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Original Article

DNA fragmentation index and human papilloma virus in males with previous assisted reproductive technology failures

Semih Tangal[1], Yasemin Taşçı[2], Emre Göksan Pabuçcu[3], Gamze Sinem Çağlar[3], Ahmet Hakan Haliloğlu[1], Kanay Yararbaş[4]

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ABSTRACT

Objective: This study was designed to evaluate the prevalence of Human Papilloma Virus (HPV) in semen and document the cycle outcomes in couples with previous intra-cytoplasmic sperm injection (ICSI) failures.

Material and methods: One hundred and seventeen couples with at least two ICSI attempts were included in the study. HPV infection in semen and DNA fragmentation in samples were analyzed by commercially available kits. The percentage of spermatozoa with fragmented DNA (DNA fragmentation index: DFI) was determined during fluorescence microscopic examination as previously described. The cycle outcomes of couples with or without HPV infected male partners were recorded.

Results: According to our results, the prevalence of HPV was 7.7% in asymptomatic males with at least two previous ICSI failures. The increased DFI (>30%) was observed in 82.9% of the cases. In HPV-positive cases significantly lower number of good quality embryos were obtained. The implantation and pregnancy rates were similar in infected and non-infected males (p>0.05). The early miscarriage rate was slightly higher in HPV- positive group (33% vs. 10%, p>0.05).

Conclusion: In cases with previous ICSI failures, the prevalence of HPV infection in semen is not higher than previously reported infertile populations. The reproductive outcome might be impaired in HPV-positive semen due to lower number of good quality embryos, which needs to be clarified by further large population-based studies.

Keywords: DNA fragmentation; human papilloma virus, intracytoplasmic sperm injection; spermatozoa.

ORCID IDs of the authors:
S.T. 0000-0003-1394-2454;
Y.T. 0000-0002-6612-7042;
E.G.P. 0000-0001-9015-5978;
G.S.Ç. 0000-0003-1956-0908;
A.H.H. 0000-0002-6008-2509;
K.Y. 0000-0002-5314-3406.

[1]Department of Urology, Ufuk University School of Medicine, Ankara, Turkey

[2]Department of Obstetrics and Gynecology, Zekai Tahir Burak Training and Research Hospital, Ankara, Turkey

[3]Department of Obstetrics and Gynecology, Ufuk University School of Medicine, Ankara, Turkey

[4]Department of Genetic, Acıbadem University School of Medicine, İstanbul, Turkey

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Corresponding Author:
Semih Tangal
E-mail: semihtangal@mynet.com

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Introduction

The overall prevalence of human papilloma virus (HPV) is 40% in general population.[1] This viral infection is one of the most common sexually transmitted diseases worldwide. As HPV infection in men was considered transient and without clinical consequences, males have negligibly been included in HPV programs. However, recent evidence has raised concerns about its possible role in male infertility. The literature review of the last two decades reported higher percentages of HPV infection in infertile men compared with fertile controls. Moreover, a possible correlation between HPV sperm infection and unexplained male infertility has also been suggested.[2] HPV infection is also associated with reduced sperm motility and idiopathic asthenozoospermia.[2,3] In addition, in infected patients there is significantly higher mean percentage of antisperm antibody- positive sperm cells.[4]

During the recent years, the investigations on HPV implications in couples seeking fertility have indicated poor reproductive outcomes. Numerous authors have suggested decreased fertility rates through different mechanisms acting at various steps of the human embryo development in couples with HPV infected

males.[5-8] Active viral genome expression at blastocyst stage and in trophoblastic cells is associated with negative implications on early embryo development.[9] Moreover, internalization of the viral pathogen in the sperm head may cause DNA damage. Up to today, two studies have found an increased DNA fragmentation in HPV-infected spermatozoa sampes whereas Kaspersen et al. failed to detect increased sperm DNA fragmentation in HPV-positive samples.[10-12]

All these findings raise concerns about the consequences of HPV infection in males undergoing Assisted Reproductive Technologies (ART). This study was designed to evaluate the prevalence of HPV in semen in couples with previous intracytoplasmic sperm injection (ICSI) failures. Also, the DNA fragmentation index (DFI) and cycle outcomes were evaluated.

