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Original Article

Impact of hepatitis B virus carrier serostatus on neonatal outcomes after IVF-ET

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Abstract: An increasing number of infertile, hepatitis B virus-infected individuals have opted for assisted reproductive technology. However, the impact of the hepatitis B virus carrier serostatus on neonatal outcomes has not been evaluated. Data from 504 patients who delivered singletons were analyzed. In females, hepatitis B surface antigen, hepatitis B e antigen, and antibody to hepatitis core antigen seropositivity significantly decreased the gestational age at delivery. In contrast, the male hepatitis B virus serostatus did not affect the gestational age at delivery. Multiple linear regression analysis showed that maternal weight, gestational age at delivery, and infant gender were significantly related to birth weight. The present retrospective study showed that in females, hepatitis B surface antigen, hepatitis B e antigen, and antibody to hepatitis core antigen seropositivity was significantly associated with the gestational age at delivery after *in vitro* fertilization-embryo transfer (IVF-ET).

Keywords: IVF-ET, chronic hepatitis B infection, neonatal outcomes

Introduction

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]. Worldwide, 2 billion people have been infected with hepatitis B virus (HBV), and > 240 million have chronic liver infections. Up to 15% of HBV carriers eventually develop hepatocellular carcinoma. Infection with HBV is particularly endemic in China where approximately 300,000 people die every year because of the acute or chronic consequences of hepatitis B [2].

HBV is transmitted by exposure to infectious blood or body fluids such as semen, vaginal fluids, and saliva. It is well known that bacterial and viral infections have a negative effect on human fertility [3, 4]. Studies have demonstrated that HBV can enter male germ cells and is capable of integrating into the human genome, causing mutagenic effects on chromosomes [5]. HBV-infected males exhibit a lower total sperm count and poorer sperm motility and morphology [6]. 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 [7].

An increasing number of infertile HBV-infected individuals have turned to assisted reproductive technology (ART) such as *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), and embryo transfer (ET). Recent studies have reported that HBV-positive patients exhibit lower fertilization, implantation, and pregnancy rates during IVF-ET [8-10]. However, the impact of the HBV carrier serostatus on neonatal outcomes has not been evaluated. The objective of this retrospective study was to determine the effect of maternal and paternal chronic HBV infection on neonatal outcomes after IVF-ET.

Materials and methods

Patients

This retrospective study was approved by the Ethics Committee of Peking University Third Hospital. Anonymity of patient information was

Table 1. Subfertility causes, subfertility duration, subfertility types, fertilization methods and patient characteristics among groups

Characteristic	HBV negative	Male HBsAg	Female HBsAg	Male HBeAg	Female HBeAg	*P*-value
Primary indication for IVF						
Male factor	45 (22.61%)	30 (23.26%)	19 (21.11%)	13 (27.08%)	7 (18.42%)	0.898
Tubal factor	75 (37.69%)	54 (41.86%)	41 (45.56%)	17 (35.42%)	18 (47.37%)	0.565
Male and tubal factors	73 (36.68%)	36 (27.91%)	25 (37.78%)	17 (35.42%)	9 (23.68%)	0.157
Unexplained	6 (3.02%)	9 (6.98%)	5 (5.56%)	1 (2.08%)	4 (10.53%)	0.191
Subfertility duration (years)	4.86 ± 3.30	4.95 ± 3.14	5.12 ± 3.28	4. 13 ± 2.95	5.44 ± 3.44	0.364
Primary subfertility	110 (55.28%)	68 (52.71%)	53 (58.89%)	30 (62.50%)	26 (68.42%)	0.413
Secondary infertility	89 (44.72%)	61 (47.29%)	37 (41.11%)	18 (37.50%)	12 (31.58%)	0.413
Cycles with ICSI	87 (43.72%)	58 (44.96%)	28 (31.11%)	23 (47.92%)	17 (44.74%)	0.209
Maternal characteristics						
Age (years)	31.76 ± 3.80	31.93 ± 3.25	32.13 ± 3.28	31.02 ± 3.28	30.87 ± 3.35	0.206
Age ≥ 38 years	11 (5.53%)	7 (5.43%)	6 (6.67%)	1 (2.08%)	1 (2.63%)	0.751
Height (cm)	161.49 ± 4.45	161.33 ± 4.19	161.28 ± 4.96	161.40 ± 5.37	161.24 ± 4.14	0.994
Weight (kg)	57.85 ± 8.05	56.44 ± 7.13	56.74 ± 7.20	56.46 ± 6.42	56.47 ± 7.24	0.433
Body mass index (kg/m^2)	22.17 ± 2.83	21.68 ± 2.60	21.83 ± 2.82	21.67 ± 2.23	21.71 ± 2.66	0.509
Paternal characteristics						
Age (years)	32.29 ± 3.99	33.43 ± 3.99	33.08 ± 3.74	32.21 ± 3.72	32.97 ± 3.77	0.437
Height (cm)	173.07 ± 5.34	172.88 ± 5.24	173.10 ± 5.22	173.22 ± 4.94	173.62 ± 4.71	0.965
Weight (kg)	72.76 ± 9.89	72.92 ± 8.93	73.86 ± 9.10	72.93 ± 9.39	72.00 ± 9.61	0.874
Body mass index (kg/m^2)	24.26 ± 2.83	24.36 ± 2.46	24.63 ± 2.69	24.27 ± 2.62	23.88 ± 2.96	0.701

