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Influence of Human PapillomaVirus Infection Detected by Cervical Swap on In-vitro Fertilization Outcomes

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Human papilloma virus (HPV) infection had been suspected of harmful effect on reproduction by increasing blastocyst apoptosis and reducing the endometrial implantation of trophoblastic cells. However, the available evidence is conflicting concerning the impact of HPV in female reproductive system on the outcome of in-vitro fertilization (IVF) The aim of study was to evaluate IVF outcomes regarding the cervical HPV infection. Retrospective case-control study conducted in women suffered IVF cycle. All women were evaluated the HPV infection using PCR by cervical swap before the initiation of IVF cycle. One hundred thirty six cycles were evaluated. The harvested oocytes number, top quality embryo number, fertilization rate, implantation rate, clinical pregnancy rate, live birth rate and miscarriage rate were analyzed depend on HPV infection. Mean age of enrolled women was 35.20±4.79 years-old, mean parity was 0.17±0.57, and mean number of miscarriage was 0.81±1.49. Basal FSH was 5.42±2.59 IU/L and AMH level was 5.07±11.01 ng/mL, which was not different between two groups. Mean harvested oocytes was 6.36±4.32 in HPV infected women compared with 8.63±5.95 in women without infection, statistically insignificant. Top quality embryos of each group were not different (2.67±0.82, 2.53±1.53 separately). In women with HPV infection, ongoing pregnancy rate (27.3%) and live birth rate (18.2%) were similar with women without infection (23.7%, 14.0%). And also, fertilization rate and implantation rate were similar. Miscarriage rate of each group was 33.3%, and 40.7%, which was statistically insignificant. The HPV infection in women did not make the adverse influence on IVF outcomes.

Keywords: Clinical pregnancy rate, Human papilloma virus, In-vitro fertilization, Live birth rate, Miscarriage rate

Introduction

Human papilloma virus (HPV) is a common sexually transmitted virus responsible for the formation of warts, cervical intraepithelial neoplasia, and cervical cancer. Recent studies propose HPV infection is related with infertility, sperm dysfunction and the outcome of the assisted reproductive technology [1]. Viral infection in men may lead to bad sperm quality such as asthenozoospermia, increased sperm DNA fragmentation, increased sperm aneuploidy, and increased rates of antisperm antibodies [1,2]. HPV is attached to the spermatozoa in two distinct sites along the equatorial region of the spermatozoon's head, which is the site of the spermatozoa that binds and subsequently fuses with the plasma membrane of the oocyte [2]. Infected spermatozoa could be a vector for HPV transmission into fertilized oocytes and the infected zygote is able to perpetuate the viral genome expression at blastocyst stage and subse-

quently in trophoblastic cells [3]. When semen was infected with HPV in the cycle of intrauterine insemination (IUI) or in vitro fertilization (IVF), clinical pregnancy rate was decreased [4,5]. Moreover, an increased risk of pregnancy loss and preterm delivery was reported in HPV infected male partners [4,6].

Even though HPV is common in both men and women and also highly contagious between sexual partners, most studies about infertility, miscarriage and preterm birth were conducted regarding to male infection. Recently, Moragianni et al. reported female genital tract infection may contribute to infertility [7]. However, there were some reports that HPV infection in women did not influence the outcomes of assisted reproductive technology [6,8]. Contrary to HPV infection in men, the effect of HPV infection in women is still controversial and inadequate to draw the conclusions. The aim of this study is to evaluate the influence of HPV infection by cervical swap on IVF outcomes and also evaluate the miscarriage rate and preterm birth rate regarding to cervical HPV infections.

Materials and Methods

Retrospective case-control study was conducted in women suffered IVF cycle at infertility clinic in Keimyung university hospital between January 2012 to April 2018. Among the five hundred twenty three IVF cycles, we enrolled 136 cases in which cervical swaps were taken within 3 months before the initiation of IVF cycle. All samples were tested for HPV DNA using PCR according to the manufacture's recommendation. Genotyping of HPV was also evaluated such as HPV high risk type 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82 and low risk type 6, 11, 40, 42, 43, 44, 54, 61, 70.

Controlled ovarian hyperstimulation (COH) was initiated with daily injection of recombinant FSH 225-300 IU from day 3 of the menstrual cycle after pituitary desensitization with GnRH agonist 0.2cc from the previous menstrual mid-luteal phase. Human chorionic gonadotropin 10,000 U was injected when the largest follicular size was reached over 18mm. Oocyte retrieval was performed via the transvaginal approach with sonographic guidance 35 hours after hCG administration. All harvested oocytes were fertilized with swim-up sperm and cultured in the incubator. Two to three cleavage embryos were transferred into the uterine cavity by Korean Guideline. Luteal support was performed by progesterone. After 14 days later from embryo transfer, β-hCG was checked for confirming the pregnancy.

