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Original Article

Effect of male hepatitis B virus infection on outcomes of in vitro fertilization and embryo transfer treatment: insights from couples undergoing oocyte donation

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Abstract: It is common to see HBV infected couple seeking fertility treatment in reproductive medical centers. However, it is still unclear whether HBV infection has any relationship with IVF outcome. To assess the impact of male HVB infection on the outcomes of IVF, we retrospectively analyzed data from two hundred and seventy-seven subfertile couples undergoing oocyte donation cycles in our center. Twenty men (7.2%) were HBV seropositive in 277 couples. 20 couples with seropositive husbands had similar semen parameters and fertilization rate when compared with their controls. Among the 215 couples undergoing their first oocyte donation cycles, 19 couples with seropositive husbands/seronegative wives had lower implantation rate (26.7% vs. 40.6%; $P > 0.05$), and lower clinical pregnancy rate (42.1% vs. 63.8%; $P > 0.05$), but the difference was not statistically significant. In binary regression model, male HBV infection had no association with clinical pregnancy. Our study shows that male HBV infection has little impact on IVF outcomes.

Keywords: Hepatitis B virus, oocyte donation, *in vitro* fertilization, male infertility

Introduction

Hepatitis B virus (HBV), one of the most common viruses threatening health of human, causes hepatic inflammation and severe liver diseases in patients. According to literature published in 2005 [1], approximately 2 billion people worldwide have been infected with HBV, and around 400 million live with chronic infection. China, with a prevalence of HBV infection being 9%, has the highest rate of HBV infection in the world.

Nowadays, many HBV seropositive couples are seeking *in vitro* fertilization-embryo transfer (IVF-ET) or intracytoplasmic sperm injection (ICSI) in reproductive medical centers, raising concerns about the association of HBV infection and IVF outcomes, since viral infections may be deleterious to human fertility [2].

Many laboratory studies showed that HBV DNA can be detected in urine, saliva, ovarian tissues and seminal plasma [3, 4]. Moreover, HBV infection was found to be associated with reduced sperm function [5], instability of sperm chromosome, and impaired sperm viability and normal morphology [6, 7]. Recently, another two studies showed that HBV DNA and RNA was detected in oocytes and embryos from HBV discordant couples, and that rates of positivity in oocytes and embryos were positively associated with serum HBV DNA levels, confirming the possibility of vertical transmission of HBV via germ line during IVF [8, 9]. However, the impact of HBV infection on final outcomes of IVF, such as implantation rate, pregnancy rate and live birth rate are still controversial.

The earliest clinical study concerning effect of HBV infection and IVF outcomes showed a significantly lower pregnancy rate (7.7% vs. 40.7%; $P < 0.01$) in HBV discordant couples compared with age-matched controls [10]. In contrast, another retrospective study with larger sample size demonstrated higher ongoing pregnancy rate or live birth rate (53.3% vs. 24.2%; $P < 0.01$) and implantation rate (43.3% vs. 18.4%;

$P < 0.01$) in HBV group. Moreover, in the regression analysis after correction for the confounding effect, successful pregnancy was positively associated with HBV seropositive stats ($P < 0.01$) [11]. Later than that, a study conducted by Lee from Hong Kong reported significantly lower percentage of normal sperm morphology in HBV seropositive males than that of seronegative controls (5.0% vs. 10.0%; $P < 0.01$). However, no adverse effect of HBV infection on the implantation rate or pregnancy rate was observed [12].

Most of the retrospective clinical studies were focusing on the effect of HBV infection from couples with discordant HBV serostatus, but not from seropositive women or seropositive men alone. Always being asked by seropositive husbands about the impact of male HBV infection on IVF outcomes, we are using oocyte donation model to explore the impact of HBV infection from male side.

In oocyte donation cycles, young women can provide a homogenous pool of high quality oocytes. Thus, it offers an opportunity to get rid of impacts of variations from female recipients on IVF outcomes, leaving variations from male as a major independent variable. In this study, with only oocyte donation cycles included, we sought to examine the impact of HBV from seropositive male on semen quality, fertilization rate in oocyte donors, implantation rate and pregnancy rate in oocyte recipients.

