Annals of West University of Timişoara, ser. Biology, 2020, vol. 23 (2), pp.159-168

THE EFFECT OF HUMAN PAPILLOMA VIRUS ON SPERMATOGENESIS

***Carla-Petra PORGE*[1], *Darius-George OANEA*[2], *Andrei NICA*[3], *Delia HUŢANU*[1*]**
[1] West University of Timişoara, Faculty of Chemistry, Biology, Geography, Department of Biology-Chemistry
[2] West University of Timişoara, Faculty of Math and Informatics, Department of Informatics
[3]National Transplant Agency
**Corresponding author's e-mail: delia.hutanu@e-uvt.ro*
Received 14 July 2020; accepted 20 November 2020

ABSTRACT
The degree of male infertility has risen sharply following studies in recent years. One of the most well-known viruses for its negative effect on the reproductive system is human Papillomavirus. In the male reproductive system, the virus produces changes in spermatogenesis by decreasing the number of sperm, reducing their motility and morphological changes in sperm. From the analysis of 14 studies, there are three hypotheses according to which the parameters of the spermogram are not changed at extreme levels with the presence of infection with Papillomavirus, the parameters of the spermogram, especially motility are strongly affected due to disease and the introduction of an enzymatic treatment encouraging. The practical part was supported by statistical mechanisms for interpreting the results using Python (scikit learn library), thus bringing reliable numerical reports that support the theory. This paper intended to study the scientific literature. The results obtained after analyzing the works advocate for the introduction in human assisted reproduction laboratories as a routine test to identify the presence of HPV (Human Papillomavirus) in men.
KEY WORDS: *spermatogenesis, male infertility, assisted human reproduction, human Papillomavirus*

INTRODUCTION

HPV infection is the best known viral infection with direct transmission through sexual contact and indirect skin contact. There are currently over 100 types of HPV, of which HPV16 and HPV18 have a high degree of infectivity. In recent years, in addition to infection with this virus on the female genital tract, it has been discovered that there is increased activity in the case of males. Following research conducted in recent years, the hypothesis was presented in which the human *Papillomavirus* influences the survival rate of sperm.

Spermogram parameters such as sperm motility, sperm viscosity, pH, and sperm morphology were negatively affected by infection onset. The virus is not genetically transmissible, but a standard treatment is not available yet, but only the prevention of infection by vaccination, but the two vaccines do not provide 100% protection against all strains of this virus. This paper has focused on the possible detrimental effect on male infertility as a single note. In the case of patients

undergoing assisted reproduction, it can be argued that a possible identification of the presence of the virus in men should introduce a screening test.

MATERIALS AND METHODS

The research in specialized literature was performed by systematically classifying the information in the databases Fertility and Sterility, Human Reproduction, PubMed, and BMC Med, for articles published starting from the first available data until 15.06.20. The search for information was limited to studies representative of the human species, and English was predominantly active in the information used. Searches of bibliographic lists of peer-reviewed articles identified by searches for additional reference material were also performed.

Several programming environments, programming languages, and software frameworks were used to compile the statistics. One of these is Python, a powerful language, OOP (Object Oriented Programming), designed by Guido van Rossum in 1989. Applications written in Python are mainly used for statistics and artificial intelligence, but you can also write Web applications and frameworks such as Django. The IDE (Integrated Development Environment) used is Jupyter Notebook and is a very convenient way to manage Python files. Anaconda was used to integrate the Jupyter Notebook into the environment. The "Jupiter Notebook" command creates a local server. Once the server is hosted on localhost, new files could be created; old ones could be edited, and so on. With Jupyter Notebook, it is easy to redistribute a notebook's contents, as it supports HTML5 preview. Git is a revision control system for the UNIX platform. Like Mercurial, Git is a distributed system and does not maintain a primary database. It is used in development teams. Matplotlib is a Python library with which graphics were created. NumPy and Pandas are also two Python libraries that have been used predominantly in compiling the work's statistics. They help to manipulate vectors and matrices.

RESULTS AND DISCUSSION

From the information research conducted on 14 studies, only one proposes treating human *Papillomavirus* infection by applying the enzyme Heparinase-III.

