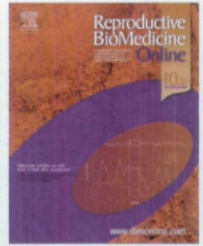




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ARTICLE

Impact of positive hepatitis B surface antigen on the outcome of IVF treatment


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Abstract There has been controversy about the effect of hepatitis B virus (HBV) infection on pregnancy outcome after IVF treatment. A total of 1676 couples undergoing their first IVF cycle were included in this study. The prevalence of HBV infection in the female partners of the subfertile population seeking treatment with assisted reproductive technology was 7.8–9.6% during the study period. The ongoing pregnancy rate was not significantly different between couples with HBV-seropositive wives and seronegative ones (26.7% versus 30.2%). The ongoing pregnancy rate and the live-birth rate of couples with both partners being HBV surface antigen positive was not significantly different from couples with discordant HBV serostatus and those couples with both partners being HBV surface antigen negative (23% versus 29% versus 30%, respectively; 23% versus 27% versus 27%, respectively). The percentage of normal sperm morphology in HBV-seropositive husbands was significantly lower than that of seronegative counterparts (5.0% versus 10.0%, $P = 0.009$). In conclusion, there was no adverse effect of HBV infection on the assisted reproduction outcomes. 

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KEYWORDS: hepatitis B surface antigen, IVF treatment

Introduction

Worldwide, more than 400 million people are chronically infected by the hepatitis B virus (HBV) (Lee, 1997) and three-quarters of chronic HBV carriers are Chinese. HBV infection is endemic in China as 9% of the general population was found to be positive for HBV surface antigen (HBsAg) in

a national survey in 2002 (Liang et al., 2005). The prevalence of positive HBsAg in Hong Kong was very similar: 9.5% as shown by Chang and Yeoh (1985). On the other hand, HBV was much less common in the Caucasian population, about 0.5% positive for HBsAg (Abusheikha et al., 1999).

Chronic HBV infection can cause cirrhosis and liver cancer. Many carriers would remain asymptomatic until they present with cirrhosis-related complications or hepatocellu-

lar carcinoma. More than a quarter of people with chronic infection will die of liver disease and more than 1 million people with this infection are estimated to die every year (Lai et al., 2003). 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 (Lai et al., 2003). The risk of chronicity varies greatly with the age at which the infection is acquired. For neonates and children younger than 1 year who acquire the infection, the risk of becoming chronically infected is 90%, while for adults, the risk decreases to about 2% (Hyams, 1995). This risk could be reduced with effective vaccination against the virus.

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. As recommended in guidelines by UK's National Institute of Health and Clinical Excellence (NICE) and European Society of Human Reproduction and Embryology (ESHRE), subfertile couples seeking assisted reproduction treatment should be checked for HBV serostatus in view of the success of prevention of vertical transmission by passive immunization with immunoglobulin for babies born from seropositive mothers (Van den Eede, 1995). Embryos from the couples with positive HBsAg should be frozen separately from those with negative HBsAg to avoid cross-contamination as it has been shown that the transmission of hepatitis C virus (HCV) infection could happen during storage and manipulation of cryopreserved semen (Lesourd et al., 2000).

The effect of HBV infection on the outcomes of IVF treatment remains controversial. Pirwany et al. (2004) showed that HBV-positive discordant couples had a significantly lower pregnancy rate compared with age-matched controls. Another study showed that the outcomes of IVF treatment were not different in terms of the number of good-quality embryos, the implantation rate, the clinical pregnancy rate and the delivery rate in subfertile couples with husbands being HBsAg positive (Zhao et al., 2007). In contrast, Lam et al. (2010) recently demonstrated a significantly higher pregnancy rate of HBV-seropositive patients.

The study centre's IVF programme has routinely screened both partners for antibodies for HBV, HCV and human immunodeficiency virus prior to the first IVF cycle since 2004. In view of the controversy on the effects of HBV infection on the IVF outcome, this retrospective study was conducted to evaluate the prevalence of positive HBsAg in centre's population and to compare the outcomes of IVF treatment between couples with and without positive reactions for HBsAg.

Materials and methods

A retrospective study of subfertile patients attending the Centre of Assisted Reproduction and Embryology (CARE) at The University of Hong Kong Queen Mary Hospital for the first IVF cycle between January 2004 and December 2008 was undertaken. The study was approved by the Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster. Details of the IVF

treatment including HBV and HCV serostatus were retrieved from the database for analysis.

