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Prevalence of human papillomavirus infection in oocyte donors and women treated for infertility: An observational laboratory-based study[☆]



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ABSTRACT

Objective: The aims of this study were to determine the prevalence of human papillomavirus (HPV) infection in women treated for infertility and oocyte donors, and to investigate the possible influence of HPV infection on reproductive outcomes.

Study design: In this observational laboratory-based study, cervical swabs were collected from oocyte donors (n = 207), and women treated for infertility (n = 945) and analysed for the presence of high-risk HPV (hrHPV) genotypes using the cobas[®] 4800 HPV Test and PapilloCheck[®] HPV-Screening. Associations between hrHPV positive status and fertility outcome or socio-behavioral and health characteristics were evaluated using R statistical software.

Results: HrHPV prevalence was significantly higher in oocyte donors than in women treated for infertility (28.0% vs. 16.1%, $P < 0.001$). Women who became pregnant spontaneously (19.6%) and women not treated with *in vitro* fertilization (IVF, 18.1%) were more frequently hrHPV positive than women treated with IVF (12.7%, $P = 0.077$). Despite the high prevalence of hrHPV in both oocyte donors and infertile women, no associations between hrHPV positive status and pregnancy or abortion rates were found in IVF treated women or in oocyte recipients. Moreover, no associations between hrHPV positive status and abortion rates were found in spontaneously pregnant women.

Conclusion: Despite the high prevalence of hrHPV in both oocyte donors and infertile women, HPV infection did not influence the outcomes of assisted reproductive technology.

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1. Introduction

Infertility remains a highly prevalent global problem, affecting about 10% of reproductive-aged couples worldwide in the twenty-first century [1,2]. Sexually transmitted infections (STIs) like *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Treponema*

pallidum are widely believed to cause fertility alternations [3]. Reproductive alternations may also be associated with viral STIs including human immunodeficiency virus (HIV), human cytomegalovirus (HCMV), human herpes virus (HSV), adeno-associated viruses, and human papillomavirus (HPV). The impact of viral STIs on human fertility is not well understood [4,5].

STIs could also be a problem in oocyte donors who are routinely screened for the HIV1/2, Hepatitis B/C and *Treponema pallidum* infections according to the European Commission Directive 2006/17/EC of the Czech Republic. In cases of suspicious infection, additional testing may be required (e.g. HCMV, malaria, *Trypanosoma cruzi* and human T-lymphotropic virus I). However, testing for HPV infection is not demanded.

HPV infections are prevalent STIs with a global prevalence of about 12%. The highest prevalence is observed in sexually active women under 25 years of age [6]. Low-risk HPV (LrHPV) genotype

[☆] The study was conducted in: Olomouc, Czech Republic, Kostelec nad Orlici, Czech Republic.

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infection causes only benign lesions like genital warts. The high-risk HPV (hrHPV) genotypes causes several premalignant and malignant lesions in the anogenital and aero-digestive tracts [7,8]. Moreover, the potential influence of HPV infection to human fertility alterations was suggested by recent studies. Nonetheless, the exact impact of HPV infection on human fertility remains uncertain [9,10].

The aim of this study was to systematically investigate the prevalence of cervical HPV infection in two groups, females treated for infertility (IW) and oocyte donors (OD). The second objective was to clarify the influence of HPV infection on pregnancy and abortion rates in women undergoing *in vitro* fertilization (IVF) or in recipients of donated oocytes.

2. Material and methods

2.1. Ethical considerations

Study proposals were approved by the Ethics Committee of the Faculty of Medicine and Dentistry of Palacky University and the University Hospital Olomouc in compliance with the Helsinki Declaration. All study participants provided signed informed consent for the use of their collected samples and completed a questionnaire on their health status and sexual behaviour.

2.2. Clinical specimen collection

Samples were collected from women from March 2013 to October 2015 in two Czech fertility centres; Fertimed Ltd. in Olomouc and Arleta IVF Ltd. in Kostelec nad Orlici which operate in the same geographical region. The inclusion criteria for oocyte donors were according to the European Commission Directive 2004/23/ES and the Czech Directive 296/2008, as amended. Inclusion criteria for women from infertile couples were: duration of infertility longer than one year, infertility due to various causes, and age between 18 and 49 years of age.

