Asian Journal of Andrology (2011) 13, 465–469

www.nature.com/aja

ORIGINAL ARTICLE

Comparison of semen quality and outcome of assisted reproductive techniques in Chinese men with and without hepatitis B

Xu-Ping Zhou[1], Xiao-Ling Hu[1], Yi-Min Zhu[2], Fan Qu[3], Sai-Jun Sun[2] and Yu-Li Qian[2]

In this study, we aimed to determine the effects of hepatitis B virus (HBV) infection on sperm quality and the outcome of assisted reproductive technology (ART). A total of 916 men (457 HBV-positive and 459 HBV-negative) seeking fertility assistance from January 2008 to December 2009 at the Women's Hospital in the School of Medicine at Zhejiang University were analysed for semen parameters. Couples in which the men were hepatitis B surface antigen (HBsAg)-seropositive were categorized as HBV-positive and included 587 *in vitro* fertilisation (IVF) and 325 intracytoplasmic sperm injection (ICSI) cycles from January 2004 to December 2009; negative controls were matched for female age, date of ova retrieval, ART approach used (IVF or ICSI) and randomized in a ratio of 1:1 according to the ART treatment cycles (587 for IVF and 325 for ICSI). HBV-infected men exhibited lower semen volume, lower total sperm count as well as poor sperm motility and morphology ($P<0.05$) when compared to control individuals. Rates of two-pronuclear (2PN) fertilisation, high-grade embryo acquisition, implantation and clinical pregnancy were also lower among HBV-positive patients compared to those of HBV-negative patients after ICSI and embryo transfer ($P<0.05$); IVF outcomes were similar between the two groups ($P>0.05$). Logistic regression analysis showed that HBV infection independently contributed to increased rates of asthenozoospermia and oligozoospermia/azoospermia ($P<0.05$) as well as decreased rates of implantation and clinical pregnancy in ICSI cycles ($P<0.05$). Our results suggest that HBV infection in men is associated with poor sperm quality and worse ICSI and embryo transfer outcomes but does not affect the outcome of IVF and embryo transfer.
Asian Journal of Andrology (2011) **13,** 465–469; doi:10.1038/aja.2010.164; published online 14 March 2011

Keywords: hepatitis B virus; infection; infertility; intracytoplasmic sperm injection; *in vitro* fertilisation; male infertility; sperm; sperm motility

INTRODUCTION

Hepatitis B virus (HBV) is one of the most prevalent blood-borne viruses worldwide. China, in particular, has a high rate of hepatitis B surface antigen (HBsAg) prevalence (7.2%) among individuals between the ages of 1 and 60 years, which affects approximately 10% of the child-bearing population.[1] Although it is a hepadnavirus, HBV has also been found in extrahepatic tissues such as kidneys, parotid glands, ovaries[2,3] and testes[4] as well as in semen.[5] The relationship between HBV infection and male reproductive performance has been of considerable interest, as HBV infection was reported to increase chromosomal instability in sperm[6] and impair sperm quality in HBV-infected males.[7–9]

Nowadays, an increasing number of infertile HBV-infected individuals have turned to assisted reproductive technology (ART), including *in vitro* fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) and embryo transfer (ET) treatments. However, little is currently known about the impact of HBV infection on ICSI outcome. Two recent studies examined the outcomes of IVF and embryo transfer treatments in couples with at least one HBV-seropositive partner and reported conflicting results. Pirwany *et al.*[10] observed lower implantation and pregnancy rates in HBV-positive individuals (10 couples with HBV-positive men and 3 couples with HBV-positive women) compared to a healthy control group. In contrast, Lam *et al.*[11] reported higher implantation and pregnancy rates in the HBV-seropositive group compared to those in the HBV-seronegative group.

HBV is routinely and systematically screened prior to IVF/ICSI and embryo transfer treatments. We performed a retrospective study to examine the impact of HBV infection on sperm quality and ART treatment outcome, focusing specifically on ICSI–ET outcomes.