Garolla et al (2012) support testing by modified application, with the addition of Heparinase-III, to try to remove HPV from infected sperm samples. This treatment was to split the connection between HPV and its receptor, syndecan-I, on the surface of the sperm. It was confirmed that naturally, infected sperm show a significant reduction in progressive motility, and the same finding was demonstrated in artificially infected semen obtained by incubating sperm with viral capsid protein. Parameters such as pH, vascularity, and semen volume showed normal values. Moreover, the FISH analysis showed that patients with an HPV infection have a high prevalence (20%) of infected sperm. The tenacity of HPV binding in sperm has been demonstrated, and direct application has not effectively eliminated sperm infection.

Analysis for HPV in sperm collected before incubation with HPV 16-L1 (incubated L1) and after incubation, with or without treatment with Heparinase III (Hep-III), can be seen in Figure 1.

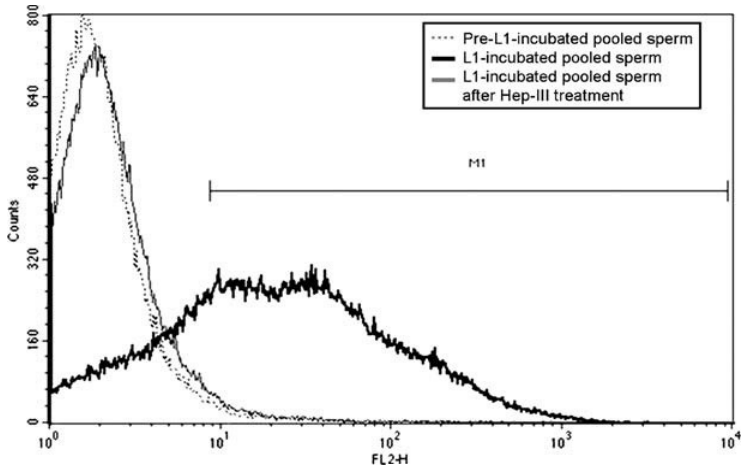

FIGURE 1. HPV Analysis (after Garolla et al. 2012)

Two of the analyzed studies claimed that the parameters of the spermogram are not changed at maximum levels. However, the presence of the *Papillomavirus* was detected in the analyzed samples. Schillaci et al. (2013) state that most genotypes were from the high-risk group, and an example of HPV-52 was found. Of the 24 infected patients, 83.3% had high-risk HPV and 16.7% low-risk HPV; this can be seen in Figure 2. The evaluation was done by PCR, polymerase chain reaction using of which millions of copies of sperm DNA samples were made for better study.

Luttmer et al. (2015) present a study conducted with the help of 430 men, of which 64, 14.9% were tested positive for human *Papillomavirus*. A comparison of spermogram parameters in samples of HPV-infected patients can be seen in Figure 3. In this group of male partners in couples wishing to assess fertility, viral DNA frequently fragments in semen were confirmed. No change in spermogram values could be demonstrated.

The studies that demonstrated the negative influence on the parameters of the spermogram by infection with the human *Papillomavirus* are 11 in number and are located on a chronological axis from 1997 to 2019.

In particular, sperm motility was affected, and other parameters such as sperm volume, pH, sperm morphology, and their vitality were altered with the occurrence of human *Papillomavirus* infection. These parameters were evaluated according to the protocol within the "World Health Organization" for spermatographic analysis.

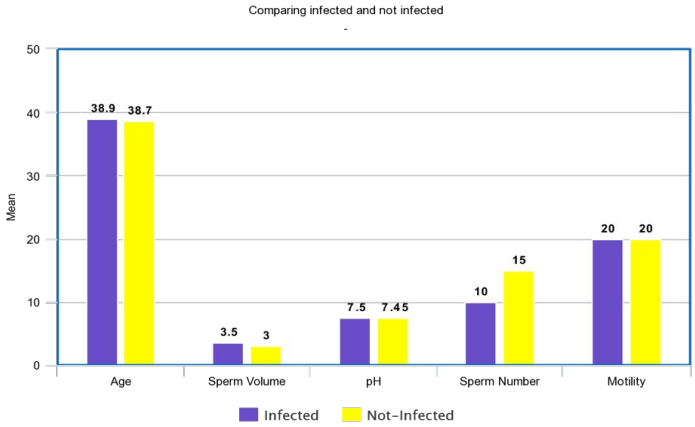

FIGURE2. Comparison of the samples (Link accessed on 20.06.20)

