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Original Article

Human papillomaviruse infection in women undergoing artificial insemination by donor: a retrospective analysis

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Abstract: The aim of this retrospective study was to demonstrate the association of HPV infection and pregnancy, delivery and neonatal outcome in women undergoing artificial insemination by donor (AID). From July 2015 to June 2016 in Tongji Reproductive Medicine Center, 329 women undergoing AID were divided into two sub-groups, HPV positive group and HPV negative group. All the couples were followed up to determine pregnancy, delivery and neonatal outcome. Forty-four (13.4%) women was detected HPV infection. HPV infection could reduce clinical pregnancy rate and live birth rate significantly of women undergoing AID (P=0.008; P=0.008). However, for miscarriage rate, there was no significant difference (0.6% vs. 2.7%; P=0.446). There were no significant differences of pregnancy outcome among various HPV type groups. Moreover, we found that only gestational weeks showed significant difference between two groups (36.00±0.71 vs. 38.74±1.65; P=0.023). No difference was noted in neonatal weight (5.10±0.14 vs. 6.45±1.01; P=0.066), neonatal sex (P=0.490), delivery mode (P=0.655). The infection of HPV could reduce the clinical pregnancy rate and live birth rate, without improving miscarriage rate. Pregnant HPV exposure for the women undergoing AID could short the gestational weeks.

Keywords: HPV infection, AID, pregnancy outcome, delivery and neonatal outcome

Introduction

Human papillomavirus (HPV) had more than 100 types [1, 2]. Type 16, 18, 31, 33, 35, 39, 51, 56, 58 and 68 were high risk types. In a general way, high risk types HPV can be divided into three type groups, A5/A6 including 51, 56, 66, A7 including 18, 39, 45, 59, 68, and A9 including 16, 31, 33, 35, 52, 58 [3]. These high risk types HPV could cause all cases of cervical carcinoma.

Recently, new insights have suggested a role for HPV in human reproduction. Prevalence of HPV infections was 5% in asymptomatic women in the general population [4]. However, the infection rate of fertility women raise to 24% [5]. Some studies suggested that HPV infection may be a cause of spontaneous abortions and major birth defects in couples undergoing assisted reproduction technology (ART) cycles [6, 7]. These studies were included male infection and female infection. Because the transmission route of HPV was sex, male HPV infection may cause his sex-partner infection. Whose infection of HPV was the key factor, this question remain contentious. So, well defined studies are needed to clarify this [8].

Some systematic review revealed that HPV semen infection in male can cause infertility mainly [9]. At the same time, a recent study showed that sperm infection of HPV may reduce assisted cumulative pregnancy rate and increase miscarriage rate. These research may revealed that semen HPV infection was a main factor to impair fertility [10]. However, some study held another opinion that HPV infection of women may be the main reason of infertility. Because HPV exposure was linked to two-cell embryo demise and delaying the exposure of HPV until later embryo stages permitted embryo development [11]. On the other hand, little is known about the effect of different HPV type group infection. Andrew's study was provided that HPV type 16 was shown to decrease blastocyst formation while HPV type 18 inhibited the blas-

tocyst hatching process. More data was needed in future.

At present, ART was received by more and more couples in China. HPV test had been held in more and more reproductive medicine center. So, to reveal the association of the HPV infection and pregnancy outcome was more important than before. The aim of this retrospective study was to evaluate the pregnancy, delivery and neonatal outcome of HPV infection in women undergoing artificial insemination by donor (AID). In China, semen donors were need to examination. Most semen donors were the young college students, who had very low HPV infection. In addition, no matter in which group, the semen was got from donors. So this study in vivo could remove interference from HPV positive sperm or semen from their husbands. Moreover, our study will firstly compare the difference on the effect of HPV infection in HPV A5/A6 type group, HPV A7 type group and HPV A9 type group in women undergoing AID.

Materials and methods

A retrospective study was performed to identify couples who are undergoing AID in Tongji Reproductive Medicine Centre from August 2015 to July 2016 with the approval and support of the Ethics or Institutional Review Board of Tongji Hospital, Huazhong University of Science and Technology in Hubei Province. Inclusion criteria was including: the male patients of non-obstructive azoospermia and a few cases of obstructive azoospermia and severe oligo-asthenoterato-zoospemia were included for AID. The women were sensitive with Letrozole. Exclusion criteria was including: Positive of hepatitis B virus, hepatitis C virus, human immunodeficiency virus, syphilis, gonorrhoea, mycoplasma, chlamydia and transmissible genetic disorders. Women with uterus diseases should be excluded. Medical charts, including admission and examination were reviewed. All participants were provided informed consent. Patient characteristics, detailed history of symptoms, menstrual history, situation of HPV infections were noted.