MATERIALS AND METHODS

Subjects

Our study included 916 male patients seeking fertility assistance at the Women's Hospital in the School of Medicine at Zhejiang University (Hangzhou, China) from January 2008 to December 2009. Semen parameters were analysed, and 457 men (26–43 years of age) were found to be HBsAg-seropositive (designated as the HBV group); the other 459 men (25–45 years of age) served as the control group.

[1]Key Laboratory of Women's Reproductive Health of Zhejiang Province, Hangzhou 310006, China; [2]Department of Reproductive Endocrinology, Women's Hospital, School of Medicine, Zhejiang University, Hangzhou 310006, China and [3]Department of Reproductive Endocrinology, The First Affiliated Hospital of Zhejiang Medical University, Hangzhou 310006, China
Correspondence: Dr YM Zhu (zhuyim@zju.edu.cn)
Received: 26 July 2010; Revised: 13 October 2010; Accepted: 16 November 2010; Published online: 14 March 2011

Controls included men who were negative for serum HBsAg, hepatitis B surface antibody (HBsAb), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb) and hepatitis B c antibody (HBcAb); control patients were also matched for days of sexual abstinence as well as time seeking fertility assistance.

Patients with chromosomal abnormalities, concomitant varicocele, a history of surgery or congenital defects (urological or related to reproductive organs), long-term drug use and/or toxic or radiation exposure were all excluded from our study. In addition, those with a history of parotitis or genital tract infections as well as those receiving any antiviral therapy for fertility during the study period were also excluded. Men with increased aminotransferase levels but normal hepatic function (i.e., normal levels of bilirubin, albumin and other coagulative parameters) as well as normal ultrasonographic features of portal hypertension were enrolled in the study.

We concurrently carried out an additional retrospective review on IVF/ICSI and embryo transfer outcomes using 1824 cycles from January 2004 to December 2009. The HBV groups included a total of 587 IVF cycles and 325 ICSI cycles from HBsAg-seropositive husbands and HBsAg-seronegative wives. Control individuals were matched for the date of ova retrieval ($\pm$1 day), female age ($\pm$1 year) and ART approach used (IVF or ICSI) and randomized using the SPSS statistical software, version 16.0 (SPSS Inc., Chicago, IL, USA) in the ratio of 1:1 according to the ART treatment cycles. Both husbands and wives in the control group were HBsAg-seronegative. We excluded cycles with donor's semen or cycles with chromosomal abnormalities as well any couples who were seropositive for HCV and/or HIV. None of the patients were diagnosed with acute hepatitis or received any antiviral treatment for fertility.

Andrological patient history and physical examinations

All male patients were questioned on the use of alcohol or cigarette, long-term drug use and toxic/radiation exposure. Information on patient height, weight and occupation was obtained, as was any history of disease (parotitis or tuberculosis) or urogenital surgery. Body mass index (BMI) was defined as weight (kg)/height (m^2). Physical examinations included routine assessment of secondary sex characteristics and genitourinary organs.

Microbiological examination

Venous blood samples were obtained for screening of hepatitis virus (HAV, HBV, HCV and HDV) and HIV. HBsAg, HBsAb, HBeAg, HBeAb and HBcAb were detected using enzyme-linked immunosorbent assays. The presence of treponema pallidum, gonococcus, mycoplasma and chlamydia in the genital tract was also tested.

Semen analysis

Semen samples (ejaculates) were obtained by masturbation after 3–7 days of sexual abstinence and collected into sterile plastic containers. All samples were allowed to liquefy at room temperature for 30 min prior to analysis. Semen parameters including liquefying time, volume, concentration, morphology, motility and PH were assessed following the WHO 1999 criteria, which were described in detail in the joint European Society of Human Reproduction and Embryology–Nordic Association for Andrology (ESHRE–NAFA) manual.[12] All infected samples were analysed in a separate laboratory for safety.