Cervical swabs were taken from oocyte donors ($n = 207$) and from women before planned IVF/IVF + ICSI treatments ($n = 945$) to test the presence of a spectrum of hrHPV and lrHPV. Oocyte recipients ($n = 87$) were not tested for HPV DNA presence. Cervical brushes were rinsed in cobas®PCR Cell Collection Media (Roche Diagnostics GmbH, Mannheim, Germany). All samples for molecular testing were stored and transported at room temperature according to the manufacturer's recommendations.

The analysis included women who underwent IVF/IVF + ICSI ($n = 362$), or who became pregnant spontaneously ($n = 46$) within 6 months after sampling without any reproductive treatment. The numbers of pregnancies (documented by vaginal ultrasound) and abortions were evaluated. In the IVF/IVF + ICSI group, only women with a transfer of one or two fresh embryos from own oocytes were included. Only 45 (21.7%) out of 207 oocyte donors were included in the analyses since HPV screening was performed within 6 months after HPV sampling. All study participants tested negative for HIV1/2, Hepatitis B and C, *Chlamydia trachomatis*, and *Treponema pallidum*. No clinical symptoms of herpes or HPV infection were detected in these patients.

2.3. HPV DNA detection

All samples were tested for HPV DNA using the cobas® 4800 HPV Test (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's recommendations [11]. After analysis, DNA extracted using a cobas x 480 instrument was subjected to HPV DNA detection and genotyping using PapilloCheck® HPV-Screening (Greiner Bio-One, Frickenhausen, Germany) [13]. In 40 samples where cobas® 4800 HPV Test and PapilloCheck® HPV-Screening were not concordant, LMNX

Genotyping Kit HPV GP (Diassay, Rijswijk, The Netherlands) [14] was used for confirmation as described previously [12].

Concordant HPV result for a given sample was obtained when at least two methods gave consistent results for HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. Positive detection of HPV53, 70, 73, 82, 6, 11, 40, 42, 43 and 44/55 was based only on PapilloCheck HPV-Screening results. Samples positive for HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, or 82 were considered hrHPV positive.

2.4. Statistical analysis

The R statistical software (version 3.5.0; R Core Team, R Foundation for Statistical Computing [<http://www.r-project.org>]) was used for data evaluation. Any associations between hrHPV positive status and fertility outcomes or social, behavioral and clinical characteristics were assessed using Fisher's exact test, Pearson's chi-squared test or Wilcoxon exact test, as appropriate. Data from questionnaires were analysed only if available. Multivariate analysis was performed using multivariate logistic regression model with adjustment to categorized age. A P -value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Population demographics

Cervical samples were collected from 945 women treated for infertility (IW) and 207 healthy oocyte donors (OD). The median ages of IW and OD were 33 years (range, 19–48 years) and 26 years (range, 18–35 years) respectively. The median age of oocyte recipients was 39 (range, 27–49 years). Of the 207 participants who were OD, 45 (21.7%) had donated oocytes.

3.2. HPV positivity rates

We detected the DNA of at least one of the 18 hrHPV genotypes or the 6 lrHPV genotypes in 20.3% of all samples (234/1152), 30.9% (64/207) of OD samples, and 18.0% (170/945) of IW samples. Of the 234 HPV positive samples, 210 (89.7%) were hrHPV positive, 38 (16.2%) were lrHPV positive, and 54 (23.1%) tested positive for hrHPV and lrHPV co-infection (Table 1).

Of the 54 HPV co-infected samples, 38 (70.4%) were infected with two HPV genotypes, 13 (24.1%) were infected with three HPV genotypes, and 3 (5.56%) were infected with four HPV genotypes. At least one hrHPV genotype was detected in all co-infection samples. HPV16 was the most frequent HPV genotype in both single-genotype infection and co-infections of OD and IW (Table 1).