Material and methods

This prospective observational study was conducted in a private In Vitro Fertilisation (IVF) center between February 2014 and April 2015. The couples with at least two ICSI attempts that either failed or ended with abortions were asked to participate in the study. Written informed consent was obtained from the participants. The inclusion criteria were as follows; (1) at least two unsuccessful ICSI attempts performed with ejaculated spermatozoa; (2) women between 18 and 40 years old; (3) male subjects with no obvious abnormalities noted in the medical history, and physical examination, (4) no evidence of subclinical genital infections, leukocytospermia, cryptorchidism, cancer or varicocele. Exclusion criteria included: (1) azoospermia; (2) women with history of poor response to ovarian stimulation or fulfilling the Bologna criteria for expected poor responders (25); (3) preimplantation genetic screening (PGS), cryopreserved/thawed embryo transfer cycles (4) uterine or tubal pathology; (5) subjects with genetic disorders; (6) male subjects defined as heavy smokers (>20 cigarettes/day). The patients (n=117) that fulfilled the inclusion criteria and accepted to participate were included in the final analysis. Ethics committee approval was received for this study from the ethics committee of Ufuk University (RN: ACTRN12614000188639-2014).

Sperm collection and DNA fragmentation analysis

Semen samples from patients were obtained by masturbation after 2-5 days of sexual abstinence and stored in sterile containers. All sperm parameters were evaluated at initial admission. Basic sperm parameters (concentration, motility and morphology) were evaluated in all samples according to World Health Organization criteria (WHO, 2010). The materials were then processed for the terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assay, which was performed using a Cell Death Detection Kit with tetramethylrhodamine-labelled dUTP (Roche, Turkey) and according to the manufacturer's instructions. The percentage of spermatozoa with fragmented DNA (DNA fragmentation index: DFI) was determined by fluorescence microscopic examination.

HPV analysis

Human papilloma virus genotyping was performed using an IVD validated ready to use commercial kit (PapilloCheck, Greiner Bio-One, GmbH, Germany) according to manufacturer's instructions. Microarray hybridization methodology is used by the procedure and 24 types of HPV (6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44/55, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, and 82) were detected.

Ovarian stimulation protocol

All couples were subjected to ICSI and all sperm injections were performed with fresh specimens. One ART cycle of each patient was included in the study. Ovarian stimulation was carried out with Human Menopausal Gonadotropin (hMG) (Menopur, Ferring, İstanbul, Turkey) administration beginning from the second day of the menstrual cycle with starting dose ranging between 150-300 IU/day according to patient's ovarian reserve. The GnRH antagonist (Cetrotide, Merck Serono, Istanbul) was introduced (0.25 mg/day) on the sixth day (fixed antagonist protocol) and continued throughout ovarian stimulation. When at least three follicles were ≥18 mm, rhCG (250 μg; Ovitrelle, Merck Serono, Istanbul) was used for final oocyte maturation. Transvaginal ultrasound-guided oocyte retrieval and embryo transfer procedure was performed. A biochemical pregnancy was defined as hCG concentration >20 IU/L on the twelfth day of the embryo transfer. A clinical pregnancy was defined as the presence of an intrauterine gestational sac with a heartbeat.

Statistical analyses

For statistical analysis IBM Statistical Package for Social Sciences (IBM SPSS Statistics; Armonk, NY, USA) Statistics Software 22 program was used. Normality test was performed using Shapiro-Wilk test. For the comparison of variables between two groups, Mann-Whitney U test were used. Statistical significance was accepted as $p<0.05$.

Results

The etiology was unexplained infertility in 67.5% (n=79) and male factor infertility in 32.5% (n=38) of the couples. The mean age and Anti-Mullerian Hormone (AMH) level of the female partners were 32.9±4.7 years and 2.6±1.8 ng/mL (median 2.1, min 1.0-max 13 ng/mL), respectively. The mean number of previous cycles was 3.8±2. The previous history confirmed early pregnancy loss in 27 (23%) of the couples (18 couples had ≥2 abortions).

