

ORIGINAL ARTICLE

Prevalence of Human Papilloma Virus and Adeno-associated Virus in Semen of Infertile Undergoing Assisted Reproduction

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ABSTRACT

Key words:

Male infertility; semen; HPV; AAV; ICSI

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Background: HPV and AAV infection are suggested to be a risk factor for infertility in men. **Objective:** To detect the prevalence of human papilloma virus (HPV) and adeno associated virus (AAV) in semen of men suffering from infertility. **Methodology:** One hundred and thirty-eight Egyptian men were included in the study, 69 infertile patients and 69 fertile men. Conventional PCR was done for HPV genome determination, and nested PCR for AAV detection from the semen samples. **Results:** 23.2% infertile men samples were containing virus, compared with 4.3% positive semen samples among fertile men with a significant difference. Among infertile patients, 10.1% of the samples had HPV genome, 8.7% had AAV and 4.3% showed co-infection with both viruses. Regarding semen parameters, 52.2% of infertile patients showed normal parameters, 47.8% showed asthenozoospermia, oligoasthenoteratozoospermia (OAT) in 33.3%, oligoasthenospermia (OA) in 27.3 %, and oligozoospermia in 6.1%. All infertile patients underwent ICSI, neither of HPV, AAV or co-infection affected the success rate. Out of 16 patients positive for viral infection, 9 (56.3%) showed successful ICSI and 7 patients (43.8%) had failed outcome with nonsignificant difference. **Conclusions:** HPV and AAV must be excluded in male infertility, due to their high prevalence among this group with negative effects on semen parameters.

INTRODUCTION

Infertility is the inability to contribute conception after 12 months of unprotected intercourse; this may be due to male or female factors. Male infertility is multifactorial as any alteration to normal physiology of reproductive organs may affect sperm functions that causes problem for a successful fertilization^{1,2}.

Many studies showed that infection is a major cause for men infertility. Pellati, (2008) reported that infections of male genito-urinary tract accounts for 15% of the infertility causes³. Also, Ochsendorf (2008) reported that sexually transmitted infections have role in both acute and chronic diseases that could lead to infertility⁴.

Semen revealed several pathogens whether the patient is symptomatic or not⁵. Viruses as human immune deficiency viruses (HIV), hepatitis viruses (HBV or HCV) can infect spermatozoa and may cause infertility by changing the sperm character, nucleic acid integrity, and reducing the sperm motility⁶. In contrast, there isn't a lot of information about seminal infection due to human papilloma virus (HPV), human herpes

virus (HHV), cytomegalovirus (CMV), and adeno-associated virus (AAV).

European Union directives for viral screening of couples undergoing assisted reproduction techniques (ART) request only evaluation of hepatitis and HIV viruses. However, growing evidences suggested that HPV, HHV, and CMV may cause infertility. Aside from the risk of horizontal or vertical transmission, the adverse effect of viral infection on male reproduction seems to be alarming^{6,7}.

HPV is a non-enveloped double-stranded DNA genome that has a hazardous impact on sperms, fertilization process and abortion rate^{8,9}. AAV cannot replicate alone and requires co-infection with helper virus such as: herpes virus, or HPV. Also, AAV was reported in the seminal fluid of men suffering from infertility in a high percentage compared with semen of fertile men which suggested a harmful effect on sperm maturation^{6,10,11}.

Intracytoplasmic sperm injection (ICSI) is used mainly in infertile men with too few motile sperms or when eggs cannot easily be penetrated by sperm. Even within severe teratozoospermia, microscopic selection

of normal sperm occurs resulting in a high successful ICSI (80%)¹²⁻¹⁴.

METHODOLOGY

This study included 138 Egyptian men that were recruited from the University Hospitals and an Integrated Fertility Centre after IRB approval and informed consent over the period Dec 2014 to Mar 2016.

Two groups were enrolled; group I (n=69) infertile men participating in ICSI program (mean age 32.9 ± 6.1 years) and group II (n=69) fertile men (mean age 36.6 ± 6.9 years).

Exclusion criteria:

Azoospermia, combined male and female factor infertility, medical treatment for infertility for previous 3 months, chronic virus infection as HCV, HBV and leukocytospermia.