Materials and methods

A retrospective cohort study of 277 subfertile patients undergoing oocyte donation from January 2010 to June August 2012 in the Reproductive Medical Center, First Affiliated Hospital of Zhengzhou University for the first IVF cycle was undertaken. This study was approved by the Institutional Review Board of the First Affiliated Hospital of Zhengzhou University.

According to the regulations of the Chinese Ministry of Health, oocytes may only donated anonymously by women undergoing IVF themselves. These young healthy oocyte donors were thoroughly counseled and gave informed consents to donate any excess oocytes. All of these women were healthy and had no determined pathology. Besides, all donors underwent screening for infectious diseases including human immunodeficiency virus (HIV) and hepatitis B virus. Any reactive or positive testing resulted in donor disqualification.

For oocyte recipients, all women were screened for normalcy of the uterine cavity by hysteroscopy. They underwent oocyte donation because of low ovarian reserve, advanced reproductive age, or chromosome abnormality. Both partners were interviewed, physically examined and underwent infectious disease serology tests for HIV, syphilis, rubella and hepatitis B virus.

Donors

In all the oocyte donors, long protocol of ovarian stimulation was used. Ovarian stimulation was achieved by pituitary desensitization using gonadotrophin-releasing agonist (GnRH-a, triptorelin acetate, decapeptyl; Ipsen, France) followed by stimulation with recombination follicle stimulating hormone (Gonal-F; Serono, Geneva, Switzerland) or human menopausal gonadotrophin (hMG; Livzon Biochemistry Co., Zhuhai, China). The daily dose of gonadotrophin was adjusted according to the donor's ovarian response based on serum estradiol levels and the number and size of follicles as considered by transvaginal ultrasonography. When at least three follicles had reached 17 mm, human chorionic gonadotrophin (hCG, 10 000 IU; Serono) was injected. Oocyte retrieval was scheduled approximately 36 hours after hCG injection.

Oocyte recipients

Patients on standby to receive donated oocyte, regardless of ovarian function, were treated with 2 mg oestradiol valerate (Progynova; Schering AG, Berlin, Germany) three times a day for endometrial preparation. When the endometrial thickness reached more than 8 mm, recipients begun intramuscular injection of progesterone oil at a dose of 40 mg/day for 2 days followed by 60 mg/day for the next 2 weeks until serum β-hCG was test.

Insemination and embryo transfer

Oocytes were distributed randomly between the donor and one or more recipients. Each patient received 2-6 oocytes. Oocytes were fertilized by conventional insemination or ICSI depending on whether there was sever male factor infertility. Embryo transfer was per-

Table 1. Demographic data and semen parameters of oocyte recipients with husband being HBV seropositive and their controls

	Husband HBV (+) (n = 20)	Control group (n = 257)	*P* value
Female age (y)	34.5 (31.0-40.0)	35.0 (30.0-40.0)	NS
Male age(y)	35.5 (32.3-42.0)	36.0 (31.0-41.0)	NS
Duration of infertility (y)	5.5 (3.0-9.3)	4.0 (3.0-8.0)	NS
Basic FSH level (IU/L)	18.3 (7.8-50.9)	21.1 (9.0-66.7)	NS
Semen analysis parameters			
Volume (mL)	4.0 (3.0-4.6)	4.0 (3.0-5.0)	NS
Concentration ($\times 10^6$/mL)	74.2 (52.2-170.0)	66.1 (40.4-112.3)	NS
Motility (%)	62.3 (42.7-72.9)	59.5 (42.5-74.6)	NS
Normal morphology (%)	9.6 (7.9-15.0)	12.0 (7.7-17.0)	NS
Progressive motility (a+b) (%)	40.1 (32.9-59.3)	42.7 (29.5-58.8)	NS

Note: Values are median (25-75 percentile). NS = not significant; FSH = follicular stimulation hormone; HBV = hepatitis B virus.

