Hepatitis B virus infection on male partner has negative impact on in-vitro fertilization

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Abstract. It is common to see HBV-infected couple seeking for fertility treatment in reproductive medical centers. The effect of hepatitis B virus (HBV) infection on pregnancy outcome after In Vitro Fertilization (IVF) treatment has been a controversy. The study aims this was to evaluate the outcome of in vitro fertilization in couples with the male partner being HBsAg-seropositive. A retrospective analytic study was in HBV-infected and non-HBV infected male partner groups who have been treated with in vitro fertilization (IVF) from October 2016 until May 2017 in HFC IVF Center. From 101 couples, 17 (16.83%) male partners were HBV seropositive. They had similar semen parameters compared to thenon-HBV infected group. Couples with the male partner being HBsAg-seropositive had significantly lower fertilized oocytes and cleaved embryos compared to thenon-HBV infected group. We also found lower clinical pregnancy rate in infected male partner group compared to control group (23.52% vs 51% respectively). Statistically, there was a significant difference in clinical pregnancy rate between HBV-infected group and control group (p<0.05). An hbv-infected male partner may lower the clinical pregnancy rate in couple undergoing IVF treatment. Therefore, the mechanism of impact of HBV infection on IVF outcome needs further exploration.

1. Introduction
Hepatitis B (HBV) is one of the most common serious viral infection in humans, threatening the health of human, causes hepatic inflammation and severe liver diseases.[1,2] Two billion people worldwide have evidence of hepatitis B virus exposure, and an estimated 350 million people being chronic HBV carriers. One million people die annually from HBV-related diseases, including acute hepatitis, chronic hepatitis, cirrhosis of the liver, and hepatocellular carcinoma (HCC).[1,2,3,4]

Hepatitis B, caused by the double-stranded DNA Hepatitis B virus (HBV), is a well-documented cause of acute and chronic hepatitis. Chronic HBV infection develops in 2-6% of adults, 30-60% of young children and 90% of infants (<1 year).[5] Transmission of HBV is parenteral. The main sources of transmission for people who become chronically infected are at birth or in the postnatal period from infected mothers and, less commonly, through close contact with infected fathers, siblings, and relatives during early childhood.[5] The viral infections are deleterious to human’s fertility.[2,4,6,7] Because previous studies have identified HBsAg and HBV DNA in the body fluids of both men and women with HBV, including the semen of male patients, it is possible that HBV infection may influence male infertility.[1,8,9,10]

Assisted reproduction in HBV-seropositive subfertile couples raises concerns about transmission of
infection to the newborn, medical or laboratory staff and about cross contamination of other virus-free
gametes or embryos, but the effect of HBV infection on the outcomes of IVF treatment remains
controversial.[2,4,6,11] Pirwany et al. (2004) showed that HBV-positive discordant couples had a
significantly lower pregnancy rate compared to age-matched controls.[12] But another study showed
that there were not different in terms of the number of good-quality embryos, the implantation rate and
the clinical pregnancy rate in the outcomes of IVF treatment between HBV-positive discordant and
control group.[11,13] The aim of this study was to evaluate the outcome of in vitro fertilization in
couple with the male partner being HBsAg-seropositive.

2. Material and Methods
A retrospective analytic study was in HBV-infected and non-HBV infected male partner groups who
have been treated with in vitro fertilization (IVF) from October 2016 until May 2017 in Halim
Fertility Center, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics &
Gynecology, Faculty of Medicine Universitas Sumatera Utara, Haji Adam Malik General Hospital,
Medan, Indonesia. We divided the samples into two groups: couples with the male partner being
HBV-seropositive were categorized as HBV (study) group, whereas those who were screened negative
served as the control group. Semen analysis was performed on all males to ensure the presence of
sperm in the ejaculate as well as blood screening for HBV, HCV, HIV, and syphilis. Couples who
were seropositive for HCV, HIV and/or syphilis were exclusion from this study. None of the
patients were diagnosed with acute hepatitis or received any antiviral treatment before IVF
treatment. A microparticle enzyme immunoassay was used the qualitative detection of hepatitis B
surface antigen (HBsAg) in the serum.