The mean age of the male partners were 36±5.4 years. Median sperm concentration was $21x10^6$/mL (min 1-max $183x10^6$/

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mL). The median number of total progressive motile sperm and percetage of spermatozoa with normal morphology (Kruger criteria) were 23.8x10^6 (min 0.3x10^6-max 184x10^6) and 4% (min 1%-max 23%), respectively. HPV-DNA was detected in 7.7% (n=9) of male partners where in 6 cases oncogenic high-risk (33, 39, 45, 56, 59, 70) and in 3 cases low- risk HPV genotypes (6, 11 and 42) were found. The median values for DFI were 38.2% (min 4.5%-max 88.3%). DFI was ≥30% in 97, (82.9 %) and <30% in 20 (17.1%) cases.

The couples with HPV infected male partners (Group I, n=9) were compared with couples without HPV-infected male partners (Group II, n=108). The demographic and basal characteristics (the age of the male and female partners, basal AMH levels of females, number of previous IVF failures, semen parameters, and DFI) of these two groups are given in Table 1 (p>0.05). The DFI ≥30% was similar when two groups are compared (82.4% in non-infected males vs 88.9% in infected males; p>0,005). The cycle characteristics and outcomes of the couples with or without HPV-infected males are given in Table 2.

Discussion

This study documents that the prevalence of HPV in semen of males with at least two previous ICSI failures is similar to previously reported infertile populations. In couples with HPV positive semen undergoing ICSI cycles, impairment in embryo quality and slightly higher early miscarriages is a point of caution.

Recently, the prevalence of HPV was reported as 6.7% in fertile (n=523) and 17% in idiopathic infertile males (n=615).[13] In a meta-analysis (7 studies) HPV prevalence in infertile populations was 16% [95% CI: 10-23%] versus 10% (95% CI: 7-14%).[14] A systematic literature review found out that HPV prevalence ranges between 10 and 35.7% in men affected by unexplained infertility.[2] In our study, the reported prevalence of HPV was 7.7% in males with at least two previous ICSI failures. Similar to our data, the previously reported prevalence of male infection in infertile patients undergoing ART was 9.5%, in a prospective study by Perino et al.[15] and 7.8% in the study of Schillaci et al.[3] . According to our results, in couples with previous ART failures, HPV prevalence does not seem to be higher than previously reported rates but needs to be confirmed in larger populations.

Human papilloma virus is able to bind to the sperm head at or near to the equatorial segment and the infected spermatozoa are vectors for HPV transmission into fertilized oocytes.[4-8] Moreover, infected sperm is able to transfer HPV E6/E7 genes and L1 capsid protein into the oocytes.[8] The viral genome expression at blastocyst stage and in trophoblastic cells in the

Table 1. Demographic and basal characteristics of the couples with infected (Group I) and non-infected male partners (Group II)

Variable	Group I (n=9)	Group II (n=108)	p
Age of the female partner (years)	35 (32-42)	33 (19-43)	>0.05
Age of the male partner (years)	38 (28-45)	36 (24-49)	>0.05
No. of previous ART cycles	3.5 (2-10)	4 (1-13)	>0.05
AMH of the female partners (ng/mL)	1.5 (0.8-4.3)	2.2 (0.8-13)	>0.05
Concentration of spermatozoa (10^6/mL)	24 (2-65)	20 (1-183)	>0.05
TPMSC (10^6/ejaculate)	8 (0-64)	9 (0-28)	>0.05
Normal morphology (%)	4 (1-23)	4 (1-5)	>0.05
DFI (%)	38 (17-70)	38 (4-88)	>0.05

Values are presented as median (minimum-maximum). NS: statistically not significant (p>0.05)

AMH: Anti-Müllerian Hormone; ART: Assisted Reproduction Technologies; DFI: DNA fragmentation index; TPMSC: total progressive motile sperm count

Table 2. The cycle characteristics and outcomes of the couples with infected HPV (Group I) and non-infected male partners (Group II)