Indications for IVF included tubal, male, endometriosis, unexplained and mixed factors. Intracytoplasmic sperm injection (ICSI) was performed for couples with severe semen abnormalities if the morphology of the semen analysis reduced to below 3% according to strict criteria or <100,000 motile spermatozoa were recovered after sperm preparation. In case of obstructive or non-obstructive azoospermia, surgically retrieved spermatozoa from epididymis or testis, respectively, were used for ICSI. Serum basal FSH concentration was checked on day 2–3 of the cycle within 2–3 months of commencement of treatment.

The details of the long protocol of ovarian stimulation regimen, gametes handling, standard insemination and ICSI were as previously described (Ng et al., 2000). In brief, patients were pre-treated with buserelin (Suprecur; Hoechst, Frankfurt, Germany) nasal spray 150 µg four times daily from the mid-luteal phase of the cycle preceding the treatment cycle. They also received human menopausal gonadotrophin (HMG; Pergonal; Serono, Geneva, Switzerland; or Menogon; Ferring, Kiel, Germany) for ovarian stimulation. Human chorionic gonadotrophin (HCG; Profasi; Serono) was given intramuscularly when the leading follicle reached 18 mm in diameter and there were at least three follicles of ≥16 mm in diameter. Serum oestradiol concentration was measured on the day of HCG administration.

Transvaginal ultrasound-guided oocyte retrieval was scheduled 36 h after the HCG injection. Patients were allowed to have a maximum of three embryos replaced into the uterine cavity 2 days after retrieval. Excess good-quality embryos were frozen on the day of embryo transfer. All fresh embryos were cryopreserved if patients had developed symptoms suggestive of ovarian hyperstimulation syndrome (OHSS) or if serum oestradiol on the day of HCG injection was >20,000 pmol/l in order to reduce the risks of OHSS. Luteal phase was supported by two doses of HCG (1500 IU on the day of embryo transfer and 6 days after embryo transfer) or vaginal progesterone, 400 mg two times per day for 14 days after embryo transfer (Cyclogest; Cox Pharmaceuticals, Barnstaple, UK). A urine pregnancy test was done 16 days after embryo transfer. If it was positive, ultrasound examination was performed 10–14 days later to confirm intrauterine pregnancy and to determine the number of gestational sacs present. The data on live-birth were voluntarily reported by patients or traced by the staff.

The overall pregnancy rate was defined by a positive urinary pregnancy test. The implantation rate was the proportion of embryos transferred resulting in an intrauterine gestational sac. Patients with one or more intrauterine gestational sacs on scanning or the histological confirmation of gestational product in miscarriages were considered as having clinical pregnancies. Ongoing pregnancies were those pregnancies with a live fetus beyond 10 weeks of gestation when they were referred to other doctors for antenatal care. A baby born after 24 weeks of gestation was classified as a live-birth.

Statistical analysis

Based on previous studies showing a significant difference between seropositive and seronegative couples, this study

determined a sample size of 82 patients in each group would have 80% power to detect a clinically significant difference in the pregnancy rate of 20% at $\alpha = 0.05$ and $\beta = 0.2$ (Sigmatat; Jandel Scientific, San Rafael, CA, USA).

The Kolmogorov Smirnov test was used to test the normal distribution of continuous variables. Continuous variables were given as mean \pm standard deviation if normally distributed and as median (interquartile range) if not normally distributed. Statistical comparison was carried out by Student's *t*-test, Mann Whitney *U*-test, Wilcoxon signed ranks test for continuous variables and chi-squared test for categorical variables, where appropriate. Statistical analysis was performed using the Statistical Program for Social Sciences version 17.0 (SPSS, Chicago, USA). A two-tailed *P*-value <0.05 was considered statistically significant.

Results

During the study period, 1676 couples undergoing their first IVF cycle were included in the analysis: 131 (7.8%; 95% CI 6.6–9.2%) women were HBsAg positive and 161 (9.6%; 95% CI 8.2–11.1%) husbands were HBsAg positive. Only 13 (0.8%; 95% CI 0.4–1.3%) couples were both HBsAg positive. The prevalence of HBsAg in each year is shown in Figure 1.

