Prevalence of human papillomavirus infection in the female partner of infertile couples undergoing IVF/ICSI-ET and subsequent reproductive outcomes

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Research Article

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Abstract

Background

Whether human papillomavirus (HPV) infection alters the efforts of assisted reproductive technology (ART) and whether it is associated with reproductive outcomes are controversial. In this study, we investigated the prevalence of human papillomavirus infection in the female partner of infertile couples and the reproductive outcomes after in vitro fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET).

Methods

We conducted a retrospective analysis on 8117 women from infertile couples who underwent IVF/ICSI treatment at Tangdu Hospital Reproductive Medical Center in 2020 and evaluated the prevalence of HPV infection in these women. These HPV-infected female patients undergoing ART were divided into high-risk HPV (hr-HPV) (n = 130) and low-risk HPV (lr-HPV) groups (n = 94), and non-infected women patients formed the negative group (n = 126). All patients underwent a fresh-cycle embryo transfer or a frozen-embryo cycle transfer after a controlled ovarian hyperstimulation cycle. We analyzed subsequent embryonic development and reproductive outcomes.

Results

Of the 8117 cases, 747 were infected with HPV (9.2%): 529 showed hr-HPV infection (70.82%; principally genotypes 16, 52, 53, 58, and 59); 175 exhibited lr-HPV infection (23.43%; primarily genotypes 6, 43, 44, 55, 61, and 81); and 43 cases were co-infected with hr-HPV and lr-HPV (5.76%). Except for the Day-3 good-quality embryo rate, there were no differences in ovum maturation, fertilization, implantation, clinical pregnancy, live-birth, or miscarriage rates between women infected with HPV and non-infected women ($p > 0.05$); however, we noted a reduced miscarriage rate after logistic regression analyses (OR, 0.16; 95% CI, 0.03–0.84; $p = 0.041$). For single-male-factor-induced infertility in couples (sm-HPV), although we likewise observed no differences in ovum maturation, fertilization, or implantation rates ($p > 0.05$) between the sm-HPV group and the negative group, we discerned diminutions in the Day-3 good-quality embryo rate (46.01% *vs.* 70.04%, $p = 0.013$), clinical pregnancy rate (46.67% *vs.* 57.94%, $P = 0.003$), and live-birth rate (33.33% *vs.* 46.83%, $p = 0.027$); and an augmented miscarriage rate (11.11% *vs.* 4.76%, $p = 0.003$), respectively. Logistic regression analyses indicated that sm-HPV was a risk factor for decreased clinical pregnancy rate (OR, 4.17; 95% CI, 2.31–7.53; $p < 0.001$) and live-birth rate (OR, 1.83; 95% CI, 0.81–2.14; $p = 0.045$), and elevated miscarriage rate (OR, 6.83; 95% CI, 2.22–21.00 $p = 0.001$).

Conclusions

High-risk HPV infections are predominant in the female partners of infertile couples. HPV infection in these women was associated with decreased miscarriage rate, and single-male-factor infertility influenced reproductive outcomes in couples undergoing IVF/ICSI treatment—both potentially due to HPV infection in the couple.

Background

It is acknowledged that sexually transmitted diseases (STDs) are a major cause of infertility, as 20–60% of cases of infertility in women are related to *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Neisseria gonorrhoeae*, which cause cervical, tubal, and mucosal damage to the host [1, 2]. Human papillomaviruses (HPV) are double-stranded DNA viruses that constitute the most common sexually transmitted causative agent infecting humans of reproductive age worldwide [3]. Among women of reproductive age, HPV infection is a potential risk factor that predisposes them to subsequent infertility [4], and it infects skin and mucosal and cutaneous epithelial cells [5]. HPV infection is highly correlated with precancerous and cancerous lesions of the cervix uteri, vulva, vagina, penis, and anogenital areas [3, 6, 7], and some studies indicate that HPV is detectable in cervical endometriotic and ovarian lesion tissues [8, 9].

HPV infection is primarily self-limiting and can be cleared by self-immunity to the infector. However, persistent HPV infection can be carcinogenic and associated with precancerous lesions and cancer of the cervix and uterus in women, and of the anogenital mucosa in women as well as men [10]. Persistent HPV infection has been linked to chronic inflammation [11], and infectious virion production may weaken the cells residing in the endometrium in association with infertility and miscarriage [12]. HPV infection increases the risk of spontaneous abortion as well as ectopic pregnancy, and different HPV genotypes may play disparate roles in adverse reproductive outcomes when using assisted reproductive technology (ART) [13].