NOTE: The parental and cycle characteristics of 504 patients undergoing ART were analyzed. Among the 504 patients, 199 were HBV seronegative couples; 129 men were HBsAg, anti-HBe, and anti-HBc seropositive; 90 women were HBsAg, anti-HBe, and anti-HBc seropositive; 48 men were HBsAg, HBeAg, and anti-HBc seropositive; and 38 women were HBsAg, HBeAg, and anti-HBc seropositive. The basic characteristics of patients were compared using ANOVA, followed by the Student-Newman-Keuls test. Data are presented as the number (%) or mean ± SD.

assured, such as name, ID card, date of birth, and zip code. Serological screening for hepatitis B was routinely performed when patients underwent ART, and it included the following tests: hepatitis B surface antigen (HBsAg) and hepatitis B surface antibody (anti-HBs); hepatitis B e antigen (HBeAg) and hepatitis B e antibody (anti-HBe); and antibody to hepatitis core antigen (anti-HBc). Females who underwent IVF-ET cycles between 2008 and 2012 in the Reproductive Medical Centre of Peking University Third Hospital were analyzed. Females underwent controlled ovarian hyperstimulation (COH) with a GnRH agonist or GnRH antagonist protocol, as described previously [11]. Ovarian follicle development was monitored on the basis of serum estradiol (E2) levels and transvaginal ultrasonographic measurements. When at least one follicle reached a mean diameter of 18 mm and the E2 concentration exceeded 500 pg/ml, 10,000 units of urinary hCG (Serono, Aubonne, Switzerland) were administered before ultrasonography-guided oocyte retrieval. Luteal support was initiated on the day following oocyte retrieval with 60 mg of progesterone (Progesterone Injection, Xianju Pharmacy, Zhejiang, China) [12].

Laboratory protocols

IVF or ICSI was performed according to seminal parameters on the day of oocyte retrieval (D 0). The presence of two pronuclei was observed 16-18 h after insemination or injection, and the zygotes were then cultured in 25 μl of pre-equilibrated cleavage medium droplets. The embryos were cultured in incubators at 37°C under 5% or 6% CO_2, as described previously [11]. The morphology of embryos was evaluated 68-72 h following insemination with respect to cell number, fragmentation, and symmetry. Two embryos could be maximally transferred according to patients' age, number of IVF cycles, and embryo quality.

Data collection

In the present study, only the patients who were aged ≤ 40 years and had a body mass index (BMI) of < 30 kg/m^2 were analyzed. Patients were excluded if they received pre-implantation genetic diagnosis. In addition, only data from singletons born alive after the 20th week of gestation were included in data analysis. The criteria were the same as those used in previous

Table 2. Neonatal characteristics of liveborn singletons

Characteristic	HBV negative	Male HBsAg	Female HBsAg	Male	Female HBeAg	*P* value
Boys	107 (53.77%)	73 (56.59%)	47 (52.22%)	20 (41.67%)	22 (57.89%)	0.470
GA at birth (weeks)	38.55 ± 1.63	38.77 ± 1.63	38.90 ± 1.40	38.73 ± 1.69	37.47 ± 2.93	0.001*
Preterm birth (< 37 weeks)	13 (6.53%)	8 (6.20%)	4 (4.44%)	4 (8.33%)	6 (15.79%)	0.216
Birth weight (g)	3350.50 ± 484.07	3407.67 ± 595.80	3354.36 ± 496.25	3336.15 ± 460.71	3202.90 ± 638.36	0.342
Birth length (cm)	50.35 ± 1.84	50.38 ± 1.81	50.35 ± 1.60	50.57 ± 3.28	50.13 ± 1.46	0.801
LBW (< 2500 g)	6 (3.02%)	5 (3.88%)	2 (2.22%)	1 (2.08%)	2 (5.26%)	0.876
Newborn anomalies	0 (0.00%)	0 (0.00%)	1 (1.11%)	0 (0.00%)	1 (2.63%)	0.113
Pregnancy complications	4 (1.83%)	2 (1.43%)	0 (0.00%)	1 (1.85%)	1 (2.38%)	0.757