ART treatment protocols

All 1824 cycles undergoing IVF/ICSI–ET were performed following the routine downregulation protocol.[13] In brief, pituitary function was effectively downregulated (serum LH $<$3 IU l^{-1} and E2 $<$110 pmol l^{-1}) with triptorelin (Decapeptyl or Diphereline; Ipsen, Paris, France) applied on day 21 of the menstrual cycle, and ovaries were stimulated with recombinant follicle stimulating hormone (Gonal-F; Serono, Geneva, Switzerland or Puregon; Organon, Oss, The Netherlands) or human menopausal gonadotropin (Livzon Biochemistry Co., Zhuhai, China). The gonadotropin dose was given according to age, ovarian reserve function and response to stimulation in previous cycles. Follicular development was monitored by serial vaginal ultrasound and levels of serum E2. When two additional follicles reached 18 mm in mean diameter, human chorionic gonadotropin (hCG, 10 000 IU; Serono) was injected. At 34–36 h post-injection, oocytes were transvaginally retrieved under ultrasound guidance.

Semen samples were washed and prepared using the classical density gradient procedure.[14] IVF or ICSI was then carried out according to seminal parameters, and embryo culture was performed as previously described.[15] Embryo quality was evaluated and graded with respect to the number of blastomeres and percentage of fragmentation using the combined scoring system.[15] Embryos with the highest combined scoring system scores were chosen for embryo transfer on day 3 of culture (two for women $\leqslant$35 years old and three for women $>$35 years old). Cycles were cancelled in the case of no available embryos or if ovarian hyperstimulation syndrome developed. Women returned to the hospital 14 days after embryo transfer to check serum β-hCG levels, and an additional ultrasound examination was carried out 2 weeks later in β-hCG-seropositive women to check for the presence and number of gestational sacs and foetal hearts. All protocols were approved by the ethics committee of the Women's Hospital in the School of Medicine at Zhejiang University. All couples provided written informed consents prior to the start of ART treatment.

ART outcome measures

The two-pronuclear (2PN) fertilisation rate was defined as the number of confirmed 2PN embryos per oocyte retrieved in an IVF cycle or per metaphase II-arrested oocyte in an ICSI cycle 17–19 h after insemination or microinjection. High-grade embryo acquisition rate was defined as the number of embryos having $\geqslant$6 cells with a grade of I or II according to combined scoring system per divisive embryo on day 3 of culture. Implantation rate was calculated by the number of intra- or extrauterine gestational sacs per embryo transferred. Clinical pregnancy rate was defined as the number of women with intrauterine gestational sacs (upon ultrasound scan) per cycle with successful embryo transfer.

Statistical analyses

Statistical analyses were performed using the SPSS statistical software for Windows, version 16 (SPSS Inc., Chicago, IL, USA). Two-sided *t*-tests were applied for continuous variables, and all categorical data were analysed using Chi-squared tests. Logistic regression analysis was performed to assess the contribution of HBV infection on sperm quality and ART outcome. $P<0.05$ was considered statistically significant.

RESULTS

Comparison of semen parameters between HBV-positive and HBV-negative men

We analysed semen parameters of 916 men who underwent ART in our hospital unit. We observed no statistical differences with respect to age and BMI between the HBV-positive and HBV-negative groups ($P>0.05$). Sperm concentration was also not significantly different between HBsAg-seropositive and -seronegative men ($P>0.05$); however, we did observe lower mean semen volume ($P=0.015$) and mean

total sperm count (P=0.019) in HBsAg-seropositive men. In addition, the mean percentages of type a, type b and type a+b motility decreased (P=0.023, P=0.040 and P=0.002, respectively), and the number of patients with asthenozoospermia (P=0.003) or oligozoospermia (P=0.027) markedly increased among HBV-positive individuals. Furthermore, HBV-infected men showed an increase in the mean percentage of sperm with abnormal morphology compared to that of the negative control group (P=0.000). Results are summarized in **Table 1**.