While HPV infection may influence pregnancy outcome, this contention is controversial [14]; thus, the effects of HPV on women's infertility and subsequent reproductive outcome require further study. Therefore, in the present study, we investigated the prevalence of HPV infection in women from infertile couples treated with IVF/ICSI-ET and assessed their reproductive outcomes.

Methods

Study design

This was a retrospective study of women patients who had undergone IVF/ICSI-ET for infertility from January 1 to December 31, 2020, at Tangdu Hospital Reproductive Center, Xi'an, China. A total of 8117 women patients from infertile couples underwent HPV genotype testing, and based on the results, patients were grouped into HPV-infected (HPV+, 747/8,117, 9.20%) and non-infected (HPV−, 7,370/8,117, 90.80%) groups. The HPV(+) group was subsequently sorted into high-risk HPV infection (hrHPV+,

529/747, 70.82%), low-risk HPV infection (lrHPV+, 175/747, 24.42%), and high-risk and low-risk sub-groups (hrHPV+/lrHPV+, 43/747, 5.76%) (Fig. 1). Of 529 cases of hrHPV-positivity, 321 did not undergo controlled ovarian hyperstimulation (COH); in 20 cases, no oocyte was retrieved, and in 60 cases, there was no embryo transfer, which were then excluded. Only 130 cases underwent IVF/ICSI-ET, including 89 cases with IVF-ET treatment and 41 cases with ICSI-ET treatment. Of 175 lrHPV(+) patients, 56 cases did not undergo COH, eight cases did not produce an oocyte, and in 17 cases there was no embryo transfer; thus only 94 cases were included for IVF/ICSI-ET treatment, including 70 cases for IVF-ET and 24 cases for ICSI-ET. One hundred twenty-six HPV(−) patients were designated as negative controls, including 68 cases with IVF-ET and 58 cases with ICSI-ET. We excluded all patients who showed an abnormal thin-prep cytologic test (TCT). Before the COH cycle, regular vaginal discharge and bacterial vaginitis (BV) were examined to exclude mycosis, trichomoniasis, *Gardnerella*, and *Neisseria gonorrhoeae*. Cervical swabs were examined to exclude *Chlamydia trachomatis, Ureaplasma urealyticum*, and *Mycoplasma genitalium*. Regular blood tests were also conducted to exclude HIV/HBV/HCV/TP. All patients underwent a fresh-cycle embryo transfer or a frozen-embryo cycle embryo transfer after the COH cycle. We obtained detailed information on infertile patients that included age, years of infertility, body mass index (BMI), cause of infertility, baseline hormonal levels such as follicle-stimulating hormone (FSH) and anti-Müllerian hormone, and antral follicle count (AFC). We also recorded the number of retrieved oocytes, ovum maturation rate (Day-0 MII), fertilization rate (Day-1 2PN), and Day-3 good-quality embryo rate. Pregnancy outcomes that included clinical pregnancy rate, live-birth rate, and miscarriage rate were also assessed. We then performed analyses to investigate embryonic development and reproductive outcomes.

Determination of HPV genotype

Sexual activities and vaginal medications were restricted prior to HPV analyses. Cervical discharges were swabbed for HPV detection, and genotyping was performed with a BioRad 100 Amplification and Luminex® 200™ System (Thermo, USA) that detected 27 genotypes: high-risk genotypes 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, and 82, as well as low-risk genotype 6, 11, 40, 42, 43, 44, 55, 61, 81, and 83.

IVF/ICSI-ET protocol

All patients underwent a standardized gonadotropin-releasing hormone (GnRH)-agonist long protocol or GnRH-antagonist protocol with oocyte retrieval, fertilization, and embryo transfer. For the GnRH-agonist long protocol, patients who underwent the IVF/ICSI-ET protocol experienced pituitary downregulation with a GnRH agonist administered at midluteal phase. For the GnRH-antagonist protocol, patients initiated rFSH treatment on the second day of the cycle by once-daily injection. After five days of this treatment, we administered the antagonist cetrorelix acetate (Merck Serono, Switzerland) daily, and the rFSH dose was adjusted according to individual ovarian response as assessed by daily ultrasonographic examination. The antagonist treatment continued until the day of hCG injection. When at least two leading follicles reached 18 mm in diameter in the two COH protocols, ovulation was induced with recombinant α-HCG (5000 to 10,000 IU, Merck Serono, Switzerland) and oocytes were collected between

36 and 38 hours later. Oocytes were then fertilized by either conventional IVF or intracytoplasmic sperm injection (ICSI) [15], and all embryos were transferred on the third day after oocyte pickup with a standard ET protocol [16]. Vaginal progesterone (8% Crinone vaginal gel, Merck Serono, Switzerland) was used daily from the day of embryo transfer (ET) to provide routine luteal support and maintain luteal function until the 10th week of pregnancy [17].