The hrHPV prevalence was significantly higher in OD compared to IW (28.0% vs. 16.1%, $P < 0.001$). Similarly, the occurrence of hrHPV single-genotype infection was significantly higher in OD compared to IW (21.3% vs. 11.9%, $P < 0.001$) (Table 1). HrHPV positive women from both groups were significantly younger than hrHPV negative women (25 years vs. 27 years in OD; 31 years vs. 33 years in IW). HrHPV positive women had more sexual partners than hrHPV negative women (4 vs. 3 in OD; 5 vs. 4 in IW). HrHPV positive oocyte donors had younger sexual partners (27 vs. 30, $P = 0.007$) and were more frequently childless (45.6% vs. 20.0%, $P < 0.001$) than hrHPV negative OD (Table 2).

Only 60 out of all 1110 women tested (5.4%) were vaccinated against HPV (Cervarix or Silgard/Gardasil). The difference between vaccination coverage in OD and IW was not significant (4.1% vs. 5.69%, $P = 0.475$).

3.3. HPV and IVF outcome

The pregnancy rate was lower in women treated with IVF (106/362, 29.3%) than in recipients of donated oocytes (35/87, 40.2%;

Table 1

Prevalence of hrHPV and lrHPV genotypes detected in cervical smear of oocyte donors and women treated for infertility.