Comparison of ART outcomes between HBV-positive and HBV-negative groups

IVF outcomes between HBV-positive and HBV-negative groups. As shown in **Table 2**, clinical characteristics did not significantly differ between the HBV-positive and HBV-negative groups, although more cases with sperm problems were present in the HBV-positive group (P=0.025). Furthermore, ovarian stimulation and number of embryos transferred per cycle were not significantly different between the two groups. IVF and embryo transfer outcomes in HBV-positive men were comparable with those in their healthy counterparts (P>0.05), though there was a trend of lower implantation rates among HBV-positive men.

ICSI outcomes between HBV-positive and HBV-negative groups. As shown in **Table 3**, all clinical characteristics in the HBV-positive group were not significantly different from those in the negative control group. Ovarian stimulation and response as well as the number of embryos transferred per cycle were similar in both study groups. After ICSI and embryo transfer, we observed decreased rates of 2PN fertilisation (P=0.005), high-grade embryo acquisition (P=0.046), implantation (P=0.008) and clinical pregnancy (P=0.035) among HBV-positive men when compared to matched controls.

Table 1 Semen parameters in HBV-infected men and negative controls

Parameters	*Positive (n=457)*	*Negative (n=459)*	P *value*
Age (year)	33.4±4.8	33.5±4.8	NS
BMI (kg m^{-2})	23.5±3.0	23.5±3.6	NS
Semen volume (ml)	2.5±1.2	2.7±1.3	0.015
Sperm concentration (10^6 per ml)	121.8±83.5	124.1±77.3	NS
Total sperm count (10^6 per ejaculate)	284.4±246.2	323.2±252.5	0.019
Rapid progressive motility (a) (%)	9.8±10.3	11.4±11.4	0.023
Slow or sluggish progressive motility (b) (%)	24.7±11.8	26.3±11.1	0.040
Progressive motility (a+b) (%)	34.2±17.4	37.7±16.7	0.002
Abnormal morphology (%)	48.4±12.8	45.0±11.5	0.000
Rate of asthenozoospermia (*n*, %)	383 (83.8)	349 (76.0)	0.003
Rate of oligozoospermia or azoospermia (*n*, %)	41 (9.0)	24 (5.2)	0.027

Values are presented as mean±s.d.
Abbreviations: BMI, body mass index; HBV, hepatitis B virus; NS, not significant.

Logistic regression analysis

To determine the contributions of potential predicting variables to the rates of asthenozoospermia and oligozoospermia/azoospermia, we performed binary logistic regression. Age, BMI, occupation, alcohol use, cigarette smoking and HBV status were considered in our regression model. We found that HBV infection significantly contributed to the rates of both asthenozoospermia (odds ratios (OR): 1.72, 95% confidence interval (CI): 1.23–2.41, P=0.001) and oligozoospermia/azoospermia (OR: 1.75, 95% CI: 1.01–3.05, P=0.045).

In addition, we assessed the contributions of different variables to implantation rate and clinical pregnancy rate after ICSI and embryo transfer by logistic regression analysis. We considered female age, BMI, duration of infertility, number of IVF/ICSI cycles, total dose of gonadotropin used, number of oocytes retrieved, maximal E_2 levels, number of 2PN embryos, number of high-grade embryos, number of embryos transferred as well as HBV status. After correcting for

Table 2 IVF and embryo transfer outcomes for HBV-positive and HBV-negative groups