Embryonic development and reproductive outcomes

Day-3 good-quality embryo rate, ovum maturation rate, fertilization rate, implantation rate, clinical pregnancy rate, live-birth rate, and miscarriage rate were determined. Our rate calculations were as follows: ovum maturation rate = no. of D_0 MII oocytes/no. of retrieved oocytes, fertilization rate = no. of Day-1 2PN embryos/no. of D_0 MII oocytes, the good-quality embryo rate = no. of Day-3 good-quality embryos/no. of Day-1 2PN oocytes, the implantation rate = no. of implanted embryos (i.e., pregnancies)/transferred embryos, clinical pregnancy rate = pregnancy cycles/total cycles, and miscarriage rate = miscarriage cycles/total cycles.

Statistical analysis

Measurements are presented as means ± standard deviation, and we applied the Statistical Package for the Social Sciences (SPSS, version 23.0, SPSS Inc., USA) for Windows for all statistical analyses. Student's *t* test and the Chi-squared test were used to compare categorical variables, and a *P* value of <0.05 was considered to be statistically significant. We executed logistic regression analysis on reproductive outcomes, and odds ratios (ORs), 95% confidence intervals (CIs), and *P* values are reported.

Results

Prevalence of HPV infection in women from infertile couples

Of 8117 women of the infertile couples, we evaluated the DNA from at least one of the 17 hrHPV genotypes or 10 lrHPV genotypes in 9.20% (747/8117) of the total samples. HPV-cases numbered 7370 (90.2%). hrHPV genotypes were detected in 70.82% (529/747) of HPV-positive patients, principally including genotypes 16, 52, 53, 56, 58, and 59. Genotypes 16 (116/747) and 52 (114/747) were the most common hrHPV infections; 175 cases (23.43%) involved lrHPV infection, primarily including genotypes 6, 43, 44, 55, 61, and 68—and in particular genotype 61(73/747). Forty-three cases of hrHPV- and lrHPV-mixed infections were detected in 5.76% of HPV-positive patients. For the hrHPV-positive group, 130 cases underwent IVF/ICSI-ET treatment, including 89 for IVF-ET and 41 for ICSI-ET. For the lrHPV-positive group, 94 cases underwent IVF/ICSI-ET treatment, including 70 for IVF-ET and 24 for ICSI-ET. HPV-negative cases (126) were selected as the random control group, including 68 for IVF-ET and 58 for ICSI-ET (**Fig. 1**).

All enrolled women patients were designated for a COH cycle and a fresh-embryos transfer or frozen-embryo transfer cycle. Statistical indicators included age, duration of infertility, body mass index (BMI),

causes of infertility, levels of anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH), AFC, no. of retrieved oocytes, no. of Day-0 M II oocytes, no. of Day-1 2PN zygotes, and no. of good-quality embryos per cycle.

Baseline data on HPV-infected women from infertile couples

A total of 224 HPV-positive women (mean age, 32.3 ± 4.7 years) and 126 HPV-negative women (mean age, 31.9 ± 4.3 years) were enrolled in the present study (**Fig. 1 and Table 1**). We compared the baseline characteristics of HPV-infected and non-HPV-infected patients who underwent COH and IVF/ICIS ET treatment, and noted no significant differences with respect to age (32.3 ± 4.7 years *vs.* 31.9 ± 4.3 years, $p = 0.476$), duration of infertility (4.2 ± 3.3 years *vs.* 3.6 ± 2.4 years, $p = 0.123$), or BMI (22.7 ± 3.0 *vs.* 23.1 ± 3.5, $p = 0.282$). However, women 26–40 years of age exhibited a higher infection rate (especially in women 26–35 years old) at above 70%, which may be associated with frequent sexual activity (**Supplementary Table 1**). There were no differences in baseline hormone levels for FSH or AMH or in AFC ($P > 0.05$), nor in the number of retrieved oocytes, the number of mature oocytes (Day-0 M II), or the number of fertilized oocytes (Day-1 2PN) per cycle between the HPV-positive and HPV-negative groups. However, HPV-infected women manifested a lower number of Day-3 good-quality embryos (4.4 ± 3.6 *vs.* 5.5 ± 3.1, respectively; $p = 0.002$) per cycle compared with non-HPV-infected women.