Table 2. Laboratory data of oocyte recipients with husband being HBV seropositive and their controls

	Husband HBV (+) (n = 20)	Control group (n = 277)	*P* value
No. of oocytes received	3.5 (3.0-4.0)	4.0 (3.0-4.0)	NS
No. of oocytes fertilized	3.0 (2.0-3.0)	3.0 (2.0-3.0)	NS
No. of viable embryo	2.0 (2.0-3.0)	2.0 (2.0-3.0)	NS
Fertilization rate (%)	75.0 (61.8-100.0)	75.0 (67.0-100.0)	NS

Note: Values are median (25-75 percentile). NS = not significant; HBV = hepatitis B virus.

formed on day 3 after sequential estrogen-progesterone preparation of the endometrium. Progesterone in oil was used for luteal support at a dose of 60 mg per day. Pregnancy was diagnosed by serum β-hCG 14 days after embryo transfer. Clinical pregnancy was defined as identification of a gestational sac 2-3 weeks after the positive pregnancy test. The implantation rate was defined as the number of gestational sacs per embryo transferred.

Statistical analysis

The sample size and test power were estimated by PASS 11.0 software (NCSS, Kaysville, UT, USA) according to previous retrospective studies demonstrating a significant difference in pregnancy rate between HBV group and controls. In this study, the ratio of controls to study subjects was about 10:1 (196/19), thus the test power would be $\beta = 0.05$, $\alpha = 0.05$ and $\beta = 0.30$, $\alpha = 0.05$ according to the different reported effects of HBV infection on pregnancy rate (7.7% vs. 40.7%; $P < 0.01$ and 53.3% vs. 24.2%; $P < 0.01$) [10, 11].

Since the continuous variables were not normally distributed examined by the Kolmogorov Smirnov test, statistical comparison between groups was carried out by Mann-Whitney U test for continuous variables and chi-squared test for categorical variables. Binary logistic regression analysis was performed to examine the association of HBV infection in male and IVF outcomes. All statistical analyses were performed using SPSS version 13.0 software (SPSS Inc., Chicago, IL). All *P* values quoted were two-sided, and data were considered to be statistically significant with a P value < 0.05.

Results

Study population

A total of 277 couples undergoing for their first oocyte donation cycle were included in this study: 20 (7.2%) men were HBsAg positive. 1 pair of couple were HBV seropositive for both partners. Since this study was carried out in oocyte donation patients, oocyte recipient's age, FSH level, HBV serostatus and other variations had no influence on the fertilization rate and embryo development. All patients were divided into two groups according to the HBV serostatus of recipient's husband (20 husbands being HBV seropositive and 257 husbands being HBV seronegative as controls). The demographic data of the patients are summarized in **Table 1**. The ages of women and their husbands, basic FSH levels of women, and duration of subfertility were comparable between the two groups. There was a trend toward lower normal morphology (9.6% vs. 12.0%; $P > 0.05$) and lower progressive motility (40.1% vs. 42.7%; $P > 0.05$) in the husband HBV (+) group, although no statistical significance was reached.

Table 3. Clinical pregnancy and implantation rates of oocyte recipients (wife is HBV seronegative and husband is HBV seropositive) and their controls (both partners are HBV seronegative)

	Husband HBV (+); wife HBV (-) (n = 19)	Both partners HBV (-) (n = 196)	*P* value
No. of embryos transferred	2.0 (2.0-2.0)	2.0 (2.0-2.0)	NS
Implantation rate	26.3%	40.6%	NS
Clinical pregnancy rate	8/19 (42.1%)	125/196 (63.8%)	NS
Ongoing pregnancy rate	4/8 (50.0%)	58/125 (46.4%)	NS
Live birth rate	4/8 (50.0%)	67/125 (53.6%)	NS

Note: Values are median (25-75 percentile). NS = not significant; HBV = hepatitis B virus.

Table 2 shows the results of laboratory outcomes of couples with HBV seropositive and seronegative men. The numbers of oocytes received, oocytes fertilized and viable embryos were similar among husband HBV (+) and control group. Moreover, no difference was observed regarding fertilization rate.

Outcomes for couples undergoing embryo transfer in the first oocyte donation cycle

Of the 277 couples, 216 couples, 20 in the husband HBV seropositive group and 196 in the husband seronegative group, underwent their first oocyte donation cycle and embryo transfer. Other couples with no viable embryos or had all their embryos cryopreserved were excluded from analysis. In order to explore the neat effect of male HBV infection on IVF outcomes, 1 couple with both partners being HBV seropositive was also excluded. Therefore, IVF outcomes of only 215 couples (19 in husband HBV seropositive, wife HBV seronegative group and 196 in both partners HBV seronegative group) were showed in **Table 3**. Women in the two groups were transferred with similar number of embryos, the implantation rate in both partners HBV (-) group was much higher (40.6% vs. 26.7%; $P > 0.05$), though the difference was not statistically significant.