On entry examination, a cervical scrape was taken with a cytobrush from all women. Cells were performed directly for HPV testing. All cell samples were processed for DNA extraction [12]. A Mastercycler (Eppendorf) and polymerase chain reaction (PCR) products were used for analysis in 8% polyacrylamide gel. According to HPV Inno-Lipa Genotyping System (Innogenetics), HPV genotypes were grouped [13].

Letrozole (Jiangsu Hengrui Medicine Co., Ltd.; Lianyungang, China) was given in a dose of 2.5-5 mg per day for 5 days starting on days 3 or 5 of the menstrual cycle. The dose and duration of gonadotrophin injection were adjusted according to follicle response. The adjustment was flexible. Gonadotrophin was including HMG (Menotrophin for Injection, Livzon Pharmaceutical Group Inc., Guangzhou, China) or uFSH (Urofollitropin for Injection, purified, Livzon Pharmaceutical Group Inc., Guangzhou, China). If the follicles with a mean diameter of 16 mm or wider were detected, HCG administration could be taken. To improve the effectiveness of AID, double intrauterine insemination per cycle using cryopreserved semen by donors was carried out 24-36 h later. Luteal support was given for two weeks. If they did not menstruate two weeks after the insemination, a quantitative serum HCG measurement was obtained. At about 4-5 weeks after the insemination, a transvaginal ultrasound was used to confirm clinical pregnancy and pregnant location.

According to infection of HPV, the patients were divided into two sub-groups, HPV positive group and HPV negative group. All the patients were followed up to determine the biochemical pregnancy, clinical pregnancy, miscarriage, ectopic pregnancy, live birth situation, delivery and neonatal outcome.

Statistical analysis was performed using SPSS version 21.0, and all hypothesis tests were two-sided. Categorical data were presented as number and percentages, and compared using the X^2-test. As all continuous variables were normally distributed, they were presented in mean ± standard deviation and compared using the Student's t-test. *P*-value <0.05 were considered statistically significant.

Results

In total, 329 women undergoing AID were recruited in our study. According to detection of HPV, the patients were divided into two subgroups (HPV positive group and HPV negative group). Forty-four (13.4%) women was detected HPV infection by the method above (**Figure 1**).

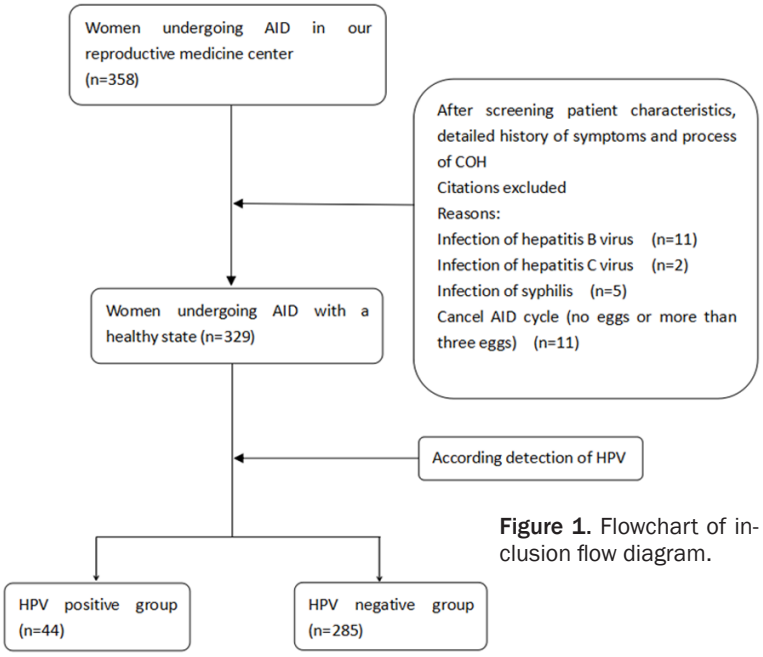

Figure 1. Flowchart of inclusion flow diagram.

Table 1. Patients' characteristics in women undergoing AID

	HPV (+)	HPV (-)	*P*-value
Age (year)	28.36±4.46	27.40±3.72	0.121
BMI (kg/cm^2)	21.61±3.24	32.14±127.29	0.584
Baseline FSH lever (mIU/ml)	7.36±1.50	7.59±3.33	0.646
Endometrial thickness (mm)#	8.62±2.44	9.74±2.89	0.006*
Diameter of follicular (mm)	20.31±2.66	20.37±2.51	0.877
Infertility (number)	44	285	0.055
Primary	36	260	
Secondary	8	25	
Regular menstrual period (number)	44	285	0.425
Yes	38	252	
No	6	33	

#On day of HCG administration; *P<0.050, statistically significant.