Table 1. Characteristics of infertile women patients who underwent IVF/ICSI-ET treatment					
Characteristics	**Positive**		**Total(n = 224)**	**Negative(n = 126)**	***p* value**
	hrHPV(n = 130)	**lrHPV(n = 94)**			
Age(years)	31.7±4.3	33.7±5.1	32.3±4.7	31.9±4.3	0.476
Duration of infertility (years)	3.9±2.8	4.6±4.0	4.2±3.3	3.6±2.4	0.123
BMI (kg/m^2)	22.8±3.0	22.6±3.1	22.7±3.0	23.1±3.5	0.282
Causes of infertility, no. (%)					
Female factors					
Fallopian tube factor	41(31.5)	38(40.4)	79(35.3)	46 36.5	
Pelvic factor	16(12.3)	10(10.6)	26(11.6)	14 11.1	
Endometriosis	0(4.3)	4(4.3)	4(1.8)	2 1.6	
Ovulatory dysfunction	5(3.8)	2(2.1)	7(3.1)	4 3.2	
PCOS	2(1.5)	0(0)	2(0.9)	4 3.2	
Mixed female factors	24(18.5)	2(2.1)	26 11.6	15 11.9	
Other female factors	8(6.2)	11(11.7)	19 8.5	2 1.6	
Male factors					
Oligospermia/asthenospermia	16(12.3)	11(11.7)	27 12.1	16 12.6	
Other male factors	11(8.5)	7(7.4)	18 8.0	17 13.5	
Mixed female and male factors	2(1.5)	4(4.3)	6 2.7	2 1.6	
Unexplained infertility	5(3.8)	5(5.3)	10 4.5	4 3.2	
Baseline hormone concentrations					
FSH(IU/L)	6.6±3.0	7.2±3.1	6.9±3.0	6.5±2.2	0.244
AMH(ng/ml)	3.0±1.9	2.6±1.6	2.9±1.8	2.8±2.0	0.888
AFC(L)	7.0±3.6	6.3±3.8	6.7±3.7	6.1±3.5	0.176
AFC(R)	7.0±3.5	6.2±3.2	6.7±3.4	6.6±3.2	0.864
No. of retrieved oocytes	11.6±5.6	10.6±3.7	11.2±4.9	11.2±5.1	0.902
No. of Day0 MII oocytes	10.2±5.2	9.6±3.7	10.0±4.6	9.8±4.3	0.723

No. of Day1 2PN oocytes	8.4±4.4	8.1±3.6	8.3±4.0	8.3±3.7	0.963
No. of Day3 good-quality embryos per cycle	4.2±4.0	4.6±2.9	4.4±3.6	5.5±3.1	0.002**

Fallopian-tube factor and pelvic factor were the main causes (nearly 50%) of infertility for both two groups, with 20% of infertility due to male factors, particularly oligospermia and asthenospermia (12.1%) in the HPV-infected group.

Notes: BMI, body mass index; PCOS, polycystic ovary syndrome; COH, controlled ovarian hyperstimulation; FSH, follicle-stimulating hormone; and AMH, anti-Müllerian hormone. Mixed female factors, mixed with two or more female infertility factors; mixed women and male factors, mixed with two or more factors of infertility for both sexes. All Day-3 good-quality embryos were developed from two pronuclear zygotes and met the following criteria: (1) more than five blastomeres; (2) a blastomere size difference of less than 20%; and (3) fragmentation of less than 50%. * $p < 0.05$, ** $p < 0.01$.