The demographic data of the subjects are shown in Table 1. The age of women, the age of their husbands and the duration of subfertility were comparable among seropositive and seronegative women. There was a statistically significant difference in causes of subfertility among seropositive and seronegative women, with more tubal factor in the HBV-seropositive women (29% versus 18.6%, $P = 0.041$). The ovarian responses in terms of the dosage of HMG used, the duration of HMG, the numbers of follicles ≥ 16 mm, the serum oestradiol concentration on the day of the ovulatory HCG administration and the number of embryos transferred were similar among seropositive and seronegative women as shown in Table 2.

The sperm concentration and forward motility of semen samples were similar among seropositive and seronegative husbands after excluding those men with azoospermia. However, the sperm normal morphology was significantly

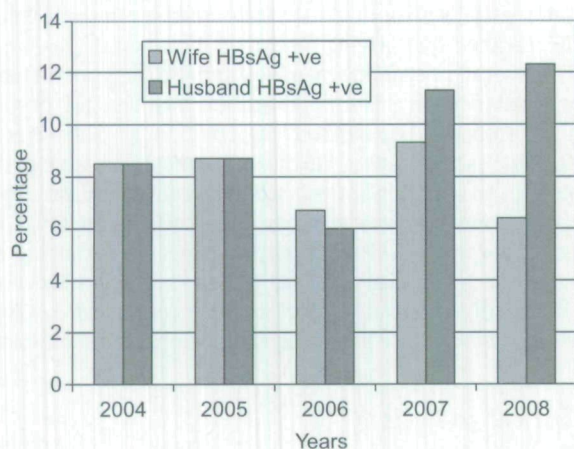


Figure 1 Prevalence of partners positive for hepatitis B surface antigen (HBsAg) in each year from 2004 to 2008.

lower in seropositive husbands than in seronegative husbands, irrespective of the criteria used (Table 3). The rate of the need for ICSI according to the unit's protocol was comparable regardless of the serostatus of husbands (36.6% versus 30.6%).

The ongoing pregnancy rates per cycle and per transfer were not significantly different among seropositive and seronegative women (26.7% versus 30.2% per started cycle; 31.5% versus 34.0% per transfer; respectively) or among seropositive and seronegative husbands (30.4% versus 29.9% per started cycle; 34.0% versus 33.8% per transfer; respectively). The ongoing pregnancy rate of couples with both partners being HBsAg positive was not significantly different from couples with discordant HBV serostatus and those couples both partners being HBsAg negative (23% versus 29% versus 30%) although the number was small as shown in Figure 2. The live-birth rate was also not significantly different among the three groups (23% versus 27% versus 27%), while it was still not significantly different between the both partners seropositive and both partners seronegative (23.1% versus 26.9%). The live-birth rate was not significantly different when the analysis was confined to the comparison of couples with both partners seropositive with those with seropositive wife or seropositive husband (23.1% versus 23.9% versus 29.2%, respectively) in serodiscordant couples.

Twenty women (15.3%) had regular liver function tests done in the medical unit and three women had abnormal liver function tests. Out of these 20 women, two were on anti-viral medications during the IVF cycle and both failed to get pregnant. The pregnancy rate of those with regular liver function tests and those without regular tests was not significantly different (data not shown).

Discussion

HBV infection is endemic in the Chinese population in Hong Kong, with the prevalence fluctuating between 9.5% in the general population in 1985 (Chang and Yeoh, 1985) and about 10% in reproductive-age women and subfertile population (Kwan et al., 1997; Lam et al., 2010; Wong and Lee, 1996). The prevalence in this study was 8–9% which seemed to be slightly lower than that reported by Lam et al. (2010) and this may reflect different proportions of immigrants from mainland China seen by the two centres in Hong Kong.

This study used data starting from 2004 when the routine screening programme for HBsAg, HCV antibody and human immunodeficiency virus antibodies started for every couple before commencing the first IVF cycle. The prevalence of seropositive women and their husband was steady throughout the 5 years, as shown in Figure 1. In Hong Kong, the government-funded vaccination programme of HBV infection for newborns was implemented since 1988 and the prevalence of HBV would be expected to decrease when these babies go into their reproductive age. However, a proportion of patients came from mainland China, where there was still no HBV vaccination programme for the newborn. This may be a reason why the prevalence remained stable during the study period.