2.1. Controlled Ovarian Hyperstimulation
The standard protocol for controlled ovarian stimulation was the short protocol, carried out using
recombinant follicle-stimulating hormone (FSH) (Gonal-F; Serono), 150-300 IU as a daily dose,
starting on day 2 of the cycle. We used a gonadotropin-releasing hormone (GnRH) antagonist
(Cetrotide; Serono) at a dose of 0,25 mg to prevent a premature luteinizing hormone (LH) surge,
starting when at least one >14 mm follicle was in visualization. Follicular growth was monitored using
a transvaginal ultrasound, starting on day 5 of the gonadotropin administration. The average of
stimulation duration was 10-12 days. Oocyte maturation was triggered with recombinant hCG
(Ovidrel; Serono) when there were at least three follicles and the lead follicles reaching 17-18 mm.
Oocytes were retrieved 36 hours after the injection of hCG by transvaginal ultrasound-guided needle
aspiration of follicles.

2.2. Method of Sperm Preparation
Semen samples were collected by masturbation into a sterile container after three days of sexual
abstinence. After 30 minutes of liquefaction, the standard analysis of semen samples was carried out
according to the World Health Organization (WHO) guidelines (WHO, 2010). Sperm-washing
involved centrifuging ejaculated semen in a 40-80% colloidal, silica-density gradient to separate
progressively motile HBV-free sperm from infected non-spermatozoa cells and seminal plasma, which
remain in the supernatant. The sperm pellet at the bottom is resuspended in fresh medium and
centrifuged twice before preparation of a final swim-up.

2.3. ICSI Procedure
The Intracytoplasmic Sperm Injection was performed in a microinjection dish prepared with 10-μL
droplets of buffered medium (G-MOPS Plus, Vitrolife) and covered with paraffin oil on the heated
stage of an inverted microscope (37±0.5°C) (Nikon Eclipse TE2000). Approximately 17 hours after
ICSI, fertilization was confirmed from the presence of two pronuclei and the extrusion of the
second PB. The embryo was cultured at 37°C with the level of CO₂ at 6% and O₂ at 5%. We used ISM1
medium (MediCult) and cultured between day 3 and day 5 (approximately 68 hours), then we performed fresh or freezing embryo transfer.

2.4. Outcome Measures

The characteristics of the patients including the age of male and female partner; type and duration of infertility; etiology of infertility, semen parameters; endometrial thickness; duration of gonadotropin stimulation; the number of >17 mm follicle; serum estradiol level on the day of hCG injection; the number of oocytes retrieved. The number of Mature oocytes retrieved (MII), fertilized oocytes, cleaved embryos and transferred embryos, were being recorded. The cleavage rate was anumber of cleaved embryos per number of fertilized oocytes. The fertilization rate was anumber of fertilized oocytes per anumber of oocytes retrieved. Clinical pregnancy rate was defined as the number of women with gestational sacs per embryo transfer cycle.

2.5. Statistical Analysis

Data were analyzed by computer applications by comparative analytical data presentation. For analyzing the outcome of IVF between HBV-infected group and control group, we used a student t-test statistical test with 95% confidence interval. A p-value of <0.05 was considered statistically significant. The statistical analysis was performed using SPSS 16.0.

3. Results

During the study period, 101 couples undergoing IVF cycle were in the analysis. As many as 17 (16.83%) male partners were HBV-seropositive. The patient demographics, type of infertility, etiology, duration of infertility and semen parameters were in Table 1. From table 1, we found that there was a significant difference between male partners being HBsAg-seropositive and control group in the age of the female, male partners and duration of infertility (p<0.05). The type of infertility and cause of infertility were similar between male partners being HBsAg-seropositive and control group (p>0.05). The semen parameters were similar among groups with male partners being HBsAg-seropositive and control group (p>0.05).