Variable	*Positive (n=587)*	*Negative (n=587)*	P *value*
Age of wives (year)	31.4±4.9	31.4±4.9	NS
Female BMI (kg m^{-2})	22.0±3.2	22.0±3.3	NS
Duration of infertility (year)	4.9±3.4	4.7±3.3	NS
Times of IVF–ET/ICSI–ET (cycle)	1.1±0.4	1.1±0.4	NS
Primary infertility (*n*, %)	181 (30.8)	183 (31.2)	NS
Distribution of infertile factors (*n*, %)			
Total number of husbands with sperm problems	415 (71.3)	379 (65.1)	0.025
Total number of wives with tubal blockage	503 (85.7)	485 (82.6)	NS
Total number of wives with ovulation failure	14 (2.4)	20 (3.4)	NS
Total number of wives with endometriosis	77 (13.1)	62 (10.6)	NS
Total number of couples with unexplained infertility	14 (2.4)	13 (2.2)	NS
Duration of stimulation (d)	9.6±2.2	9.7±2.1	NS
Total dose of gonadotropin used (IU)	2670±1085	2659±1056	NS
Serum E_2 levels on day of hCG injection (pmol l^{-1})	1 1669±7024	1 2161±7634	NS
No. of retrieved oocytes	12.5±6.4	12.6±6.4	NS
No. of embryos transferred in cycles by ET	2.1±0.5	2.1±0.5	NS
2PN fertilisation rate (%)	4508/7144 (63.1)	4803/7623 (63.0)	NS
High-grade embryo rate (%)	2757/4633 (59.5)	2907/4943 (58.8)	NS
Implantation rate (%)	284/1140 (24.9)	296/1108 (26.7)	NS
Clinical pregnancy rate (%)	217/535 (40.5)	210/521 (40.3)	NS
Cancellation rate (*n*, %)	52 (8.9)	66 (11.2)	NS

Values are presented as mean±s.d.
Abbreviations: BMI, body mass index; E_2, estradiol; ET, embryo transfer; HBV, hepatitis B virus; hCG, human chorionic gonadotrophin; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilisation; NS, not significant; 2PN, two-pronuclear.

Table 3 ICSI and embryo transfer outcomes for HBV-positive and HBV-negative groups

Variable	*Positive* (n=*325*)	*Negative* (n=*325*)	P *value*
Age of wives (year)	31.4±4.3	31.5±4.3	NS
Female BMI (kg m^{-2})	22.1±2.9	22.1±3.2	NS
Duration of infertility (year)	4.9±3.1	5.0±3.3	NS
Times of IVF–ET/ICSI–ET (cycle)	1.2±0.5	1.3±0.6	NS
Primary infertility (*n*, %)	163 (50.1)	159 (48.9)	NS
Distribution of infertile factors (*n*, %)			
Total number of husbands with sperm problems	305 (93.8)	294 (90.5)	NS
Total number of wives with tubal blockage	163 (50.2)	177 (54.5)	NS
Total number of wives with ovulation failure	12 (3.7)	14 (4.3)	NS
Total number of wives with endometriosis	28 (8.6)	19 (5.8)	NS
Total number of couples with unexplained infertility	10 (3.1)	10 (3.1)	NS
Duration of stimulation (day)	9.7±2.0	9.4±2.0	NS
Total dose of gonadotropin used (IU)	2572±1003	2570±1075	NS
Serum E_2 levels on day of hCG injection (pmol l^{-1})	1 1509±7075	1 2212±7495	NS
No. of retrieved oocytes	13.1±6.9	12.8±6.5	NS
No. of embryos transferred in cycles by ET	2.3±0.5	2.3±0.6	NS
2PN fertilisation rate (%)	2445/3448 (70.9)	2463/3328 (74.0)	0.005
High-grade embryo rate (%)	1405/2439 (57.6)	1452/2403 (60.4)	0.046
Implantation rate (%)	126/688 (18.3)	159/657 (24.2)	0.008
Clinical pregnancy rate (%)	96/308 (31.2)	118/300 (39.3)	0.035
Cancellation rate (*n*, %)	17 (5.2)	25 (7.6)	NS

Values are presented as mean±s.d.