Embryonic development and pregnancy outcomes in HPV-positive and HPV-negative women

When we analyzed evaluation indicators of embryonic development and pregnancy outcomes, we observed no difference between the HPV-positive and -negative groups in ovum maturation rate (89.13% *vs.* 87.01%, $p = 0.564$) or fertilization rate (83.27% *vs.* 84.58%, $p = 0.725$), while the HPV-positive group had a lower Day-3 good-quality embryo rate (52.72% *vs.* 70.04%, $p < 0.001$, respectively). Regarding pregnancy outcomes, there were no significant differences between the HPV-positive and HPV-negative groups in the implantation rate (44.28% *vs.* 44.06%, $p = 0.972$), clinical pregnancy rate (55.36% *vs.* 57.94%, $p = 0.587$), live-birth rate (40.63% *vs.* 46.83%, $p = 0.104$, or miscarriage rate (6.25% *vs.* 4.76%, $p = 0.556$) (**Table 2**). The hrHPV- and lrHPV-infection groups did not differ in embryonic development or pregnancy outcome relative to the HPV-negative control group ($p > 0.05$), except for the Day-3 good-quality embryo rate ($p < 0.001$) (**Supplementary Table 2**)

Table 2 Embryonic development and pregnancy outcomes in HPV positive group and HPV negative group

Embryonic development and pregnancy outcomes	HPV positive(n = 224)	HPV negative(n = 126)	*p* value
Ovum maturation rate [a]	89.13% (2230/2502)	87.01% (1232/1416)	0.564
Fertilization rate [b]	83.27% (1857/2230)	84.58% (1042/1232)	0.725
Good-quality embryo rate [c]	52.72% (978/1855)	70.04% (699/998)	<0.001**
Implantation rate [d]	44.28% (151/341)	44.06% (89/202)	0.972
Clinical pregnancy rate [e]	55.36% (124/224)	57.94% (73/126)	0.587
Live-birth rate [f]	40.63% (91/224)	46.83% (59/126)	0.104
Miscarriage rate [g]	6.25% (14/224)	4.76% (6/126)	0.156

Notes: [a] Ovum maturation rate was defined as the no. of Day-0 MII oocytes per cycle/the no. of retrieved oocytes per cycle. [b] Fertilization rate was defined as the no. of Day-1 2PN embryos per cycle/the no. of Day-0 MII oocytes per cycle. [c] The good-quality embryo rate was defined as the no. of Day-3 good-quality embryos per cycle/the no. of Day-1 2PN embryos per cycle. [d] Implantation rate was defined as the no. of implanted embryos (i.e., pregnancies) per cycle/the no. of transferred embryos per cycle. [e] Clinical pregnancy rate was defined as clinical pregnancy cycles/total cycles. [f] Miscarriage rate was defined as miscarriage cycles/total cycles. [g] Live-birth rate was defined as live-birth cycles/total cycles. *$p < 0.05$, **$p < 0.01$.

To assess whether infection with HPV in women was associated with reproductive outcomes, we executed binary logistic regression analyses for HPV infection compared with uninfected status. Our results indicated that female infection with HPV was an independent risk factor for increased miscarriage rate (OR, 0.16; 95% CI, 0.03–0.84; $p = 0.041$). Women with HPV infection also showed a diminished clinical pregnancy rate (OR, 0.25; 95% CI, 0.08–0.77; $p = 0.216$) and live-birth rate (OR, 0.31; 95%CI, 0.11–0.93; $p = 0.437$) in infertile couples. However, this difference was not significant ($p > 0.05$) (**Table 3**).

Table 3 Logistic regression analyses for reproductive outcomes between HPV positive group and HPV negative group.

Variable	Clinical pregnancy rate		Miscarriage rate		Live-birth rate	
	OR(95% *CI*)	*p* value	OR(95% *CI*)	*p* value	OR(95% *CI*)	*p* value
HPV-positive *vs*. HPV-negative	0.25 0.08–0.77	0.216	0.16(0.03–0.84)	0.041*	0.31(0.11–0.93)	0.437

Notes: Factors were adjusted for age, duration of infertility, BMI, causes of infertility, baseline hormone levels, and the number of good-quality embryos per cycle. *OR*, odds ratio; *CI*, confidence interval. *$p < 0.05$, **$p < 0.01$.

Embryonic development and pregnancy outcomes in the smHPV and HPV-negative groups