| Characteristics          | Male HBV group (n=17) | Control group (n=84) | p-value |
|--------------------------|-----------------------|----------------------|---------|
| Female age (y)           | 35.35 ± 3.42          | 32.38 ± 4.70         | 0.015a  |
| Male age (y)             | 37.58 ± 4.01          | 34.70 ± 5.64         | 0.048a  |
| Type of infertility (%)  |                       |                      |         |
| Primary                  | 15 (88.23)            | 81 (96.42)           | 0.159   |
| Secondary                | 2 (11.76)             | 3 (3.57)             | 0.159   |
| Cause of infertility (%) |                       |                      |         |
| Female factors           | 3 (17.64)             | 15 (17.85)           | 0.984   |
| Male factors             | 6 (35.29)             | 22 (26.19)           | 0.449   |
| Mixed factors            | 8 (47.05)             | 46 (54.76)           | 0.566   |
| Duration of infertility (y) | 9.88 ± 3.25       | 5.92 ± 3.36          | 0.00a   |
| Semen parameters         |                       |                      |         |
| Sperm density (x10⁶/mL)  | 14.49 ± 14.91         | 15.30 ± 12.51        | 0.814   |
| Sperm motility (%)       | 41.53 ± 12.54         | 40.63 ± 15.92        | 0.846   |

*aStudent t-test

| Ovarian stimulation variables | Male HBV group (n=17) | Control group (n=84) | p-value |
|------------------------------|-----------------------|----------------------|---------|
| Duration of gonadotropin stimulation (d) | 9.29 ± 1.35         | 9.14 ± 1.13          | 0.628   |

Table 1. Characteristics of HBsAg-seropositive and control groups.

Table 2. Ovarian stimulation variables of HBsAg-seropositive and control groups.
No. of follicle size > 17 mm & 4.70 ± 2.61 & 4.66 ± 2.62 & 0.955 \\
Serum Estradiol level on day of hCG injection (pg/mL) & 2930 ± 2711.35 & 2738 ± 2321.91 & 0.776 \\
Endometrium thickness (mm) on day of hCG injection & 10.47 ± 2.18 & 9.97 ± 1.91 & 0.347

In table 2, there were no significant differences in duration of gonadotropin stimulation, the number of follicle size, serum estradiol level on the day of hCG injection and endometrial thickness on day of hCG injection between male partners being HBsAg-seropositive and control group (p>0.05).

Table 3. Outcomes of in vitro fertilization in HBsAg seropositive and HBsAg-seronegative groups.

| Parameter                                | Male HBV group (n=17) | Control group (n=84) | p-value |
|------------------------------------------|-----------------------|----------------------|---------|
| No. of oocytes retrieved                 | 10.88 ± 6.32          | 14.30 ± 7.57         | 0.084   |
| No. of Mature oocyte retrieved (MII)     | 8.05 ± 4.73           | 10.54 ± 5.36         | 0.079   |
| No. of fertilized oocytes                | 5.58 ± 3.58           | 7.85 ± 3.97          | 0.032*  |
| No. of Cleaved embryos                   | 5.47 ± 3.60           | 7.75 ± 3.92          | 0.029*  |
| No. of Embryos transferred               | 2.52 ± 0.71           | 2.51 ± 0.70          | 0.926   |
| Cleavage rate (%)                        | 98.03 ± 8.08          | 98.82 ± 4.31         | 0.566   |
| Fertilization rate (%)                   | 74.54 ± 24.79         | 76.69 ± 17.21        | 0.665   |
| Clinical pregnancy rate (%)              | 4/17 (23.52)          | 43/84 (51)           | 0.037*  |

*aStudent t-test

Table 3 showed that there were no significant differences in the number of oocytes retrieved, mature oocytes retrieved (MII) and someembryones transferred between male partners being HBsAg-seropositive group and control group (p>0.05). There was a significant difference in the number fertilized oocytes (5.58±3.58 vs. 7.85±3.97, p<0.05) and cleaved embryos (5.47 ± 3.60 vs. 7.75 ± 3.92, p<0.05) between male partners being HBsAg-seropositive and control group. The cleavage rates in the male partners being HBsAg-seropositive group were similar compared to control group (98.03 ± 8.08 vs. 98.82 ± 4.31, p>0.05). The fertilization rates were similar among male partners being HBsAg-seropositive group and control group (74.54 ± 24.79 vs. 76.69 ± 17.21, p>0.05). In table 3, the clinical pregnancy rates in male partners being HBsAg-seropositive group were significantly lower than the control group (23.52% vs. 51%, p<0.05).