| HPV genotype | Oocyte donors (n = 207) | | | Women treated for infertility (n = 945) | | | P-value (single-genotype infection) | P-value (co-infection) | P-value (total) |
|--------------|----------------------------------|---------------------|--------------|---|---------------------|--------------|-------------------------------------|------------------------|------------------|
| | Single-genotype Infection, n (%) | Co-infection, n (%) | Total, n (%) | Single-genotype Infection, n (%) | Co-infection, n (%) | Total, n (%) | | | |
| HPV16 | 8 (3.86) | 6 (2.90) | 14 (6.76) | 23 (2.43) | 13 (1.38) | 36 (3.81) | 0.360 | 0.131 | 0.089 |
| HPV18 | 1 (0.483) | 1 (0.483) | 2 (0.966) | 2 (0.212) | 1 (0.106) | 3 (0.317) | 0.448 | 0.327 | 0.221 |
| HPV31 | 6 (2.90) | 2 (0.966) | 8 (3.86) | 14 (1.48) | 11 (1.16) | 25 (2.65) | 0.151 | 1.000 | 0.470 |
| HPV33 | 1 (0.483) | 3 (1.45) | 4 (1.93) | 7 (0.741) | 2 (0.212) | 8 (0.847) | 1.000 | 0.043 | 0.245 |
| HPV35 | 0 (-) | 0 (-) | 0 (-) | 0 (-) | 0 (-) | 0 (-) | NA | NA | NA |
| HPV39 | 5 (2.41) | 3 (1.45) | 8 (3.86) | 8 (0.847) | 8 (0.847) | 16 (1.69) | 0.067 | 0.427 | 0.059 |
| HPV45 | 1 (0.483) | 1 (0.483) | 2 (0.966) | 5 (0.529) | 4 (0.423) | 9 (0.952) | 1.000 | 1.000 | 1.000 |
| HPV51 | 4 (1.93) | 3 (1.45) | 7 (3.38) | 7 (0.741) | 5 (0.529) | 12 (1.27) | 0.118 | 0.160 | 0.062 |
| HPV52 | 4 (1.93) | 1 (0.483) | 5 (2.42) | 10 (1.06) | 6 (0.635) | 16 (1.69) | 0.295 | 1.000 | 0.563 |
| HPV53 | 1 (0.483) | 2 (0.966) | 3 (1.45) | 6 (0.635) | 5 (0.529) | 11 (1.16) | 1.000 | 0.616 | 0.726 |
| HPV56 | 2 (0.966) | 4 (1.93) | 6 (2.90) | 4 (0.423) | 8 (0.847) | 12 (1.27) | 0.295 | 0.245 | 0.114 |
| HPV58 | 4 (1.93) | 1 (0.483) | 5 (2.42) | 11 (1.16) | 2 (0.212) | 13 (1.38) | 0.327 | 0.448 | 0.347 |
| HPV59 | 0 (-) | 0 (-) | 0 (-) | 3 (0.317) | 0 (-) | 3 (0.317) | 1.000 | NA | 1.000 |
| HPV66 | 1 (0.483) | 1 (0.483) | 2 (0.966) | 2 (0.212) | 3 (0.317) | 5 (0.529) | 0.448 | 0.548 | 0.616 |
| HPV68 | 4 (1.93) | 0 (-) | 4 (1.93) | 3 (0.317) | 4 (0.423) | 7 (0.741) | 0.023 | 1.000 | 0.118 |
| HPV70 | 0 (-) | 0 (-) | 0 (-) | 4 (0.423) | 2 (0.212) | 6 (0.635) | 1.000 | 1.000 | 0.599 |
| HPV73 | 0 (-) | 1 (0.483) | 1 (0.483) | 1 (0.106) | 1 (0.106) | 2 (0.212) | 1.000 | 0.327 | 0.448 |
| HPV82 | 1 (0.483) | 2 (0.966) | 3 (1.45) | 2 (0.212) | 1 (0.106) | 3 (0.317) | 0.448 | 0.085 | 0.075 |
| HPV6 | 2 (0.966) | 1 (0.483) | 3 (1.45) | 1 (0.106) | 1 (0.106) | 2 (0.212) | 0.085 | 0.327 | 0.043 |
| HPV11 | 0 (-) | 0 (-) | 0 (-) | 0 (-) | 0 (-) | 0 (-) | NA | NA | NA |
| HPV40 | 0 (-) | 1 (0.483) | 1 (0.483) | 0 (-) | 0 (-) | 0 (-) | NA | 0.180 | 0.180 |
| HPV42 | 3 (1.45) | 2 (0.966) | 5 (2.42) | 8 (0.847) | 4 (0.423) | 12 (1.27) | 0.427 | 0.295 | 0.209 |
| HPV43 | 0 (-) | 1 (0.483) | 1 (0.483) | 3 (0.317) | 0 (-) | 3 (0.317) | 1.000 | 0.180 | 0.548 |
| HPV44/55 | 1 (0.483) | 0 (-) | 1 (0.483) | 6 (0.635) | 4 (0.423) | 10 (5.88) | 1.000 | 1.000 | 0.700 |
| lrHPV | 6 (2.90) | 5 (2.42) | 11 (5.31) | 18 (1.90) | 9 (0.952) | 27 (2.86) | 0.416 | 0.150 | 0.115 |
| hrHPV | 44 (21.3) | 14 (6.76) | 58 (28.0) | 112 (11.9) | 40 (4.23) | 152 (16.1) | <0.001 | 0.168 | <0.001 |
| lr + hrHPV | 50 (24.2) | 14 (6.76) | 64 (30.9) | 130 (13.8) | 40 (4.23) | 170 (18.0) | <0.001 | 0.168 | <0.001 |

Statistically significant data (P -value < 0.05) are highlighted in bold.

High risk HPV (hrHPV) includes HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, and 82 genotypes. HPV genotypes detectable by all test methods are highlighted in bold.

Low risk HPV (lrHPV) includes HPV6, 11, 40, 42, 43, and 44/55 genotypes.

The HPV result for a given sample was obtained when at least two detection methods were concordant. The presence of hrHPV genotypes HPV53, 70, 73, 82 and lrHPV genotypes (HPV6, 11, 40, 42, 43, 44/55) were evaluated using only PapilloCheck® HPV-Screening.

NA- not available.

$P = 0.065$). The abortion rate was lower in spontaneously pregnant women (1/46, 2.17%), and women treated with IVF (24/106, 22.6%) than in recipients of donated oocytes (15/35, 42.9%, $P < 0.001$).