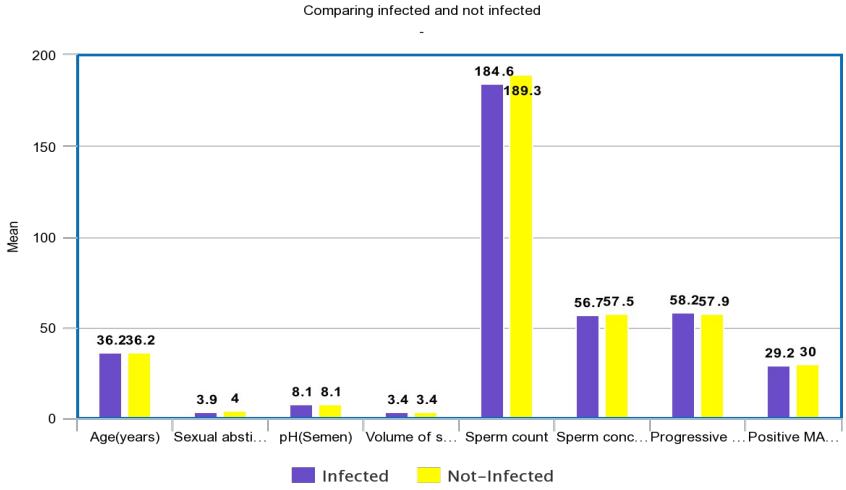

FIGURE 3. Comparison of spermogram parameters (Link accessed on 20.06.20)

Lai et al. (1997) support a significant reduction in the rate of advance of infected sperm, but the frequency of sperm beats has not changed. The study was performed on 27 male patients, 11 of whom were tested positive for HPV. The following sperm motility parameters were measured: linear velocity, curvilinear velocity, mean amplitude of lateral displacement of the head, linearity, cross-beat frequency, and rectilinear direction. For each measurement, at least 100 sperm were

studied. All sperm analyzes were performed using the CASA method. The method involves placing the sperm sample on the microscope plate. This microscope has a high-resolution video camera attached. The camcorder enters the data into the computer where it is analyzed by software. A computerized analysis of the semen will provide several parameters that are useful to the fertility specialist. Determining the number of rapidly moving sperm is one of the most important factors in assessing male fertility. It can be seen in Table 1 that none of the 24 patients had a history of teratozoospermia. It was found that viral DNA can be found in human sperm and that the presence of *Papillomavirus* in sperm appears to affect some of the motility parameters of the examined sperm; and that the incidence of asthenozoospermia may be associated with the presence of HPV in sperm.

TABLE 1. The presence of HPV16 and HPV18 located in the DNA and RNA of sperm. Incidence of oligozoospermia and asthenozoospermia (Lai et all. 1997)

	HPV	Oligozoospermia	Astenozoospermie
HPV16 **DNA** **ARN**	6/24(25) 2/24(8)	2/6(33)	5/6(83)
HPV18 **DNA** **ARN**	11/24(46) 5/24(21)	2/11(18)	8/11(73)

Bezold et al. (2006) state that sperm count was reduced due to human *Papillomavirus* infection and that sperm motility was reduced without statistically significant data. The study's action was performed with the help of 179 men who were infected with HPV. Leukocytospermia (LCS), also known as leukospermia, pyospermia, or pyosemia, is a term used to refer to abnormally high concentrations of white blood cells (WBCs) in sperm and has been defined by the World Health Organization as 10^6 WBC per milliliter of semen. The prevalence of LCS in patients with male infertility ranges from 2% to 40% in published reports, and elevated WBC concentrations in sperm have been associated with low sperm quality. Quantification of the number of pre-PCR copies was performed using a standard external curve and internal controls of a similar size (mimicry). Internal controls were performed using PCR-MIMIC-Construction Kit. In conclusion, using molecular analyzes, an unexpectedly high prevalence of sexually transmitted pathogens in sperm was detected in patients with asymptomatic infertility. The pathogenic DNA was not associated with LCS or other inflammation markers but was associated with a decrease in the analyzed parameters.

Foresta et al. (2009) state the analysis of 200 samples from men, of which 100 had HPV infection, and 100 did not have the infection. After liquefaction at room temperature, sperm volume, pH, sperm concentration, viability, motility, and normal morphology were determined following the guidelines of the "World Health

Organization" for semen analysis. Virus identification for primary study patients was made by amplifying PCR of HPV DNA and real-time PCR for HPV-16 DNA, performed on the DNA of sperm from young men who had previously unprotected sexual contact.