Variable	Group I (n=9)	Group II (n=108)	p
Cycle duration (days)	10 (9-15)	11 (8-18)	>0.05
Estradiol levels* (pg/mL)	1667 (355-2264)	2218 (732-6897)	>0.05
Endometrial thickness (mm)	12 (8-14)	11 (7-15)	>0.05
No. of retrieved oocytes	7 (1-11)	8 (1-26)	>0.05
No. of MII oocytes	6 (1-10)	7 (1-20)	>0.05
Fertilization rate (%)	82.5 (25-100)	76.5 (33-100)	>0.05
No. of Grade I embryos	1 (0-2)	2 (0-9)	<0.003[a]
Implantation rate (%)	29	33	>0.05
Positive HCG, n (%)	4 (44)	44 (40)	>0.05
Clinical pregnancy rate, n (%)	1 (11)	31 (28)	>0.05
Pregnancy loss, n (%)	3 (33)	11 (10)	>0.05

Values are presented as median (minimum-maximum). NS: statistically not significant (p>0.05). astatistically significant

infected embryos negatively effect early development.[5,16]. The oncogenic HPV types 16 and 18 are able to inhibit the embryo development with a 25% reduction in blastocyst formation and impaired embryo development.[17,18] In a recent study in patients undergoing ART, significantly reduced blastocyst formation was found in infected (54%) vs non-infected couples (27%), p<0.05).[19] Also, in our study, embryos obtained from HPV-positive semen samples yielded significantly lower number of good quality embryos on the day of transfer. Therefore, the possible negative role of HPV infection in embryo development needs to be clarified with further in-vitro studies as the causal effects start within the very early stages of development.

Regarding implantation rates, 37% reduction was observed in murine embryos in case of HPV infection.[20] Supporting this data, in ART cycles cumulative pregnancy rates were significantly lower (38% in non-infected vs. 14%, in infected couples).[15,19] In our study neither the implantation nor the pregnancy rate was significantly different. However, in our study, the number of HPV positive cases was quite low to reach definitive conclusions, which is a limitation of this study. Another point is that HPV was detected in seminal plasma not in spermatozoa. So, the consequences of HPV infection in ART might be more serious when the infection is detected at the sperm level.

In addition, early pregnancy loss is another possible negative impact of HPV infection.[15,19] Remarkably high abortion rates were reported in infertile couples (62.5% in infected vs. 16.7% in non-infected couples, p<0.05) and in HPV infected males (66% vs. 15% in non-infected males).[15,19] Apoptosis of embryonic cells through fragmentation in infected embryos is suggested as the probable cause of pregnancy loss.[16,20,21] However, the limited number of HPV positive cases and undocumented HPV viral genome expression in abortion materials was a handicap in this study.

Finally, whether HPV infection in semen is or is not associated with increased DNA fragmentation of the spermatozoa is unknown. Up to today, two studies reported increased DNA fragmentation in HPV infected spermatozoa. The first study evaluated DNA fragmentation by Commet assay and found a significant increase in apoptotic phenomena in spermatozoa exposed to HPV type 16 and 31.[10] Similarly, Lee et al.[11] demonstrated fragmentation of sperm DNA including exons of p53 gene. On the other hand, others failed to detect an increase in sperm DNA fragmentation by sperm chromatin structure assay in HPV positive samples.[12] Due to a limited number of cases with HPV-positive semen in our study, our results might not contribute to this point.

In conclusion, regarding the results obtained from this study, we may conclude that HPV infection in semen is not more prevalent in men with two previous ICSI failures than other infertile populations. In addition, the impact of seminal HPV infection on assisted fertilization, and abortions still remains as an unresolved issue.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ufuk University (RN: ACTRN12614000188639).

Informed Consent: Written informed consent was obtained from participants who participated in this study.