Depending on HPV infection, the harvested oocytes number, top quality embryo number, fertilization rate, implantation rate, clinical pregnancy rate, live birth rate and miscarriage rate were analyzed. Statistical analysis were performed by Mann-Whitney test, Fisher's exact test, Pearson's chi-square test with SPSS 12.0. A p-value ≤ 0.05 was considered statistically significant.

Results

One hundred thirty six cycles were evaluated. The Mean age of enrolled women was 35.20 ± 4.79 years old, mean parity was 0.17 ± 0.57, and mean number of miscarriage was 0.81 ± 1.49. By the cervical swap before IVF cycle, HPV infection was found 11 cases out of 136 cycles (8.08%). Genotyping of HPV in infected cases were 6 cases of high risk type (16, 18, 53, 52, 56, and 59), 2 cases of low risk type (11, and coinfection of 44 and 61), and 3 cases of mixed infection (16, 31 and 54, 16 and 6, 51 and 54). The clinical and biological characteristics of women with HPV infection were compared with those of women without HPV infection. Mean age, parity and miscarriage were not different between two groups (Table 1). Depending on the HPV infection, basal FSH levels (6.23 ± 2.73 vs. 5.34 ± 2.58 mIU/ml), basal estradiol levels (70.50 ± 12.82 vs. 75.17 ± 39.54 pg/ml) or AMH (4.06 ± 4.72 vs. 5.18 ± 11.51 ng/ml) were not different either.

After controlled ovarian hyperstimulation cycles, no statistically significant difference was found in COH parameters between HPV infected and non-infected women (Table 2). Total dose of Gonadotropin in each groups was 2725.00 ± 773.22 IU and 3118.85 ± 681.96IU in HPV positive and HPV negative women, respectively (p-Value = 0.09). Mean harvested oocytes in women with HPV infection was 6.36 ± 4.32, similar with 8.63 ± 5.95 in women without HPV infection (p-Value = 0.26). Fertilization rate was not different between two groups (33 out of 70 eggs (47.1%) vs. 523 out of 1053 eggs (49.7%), p-Value = 0.54). In women with HPV in-

Table 1. Demographic data of enrolled women

	HPV +	HPV -	p-Value
Age	34.00 ± 4.41	35.30 ± 4.84	0.34
Parity	0.18 ± 0.60	0.17 ± 0.56	0.88
Miscarriage	0.55 ± 0.93	0.83 ± 1.53	0.49
Basal FSH (mIU/ml)	6.23 ± 2.73	5.34 ± 2.58	0.26
Basal E2 (pg/ml)	70.50 ± 12.82	75.17 ± 39.54	0.74
AMH (ng/ml)	4.06 ± 4.72	5.18 ± 11.51	0.76

AMH, antimullerian hormone; FSH, follicle stimulating hormone; E2, estradiol; HPV, human papilloma virus

Table 2. Characteristics of controlled ovarian hyperstimulation and IVF cycles in women with HPV infection compared without HPV infection

	HPV +	HPV -	*p*-Value
Gonadotropin (total dose, IU)	2725.00 ± 773.22	3118.85 ± 681.96	0.09
Oocytes retrieved (n)	6.36 ± 4.32	8.63 ± 5.95	0.26
Embryos obtained (n)	3.0 ± 1.95	4.29 ± 3.25	0.09
Fertilization rate	33/70 (47.1%)	523/1053 (49.7%)	0.54
Top quality embryos obtained (n)	2.67 ± 0.82	2.53 ± 1.53	0.58
Embryos transferred (n)	2.18 ± 0.75	2.11 ± 0.86	0.96

Table 3. IVF outcomes of patients with HPV infection and without HPV infection

	HPV +	HPV -	*p*-Value
Implantation rate	4/24 (16.7%)	35/264 (13.3%)	0.48
Clinical pregnancy rate per cycle	3/11 (27.3%)	27/125 (21.6%)	0.45
CPR per transfer cycle	3/11 (27.3%)	27/114 (23.7%)	0.52
Live birth rate per cycle	2.11 (18.2%)	16/125 (12.8%)	0.44
LBR per transfer cycle	2/11 (18.2%)	16/114 (14.0%)	0.49
Miscarriage rate	1/3 (33.3%)	11/27 (40.7%)	0.26

fection, mean number of fertilized embryos (3.0 ± 1.95) and top quality embryos (2.67 ± 0.82) were not deteriorated compared with those of women without HPV infection (4.29 ± 3.25, 2.53 ± 1.53, respectively).

HPV infected and non-infected women had similar implantation rates (16.7% vs. 13.3%, $p=0.48$), clinical pregnancy rates (27.3% vs. 21.6%, $p=0.45$) and live birth rates (18.2% vs. 12.8%, $p=0.44$). Considering only the transferred cycles, clinical pregnancy rates and live birth rates were not statistically different between two groups (Table 3). Miscarriage rate was found as 33.3% in women with HPV infection, which was not increased compared with non-infected women.