4. Discussion

Hepatitis B is a viral infection that attacks the liver and causes acute illness. Symptoms include yellowing of the skin and eyes, dark urine, extreme fatigue, nausea, vomiting and abdominal pain. [1,3,16] Hepatitis B virus belongs to the Hepadnaviridae family: a family of DNA viruses with circular and incomplete double helix-strand DNA; this family of virus infects preferably hepatocytes (kidney, pancreas and mononuclear cells too). Hepatitis B is made up of: external envelope (surface antigen) and central particle or core (nucleocapsid proteins, viral genome and a polymerase complex). [3,14] HBV is transmitted by exposure to infectious blood or body fluids such as semen, vaginal fluids, and saliva. The incubation period for acute Hepatitis B is 45–160 days. [1,8,14] The impact of HBV infection on the outcome of IVF and embryo transfer treatment remains controversial. [1,2,4,11] From 101 couples included in this study, we found 17 (16.83%) male partners were HBV-seropositive. They had similar semen parameters compared to non-HBV infected group. Couples with the HBV-infected male partner had significantly lower fertilized oocytes and cleaved embryos compared to non-HBV infected group. We also found lower clinical pregnancy rate in couples with the HBV-infected male partner compared to control group (23.52% vs. 51% respectively). Statistical analysis showed a significant difference in clinical pregnancy rate between HBV-infected group and control group.
HBV-infected males exhibit a lower total sperm count, poorer sperm motility, and morphology. Our study was similar with another study showing that HBV-positive couples had much lower implantation and pregnancy rate than the age-matched HBV-negative controls and no significant differences were found in semen parameters or fertilization rate between two groups (Pirwany et al., 2004). But some study reported unexpected higher implantation and pregnancy rates in the HBV-seropositive group compared to those of HBV-seronegative group. Chronic viral infections can infect sperm and are considered a risk factor for male's infertility. Recent studies have shown that the presence of HIV, HBV or HCV in semen impairs sperm parameters, DNA integrity and in particular reduces forward motility. The sperm carrying HBV genes can pass through the oolemma and enter the cytoplasm of oocytes. After fertilization, HBV genes can be expressed at the mRNA and protein levels in the early embryonic cells. Our study was similar with another study showing that HBV-positive couples had much lower implantation and pregnancy rate than the age-matched HBV-negative controls and no significant differences were found in semen parameters or fertilization rate between two groups (Pirwany et al., 2004).

Our study also found that sperm density in male partner being HBsAg-seropositive was lower than the control group (14.49 ± 14.91 vs 15.30 ± 12.51), but there was no significant difference between the group statistically. Our findings were consistent with data from another study showing sperm quality did not compromise with HBV-infected male partner. Nie R et al. (2011) found that there was HBV present in oocyte and embryos that suggest the possibility of vertical transmission of HBV via the germ line. The percentage of HBV-positive embryos was 40% after ICSI and 38% after conventional IVF in male HBV carriers. There seems to be a risk of HBV transmission through oocytes and embryos from chronic HBV carriers during the ART procedure. Huang et al. (2003) provided direct evidence that HBV DNA can integrate into the chromosomes of the patient’s sperm, and that such HBV-carrying sperm can fertilize oocytes. Lee VCY et al. (2010) stated that there was no adverse effect of HBV infection on the assisted reproduction outcomes. The ongoing pregnancy rate and the live-birth rate was not significantly different from couples with discordant HBV serostatus and those couples with both partners being HBV-surface antigen-negative (23% vs. 29% vs 30%, respectively; 23% vs. 27% vs. 27%, respectively). Shi L et al. (2014) found that the fertilization rate in groups with male or female partners being HBsAg-seropositive was significantly lower than control and it suggested that chronic HBV infection is likely to represent a significant cause of infertility. Jin L et al. (2015) found that the fertilization rate in groups with male or female partners being HBsAg-seropositive was significantly lower than control and it suggested that chronic HBV infection is likely to represent a significant cause of infertility.

Detection of HBV genome in gametes and embryos need focus in the future study. With more knowledge on the vertical transmission of HBV, proper preconception counseling can be an offer to HBV-seropositive couples.

5. Conclusion
HBV infected male partner may lower the clinical pregnancy rate in couple undergoing IVF treatment. The mechanism of HBV infection in deteriorating the outcome of IVF needs further exploration.

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