History taking and clinical examination:

Included; occupation, residence, duration of marriage, female factor of infertility, systemic diseases, genitourinary tract infection, surgery or injury and infertility treatment. Clinical examination was done to exclude varicocele by Doppler US and hormonal disorders was done.

Semen samples collection:

Samples were obtained by masturbation and transported within half an hour for computerized semen analysis according to WHO guidelines¹⁵.

DNA extraction

Semen sample were processed by centrifuge at 3500 rpm for 15 minutes, the supernatant was removed, and DNA extraction was done from the pellet by using GeneJET Gel Extraction and DNA Cleanup Micro Kit (Sigma, EU) as recommended by manufacturer's instructions.

Detection of HPV DNA by conventional PCR

L1 gene was amplified by (CGT CCA AGA GGA TAC TGA TC) and (GCA CAG GGA CAT AAT AAT GG) primers¹⁶

Detection of AAV DNA by nested PCR

Rep gene was amplified by the first round PCR primers (ACA CCA TCT GGC TGT TTG GG) and (AAA AAG TCT TTG ACT TCC TGC TT), and (GAG GCC ATA GCC CAC ACT GT) and (GAG AAT GGC TTT GGC CGA CT) for the nested PCR¹⁶.

The agarose gel was examined by using UV transilluminator for bands visualization at 450 bp for L1 gene and 151 bp for Rep gene (Figures 1,2).

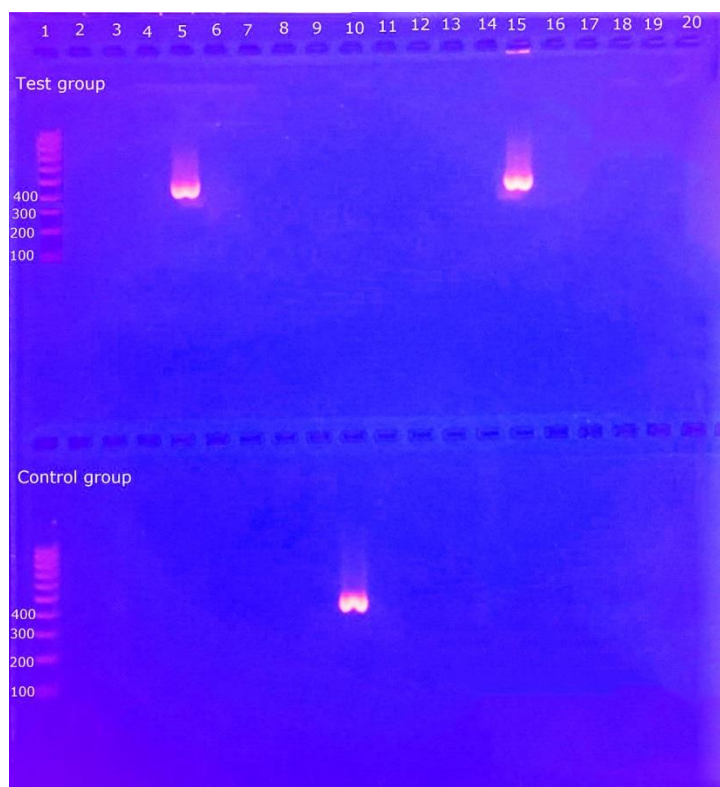


Fig. 1: PCR using primers for L1 gene. Upper part: lane (1) 1000 bp ladder, lanes (2-20) semen samples of infertile men, lanes (5 and 15) were positive 450 bp DNA band (positive samples for human papilloma virus). Lower part: lane (1) 1000 bp ladder, lanes (2-20) semen samples of control group, lane (10) was positive 450 bp DNA band (positive sample for human papilloma virus).