Of the HPV-infected group, infertility in 45 infertile couples was caused by single male factors such as oligospermia and asthenospermia. Embryonic development and pregnancy outcomes of the single-male-factor group (defined as the smHPV group) were also evaluated, and we noted no difference in ovum maturation rate (88.20% *vs.* 87.01%, $p = 0.418$) or fertilization rate (83.94% *vs.* 84.58%, $p = 0.217$) between the smHPV group and the HPV-negative group. Implantation rate tended to be lower in the smHPV group relative to the HPV-negative group, but this was not significant (31.58% *vs.* 44.06%, $p = 0.089$). The smHPV group also exhibited a reduced Day-3 good-quality embryo rate (46.01% *vs.* 70.04%, $p = 0.013$), clinical pregnancy rate (46.67% *vs*. 57.94%, $p = 0.003$), live-birth rate (33.33% *vs*. 46.83%, $p = 0.027$), and increased miscarriage rate (11.11% *vs.* 4.76%, $p = 0.003$) compared with HPV-negative group (**Table 4**). Our logistic regression analysis results indicated that single male factors comprised an independent risk for decreased clinical pregnancy rate (OR, 4.17; 95% CI, 2.31–7.53; $p < 0.001$), decreased live-birth rate (OR, 1.83; 95% CI, 0.81–2.14; $p = 0.045$), and increased miscarriage rate (OR, 6.83; 95% CI, 2.22–21.00; $p = 0.001$) in infertile couples after infection of the female partner with HPV (**Table 5**).

Table 4 Embryonic development and pregnancy outcomes for single-male-factor HPV-positive and HPV-negative groups

Embryonic development and pregnancy outcomes	smHPV-positive (n = 45)	HPV-negative (n = 126)	*p* value
Ovum maturation rate	88.2% (523/593)	87.01% (1232/1416)	0.418
Fertilization rate	83.94% (439/523)	84.58% (1042/1232)	0.217
High quality embryo rate	46.01% (202/439)	70.04% (699/998)	0.013*
Implantation rate	31.58% (23/69)	44.06% (89/202)	0.089
Clinical pregnancy rate	46.67% (21/45)	57.94% (73/126)	0.003**
Live-birth rate	33.33% (15/45)	46.83% (59/126)	0.027*
Miscarriage rate	11.11% (5/45)	4.76% (6/126)	0.003**

Notes: smHPV, single-male-factor for infertility. *$p < 0.05$, **$p < 0.01$.

Table 5 Logistic regression analyses of reproductive outcomes between single-male-factor HPV-positive and HPV-negative groups

Variable	Clinical pregnancy rate		Miscarriage rate		Live-birth rate	
	OR(95% *CI*)	*p* value	OR(95% *CI*)	*p* value	OR(95% *CI*)	*p* value
smHPV-positive *vs.* HPV-negative	4.17(2.31–7.53)	<0.001**	6.83(2.22–21.00)	0.001**	1.83 0.81–2.14	0.045*

Notes: Factors were adjusted for age, duration of infertility, BMI, causes of infertility, baseline hormone levels, and the number of good-quality embryos per cycle. *OR*, odds ratio; *CI*, confidence interval. *$p < 0.05$, **$p < 0.01$.

Discussion

HPV infection can be spontaneously cleared within one to two years, but repeated infection is associated with multiple malignancies that include cervical, anogenital, and oropharyngeal cancers [18]. Over 200 different HPV genotypes have been identified [19], and the prevalence of HPV differs with respect to geographic location and socioeconomic status [14, 20–23]. Several authors indicated that HPV prevalence was higher in pregnant women than in non-pregnant women, and demonstrated overall HPV prevalence rates of 16.82% and 12.25%, respectively [24]. A case-control study suggested that HPV prevalence was 24.2% in pregnant women *vs.* 14.8% in non-pregnant women, and that HPV prevalence was age and genotype dependent [25]. In our study, HPV prevalence in women from infertile couples was

9.2%, and hrHPV was detected in 78.82% of all HPV-positive women. The predominant hrHPV genotypes were 16, 52, 53, 56, 58, and 59; and the predominant lrHPV genotypes were 6, 43, 44, 55, 61, and 68. Types 16 and 52 were the most common genotypes we observed in the infertile women, congruent with a recent report [25]. Genotype 61 is the predominant type in lrHPV, occupying 9.77% of the total HPV infection (73/747), and higher than genotypes 6 and 11 (25/747). Our results also indicated that there was an elevated infection rate in women of infertile couples who were 26–40 years old (and particularly in women 26–35 years of age), accounting for 70% of total infections and potentially associated with frequent sexual activity in this age group.