The seropositive group had significant more tubal factor and this finding was consistent with the study of Lam et al. (2010). It may be due to the common transmission route of

Table 1 Demographic data.

<i>Parameter</i>	<i>Wife HBsAg +ve</i> (n = 131)	<i>Wife HBsAg -ve</i> (n = 1545)	<i>P-value</i>
Wife's age (years)	35.0 (32–37)	35.0 (33–37)	NS
Husband's age (years)	38.0 (34–42)	38.0 (35–42)	NS
Duration of subfertility (years)	4.0 (2.5–6)	4.0 (3–6)	NS
Causes of subfertility			0.041
Tubal	38/131 (29)	288/1545 (18.6)	
Endometriosis	10/131 (7.6)	150/1545 (9.7)	
Male	57/131 (43.5)	730/1545 (47.3)	
Unexplained	6/131 (4.6)	133/1545 (8.6)	
Mixed causes	20/131 (15.3)	244/1545 (15.8)	

Values are median (25–75 percentile) or number/total (%).
HBsAg = hepatitis B surface antigen; NS = not significant.

Table 2 Details of ovarian stimulation.

<i>Parameter</i>	<i>Wife HBsAg +ve</i> (n = 131)	<i>Wife HBsAg -ve</i> (n = 1545)
Dosage of HMG (IU)	1950 (1650–2700)	1950 (1650–2700)
Duration of HMG (days)	12.0 (10–13)	12.0 (10–13)
Oestradiol concentration on the day of HCG administration (pmol/l)	9787.5 (6463–15,597)	9202 (5693–14,609)
No. of follicles ≥ 16 mm	6.0 (4–8)	6.0 (4–8)
No. of oocyte retrieved	10.0 (5–14)	9.0 (5–13)
Number of fertilized oocytes	7.0 (4–10)	6.0 (4–10)
Number of good-quality embryos	4.0 (2–7)	4.0 (2–7)
Number of transferred embryos		
0	20/131 (15.3)	171/1545 (11.0)
1	12/131 (9.2)	156/1545 (10.1)
2	97/131 (74.0)	1203/1545 (77.9)
3	2/131 (1.5)	15/1545 (1.0)

Values are median (25–75 percentile) or number/total (%).
HBsAg = hepatitis B surface antigen; HCG = human chorionic gonadotrophin; HMG = human menopausal gonadotrophin; NS = not significant.

There were no statistically significant differences between the two groups.

Table 3 Semen parameters of husbands.

<i>Semen parameter</i>	<i>Husband HBsAg +ve</i> (n = 154)	<i>Husband HBsAg -ve</i> (n = 1473)
Sperm concentration (million/ml)	53.0 (20.5–88.6)	47.6 (18.9–88.6)
Forward motility (%)	44 (32–55)	44 (28–55)
Normal morphology (strict criteria)	5.0 (2.3–8)	10.0 (6–16)

Values are median (25–75 percentile).

HBsAg, hepatitis B surface antigen.

The data in the morphology was significantly different between the two groups with the P value of 0.009.

HBV and the common pathogens causing pelvic inflammatory disease and tubal subfertility. The detection of DNA of sexually transmissible pathogens in semen from a high

percentage of asymptomatic male infertility patients also suggested the underlying mechanism (Bezold et al., 2007). However, it was revealed by retrospective analysis that

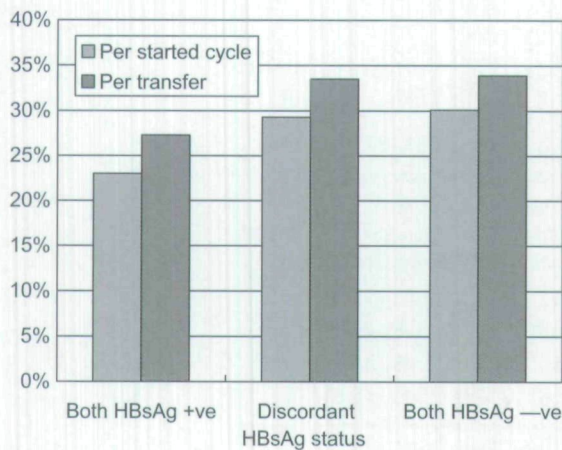


Figure 2 Ongoing pregnancy rate in couples according to serostatus for hepatitis B surface antigen (HBsAg).

there was no significant differences in live-birth rate per treatment cycle by cause of subfertility (Templeton et al., 1996).