In regard to clinical pregnancy, 8 couples (42.1%) in husband HBV seropositive and wife HBV seronegative group achieved clinical pregnancy. Four ended up live birth and the other 4 still being ongoing pregnancies. In both partners HBV seronegative group, clinical pregnancy rate was more than fifty percent (125/196 = 63.8%; 67 live births and 58 ongoing pregnancies). However, the difference concerning clinical pregnancy was not statistically significant between these two groups either.

To assess the contributions of different variables to pregnancy rate, we performed binary logistic regression. We found that HBV infection of male contributed little to clinical pregnancy (Odd ratios, OR=0.4, 95% Confidence interval, CI = 0.2 - 1.1; $P = 0.07$). After adjustment for potential confounding factors, such as number of embryos transferred, male age, parameters of semen, only number of embryos transferred was found to be associated with clinical pregnancy (OR = 6.2, 95% CI = 2.2 - 17.3; $P = 0.001$). However, male HBV infection was still not associated with lower pregnancy rate.

Discussions

HBV infection is a serious public health problem in the world. People who are chronically infected with HBV are mainly from China and other Asian countries. In this study, which was conducted in central China, the prevalence of male HBV infection was 7.2%, which was slightly lower than that reported by other investigators from China's southern provinces [8, 11]. The reasonable explanations for this result included that, being China's biggest and most prosperous region, southern China has a huge floating population. Thus, people living there are more likely to get infected.

As early as 1985, investigators noticed the presence of HBV DNA in spermatozoa from HBV carriers [13]. Later than that, HBV was found to have the ability of integrating into human sperm chromosomes, suggesting the possibility of vertical transmission of HBV to the offspring via germ line [14]. All these findings remind us to highlight the impact of male HBV infection on fertility, because any alterations from germ cells could affect fertilization, implantation and embryo development.

If male HBV infection does have impacts on reproduction, semen and sperm that directly come from men should be affected firstly. Clinical retrospective studies reported that compared with controls, HBV seropositive male

exhibited lower sperm motility and total sperm count as well as poor morphology and sperm motility [15, 16]. However, our study showed that semen parameters were comparable between groups. Sperm from HBV infected men had lower normal morphology and progressive motility, but higher concentration and motility compared with that from controls, but the differences were not statistically significant. Our findings were consistent with data from another study showing sperm quality was not compromised in patients with chronic HBV [17]. The discrepancy may be due to the retrospective nature of these studies, and relatively small sample-size of HBV seropositive men.

Fertilization, which involves the fusion of an ovum with a sperm, eventually leads to the development of an embryo. It is well-known that HBV has the abilities not only of penetrating blood-testis barrier, entering male germ line and integrating into their genome, but also possibly of affecting sperm function. It is believed that these changes caused by HBV can affect the process of fertilization during IVF. However, studies on this topic are scarce.

Zhao and the colleagues were the first investigators who explore the outcomes of IVF in couples with seropositive husbands. Their study showed that 102 couples with HBV infected husbands (but not wives), had similar fertilization rate, cleavage rate and rate of good quality embryos with 204 matched controls [18]. A few years later, after matched for age, time period, cycle rank and sperm parameters on the day of oocyte retrieval, another study with fewer subjects (32 IVF cycles in couples with male HBV infection) demonstrated that HBV infected male had a higher risk of having low fertilization rate during IVF, indicating that HBV had a deleterious effect on IVF outcomes [15]. Interestingly, a recent larger sample-size study observed male HBV infection was associated with poor sperm quality and fertilization rate during ICSI, but did not affect the outcomes of IVF. In the 587 IVF cycles, fertilization rate and high grade embryo rate were comparable between groups. However, final ICSI outcomes including fertilization rate, implantation rate and clinical pregnancy rate were significantly lower in HBV seropositive men compared with those of HBV seronegative men in 325 ICSI cycles [16].