Foresta et al. (2010) present a study involving over 200 men who were divided into four groups: the first group included 64 men with genital warts (group A), 66 men had a positive female partner with HPV (group B), and 108 men showed infertility (group C). Ninety fertile men with an average age of 34 years were used as control subjects (group D). Figure 4 shows no significant differences between the frequency of sexual intercourse and the age of first sexual contact in the four groups. Sperm motility was negatively affected, and HPV DNA was localized to the sperm head, but it is unclear whether it enters the nucleus.

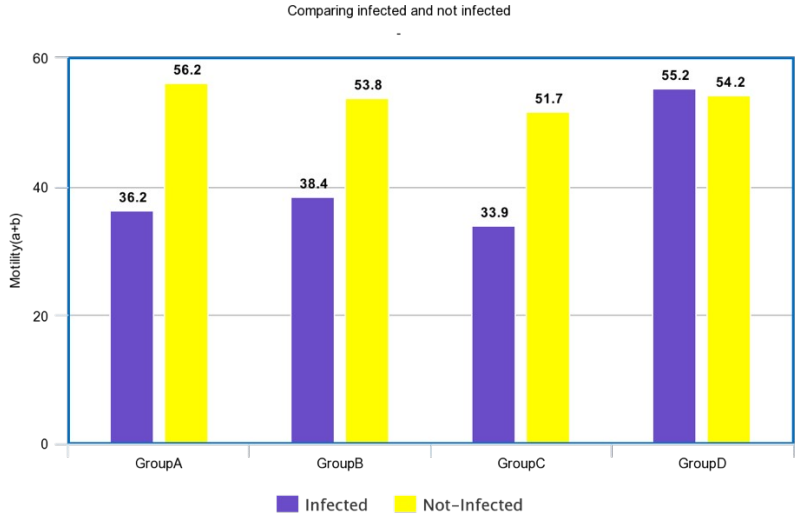

FIGURE 4. Percentage comparison between samples of infected and non-infected patients from 4 distinct groups (Link accessed on 20.06.20)

Yanget et al. (2013) state the analysis of 1138 samples from men, of which 142 tested positive for HPV infection, and 523 of them were diagnosed as fertile and 615 infertile. The infection rate was 6.70%. Of the 107 HPV-positive subjects, 29 (27.1%) were infected with several HPV genotypes. Of the 35 HPV-positive subjects, two (5.7%) were infected with several HPV genotypes. The difference between the two groups was statistically significant.

In 2013, Garolla et al. state the analysis of 257 samples from men, of which 61 were tested positive. The average percentage of ASA (anti-sperm antibodies) was significantly higher in infected people than in uninfected patients (average percentage

34.7 `19.8 and 15.8 '9.6, respectively).Pedros et al. (2013) support through a study, the analysis of sperm samples obtained from 1,500 men randomly selected from couples who went to an infertility clinic. The analyzes were performed using Spearman's correlation and Mann-Whitney tests. Figure 5 shows that the age groups were made up of men ≤35 years, between 36-45 years and the age of 45 years. This study aimed to evaluate the effect of paternal age on reproductive outcomes. There was an association between advancing paternal age and changing spermatogram parameters.