Nine of 46 women (19.6%) that had experienced spontaneous pregnancy were hrHPV positive. Similarly, ninety-seven of 535 women (18.1%) that had not received IVF treatment were hrHPV positive. Forty-six hrHPV positive women were found in the group of 362 women who received IVF treatment (12.7%; $P = 0.077$).

No association between hrHPV infection of OD and lower pregnancy rate or higher abortion rate was identified in recipients of donated oocytes (Table 3). Furthermore, no associations were identified between pregnancy or abortion rates and hrHPV infection or cause of infertility in IW subjected to IVF (Table 4). Similarly, no associations were identified between abortion rate and hrHPV positivity or cause of infertility in spontaneously pregnant women (Table 5). Finally, no association of hrHPV positivity with fertility outcome was confirmed by multivariate analysis (Tables 4 and 5).

4. Comments

This study investigates the prevalence of cervical HPV infection in oocyte donors, and women treated for infertility, focusing on the influence of hrHPV infection on fertility outcomes. Only a few studies evaluate HPV prevalence in women undergoing assisted reproduction [15–20], but no published study systematically investigates HPV prevalence in women treated for infertility in general. It is important to emphasize that two independent HPV

detection methods were used for reliable HPV evaluation in all samples. Moreover, a third HPV detection method was used to confirm discordant results.

In our study, hrHPV infection was detected in 16.1% (152/945) of women treated for infertility, an incidence similar to the high hrHPV prevalence in cytologically negative findings reported in Czech women (15.6%, 203/1302) [21]. Nevertheless, hrHPV prevalence in oocyte donors was significantly higher than in women from infertile couples in this study and in the Czech women in the Tachezy study [21] (28.0%, 58/207, $P < 0.001$ [OD vs. IW]; $P < 0.001$ [OD vs. cytologically negative findings in Czech women]). The difference in hrHPV prevalence is unaffected by vaccination coverage (4.1% vs. 5.69%, $P = 0.475$) and could be caused by younger age of OD as compared to IW (26 years vs. 33 years, $P < 0.001$).

Higher HPV prevalence in women treated for infertility was observed in several studies. Perino et al., [18] reported that 17.5% (35/199) of women undergoing IVF tested HPV positive, with no distinction between lrHPV and hrHPV. Similarly to our findings, Spandorfer et al., 2006 [16] reported that 16.0% (17/106) of women undergoing IVF tested HPV positive. From this group, 14.1% were hrHPV positive and 7.6% were lrHPV positive.

In our study, women who became pregnant spontaneously (19.6%) and women not treated with IVF (18.1%) were more frequently hrHPV positive than women treated with IVF (12.7%, $P = 0.077$). Previous studies reported lower hrHPV prevalence in women undergoing IVF than in the cervical screening population (7.8% [23/294], and 7.0% [15/214] vs. 8.4% [192/2262] and 9.1% [18/197]) [15,17].

Table 2
Evaluation of questionnaires in the context of hrHPV positive status.