The inconsistency of these studies can be explained. All these three conclusions above were made basing on retrospective analysis of people from different regions in the world. More importantly, the variations in oocyte quality and other factors from women side were not well controlled, although patients of two groups had similar age, basic follicular stimulation hormone (FSH) level, and controlled ovary hyperstimulation protocols in these studies. In the present study, using oocyte donation as a model, which allows us to extremely minimize variations from women side, our data showed that fertilization rate and number of viable embryo were comparable between groups during IVF/ICSI. Thus, there seems no association between male HBV infection and low fertilization rate during IVF/ICSI.

Embryo transfer is an important last step during the process of IVF-ET. After this procedure, fates of transferred embryos are out of our control. Over years, we have given much attention and time to this crucial step, hoping to help embryos implant. However, successful implantation needs not only high quality embryo, but also the appropriate preparation of endometrium, indicating that no single factor can completely change implantation or clinical pregnancy. Thus, it is no wonder that, in the three retrospective studies regarding to impact of male HBV infection on final IVF outcomes, only one study showed decreased rates of implantation and clinical pregnancy in HBV seropositive men during 325 ICSI cycles [15, 16, 18]. After reducing the variability of oocyte quality associated with female infertility and also reducing endometrial receptivity variability associated with controlled ovarian stimulation protocols, we observed comparable implantation rate and clinical pregnancy between couples with seropositive men/seronegative women and couples with both seronegative partners. In the binary logistic regression model, only number of embryo transferred was found to be associated with clinical pregnancy. However, male HBV infection status, did not contribute to clinical pregnancy before or after correcting variables that have a potential of affecting IVF outcomes.

During IVF/ICSI-ET, from sperm quality, fertilization to implantation and pregnancy, male factor plays very important roles in these procedures.

Our study showed that male HBV infection had little impact on the outcomes of these three steps, even we used oocyte donation as a model, which is a useful tool for studying external variables from men affecting assisted reproduction treatments. In addition, after reviewing other studies on this topic, we still could not get a clear conclusion. Maybe we can argue this with several explanations: First, as we mentioned earlier, all those studies were retrospective in nature, and a more convincing conclusion needs a prospective study consisting of large number of subjects; Second, patients included were from different regions of the world. In Asia and Africa, the predominant HBV genotypes are E and B/C, respectively. This difference could contribute to the conflicting findings [15, 19]; Third, since sperm used for IVF/ICSI were washed to effectively reduce virus transmission in reproductive medical centers [20], inappropriate ways of washing may reduce its efficiency. These theories above may be the true reasons behind the controversial findings, but we believe that there should be a better one.

Studies showed that HBV had the potential of penetrating blood-testis barrier, and could bring mutagenic effects of sperm chromosomes by integrating into them, but not all the sperms were affected. In HBV infection men, the total frequency of sperm chromosome aberrations was 14.8%. Meanwhile, this aberration could also be found in 4.3% of normal subjects [14]. Indeed, embryos from HBV seropositive men/seronegative women had the possibility of getting infected. According to two similar studies, the rates of HBV DNA positive embryos were only 16.6% and 21.3% in couples with HBV seropositive husbands, respectively [8, 9]. Supposing that the entering of HBV DNA into germ cells adversely affected the sperm's ability to fertilize, and that those infected embryos could not finish implantation, but the HBV seropositive men still have a good chance of getting uninfected sperm and embryos, which have normal ability to fertilize and implant, respectively. Thus, fertilization rate and clinical pregnancy rate are depended on the HBV infection status of embryo transferred. If HBV free embryos are transferred, rates of fertilization and clinical pregnancy will not be affected. Otherwise, IVF outcomes can be changed. Since rates of implantation and clinical pregnancy in couples with HBV seropositive men were slightly lower than that in normal controls in our study, we speculate that male HBV infection has a deleterious impact on fertilization and clinical pregnancy during IVF. However, whether male HBV infection has an adverse impact on the fertilization of oocytes and the implantation of embryos still needs us to explore. Maybe further studies examining the impact of HBV infection and IVF outcomes can compare the development and implantation competency of HBV infected embryos and HBV free ones.

To the best of our knowledge, this is the first report about the impact of male HBV infection on the IVF outcomes concerning fertilization rate and clinical pregnancy using oocyte donation model in the world. It was found in this study that couples with HBV seropositive husbands had similar semen parameters, rates of fertilization, implantation, and clinical pregnancy compared with their control. Male HBV infection has little impact on outcomes of IVF-ET.

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Disclosure of conflict of interest

None.

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