Abbreviations: BMI, body mass index; E_2, estradiol; ET, embryo transfer; HBV, hepatitis B virus; hCG, human chorionic gonadotrophin; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilisation; NS, not significant; 2PN, two-pronuclear.

confounding effects, we found that HBV infection significantly contributed to both implantation rate (OR: 0.57, 95% CI: 0.48–0.99, $P=0.044$) and clinical pregnancy rate (OR: 0.66, 95% CI: 0.45–0.95, $P=0.036$).

DISCUSSION

Recently, much interest has been focused on the relationship between HBV infection and male reproductive performance. In this study, we found that HBV-infected men had decreased semen volume, lower total sperm count and poorer progressive sperm motility and morphology when compared to matched control individuals. Although both mean semen volume (2.5 ml) and mean total sperm count (121.8×10^6/ejaculate) in HBV-infected men were within the normal range ($\geqslant$2 ml, $\geqslant40\times10^6$/ejaculate, WHO 1999 criteria), we observed an increased number of patients with oligozoospermia or azoospermia (total sperm count $\leqslant40\times10^6$/ejaculate) among HBV-infected individuals. Although our study population had a mean percentage of progressive motility (a+b) below the normal reference values (50%, WHO 1999 criteria) in both HBV-positive (34.2%) and HBV-negative men (37.7%), the rate of asthenozoospermia in the HBV-positive group was much higher than that in the control, as confirmed by logistic regression analysis. Thus, our findings indicate that HBV infection negatively affects semen quality.

These observations are consistent with previous data reported on sperm quality in HBV-infected men. Vicari *et al.*[7] had compared 23 HBV-infected men with 19 HCV-infected men and observed overall worse sperm parameters in the HBV-infected group. Similarly, Lorusso *et al.*[8] had reported decreased sperm motility and viability but normal morphology in HBV-seropositive men ($n=30$) when compared to a negative control group ($n=130$), and Moretti *et al.*[9] observed a lower fertility index (expressed as the number of 'healthy' spermatozoa) among HBV-infected men when compared to healthy men. All three reports demonstrated a negative correlation between HBV infection and semen quality based on a limited sample number. However, the latter two studies only selected fertile men (those without any infertility issues or oligoasthenoteratozoospermia) for the control group, which would bias results towards better sperm parameters in the control. Our study avoids this bias by comparing 457 HBV-infected men with 459 matched HBV-negative patients who were all seeking fertility assistance.

HBV is able to integrate into sperm chromosomes,[16] causing chromosomal instability and metaphase chromosome stickiness, hard to be stained in HBV-infected men.[6] Thus, HBV infection can induce chromosome aberrations, leading to hereditary defects in male germinal cells and problems in spermatogenesis. Consistent with this idea, Moretti *et al.*[9] had observed that HBV infection increased the percentage of phenotypic sperm pathologies, such as necrosis, apoptosis and immaturity. In addition, a recent *in vitro* study on HBV S protein and sperm function demonstrated that sperm treated with S protein exhibited lower fertilisation rate and index due to reduced sperm motility from mitochondrial injury.[17] It is also possible that immunological, inflammatory or direct toxic effects caused by viral infection impaired fertility performance,[18] which would all contribute to poor sperm quality in HBV-infected men.

As HBV infection was reported to reduce male fertility, we aimed to evaluate the ART outcomes of HBV-infected men. To our knowledge, we are the first to demonstrate that HBV infection in men negatively affects the outcome of ICSI cycles but not IVF cycles. Our results contradict previous observations in which implantation and pregnancy rates in HBV-positive IVF cycles (10 HBV-positive men and 3 HBV-positive women) were found to be lower than those in age-matched controls.[10] Interestingly, another study comparing HBV-seropositive couples ($n=34$) with age-matched female controls showed higher implantation and pregnancy rates in HBV-positive individuals.[11] These discrepancies may be due in part to differences in the matched control groups used for comparison as well as differences in overall sample size. In contrast to previous studies, we specifically matched controls according to the ART approach used, female age

(±1 year) and oocyte retrieval period. In addition, we examined a substantial number of HBV-seropositive patients to assess ART outcomes in HBV-positive cycles.

