV infection in Europe. Eur J Cancer. 2009;45(15):2632-9.
8. Desai S, Chapman R, Jit M, et al. Prevalence of human papillomavirus antibodies in males and females in England. Sex Transm Dis. 2011;38(7):622-9.
9. Garolla A, Pizzol D, Bertoldo A, Menegazzo M, Barzon L, Foresta C. Sperm viral infection and male infertility: focus on HBV, HCV, HIV, HPV, HSV, HCMV, and AAV. J Reprod Immunol. 2013;100(1):20-9.
10. Dunne EF, Nielson CM, Stone KM, Markowitz LE, Giuliano AR. Prevalence of HPV infection among men: A systematic review of the literature. J Infect Dis. 2006;194(8):1044-57.
11. Foresta C, Pizzol D, Moretti A, Barzon L, Palù G, Garolla A. Clinical and prognostic significance of human papillomavirus DNA in the sperm or exfoliated cells of infertile patients and subjects with risk factors. Fertil Steril. 2010;94(5):1723-7.
12. Capra G, Schillaci R, Bosco L, Roccheri MC, Perino A, Ragusa MA. HPV infection in semen: results from a new molecular approach. Epidemiol Infect. 2019;147:e177.

13. Lai YM, Lee JF, Huang HY, Soong YK, Yang FP, Pao CC. The effect of human papillomavirus infection on sperm cell motility. Fertil Steril. 1997;67(6):1152-5.
14. Foresta C, Garolla A, Zuccarello D, et al. Human papillomavirus found in sperm head of young adult males affects the progressive motility. Fertil Steril. 2010;93(3):802-6.
15. Moghimi M, Zabihi-Mahmoodabadi S, Kheirkhah-Vakilabad A, Kargar Z. Significant correlation between high-risk HPV DNA in semen and impairment of sperm quality in infertile men. Int J Fertil Steril. 2019;12(4):306-309.
16. Ochsendorf FR. Sexually transmitted infections: impact on male fertility. Andrologia. 2008;40(2):72-5.
17. Rusz A, Pilatz A, Wagenlehner F, et al. Influence of urogenital infections and inflammation on semen quality and male fertility. World J Urol. 2012;30(1):23-30.
18. Heidereich A, Bonfig R, Wilbert DM, Strohmaier WL, Engelmann UH. Risk factors for antisperm antibodies in infertile men. Am J Reprod Immunol. 1994;31(2-3):69-76.
19. Piroozmand A, Mousavi Nasab SD, Erami M, et al. Distribution of human papillomavirus and antisperm antibody in semen and its association with semen parameters among infertile men. J Reprod Infertil. 2020;21(3):183-8.
20. Cao X, Wei R, Zhang X, Zhou J, Lou J, Cui Y. Impact of human papillomavirus infection in semen on sperm progressive motility in infertile men: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2020;18(1):38.
21. Capra G, Notari T, Buttà M, Serra N, Rizzo G, Bosco L. Human papillomavirus (HPV) infection and its impact on male infertility. Life (Basel). 2022;12(11):1919.
22. Foresta C, Noventa M, De Toni L, Gizzo S, Garolla A. HPV-DNA sperm infection and infertility: from a systematic literature review to a possible clinical management proposal. Andrology. 2015;3(2):163-73.
23. Garolla A, Engl B, Pizzol D, et al. Spontaneous fertility and in vitro fertilization outcome: New evidence of human papillomavirus sperm infection. Fertil Steril. 2016;105(1):65-72.e1.
24. Lyu Z, Feng X, Li N, et al. Human papillomavirus in semen and the risk for male infertility: a systematic review and meta-analysis. BMC Infect Dis. 2017;17(1):714.
25. Depuydt CE, Donders G, Verstraete L, et al. Time has come to include Human Papillomavirus (HPV) testing in sperm donor banks. Facts Views Vis Obgyn. 2018;10(4):201-5.
26. Siristatidis C, Vaidakis D, Sertedaki E, Martins WP. Effect of human papilloma virus infection on in-vitro fertilization outcome: systematic review and meta- analysis. Ultrasound Obstet Gynecol. 2018;51(1):87-93.
27. Xiong YQ, Chen YX, Cheng MJ, He WQ, Chen Q. The risk of human papillomavirus infection for male fertility abnormality: a meta-analysis. Asian J Androl. 2018;20(5):493-7.
28. Boeri L, Capogrosso P, Ventimiglia E, et al. High-risk human papillomavirus in semen is associated with poor sperm progressive motility and a high sperm DNA fragmentation index in infertile men. Hum Reprod. 2019;34(2):209-17.