NOTE: GA was significantly shorter in the female HBeAg group than in the other groups ($P < 0.05$). Categorical variables were evaluated with chi-square tests. Data are presented as the number (%) or mean ± SD, $^*P < 0.05$.

Table 3. Results of multiple linear regression analysis among live born singletons

Characteristic	Birth weight (g)		
	β	t	*P* value
Paternal HBsAg	0.031	0.461	0.645
Maternal HBsAg	-0.138	-1.774	0.077
Paternal HBeAg	0.047	0.496	0.620
Maternal HBeAg	0.000	0.004	0.997
Maternal age (per year)	-0.034	-0.551	0.582
Paternal age (per year)	0.009	0.154	0.877
Maternal height (per cm)	-0.026	-0.585	0.559
Paternal height (per cm)	0.097	1.922	0.055
Maternal weight (per kg)	0.241	5.476	0.000*
Paternal weight (per kg)	-0.056	-1.159	0.247
Subfertility causes	-0.017	-0.408	0.684
Subfertility types	-0.068	-1.570	0.117
Subfertility duration (per year)	0.014	0.304	0.761
ICSI (versus IVF)	0.011	0.254	0.800
Gestational age at birth (per week)	0.389	9.448	0.000*
Gender (male versus female)	-0.100	-2.424	0.016*
Pregnancy complications	0.004	0.093	0.926

NOTE: Multiple linear regression was used to determine the relationship between the maternal and paternal HBV serostatus and birth weight in neonates with maternal age, paternal age, maternal height, paternal height, maternal weight, paternal weight, subfertility types, subfertility duration, subfertility cause, fertilization methods, GA, gender, and pregnancy complications. β is the regression coefficient; $^*P < 0.05$.

reports; in particular, gestational age (GA) and low birth weight (LBW) were defined as described previously [13]. In brief, preterm birth was defined as delivery before 37 completed weeks of gestation and LBW was defined as birth weight of < 2500 g.

Statistical analysis

All statistical analyses were performed with SPSS software (version 16, Inc., Chicago, IL, USA). The basic characteristics of patients were compared using analysis of variance (ANOVA), followed by the Student-Newman-Keuls test. Categorical variables were evaluated with chi-square tests. $P < 0.05$ was considered statistically significant. Multiple linear regression analyses were used to evaluate the association between the HBV serostatus and birth weight, while controlling for the effects of possible confounding factors, including the HBV carrier serostatus, maternal age, paternal age, maternal height, paternal height, maternal weight, paternal weight, subfertility types, subfertility duration, subfertility cause, fertilization methods (IVF or ICSI), GA, infant gender, and pregnancy complications.

Results

Data from 504 patients who delivered singletons were analyzed. Among the 504 patients, 199 were seronegative couples (HBV-negative group); 129 males were HBsAg, anti-HBe, and anti-HBc seropositive (male HBsAg group); 90 females were HBsAg, anti-HBe, and anti-HBc seropositive (female HBsAg group); 48 males were HBsAg, HBeAg, and anti-HBc seropositive (male HBeAg group); and 38 females were HBsAg, HBeAg, and anti-HBc seropositive (female HBeAg group). The subfertility causes, subfertility duration, subfertility types, fertilization methods, maternal age, maternal height, maternal weight, maternal BMI, paternal age, paternal height, paternal weight and paternal BMI are shown in **Table 1**. No significant differences were found among the groups.

Following this, the birth outcomes of singletons were compared, as shown in **Table 2**. GA was

38.55 ± 1.63 weeks in the HBV-negative group, 38.77 ± 1.63 weeks in the male HBsAg group, 38.90 ± 1.40 weeks in the female HBsAg group, 38.73 ± 1.69 weeks in the male HBeAg group, and 37.47 ± 2.93 weeks in the female HBeAg group. Female HBsAg, HBeAg, and anti-HBc seropositivity was significantly associated with delivery GA.