Table 2. Comparison of pregnancy outcome in women undergoing AID

	HPV (+)	HPV (-)	*P*-value
Clinical pregnancy (%)	9.1	26.0	0.008*
Live birth (%)	5.4	22.5	0.008*
Miscarriage (%)	0.6	2.7	0.446

*P<0.050, statistically significant.

The characteristic of women undergoing AID with respect to the groups were presented in **Table 1**. The baseline variable of women in two groups was comparable between the HPV positive group and HPV negative group. The mean age (P=0.121), body mass index (P=0.584), baseline FSH lever (P=0.646), endometrial thickness (P=0.006), diameter of follicular (P=0.877), number of different infertility type (P=0.055), number of women with regular menstrual period (P=0.425) did not differ between two groups, except endometrial thickness on day of HCG administration.

In **Table 2**, pregnancy outcome of women undergoing AID, including clinical pregnancy rate, live birth rate and miscarriage rate, were compared between the HPV positive group and HPV negative group. Our result showed that HPV infection could significantly reduce clinical pregnant rate and live birth rate of women undergoing AID (P=0.008; P=0.008). In the HPV positive group, the clinical pregnant rate and live birth rate were 9.1% and 5.4%, which was significantly lower than 26.0% and 22.5% in the HPV negative group (P=0.008; P=0.008). However, for miscarriage rate, there was no significant difference between this two groups (0.6% vs. 2.7%; P=0.446).

In our study, 46% of women undergoing AID tested positive for A9 HPV type group. This prevalence rate is significantly higher than A5/A6 HPV type group, A7 HPV type group and mixed infection of A5/A6, A7, A9. In addition, our result firstly revealed that there were no significant difference of pregnancy outcome, including clinical pregnant rate, live birth rate and miscarriage rate, among these four groups. As shown in **Table 3** and **Figure 2**, the clinical pregnant rate were respectively 10.0% in A5/A6 HPV type group, 6.0% in A7 HPV type group, 4.0% in A9 HPV type group, 0% in mixed infection of A5/A6, A7, A9 (P=0.081). The live birth rate were respectively 7.0% in A5/A6 HPV type group, 4.7% in A7 HPV type group, 2.3% in A9 HPV type group, 0% in mixed infection of A5/A6, A7, A9 (P=0.343). At

Table 3. Comparison of pregnancy outcome in women undergoing AID with different HPV type groups

	A5/A6	A7	A9	A5/A6, A7, A9	*P*-value
Total (%)	24.0	22.0	46.0	8.0	
Clinical pregnancy (%)	10.0	6.0	4.0	0	0.081
Live birth (%)	7.0	4.7	2.3	0	0.343
Miscarriage (%)	2.0	2.0	2.0	0	0.881

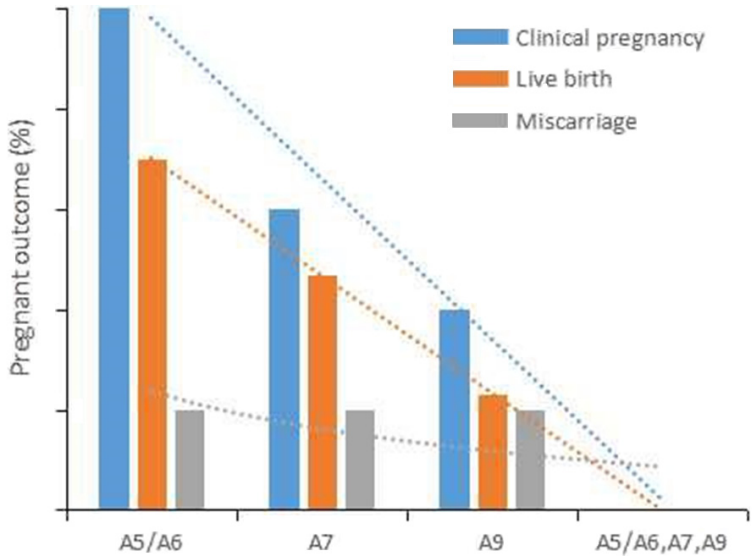

Figure 2. Pregnant outcome of women with different HPV type groups.