Because ICSI could aggravate HBV transmission into the oocyte,[19] the Dutch-Belgian Association for Artificial Insemination advised against ICSI treatment for chronic HBV carriers.[20] Here, we also observed suboptimal ICSI and embryo transfer outcomes in the HBV-positive group (decreases in the rates of 2PN fertilisation, high-grade embryos acquisition, implantation and clinical pregnancy per cycle of embryo transfer). However, we obtained positive results using IVF cycles on HBV-positive patients. One possible explanation for this difference is that sperm infected with HBV could have also been injected into the oocyte during ICSI. This is unlikely though because HBV-infected sperm generally have a lower probability of successful fertilisation by either IVF or natural conception,[9] as sperm infected with HBV S protein was shown to have decreased fertilising ability.[17] Moreover, HBV DNA could be detected in the semen of HBV-infected patients,[5] and even after thoroughly washing sperm to reduce the risk of viral transmission, about 10% remained HBV-positive.[21] Thus, another explanation for poor ICSI and embryo transfer outcomes in HBV-positive men would be that extracellular HBV was introduced into the oocyte during ICSI. In fact, HBV transmitted into oocytes or embryos was found to replicate and express S protein,[22] which would lead to loss of mitochondrial membrane potential.[17] We speculate that HBV infection negatively affects the overall course of fertilisation, zygote development and implantation. By contrast, microinjection and consequent injury rarely occur in IVF cycles. Thus, similar outcomes are observed between HBV-positive and HBV-negative groups after IVF and embryo transfer.

Semen quality and ART outcomes of HBV-seropositive men were examined in this study, but the underlying molecular mechanisms still remain to be elucidated. In addition, we did not evaluate the potential developmental effects on children borne to HBV-positive fathers undergoing ART treatment. Therefore, future studies on the offspring of HBV-positive men receiving ART treatment are needed to follow up on these findings.

We conclude that HBV infection in men is associated with impaired ICSI and embryo transfer outcomes as well as impaired sperm quality. Further studies are needed to confirm these findings and to understand the molecular mechanisms responsible for these effects on reproductive performance.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

YMZ designed and supervised the study. XPZ analysed the data and wrote the paper. SJS gathered data. XLH, FQ and YLQ edited the manuscript. All authors discussed the results and implications and commented on the manuscript at all stages.

ACKNOWLEDGMENTS

This work was supported by a grant from the Natural Science Foundation of Zhejiang Province, China (Y206148). The authors gratefully acknowledge the members of the Department of Reproductive Endocrinology in the Women's Hospital at Zhejiang University for their continued assistance.