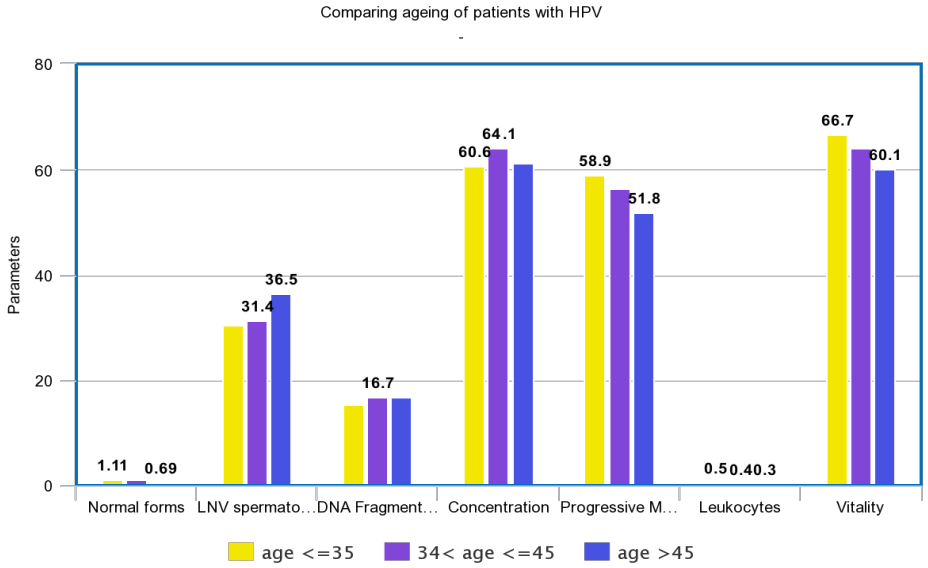

FIGURE 5. Comparison of spermogram values according to the age of HPV-infected patients (Link accessed on 20.06.20)

In 2017, Damke et al., state that *Papillomavirus*'s presence negatively influences sperm parameters such as volume, concentration, and motility, and a year later, in 2018, another study coordinated by Xiong et al. states the same thing as the negative effect on sperm motility. The 2017 study was conducted by taking samples from 229 men, 38 of whom were tested positive for *Papillomavirus*. Overall, 16.6% of the total study population was HPV positive. Single HPV infections occurred in 10.5% of samples and 63.2% of HPV-positive sperm samples. Infections with several types of HPV occurred in 6.1% of samples and 36.8% of HPV positive sperm samples. The detection of the basic seminal parameters was performed according to the criteria published by the "World Health Organization".

In the 2018 study, Xiong et al., a meta-analysis was performed in which eight articles were included, providing data on 1955 participants. In 5 reviews, the authors

detected both high-risk HPV (HR-HPV) and low-risk HPV (LR-HPV) genotype) in semen. In one study, men were defined as infertile when their sperm concentration was <20 × 106 ml-1 or when sperm motility was <50%, while in another study, all participants in the infertile male groups were further affected by infection of the male accessory gland. Two studies did not report a detailed definition of male infertility. HPV semen infection was identified as a risk factor for sperm abnormality.

Boeri et al. (2019) stated in a study that the type of *Papillomavirus* present could influence the qualitative index of samples. Samples from 729 infertile men were analyzed, of which 113 (15.5%) were tested positive for HPV. The HPV viral DNA ratio determined by real-time PCR can be seen in Figure 6. The location of the virus was observed at the sperm level as well as in semen. After liquefaction at room temperature, sperm volume, sperm concentration, motility, and normal morphology were assessed according to the World Health Organization's recommendations for sperm analysis. The swimming technique then separated the sperm. This technique allows the selection of sperm for their ability to migrate from seminal plasma to the culture medium. Motility was affected especially in those with an active infection, and there is a possibility of amounts of fragmented DNA.

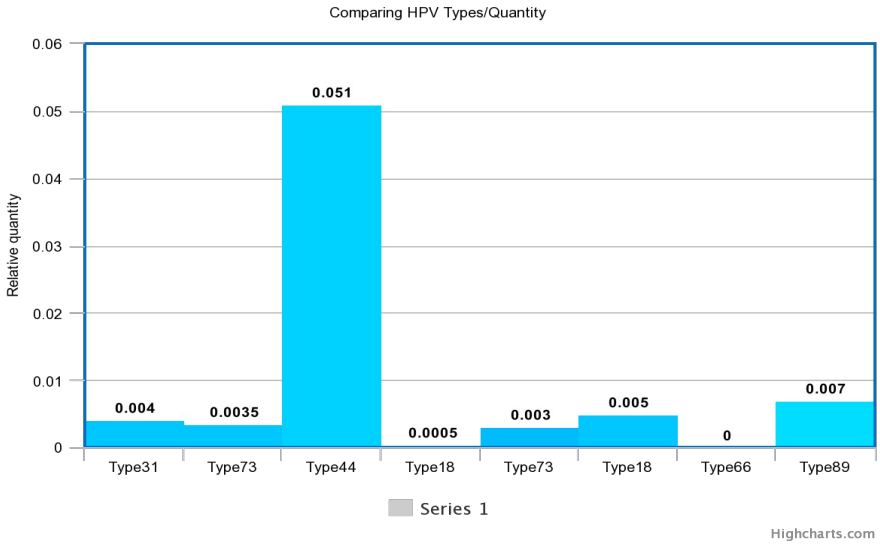

FIGURE 6. Comparison of samples according to HPV type (Link accessed on 20.06.20)

CONCLUSION

Research on 14 studies has shown in 11 of them the negative effect of human *Papillomavirus*, especially on sperm motility, a process that could be responsible for the higher rate of infertility in infected men. Two of the studies showed that the

parameters of the spermogram are not changed, even though the virus was present in the analyzed samples. One study demonstrated a possible treatment hypothesis by adding Heparinase III during positive sperm samples processing with encouraging results.