The HBsAg-positive husbands showed a significantly lower percentage of normal sperm morphology when compared with HbsAg-negative husbands. In a recent report, the sperm quality was not impaired in patients with chronic HBV but the number of patients was small, i.e. 34 patients (Moretti et al., 2008). There were also studies verifying the detection of HBV DNA in semen and spermatozoa (Davison et al., 1987; Hadchouel et al., 1985). HBV is able not only to pass through the blood–testis barrier and enter male germ cells but also to integrate into their genome, which could create extensively hereditary effects by alteration of genetic constituent and induction of chromosome aberrations (Huang et al., 2003). The replication of the virus within the embryos after introduction of spermatozoa would be a way of father-to-infant vertical transmission occurring during the fertilization period (Zhou et al., 2009). In the present study, despite a lower percentage of sperm normal morphology in the semen, HBsAg-positive husbands had an ongoing pregnancy rate similar to that of HBsAg-negative husbands. The unaffected ongoing pregnancy rate could be due to the centre's policy of using ICSI in couples with low normal sperm morphology (less than 3%) which may compensate for any defect in fertilization due to impaired morphology. The percentage of ICSI required was slightly higher in seropositive husband compared with seronegative husband, though it did not reach statistical significance. The present results are in agreement with those of Zhao et al. (2007).

The effect of HBV on the outcome in IVF treatment cycle remains controversial and as far as is known this is the first report of live-birth rates in the couples with HBV infections. The pregnancy rate of HBV-serodiscordant couples was significantly lower than that of the control in one report (Pirwany et al., 2004) and the authors suggested that the extra precaution on handling the embryos from HBV-positive couples embryos may be the cause of the lower pregnancy rate. Another small study (Zhao et al., 2007) showed there was no significant difference in the pregnancy rate and delivery rate in couples with husbands

who were chronic carriers of HBV. The latest study (Lam et al., 2010) reported a significantly higher pregnancy rate in the serodiscordant couples with a seropositive wife but not in serodiscordant couples with a seropositive husband or in couples with both partners seropositive. All of the above studies included small numbers of subjects and it may be difficult to draw any firm conclusion. The present study had a much larger sample size. However, it could not find significant differences in the ongoing pregnancy and live-birth rates among seropositive and seronegative women and among serodiscordant couples and seronegative couples. There was a trend of a lower live-birth rate of couples who were both HBsAg positive when compared with those who were both HbsAg negative, but the number of patients was too small to reach statistical significance. Only a proportion of HBsAg-positive women had regular liver function tests and it is difficult to evaluate the impact of abnormal liver function tests on the outcome of IVF treatment. A large multicentre study is urgently needed to clarify this important issue.

In endemic regions such as China, the vertical transmission from mothers to children plays a major role in the transmission of HBV causing chronic carrier status (Lai et al., 2003). The vicious cycle of transmission to their own offspring continues and thus the understanding of the mechanism of vertical transmission is vital in primary prevention. Infection during the peripartum period could be prevented by HBV vaccination together with HB immunoglobulin injection after delivery. There are reports on the other possible mechanisms of vertical transmission, including the infection of the ovum by HBV at different stages and the replication of the virus in the ovum (Ye et al., 2006) and the vertical transmission from father to fetus proven by direct sequencing (Wang et al., 2003). Therefore, children conceived from HBsAg-positive couples should be followed up to evaluate the effectiveness of prevention by HBV vaccination of newborn.

There were several limitations of the present study. It was retrospective in nature and the data need a prospective study consisting of a larger number of patients to be confirmed. Liver function test and HBeAg serum concentration were not checked for those with HBV-seropositive infection as this was a retrospective study. Other HBV markers are not checked especially sensitive markers. The numbers of women having abnormal liver function tests and that on anti-viral treatment for HBV were too small to reveal any difference in the pregnancy rate. The biological mechanism of a lower percentage of normal morphology in HBV-seropositive husbands remains unclear.

In conclusion, 8–9% of the subfertile couples were chronic HbsAg carriers. There were no significant differences in ongoing pregnancy rate and live-birth rate among HbsAg-positive and-negative couples. This piece of information is of importance in the counselling of seropositive couples undergoing IVF treatment.

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