Discussion

Human papilloma virus (HPV) is one of the common sexual transmitted pathogens and is believed as a responsible factor for both male and female cancer development. Nowadays, evidence about HPV infection in human reproduction impairment and infertility is emerging. Sexually transmitted infections (STI) such as Chlamydia trachomatis, and Neisseria gonorrhea has been widely believed to cause infertility by tubal obstruction, pelvic inflammatory disease and adhesions [9]. As the bacterial infection, viral STIs including human immunodeficiency virus (HIV), cytomegalovirus (CMV), herpes virus (HSV), and HPV may also be associated with human fertility [8]. Nielson et al. [10] tested HPV infection at the glans, penile shaft, scrotum, urethra, perianal area, anal canal, and in a semen sample, and reported that 51.2% of men were positive for at least one oncogenic or non-oncogenic HPV type from any sampling site. Although the penile shaft was the site most likely to be HPV positive, HPV were positive 5.3% in the semen samples. In the meta analysis, the prevalence of HPV infection in semen is 16% (95% CI: 10-23%) in men affected by unexplained infertility, more common than 10% (95% CI: 7-14%) in general male population [11]. HPV infected semen could be associated with an impairment of sperm parameters especially a reduction of sperm motility. And also, infected sperm is able to penetrate the oocyte, to deliver HPV genome in the oocyte and to increase the risk of pregnancy loss [2]. In vivo study, cumulative pregnancy rates after IUI and ICSI treatments showed significantly lower in HPV infected couples compared with non-infected couples, and also miscarriage rates were higher in HPV infected couples [4-6]. Hermonat et al. [12] reported that spontaneous miscarriage products were more prevalent (60%) for HPV-DNA sequences compared to elective aborted products (20%). Although confounding contamination from the cervix and vagina could not be ruled out, these data suggest that HPV infection might be related with early pregnancy losses.

Although current evidence regarding HPV infection in semen to negative effect on semen parameters and reproductive outcomes has been accumulated, the impact of HPV on female fertility has not been thoroughly studied. There is a lack

of studies on HPV effects in female fertility parameters such as hormonal balance or oocyte production [13]. Furthermore, there is an ongoing debate whether HPV in female reproductive system alters the efficacy of assisted reproductive technologies or pregnancy outcomes. In this study, basal FSH, estradiol and AMH levels were not different whether the HPV infection on cervix was or not. We found that mean harvested oocytes, mean number of fertilized embryos, or top quality embryos was not different in women with HPV infection. Fertilization rate was not different either between two groups. We believed women with HPV infection was not influenced on hormonal balance or oocyte/embryo production.

In vitro study using extravillous trophoblast cells transfected with plasmid harboring HPV16 genome, rates of apoptosis were 3-6 fold greater in transfected cells than in non-transfected cells. And also, invasion of transfected cells through extracellular matrices was 25-58% lower than that of the controls [14]. Spandorfer et al. [15] reported that women with HPV infection had a lower pregnancy rate (23.5%) compared to women without HPV infection (57.0%) even though no difference was found in the number of oocytes retrieved, number of embryos transferred, embryo quality. However, meta-analysis showed no significant difference between HPV-infected and non-infected women in rates of live birth/ongoing pregnancy, clinical pregnancy, or miscarriage [6]. Our study is also consistent with that study, which means that implantation rate, clinical pregnancy rate and live birth rate are similar regardless of HPV infection on cervix. The studies about the relation between HPV infection in female reproductive system and spontaneous abortions or premature rupture of membranes after IVF are still conflicting [6,13,16]. Our study showed miscarriage rate was not increased even in women with HPV infection.

Recently, Jaworek et al. [8] reported that HPV prevalence was significantly higher in oocyte donors than in women treated for infertility, and women who became pregnant spontaneously than women treated with IVF. Our study is consistent with previous study. In this study, cervical HPV infection was found only in 8.08% of women, much less prevalent than general population. It made the sample size of study group was getting smaller than we expected. That is one of the limitation of this study. Like for the risk of carcinogenesis, some genotype of HPV might be related with the risk of fertility alteration [13]. With the limited number of cases, we could not investigate it. Further large scale and deep investigations should be considered. Vaccination of both genders against HPV can reduce the prevalence of HPV infection, the study will be needed in the future whether the vaccination eliminate its implications on human fertility or not [1].

To our acknowledgement, this is the first study in Korean women regarding the relation of cervical HPV infection and reproductive outcomes after IVF. In this study, HPV infection on cervix may not make the negative impact on IVF outcomes. Considering the studies about the HPV effect on female fertility are still lack, we believe further researches are needed.

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Conflict of interest

All authors declare no conflicts-of-interest related to this article.

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