The present paper is intended to be a study of the scientific literature, the results obtained after analyzing the works advocate for the introduction in human assisted reproduction laboratories as a routine test to identify the presence of HPV in men. Although the data are still in small numbers, as practical applicability can be taken into account, the recommendation of vaccination in men as a prevention method, and in infected samples, the possibility of treating samples with Heparinase III.

REFERENCES

- Garolla A., Pizzol D., Bertoldo A., De Toni L., Barzon L., Foresta C. 2013.Association, prevalence, and clearance of human papillomavirus and antisperm antibodies in infected semen samples from infertile patients. Fertility and Sterility. 99.
- Garolla A., Lenzi A., Palu` G., Pizzol D., Bertoldo A., De Toni L., Foresta C. 2012. Human papillomavirus sperm infection and assisted reproduction: a dangerous hazard with a possible safe solution. Human Reproduction. 27(2): 967–973.
- Foresta C., Garolla A., Zuccarello D., Pizzol D., Moretti A., Barzon L., Palu G. 2010.Human papillomavirus found in sperm head of young adult males affects the progressive motility. Fertility and Sterility. 93.
- Foresta C., Pizzol D., Moretti A., Barzon L., Palu G., Garolla A. 2009. Clinical and prognostic significance of human papillomavirus DNA in the sperm or exfoliated cells of infertile patients and subjects with risk factors. Fertility and Sterility. 94.
- Pedros D., Oliveira J., Petersen C., Mauri A., Nascimento A., Vagnini L., Nicoletti A., Massaro F., Cavagna M., Martins A., Baruffi R., Franco J. 2013.Lifestyle dangers for men's fertility. Human Repoduction. 0:71-72.
- Damke E., Kurscheidt F., Balani A., Takeda K., Irie M., Gimenes F., Consolaro M. 2017. Male Partners of Infertile Couples with Seminal Infections of Human Papillomavirus Have Impaired Fertility Parameters.PubMed. 10.
- Bezold G., Politch A., Kiviat N., Kuypers J., Wolff H., Anderson D. 2006. Prevalence of sexually transmissible pathogens in semen from asymptomatic male infertility patients with and without leukocytospermia . Fertility and Sterility.87.
- https://github.com/odariusgeorge/Thesis_BioChem/blob/master/BioChemThesis.ipynb, available on 20/06/20 @ 17.35 pm
- Boeri L., Capogrosso P., Ventimiglia E., Pederzoli F., Cazzaniga W., Chierigo F., Pozzi E., Clementi M., Viganò P., Montanari E., Montorsi F., Salonia A. 2019. High-risk human papillomavirus in semen is associated with poor sperm progressive motility and a high sperm DNA fragmentation index in infertile men. Human Reproduction. 34: 209–217.
- Luttmer R., Dijkstra M., Snijders P., Hompes P., Pronk D., Hubeek I., Berkhof J., Heideman D., Meijer C. 2015. Presence of human papillomavirus in semen in relation to semen quality. Human Reproduction. 31(4): 280–286.
- Schillaci R., Capra G., Bellavia C., Ruvolo C., Scazzone C., Venezia R., Perino A. 2013.Detection of oncogenic human papillomavirus genotypes on spermatozoa from male partners of infertile couples. Fertility and Sterility. 100(5):1236-1240.
- World Health Organization. 2010. WHO laboratory manual for the Examination and processing of human semen. ed. 5.WHO Press. 287.
- Yang Y., Jia C., Ma Y., Zhou L., Wang S. 2013. Correlation between HPV sperm infection and male infertility. PubMed. 15: 529–532.
- Xiong Y., Chen Y., Cheng M., He W., Chen Q. 2018. The risk of human papillomavirus infection for male fertility abnormality: a meta-analysis. PubMed. 5: 493–497.

- Lai Y., Soong Y., Lee J., Yang F., Huang H., Pao C. 1997. The effect of human papillomavirus infection on sperm cell motility. Fertility and Sterility. 67.