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References

1. Bosch FX, Broker TR, Forman D, Moscicki AB, Gillison ML, Doorbar J, et al. Comprehensive control of human papillomavirus infections and related diseases. Vaccine 2013; 30: H1-31.
2. 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;3:163-73. [CrossRef]
3. Schillaci R, Capra G, Bellavia C, Ruvolo G, Scazzone C, Venezia R, et al. Detection of oncogenic human papillomavirus genotypes on spermatozoa from male partners of infertile couples. Fertil Steril 2013;100:1236-40. [CrossRef]
4. 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;99:125-31. [CrossRef]
5. Chan PJ, Seraj IM, Kalugdan TH, King A. Evidence for ease of transmission of human papillomavirus DNA from sperm to cells of the uterus and embryo. J Assist Reprod Genet 1996;13:516-9. [CrossRef]
6. Lai YM, Yang FP, Pao CC. Human papillomavirus deoxyribonucleic acid and ribonucleic acid in seminal plasma and sperm cells. Fertil Steril 1996;65:1026-30. [CrossRef]
7. Perez-Andino J, Buck CB, Ribbeck K. Adsorption of human papillomavirus 16 to live human sperm. PLoS One 2009;4:e5847.
8. Foresta C, Patassini C, Bertoldo A, Menegazzo M, Francavilla F, Barzon L, et al. Mechanism of human papillomavirus binding to

Turk J Urol 2019; 45(1): 12-6
DOI:10.5152/tud.2018.96393

human spermatozoa and fertilizing ability of infected spermatozoa. PLoS One 2011;6:e15036.
9. Gizzo S, Ferrari B, Noventa M, Ferrari E, Patrelli TS, Gangemi M, et al. Male and couple fertility impairment due to HPV-DNA sperm infection: update on molecular mechanism and clinical impact-systematic review. Biomed Res Int 2014;230263.
10. Connelly DA, Chan PJ, Patton WC, King A. Human sperm deoxyribonucleic acid fragmentation by specific types of papillomavirus. Am J Obstet Gynecol 2001;184:1068-70. [CrossRef]
11. Lee CA, Huang CT, King A, Chan PJ. Differential effects of human papillomavirus DNA types on p53 tumor-suppressor gene apoptosis in sperm. Gynecol Oncol 2002;85:511-6. [CrossRef]
12. Kaspersen MD, Bungum M, Fedder J, Bonde J, Larsen PB, Ingerslev H, et al. No increased sperm DNA fragmentation index in semen containing human papillomavirus or herpesvirus. Andrology 2013;1:361-4. [CrossRef]
13. Yang Y, Jia CW, Ma YM, Zhou LY, Wang SY. Correlation between HPV sperm infection and male infertility. Asian J Androl 2013;15:529-32. [CrossRef]
14. Laprise C, Trottier H, Monnier P, Coutlée F, Mayrand MH. Prevalence of human papillomaviruses in semen: a systematic review and meta-analysis. Hum Reprod 2014;29:640-51. [CrossRef]
15. Perino A, Giovannelli L, Schillaci R, Ruvolo G, Fiorentino FP, Alimondi P, et al. Human papillomavirus infection in couples undergoing in vitro fertilization procedures: impact on reproductive outcomes. Fertil Steril 2011;95:1845-8. [CrossRef]
16. 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;23:709-15. [CrossRef]
17. Henneberg AA, Patton WC, Jacobson JD, Chan PJ. Human papilloma virus DNA exposure and embryo survival is stage-specific. J Assist Reprod Genet 2006;23:255-9. [CrossRef]
18. Calinisan JH, Chan SR, King A, Chan PJ. Human papillomavirus and blastocyst apoptosis. J Assist Reprod Genet 2002;19:132-6. [CrossRef]
19. Garolla A, Engl B, Pizzol D, Ghezzi M, Bertoldo A, Bottacin A, et al. Spontaneous fertility and in vitro fertilization outcome: new evidence of human papillomavirus sperm infection. Fertil Steril 2016;105:65-72.e1.
20. Hong LJ, Oshiro BT, Chan PJ. HPV-16 exposed mouse embryos: a potential model for pregnancy wastage. Arch Gynecol Obstet 2013;287:1093-7. [CrossRef]
21. You H, Liu Y, Agrawal N, Prasad CK, Edwards JL, Osborne AF, et al. Multiple human papillomavirus types replicate in 3A trophoblasts. Placenta 2008;29:30-8. [CrossRef]