29. Weinberg M, Sar-Shalom Nahshon C, Feferkorn I, Bornstein J. Evaluation of human papilloma virus in semen as a risk factor for low sperm quality and poor in vitro fertilization outcomes: a systematic review and meta-analysis. Fertil Steril. 2020;113(5):955-969.e4.
30. Francavilla F, Santucci R, Barbonetti A, Francavilla S. Naturally-occurring antisperm antibodies in men: interference with fertility and clinical implications. An update. Front Biosci. 2007;12:2890-911.
31. Garolla A, Pizzol D, Bertoldo A, De Toni L, Barzon L, Foresta C. Association, prevalence, and clearance of human papillomavirus and antisperm antibodies in infected semen samples from infertile patients. Fertil Steril. 2013;99(1):125-131.e2.
32. Depuydt CE, Donders GGG, Verstraete L, et al. Infectious human papillomavirus virions in semen reduce clinical pregnancy rates in women undergoing intrauterine insemination. Fertil Steril. 2019;111(6):1135-44.
33. Foresta C, Ferlin A, Bertoldo A, Patassini C, Zuccarello D, Garolla A. Human papilloma virus in the sperm cryobank: An emerging problem? Int J Androl. 2011;34(3):242-6.
34. Perino A, Giovannelli L, Schillaci R, et al. Human papillomavirus infection in couples undergoing in vitro fertilization procedures: impact on reproductive outcomes. Fertil Steril. 2011;95(5):1845-8.
35. Cortés-Gutiérrez EI, Dávila-Rodríguez MI, Fernández JL, et al. The presence of human papillomavirus in semen does not affect the integrity of sperm DNA. Andrologia. 2017;49(10).
36. Pérez-Andino J, Buck CB, Ribbeck K. Adsorption of human papillomavirus 16 to live human sperm. PLoS One. 2009;4(6):e5847.
37. Foresta C, Patassini C, Bertoldo A, et al. Mechanism of human papillomavirus binding to human spermatozoa and fertilizing ability of infected spermatozoa. PLoS One. 2011;6(3):e15036.
38. Foresta C, Pizzol D, Bertoldo A, Menegazzo M, Barzon L, Garolla A. Semen washing procedures do not eliminate human papilloma virus sperm infection in infertile patients. Fertil Steril. 2011;96(5):1077-82.
39. Gomez LM, Ma Y, Ho C, McGrath CM, Nelson DB, Parry S. Placental infection with human papillomavirus is associated with spontaneous preterm delivery. Hum Reprod. 2008;23(3):709-15.
40. Henneberg AA, Patton WC, Jacobson JD, Chan PJ. Human papilloma virus DNA exposure and embryo survival is stage-specific. J Assist Reprod Genet. 2006;23(6):255-9.
41. Noventa M, Andrisani A, Gizzo S, Nardelli GB, Ambrosini G. Is it time to shift the attention on early stages embryo development to avoid inconclusive evidence on HPV-related infertility: debate and proposal. Reprod Biol Endocrinol. 2014;31(12):48.
42. Cabrera M, Chan PJ, Kalugdan TH, King A. Transfection of the inner cell mass and lack of a unique DNA sequence affecting the uptake of exogenous DNA by sperm as shown by dideoxy sequencing analogues. J Assist Reprod Genet. 1997;14(2):120-4.
43. Garolla A, Pizzol D, Vasoin F, Barzon L, Bertoldo A, Foresta C. Counseling reduces HPV persistence in coinfected couples. J Sex Med. 2014;11(1):127-35.
44. Giuliano AR, Lazcano-Ponce E, Villa LL, et al. The human papillomavirus infection in men study: human papillomavirus prevalence and type distribution among men residing in Brazil, Mexico, and the United States. Cancer Epidemiol Biomarkers Prev. 2008;17(8):2036-43.
45. Foresta C, Garolla A, Parisi S, et al. HPV prophylactic vaccination in males improves the clearance of semen infection. EBioMedicine. 2015;2(10):1487-93.
46. Garolla A, De Toni L, Bottacin A, et al. Human Papillomavirus Prophylactic Vaccination improves reproductive outcome in infertile patients with HPV semen infection: a retrospective study. Sci Rep. 2018;8(1):912.
47. De Toni L, Muscianisi F, Corsini C, et al. Serum anti-HPV antibody titer as a marker of vaccine effectiveness in males with genital infection. Vaccines (Basel). 2020;8(4):743.
48. Garolla A, Lenzi A, Palù G, et al. Human papillomavirus sperm infection and assisted reproduction: a dangerous hazard with a possible safe solution. Hum Reprod. 2012;27(4):967-73.
49. De Toni L, Cosci I, Carosso A, et al. Hyaluronidase-based swim-up for semen selection in patients with human papillomavirus semen infection. Biol Reprod. 2021;104(1):211-22.