1 Liang X, Bi S, Yang W, Wang L, Cui G *et al*. Epidemiological serosurvey of hepatitis B in China—declining HBV prevalence due to hepatitis B vaccination. *Vaccine* 2009; **27**: 6550–57.
2 Ye F, Yue Y, Li S, Chen T, Bai G *et al*. Presence of HBsAg, HBcAg, and HBVDNA in ovary and ovum of the patients with chronic hepatitis B virus infection. *Am J Obstet Gynecol* 2006; **194**: 387–92.
3 Chen LZ, Fan XG, Gao JM. Detection of HBsAg, HBcAg, and HBV DNA in ovarian tissues from patients with HBV infection. *World J Gastroenterol* 2005; **11**: 5565–67.
4 Hadchouel M, Scotto J, Huret JL, Molinie C, Villa E *et al*. Presences of HBV DNA in spermatozoa: a possible vertical transmission of HBV *via* the germ line. *J Med Virol* 1985; **16**: 61–6.
5 Qian WP, Tan YQ, Chen Y, Peng Y, Li Z *et al*. Rapid quantification of semen hepatitis B virus DNA by real-time polymerase chain reaction. *World J Gastroenterol* 2005; **11**: 5385–89.
6 Huang JM, Huang TH, Qiu HY, Fang XW, Zhuang TG *et al*. Effects of hepatitis B virus infection on human sperm chromosomes. *World J Gastroenterol* 2003; **9**: 736–40.
7 Vicari E, Arcoria D, Di Mauro C, Noto R, Noto Z *et al*. Sperm output in patients with primary infertility and hepatitis B or C virus; negative influence of HBV infection during concomitant varicocele. *Minerva Med* 2006; **97**: 65–77.
8 Lorusso F, Palmisano M, Chironna M, Vacca M, Masciandaro P *et al*. Impact of chronic viral diseases on semen parameters. *Andrologia* 2010; **42**: 121–6.
9 Moretti E, Federico MG, Giannerini V, Collodel G. Sperm ultrastructure and meiotic segregation in a group of patients with chronic hepatitis B and C. *Andrologia* 2008; **40**: 286–91.
10 Pirwany IR, Phillips S, Kelly S, Buckett W, Tan SL. Reproductive performance of couples discordant for hepatitis B and C following IVF treatment. *J Assist Reprod Genet* 2004; **21**: 2455–60.
11 Lam PM, Suen SH, Lao TT, Cheung LP, Leung TY *et al*. Hepatitis B infection and outcomes of *in vitro* fertilization and embryo transfer treatment. *Fertil Steril* 2010; **93**: 480–5.
12 Kvist U, Björndahl L, editors. Manual on Basic Semen Analysis. ESHRE Monographs. Oxford: Oxford University Press; 2002.
13 Lu XE, Huang HF, Li MG, Zhu YM, Qian YL *et al*. Resistin levels of serum and follicular fluid in non-obese patients with polycystic ovary syndrome during IVF cycles. *J Zhejiang Univ Sci B* 2005; **6**: 897–902.
14 Jedrzejczak P, Pawelczyk L, Taszarek-Hauke G, Kotwicka M, Warcho½ W *et al*. Predictive value of selected sperm parameters for classical *in vitro* fertilization procedure of oocyte fertilization. *Andrologia* 2005; **37**: 72–82.
15 Qian YL, Ye YH, Xu CM, Jin F, Huang HF. Accuracy of a combined score of zygote and embryo morphology for selecting the best embryos for IVF. *J Zhejiang Univ Sci B* 2008; **9**: 649–55.
16 Huang JM, Huang TH, Qiu HY, Fang XW, Zhuang TG *et al*. Studies on the integration of hepatitis B virus DNA sequence in human sperm chromosomes. *Asian J Androl* 2002; **4**: 209–12.
17 Zhou XL, Sun PN, Huang TH, Xie QD, Kang XJ *et al*. Effects of hepatitis B virus S protein on human sperm function. *Hum Reprod* 2009; **24**: 1575–83.
18 Keck C, Gerber-Schafer C, Clad A, Wilhelm C, Breckwoldt M. Seminal tract infections: Impact on male fertility and treatment options. *Hum Reprod Update* 1998; **4**: 891–903.
19 Lutgens SP, Nelissen EC, van Loo IH, Koek GH, Derhaag JG *et al*. To do or not to do: IVF and ICSI in chronic hepatitis B virus carriers. *Hum Reprod* 2009; **24**: 2676–78.
20 NVOG-Standpunt. Screening infectieziekten bij kunstmatige voortplanting. Nederlandse Vereniging voor Obstetrie en Gynaecologie, 2004. http://www.nvog.nl
21 Garrido N, Meseguer M, Remoh J, Simon C, Pellicer A. Semen characteristics in human immunodeficiency virus (HIV)- and hepatitis C (HCV)-seropositive males: predictors of the success of viral removal after sperm washing. *Hum Reprod* 2005; **20**: 1028–34.
22 Ali BA, Huang TH, Salem HH, Xie QD. Expression of hepatitis B virus genes in early embryonic cells originated from hamster ova and human spermatozoa transfected with the complete viral genome. *Asian J Androl* 2006; **8**: 273–9.