| Factor | Level | Oocyte donors | | | Women treated for infertility | | |
|--|---------------------------------------|--|------|-----------------------|--|-------|-----------------------|
| | | hrHPV positive/ all samples | % | <i>P</i> -value | hrHPV positive/ all samples | % | <i>P</i> -value |
| Sexually transmitted disease in past | No | 52/190 | 27.4 | 0.152* | 123/792 | 15.5 | 0.374** |
| | Yes | 5/10 | 50.0 | | 22/114 | 19.3 | |
| HPV vaccination | No | 54/187 | 28.9 | 1.00* | 138/862 | 16.0 | 0.958** |
| | Yes | 2/8 | 25.0 | | 9/52 | 17.3 | |
| Surgery | No | 22/93 | 23.7 | 0.630* | 101/609 | 16.6 | 0.600** |
| | Yes | 2/6 | 33.3 | | 44/294 | 15.0 | |
| Fertility alterations in family of treated woman | No | 14/63 | 22.2 | 1.00* | 130/814 | 16.0 | 1.00** |
| | Yes | 0/2 | 0 | | 13/81 | 16.1 | |
| Assisted reproduction in past | No | 11/50 | 22.0 | 1.00* | 128/708 | 18.1 | 0.003** |
| | Yes | 0/1 | 0 | | 17/194 | 8.8 | |
| Children | No | 31/68 | 45.6 | <0.001** | 106/659 | 16.1 | 0.847** |
| | Yes | 27/135 | 20.0 | | 39/255 | 15.3 | |
| Fertilization | Spontaneous | 27/130 | 20.8 | 1.00* | 34/209 | 16.23 | 0.077* |
| | Assisted reproduction | 0/1 | 0 | | 2/35 | 5.7 | |
| | Spontaneous and assisted reproduction | 0/2 | 0 | | 2/5 | 40.0 | |
| Abortion | No | 42/140 | 30.0 | 0.474** | 109/669 | 16.3 | 0.595** |
| | Yes | 8/36 | 22.2 | | 34/234 | 14.5 | |
| Abortion stage | No | 42/140 | 30.0 | 1.00* | 109/669 | 16.3 | 0.544* |
| | ≤6.week | 4/14 | 28.6 | | 10/54 | 18.2 | |
| | 6<N ≤ 12.week | 2/9 | 22.2 | | 10/92 | 10.9 | |
| | >12.week | 0/1 | 0 | | 3/13 | 23.08 | |
| Number of abortions | No | 42/140 | 30.0 | 0.334* | 109/669 | 16.3 | 0.911** |
| | 1 | 5/28 | 17.9 | | 23/154 | 14.9 | |
| | ≥2 | 3/8 | 37.5 | | 11/71 | 15.5 | |
| | | hrHPV positive/ hrHPV negative samples (median) | | <i>P</i>-value | hrHPV positive/ hrHPV negative samples (median) | | <i>P</i>-value |
| Median age | | 25/27 | | 0.004*** | 31/33 | | 0.007*** |
| Median age of sexual partner | | 27/30 | | 0.036*** | 34/35 | | 0.369*** |
| Number of sexual partners | | 4/3 | | 0.041*** | 5/4 | | <0.001*** |

The *P*-value was calculated using Fisher's exact test (*), Pearson's test chi-squared test (**) or Wilcoxon exact test (***), as appropriate. Statistically significant data (*P*-value < 0.05) are highlighted in bold.

Vaccine-targeted HPV16 and HPV18 are the most frequent HPV genotypes worldwide (20.4–24.0% and 7.4–9.8%, respectively) [22–24] as well as in the Czech Republic (24.2–55.0% and 4.4–10.3%, respectively) [21,25,26]. In our study, HPV16 occurred most frequently (21.4% of HPV positive samples), and it was the most prevalent HPV genotype in infertile women treated with IVF in our study (27.1%, 13/48) and in Perino et al., 2011¹⁸ report. HPV18 was most prevalent in Lundqvist et al., 2002 study [17] (40%, 6/15), and HPV16 was the second most prevalent (33.3%, 5/15). In this report, HPV18 was detected in only 2.14% of the samples.

In our study, which comprises to our knowledge the largest cohort of IW, no associations between hrHPV infection and lower pregnancy rate or higher abortion rate were found in hrHPV positive women treated with IVF or in oocyte recipients from

hrHPV positive oocyte donors. Similarly to our study, several other studies found no associations between positive HPV detection and lower pregnancy rate [17–20]. On the other hand, Spandorfer et al., [16] reported significant associations between HPV infection and reduced pregnancy rate in women treated by IVF (23.5% [4/17] in HPV+ vs. 57% [51/89] in HPV-, *P* = 0.02).