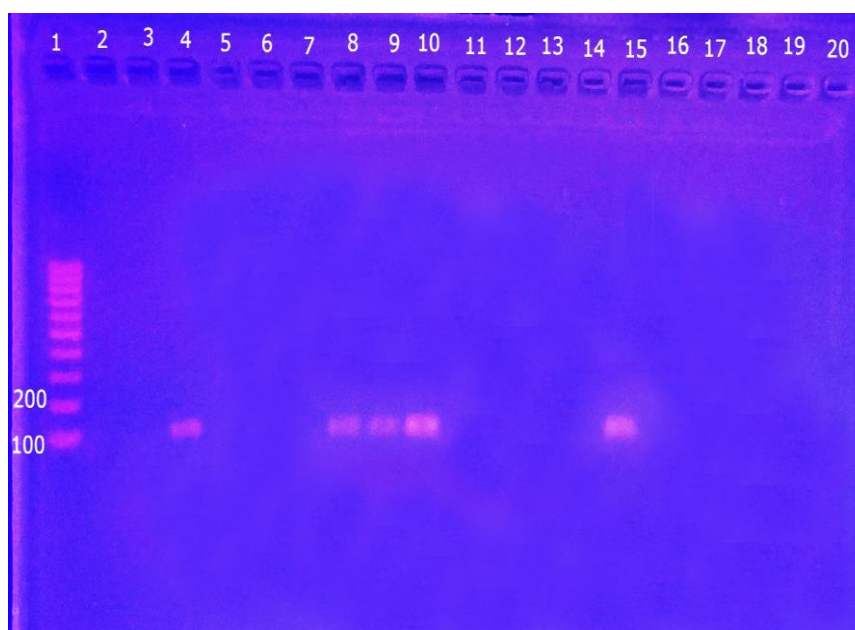


Fig. 2: Nested PCR using primers for rep gene, lane (1) 1000 bp ladder, lanes (2-20) semen samples, lanes (4,8, 9, 10 and 15) were positive 151 bp DNA band (positive samples for AAV).

Statistical analysis

The data were analyzed with SPSS program version 21 (SPSS Inc, Chicago, IL, USA). Normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square tests. Continuous variables were presented as mean \pm SD (standard deviation). The two groups were compared with Student *t* test. *P* value <0.05 was set as statistically significant.

RESULTS

Thirty-six patients (52.2%) in group I had normal semen parameters, while 33 patients (47.8%) had not; 11 asthenozoospermic samples (33.3%), 11 OAT samples (33.3%), 9 OA samples (27.3%), and 2 oligozoospermic samples (6.1%). In all, 16/69 infertile patients (23.2%) were positive for viral infection compared with 3 men (4.3%) in group II. Seven/9 infertile patients (10.1%) had HPV genome in their semen samples whereas only one semen sample was positive (1.4%) in the controls. Six semen samples (8.7%) were positive for AAV DNA in infertile patients, while 2 samples (2.8%) were positive in the controls with nonsignificant difference. Co-infection by HPV and AAV was diagnosed in 3 samples (4.3%) in group I, and no co-infection was diagnosed in the controls (Table 1).

Table 1: Prevalence of HPV and AAV in semen samples

Virus type	Group I (No.=69)		Group II (No.=69)		<i>p</i> value
	No.	%	No.	%	
HPV:	7	10.1	1	1.4	0.03*
AAV:	6	8.7	2	2.8	0.14
Co-infection:	3	4.3	0	0.0	0.08

Among the positive patients (7) for HPV, 2 samples (28.6%) showed normal semen parameters and 5 samples showed abnormal parameters; 2 OAT (28.6%), 2 OA (28.6%) were OA and 1 asthenozoospermia (14.3%). Comparing the semen parameters for the individuals with positive semen samples for HPV DNA and those without, nonsignificant difference was detected. Among the positive patients (6) for AAV infection, 1 sample (16.7%) showed normal semen parameters and 5 samples showed abnormal parameters; 2 OAT (33.3%), 2 OA (33.3%) and 1 asthenozoospermia (16.7%). Comparing the semen parameters for the patients with positive semen samples for AAV DNA and those without, nonsignificant difference was found.

Co-infection with both HPV and AAV was detected in 3 samples in group I, 1 OA (33.3%) and 2 OAT (66.7%). Nonsignificant difference was detected between samples with co-infection and those with HPV or AAV only nor between samples positive for co-infection and negative ones. No co-infection was detected in control group.

Comparing the success rate of positive and negative samples in ICSI procedure, 9/16 (56.3%) positive cases in group I, showed successful ICSI and 7 (43.8%) had failed outcome with nonsignificant difference. For 53

patients negative to viral infection, 54.7% succeeded and 45.3% failed with nonsignificant differences (Table 2).