The mean birth weight was 3350.50 ± 484.07 g in the HBV-negative group, 3407.67 ± 595.80 g in the male HBsAg group, 3354.36 ± 496.25 g in the female HBsAg group, 3336.15 ± 460.71 g in the male HBeAg group, and 3202.90 ± 638.36 g in the female HBeAg group. The neonatal mean birth weight was slightly decreased in the female HBeAg group; however, there were no significant differences. The mean birth length was 50.35 ± 1.84 cm in the HBV-negative group, 50.38 ± 1.81 cm in the male HBsAg group, 50.35 ± 1.60 cm in the female HBsAg group, 50.57 ± 3.28 cm in the male HBeAg group, and 50.13 ± 1.46 cm in the female HBeAg group. Again, there were no significant differences between the HBV serostatus and birth length. In addition, there were two newborn anomalies in the female HBsAg (mild cleft palate) and female HBeAg groups (enuresis).

Finally, multiple linear regression was used to determine the relationship between the maternal and paternal HBV serostatus and birth weight in neonates with maternal age, paternal age, maternal height, paternal height, maternal weight, paternal weight, subfertility types, subfertility duration, subfertility cause, fertilization methods, GA, gender, and pregnancy complications. As shown in **Table 3**, birth weight was associated with maternal weight, GA at birth, and gender.

Discussion

Screening for maternal and paternal HBV chronic carriers prior to IVF-ET is routinely performed in hospitals in China. The HBV serostatus is important information because hepatitis B vaccine and hepatitis B immunoglobulin are administered to newborns [14]. An increasing number of studies have focused on the association between the effects of chronic HBV infection and neonatal outcomes. Case reports have indicated that the HBV carrier status increases maternal and neonatal morbidity from spontaneous conceptions [15-17]. Despite this association, the effects of the HBV carrier serostatus on neonatal outcomes have not been evaluated for IVF-ET cycles.

The present retrospective study first analyzed the effects of the HBV carrier serostatus on neonatal outcomes after IVF-ET. The results show that in females, HBsAg, HBeAg, and anti-HBc seropositivity was significantly associated with delivery GA in singletons; however, male HBsAg, HBeAg, and anti-HBc seropositivity did not affect delivery GA. Delivery GA was also not influenced by male or female HBsAg, anti-HBe, and anti-HBc seropositivity. HBsAg carriers had higher incidences of preterm birth on the basis of a case-control study [18]. However, the HBV serostatus was not specified and the data were derived from spontaneous conceptions. In the present study, results indicate that delivery GA was lower in the female HBeAg group than in the other groups. Chronic hepatitis B infection in pregnancy is an important and pervasive issue. Vigorous metabolism and increased nutrient consumption occur during pregnancy. The metabolism and detoxification produced by the mother and fetus depend on the mother's liver. This aggravates pre-existing liver disease and exacerbates liver damage [19, 20]. Chronic HBV infection increases the risk of developing adverse outcomes, including ascites, portal hypertension, hepatic fibrosis, cirrhosis, and hepatocellular carcinoma [21]. It is possible that females who were HBsAg, HBeAg, and anti-HBc seropositive had further deterioration of the liver status, which led to a decrease in delivery GA. However, the underlying mechanism needs further study.

Resurgence in research has focused on the effects of HBV on germ lines and embryos because of the increase in couples with infertility. HBV-infected males have decreased semen volume, lower total sperm counts, and poorer progressive sperm motility [22]. HBV infection has been found to reduce the fertilization ability and increase the early abortion rate of pregnancy [23, 24]. However, the newborn outcomes have not been evaluated after IVF-ET. It is wellknown that birth weight is a commonly used measure for the assessment of perinatal outcome, which is related to morbidity and mortality [25]. Multiple linear regression analysis was performed to determine the relationship between confounding factors and birth weight. Only maternal weight, GA, and neonatal gender were significantly related to birth weight in sin-

gletons in the present study. There was no association between the HBV serostatus and newborn birth weight. It is possible that maternal weight, GA, and neonatal gender, rather than the HBV serostatus, affected the birth weight. To this end, more research remains necessary to determine whether the HBV serostatus has an influence on neonatal birth weight.

In conclusion, the present study showed that female HBsAg, HBeAg, and anti-HBc seropositivity was significantly associated with delivery GA in singletons. Because there is an increase in the number of infertile HBV-infected individuals who have opted for ART, more research needs to be conducted to evaluate the effects of chronic HBV infection on newborn outcomes after IVF-ET.

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Disclosure of conflict of interest

None.

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