As HPV DNA has not only been identified in the cervix but also in the placenta, fetal membranes, and amniotic fluid, pregnant women undergo an increased risk of HPV infection [18, 24]. However, whether HPV infection exerts adverse effects on pregnancy outcomes remains controversial. While several researchers have suggested a higher HPV prevalence among women who suffered a spontaneous abortion in relation to normal pregnancies [26–28], others uncovered no correlation between HPV infection and the risk of spontaneous abortion, miscarriage, or preterm delivery [29, 30]. In addition, attenuated HPV infection rates have been observed in patients with recurrent miscarriage, and it has been hypothesized that augmented immunoreactivity may be partially responsible for the recurrent pregnancy loss and that this may be protective against HPV infection [31]. Our results indicated that HPV infection in women of infertile couples did not alter ovum maturation or fertilization rate but reduced the Day-3 good-quality embryo rate ($p < 0.001$) regardless of whether the infection was either hr-HPV or lr-HPV. With respect to pregnancy outcomes, women's HPV infection appeared to lower the clinical pregnancy and live-birth rates, and elevate the miscarriage rates (but not to a statistically significant extent). However, we noted after logistic regression analysis that women's HPV infection increased the risk of miscarriage, which is the most common adverse pregnancy outcome [26–28, 32–34].

HPV commonly infects both the male and female partners. Men can be infected with HPV in the penis, anus, and head and neck; and it can be detected in penile swabs and semen. There are reports of a significantly higher HPV infection in infertile couples compared to the general population (20.9% *vs.* 8.2%) [35], with HPV also affecting semen parameters [36]. One study indicated a statistically significant correlation between the rate of pregnancy loss and positivity for HPV DNA in the male partner of infertile couples compared with non-infected couples (66.7% *vs.* 15%, respectively) [32]. Pregnancy rate was reduced, but the miscarriage rate was increased after HPV infection in both women and men [33]. Christophe et al. reported that the pregnancy rate with intra-uterine insemination (IUI) declined when the sperm DNA fragmentation index (DFI) exceeded 26%; and sperm samples containing HPV exhibited a significantly higher DFI compared with HPV-negative sperm samples (29.8% *vs.* 20.9%, respectively; $p = 0.011$) [37]. However, Hana et al. recently reported that men with hrHPV-positive semen samples showed altered seminal parameters that included lower semen volume, sperm concentration, and total sperm count relative to men with HPV-negative samples; but there was no association between seminal hrHPV infection and pregnancy outcomes that included spontaneous abortion [36]. Thus, the impact of HPV on male fertility and associated reproductive outcomes remains debatable.

In our study, infertility in 45 couples was caused by single male factors that included oligospermia and asthenospermia, and this was possibly associated with HPV infection. The single-male-factor group (smHPV group) manifested a lower Day-3 good-quality embryo rate, clinical pregnancy rate, and live-birth rate, and increased miscarriage rate compared with the HPV-negative group. Logistic regression analysis indicated that single male factors comprised an independent risk for decreased clinical pregnancy rate, decreased live-birth rate, and increased miscarriage rate in infertile couples in which the female partner was infected with HPV. Our results suggested that HPV infection caused semen parameters to change, giving rise to oligospermia and asthenospermia; and that it reduced clinical pregnancy and live-birth rates and increased the miscarriage rate. We did not investigate the prevalence of male-partner HPV infection and changes in semen parameters, and assert that further investigation of male-partner infection status is necessary.

Several studies indicated that HPV infection was associated with spontaneous preterm birth (sPTB), defined as delivery between 28 and 37 weeks of gestation [14]. In normal pregnancy, 17.5% of HPV infection occurs at the cervix, significantly lower than in sPTB patients; and cervical cytology shows that HPV infection generates placental abnormalities and preterm birth [38, 39]. Hr-HPV infection was also associated with a risk of premature rupture of the membranes [40], and persistent HPV-16/18 infection was related to an increased risk of preterm birth, independent of cervical treatment [41]. In our study, we observed no rise in the sPTB rate or attenuation in the live-birth rate between the HPV-infected and non-HPV-infected groups. We thus recommend that persistent HPV infection be determined clinically.

Conclusions

In summary, our results indicated that HPV-infected women of infertile couples did not show alterations in ovum maturation, fertilization, implantation, clinical pregnancy, live-birth, or miscarriage rates regardless of hr-HPV or lr-HPV infection, but did exhibit lower good-embryo rates with HPV infection. HPV infection in these women was associated with a reduced miscarriage rate, and single-male-factor-induced infertility influenced reproductive outcomes of couples undergoing IVF/ICSI treatment. However, simultaneous determination of HPV status for both female and male partners of infertile couples is required to further clarify this phenomenon.