Table 2: The ICSI outcome according to the type of the virus.

Virus	ICSI outcome				p value
	Succeeded		Failed		
	No.	%	No.	%	
Negative for the 2 viruses (n=53)	29	54.7	24	45.3	0.402
Positive for the 2 viruses (n=16)	9	56.3	7	43.8	
• HPV Positive (n=7)	3	42.9	4	57.1	
• AAV Positive (n=6)	3	50.0	3	50.0	
• Co-infection Positive (n=3)	3	100.0	0	0.0	

DISCUSSION

In the present study, 16 semen samples (23.2%) of infertile patients were positive for viral infection compared with 3 samples (4.3%) in fertile. Bezold et al.¹⁷ reported a prevalence of 19% in infertile patients. La Vignera et al.¹⁸ clarified that; chronic viral infection obstructs the male accessory gland and change in their secretory function, or release of the inflammatory mediators. Also, Monavari et al.¹⁹ reported that viral infections had a negative effect on male fertility directly by invading the cells or indirectly by causing local inflammatory/immunological responses.

In the present study, there was 7 (10.1%) positive semen samples for HPV DNA in infertile patients, and one positive sample (1.4%) in the controls. Foresta et al.²⁰ reported close results (10.2% and 2.2%) for infertile and fertile men, respectively. Perino et al.⁸ showed that the seminal fluid was positive for HPV in 9.5%. Foresta et al.²¹ reported that the prevalence of HPV sperm infection ranges 10-35.7% in men with unexplained infertility and 2-31% in men from general population. In the contrary, lower prevalences (6.7%, and 7.8%) were reported^{16, 22}. A higher HPV infection percentage were detected by many studies²³⁻²⁵.

In this study, the semen parameters were normal in 28.6% of infertile men with HPV, while the rest showed different abnormalities with increased prevalence of OAT and OA in patients with HPV infection compared with those without (28.6% vs 14.5% and 28.6% vs 11.3%). The results reported by two different studies were in line with our results^{26, 27}. In contrast, Bezold et al.²⁸ reported a significant relation between HPV infections and decreased sperm count and motility, but others studies associated between HPV semen infection and decreased sperm motility only^{20, 29, 30}.

Several factors could be responsible for this dissimilarity among studies such as the heterogeneity of study populations and/or using different techniques and differences in PCR primers. Regional differences in

HPV and AAV prevalence are other important factors as HPV is more common in the less developed countries³¹.

In our study, 6 (8.7%) positive semen samples for AAV DNA were diagnosed in infertile patients and 2 (2.8%) in the controls. Erles et al. reported a close percentage (4.6%) in the fertile group³². Higher prevalence (30%, 38%, and 19.9%) in the infertile men were reported^{11, 32, 33}. Much higher percentages (60, 14.3%) were reported by Kim et al. for both infertile men and fertile one¹⁶.

Semen parameters were normal in 16.7% of infertile men with AAV, but, 33.3% were diagnosed with OAT, 33.3% with OA, and 16.7% with asthenozoospermia. There is increase in the OAT and OA in patient with AAV infection compared with those without (33.3% vs 14.3% and 33.3% vs 11.1%) which was similar to many other studies^{11, 16, 34, 35}.

In the existing study, co-infection was in 3 samples (4.3%) from infertile patients and none in the controls parallel to Erles et al. results³². Interestingly, Kim et al. reported no co-infection among infertile men and 11.8% in the controls¹⁶.

The success rate of ICSI in cases with positive semen samples showed nonsignificant difference compared with non-positive cases (56.3% vs 54.7%) in parallel with different studies^{36, 37}. On the other hand, other studies reported that the presence of HPV genome in the sperm increase the apoptosis rate in trophoblast cells^{38, 39}. Interestingly, spermatozoa can transfer the viral genome into the oocyte⁴⁰.

CONCLUSION

Attention must be directed towards viral infection as a cause of idiopathic infertility. HPV and AAV must be excluded in cases of idiopathic male infertility, due to their high prevalence among infertile men and negative effect on semen parameters.

Further studies are required to consider all factors affecting the outcome of ICSI, to assess the role of viral infection in ICSI success. Also, follow up is needed to

compare between miscarriage rate in cases of viral infection, and in negative cases.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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