Table 4. Comparison of delivery and neonatal outcome in women undergoing AID

	HPV (+)	HPV (-)	*P*-value
Gestational weeks (week)	36.00±0.71	38.74±1.65	0.023*
Neonatal weight (500 g)	5.10±0.14	6.45±1.01	0.066
Neonatal sex (number)			0.490
Male	1	37	
Female	2	35	
Delivery mode (number)			0.655
Cesarean delivery	1	26	
Natural labour	1	38	

*P<0.050, statistically significant.

the same time, miscarriage rate were respectively 2.0% in A5/A6 HPV type group, 2.0% in A7 HPV type group, 2.0% in A9 HPV type group, 0% in mixed infection of A5/A6, A7, A9 (P=0.881).

On the other hand, following up by telephone or e-mail, we compared the delivery and neonatal factors, including gestational weeks, neonatal weight, neonatal sex, delivery mode (cesarean delivery or natural labour) of women undergoing AID. As shown in **Table 4**, we found that only gestational weeks showed significant difference between the HPV positive group and the HPV negative group (36.00±0.71 vs. 38.74±1.65; P=0.023). No difference was noted in neonatal weight (5.10±0.14 vs. 6.45±1.01; P=0.066), neonatal sex (P=0.490), delivery mode (P=0.655).

Discussion

In the present study, the possible association existing between HPV infection and fertility impairment represents indetermination and debated themes of human reproduction in recent years [14, 15]. In addition, HPV is the most common viral sexually transmitted disease, so the reproductive-aged couples could be infected by their sex-partners. Because of asymptomatic and transient infection of HPV, many couples could not found infection except by HPV-DNA test from cervical of women or semen of men. The present study had demonstrated the HPV infection of oocyte or sperm could reduce pregnancy rate, although the mechanism was not clear. The reason may be that infection could disturb the embryo development [16, 17]. However, almost all the research in vivo could not eliminate influence from his/her sex partner, because of the transmission route of HPV [18]. In our study, the women undergoing AID was included for HPV research firstly. This study could remove interference from sperm or semen. It was very important for revealing the effect of oocyte infection in vivo.

To our knowledge, in our respective study, endometrium thickness on day of HCG administration was firstly demonstrated thinner in HPV negative group than in HPV positive group

(**Table 1**). In addition to oocyte impairment by HPV infection in vivo [19], the thinner endometrium thickness might be another new factor to reduce the clinical pregnant rate of HPV positive women. The mechanism may need further study.

From our data, the infection of HPV could reduce clinical pregnant rate and live birth rate of women undergoing AID. In previous study, many researchers held the opinion that HPV infection might disturb the oocyte and embryo development [16, 17]. It was similar with our result. Of course, the thinner endometrium thickness on day of HCG administration in HPV positive group may be another reason of low clinical pregnant rate. In addition, the infection of HPV could interference pregnant environment, especially the placental function [20]. It may be one of reason of reducing live birth rate. However, for miscarriage rate, no significant difference was found between HPV positive group and HPV negative group. From the present study, the main reason of miscarriage was chromosome abnormality, but not infectious diseases. So far, there were few researches about the association of HPV infection and chromosome abnormality. That might be why the miscarriage rate could not effect by infection of HPV.

Furthermore, we found A9 type group, including HPV 16 type, was the most prevalent. However, response was no specific to the type groups of HPV. Similar effect of the various HPV type groups on pregnant outcome of women undergoing AID has been documented. Though HPV types 16 and 18 were reported deleterious to the two-cell embryo and caused embryo demise [21], the result was unlike in our study. The result was proved in vitro before, but our result was made in vivo. So our data was more certified the infection of HPV.

Moreover, new born exposed to HPV had the shorter gestational weeks, which might caused by the infection of live canal and amnion cavity. In the previous study, the infection of HPV was proved associated with premature rupture of membranes [22]. However, some research showed the infection of HPV did not cause preterm [23, 24]. The controversy still existed at present. On the other hand, neonatal weight, neonatal sex, delivery mode (cesarean delivery or natural labour) of women undergoing AID did not show significant difference between HPV positive group and HPV negative group.

In terms of limitations, the test of HPV before AID was carried out just for a short time in our center, so the number of sample was limited. Further prospective and large studies were needed.

In summary, our results showed an association between HPV exposure and pregnancy outcome of women undergoing AID. This result in vivo firstly suppressed interference from male. The infection of HPV could reduce the clinical pregnancy rate and live birth rate, but miscarriage rate. Pregnant HPV exposure for the women undergoing AID could shorten the gestational weeks. The effect on pregnancy outcome of various HPV type groups had no difference. Our results are supported by a concise study design but with a small sample size. So, more research was needed in the future.

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Disclosure of conflict of interest

None.

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