In our study, no associations among hrHPV infection and higher miscarriage risk were found. Our finding is in accordance with several other studies with large cohort of patients [16,17,19,27–29]. Perino et al., 2011 [18] found, however, higher abortion rate in HPV positive IVF-treated women as compared to HPV negative IVF-treated women (40.0% [6/15] vs. 13.7% [7/51] *P* = 0.0601). Higher abortion rate in HPV positive women was also reported by Comar et al., [20] (50.0% [1/2] vs. 18.2% [2/11]

Table 3
Fertility outcomes of oocyte recipients according to hrHPV status of oocyte donors.

| | HPV status | No. of pregnancies (total = 35) | <i>P</i> -value | No. of abortions (total = 15) | <i>P</i> -value |
|----------------------------|---------------------|---------------------------------|-----------------|-------------------------------|-----------------|
| Oocyte donors (n = 45) | hrHPV + OD (n = 10) | 8/17 (47.1%) | 0.716 | 4/8 (50.0%) | 0.954 |
| Oocyte recipients (n = 87) | Recipient (n = 17) | 27/70 (38.6%) | | 11/27 (40.7%) | |
| | hrHPV- OD (n = 35) | | | | |
| | Recipient (n = 70) | | | | |

The *P*-value was calculated using Pearson's chi-square test. OD – oocyte donor.

Table 4

Fertility outcomes in infertile women who become pregnant spontaneously according to cause of infertility, age and hrHPV status.

| Cause of infertility (total = 945) | | HPV status | | No. of spontaneous pregnancies (total = 46) | P-value* | Abortion (total = 1) | P-value* |
|------------------------------------|-----------------|------------|------------------|---|----------|----------------------|----------|
| Unexplained | 254/945 (26.9%) | hrHPV+ | 39/254 (15.4%) | 5/39 (12.8%) | 0.780 | 0/5 (%) | 1 |
| | | hrHPV- | 215/254 (84.6%) | 23/215 (10.7%) | | 1/23 (4.35%) | |
| Female | 234/945 (24.8%) | hrHPV+ | 37/234 (15.8%) | 1/37 (2.7%) | 0.697 | 0/1 (0%) | NA |
| | | hrHPV- | 197/234 (84.2%) | 11/197 (5.58%) | | 0/11 (0%) | |
| Male | 258/945 (27.3%) | hrHPV+ | 38/258 (14.7%) | 2/38 (5.26%) | 0.158 | 0/2 (0%) | NA |
| | | hrHPV- | 220/258 (85.3%) | 3/220 (1.36%) | | 0/3 (0%) | |
| Couple | 199/945 (21.1%) | hrHPV+ | 38/199 (19.1%) | 1/38 (2.63%) | 0.191 | 0/1 (0%) | NA |
| | | hrHPV- | 161/199 (80.9%) | 0/161 (0%) | | 0/0 (0%) | |
| All | 945/945 (100%) | hrHPV+ | 152/945 (16.08%) | 9/152 (5.92%) | 0.651 | 0/9 (0%) | 0.843 |
| | | hrHPV- | 793/945 (83.9%) | 37/793 (4.67%) | | 1/37 (2.70%) | |
| Age (total = 945) | | HPV status | | No. of spontaneous pregnancies (total = 46) | P-value* | Abortion (total = 1) | P-value* |
| ≤35 | 639/945 (67.6%) | hrHPV+ | 115/639 (18.0%) | 6/115 (5.22%) | 1 | 0/6 (0%) | NA |
| | | hrHPV- | 524/639 (82.0%) | 29/524 (5.53%) | | 0/29 (0%) | |
| >35 | 306/945 (32.4%) | hrHPV+ | 37/306 (12.1%) | 3/37 (8.11%) | 0.136 | 0/3 (0%) | 1 |
| | | hrHPV- | 269/306 (87.9%) | 8/269 (2.97%) | | 1/8 (12.5%) | |

The P-value was calculated using Fisher's exact test (*).

NA – not available.