Declarations

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Not applicable

Author contributions

Conception and design of the research: Sanhua Wei and Xiaohong Wang; acquisition of the data: Fang Cheng, Zhenhua Chang, Xiaoyan Ren, Mengxin Liu, and Tao Yang; analysis and interpretation of the data: Sanhua Wei and Zheng Liu; statistical analysis: Sanhua Wei, Zheng Liu, Xiaoyan Ren, Xuhui Ma, and

Xiaojuan Xie; writing of the manuscript: Sanhua Wei and Xiaohong Wang; and critical revision of the manuscript for intellectual content: Sanhua Wei. All authors read and approved the final version of this manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Independent Ethics Committee of Tangdu Hospital (approval number: TDLL-202206-07; approval date: June 17, 2022), in accordance with the Helsinki Declaration.

Consent for publication

Not applicable

Conflict of interest

None of the authors showed any personal, financial, commercial, or academic conflicts of interest.

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Figures

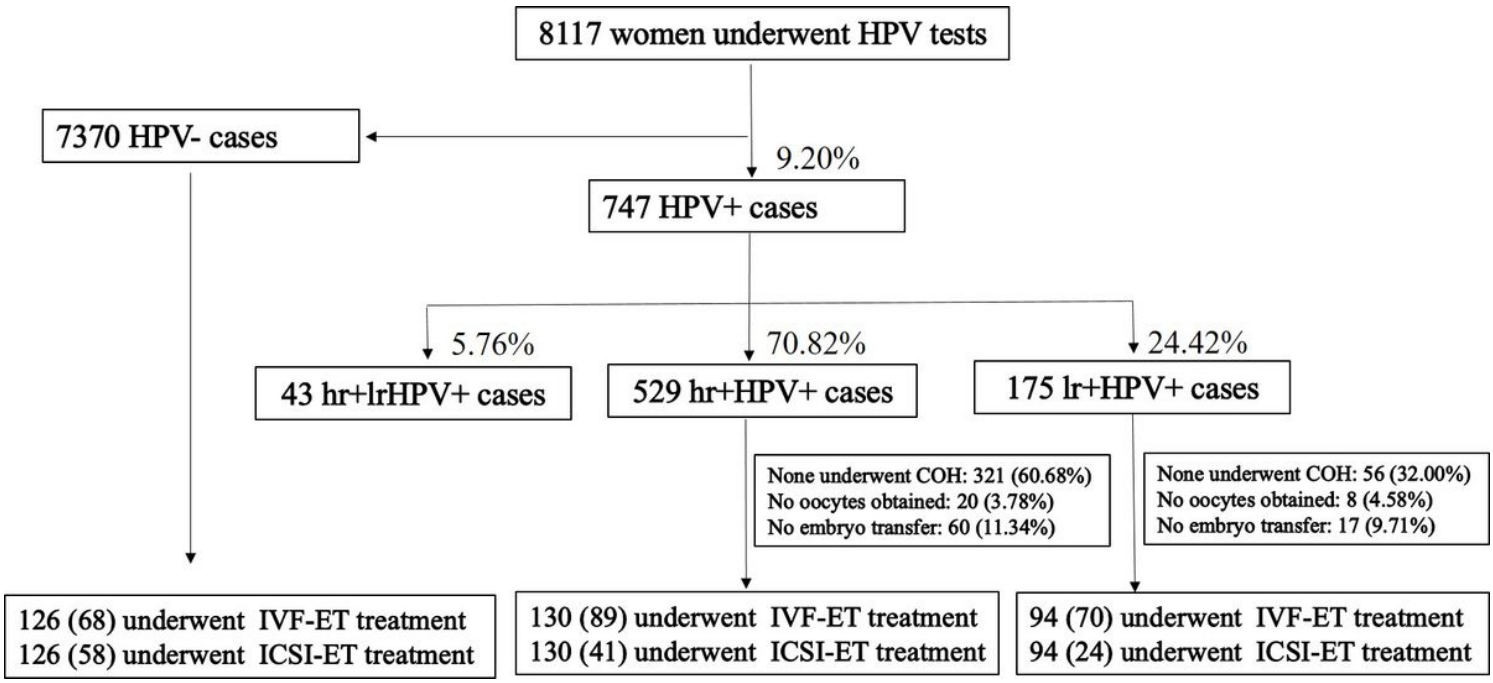

Figure 1

Study flowchart of HPV tests in women and IVF/ICSI-ET treatments. hrHPV, high-risk HPV; lrHPV, low-risk HPV; COH, controlled ovarian hyperstimulation; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; and ET, embryo transfer.

Supplementary Files

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