Table 5Fertility outcomes of *in vitro* fertilization with embryo transfer in infertile women according to cause of infertility, age and hrHPV status.

| Cause of infertility (total = 362) | | HPV status | | No. of pregnancies (total = 106) | P-value* | Adjusted OR (95% CI) ** | P-value** | No. of abortions (total = 24) | P-value* | Adjusted OR (95% CI) ** | P-value** |
|------------------------------------|-----------------|------------|-----------------|----------------------------------|----------|-------------------------|-----------|-------------------------------|----------|-------------------------|-----------|
| Unexplained | 68/362 (18.8%) | hrHPV + | 5/68 (7.35%) | 2/5 (40%) | 1 | 1.11 (0.71,1.75) | 0.648 | 1/2 (50%) | 0.395 | 1.65 (0.91,2.98) | 0.114 |
| | | hrHPV- | 63/68 (92.6%) | 21/63 (33.3%) | | | | 4/21 (19.0%) | | | |
| Female | 101/362 (27.9%) | hrHPV + | 11/101 (10.9%) | 4/11 (36.4%) | 0.733 | 1.06 (0.79,1.42) | 0.688 | 1/4 (25%) | 1 | 0.93 (0.56,1.54) | 0.775 |
| | | hrHPV- | 90/101 (89.1%) | 27/90 (30%) | | | | 8/27 (29.6%) | | | |
| Male | 102/362 (28.2%) | hrHPV + | 13/102 (12.7%) | 6/13 (46.15%) | 0.186 | 1.22 (0.95,1.58) | 0.126 | 1/6 (16.7%) | 1 | 0.89 (0.59,1.34) | 0.582 |
| | | hrHPV- | 89/102 (87.3%) | 23/89 (25.8%) | | | | 6/23 (26.1%) | | | |
| Couple | 91/362 (25.1%) | hrHPV + | 17/91 (18.7%) | 6/17 (35.3%) | 0.355 | 1.09 (0.86,1.40) | 0.473 | 0 (0%) | 0.539 | 0.80 (0.56,1.15) | 0.241 |
| | | hrHPV- | 74/91 (81.3%) | 17/74 (23.0%) | | | | 3/17 (17.6%) | | | |
| All | 362/362 (100%) | hrHPV + | 46/362 (12.7%) | 18/46 (39.1%) | 0.162 | 1.10 (0.96,1.27) | 0.182 | 3/18 (16.7%) | 0.722 | 0.95 (0.76,1.18) | 0.616 |
| | | hrHPV- | 316/362 (87.3%) | 88/316 (27.8%) | | | | 21/88 (23.9%) | | | |
| Age (total = 362) | | HPV status | | No. of pregnancies (total = 106) | P-value* | Adjusted OR (95% CI) ** | P-value** | No. of abortions (total = 24) | P-value* | Adjusted OR (95% CI) ** | P-value** |
| ≤35 | 208/362 (57.5%) | hrHPV + | 34/208 (16.3%) | 15/34 (44.1%) | 0.112 | - | - | 3/15 (20%) | 1 | - | - |
| | | hrHPV- | 174/208 (83.7%) | 52/174 (29.9%) | | | | 10/52 (19.2%) | | | |
| >35 | 154/362 (42.5%) | hrHPV + | 12/154 (7.79%) | 3/12 (25%) | 1 | - | - | 0/3 (0%) | 0.545 | - | - |
| | | hrHPV- | 142/154 (92.2%) | 36/142 (25.4%) | | | | 11/36 (30.6%) | | | |

The P-value was calculated using Fisher's exact test (*) or multivariate logistic regression model with categorized age as adjusting factor (**).

$P=0.423$). Even though the number of patients in both studies is limited, the results of these studies align with studies reporting higher HPV prevalence in placentas of spontaneous abortions as compared to placentas from voluntarily terminated pregnancies [30] or in term deliveries [31].

Despite the lack of any association between HPV infection in women and pregnancy or abortion rates observed in this and other studies, circumstantial evidence suggests that HPV could affect fertility outcome [18,32]. It is possible that male HPV infection could influence the couple's fertility outcome. Thus, future studies

should consider analyzing male HPV infection in infertile couples and sperm donors.

In conclusion, and for the first time to our knowledge, we found significantly higher HPV prevalence in oocyte donors than in women treated for infertility and in the general Czech female population. No associations between HPV positive status of oocyte donors and pregnancy or abortion rates in recipients of oocytes from these donors were found. Likewise, no associations between HPV positive status and pregnancy or abortion rates were observed in IVF-treated women.

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