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ARTICLE

Adverse effects of hepatitis B virus on sperm motility and fertilization ability during IVF

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Abstract The consequences of hepatitis B virus (HBV) infection for fertility are still unclear. Spermatozoa with decreased motility have been reported in HBV-infected patients. It has been demonstrated *in vitro* that HBV S protein has adverse effects on human sperm function with consequences for fertilization. In a case-control study design, 32 IVF cycles in couples with male HBV infection were compared with 64 cycles in non-infected couples, matched for age, time period, cycle rank and sperm parameters on the day of oocyte retrieval. Sperm motility before selection was significantly reduced in the HBV group (36.3 ± 11.6% versus 45.3 ± 14.4%, $P = 0.003$). A low fertilization rate (LFR) was more frequently observed in the HBV group (34.4% versus 15.6%, $P = 0.036$) and was associated with a decreased number of embryos available for transfer, although embryo quality on day 2 or 3 was not different. Implantation and pregnancy rates were comparable between groups. This study shows that HBV has a deleterious effect on sperm motility *in vivo* and that couples whose male partner is infected have a higher risk of LFR after IVF, a risk which is independent from the initial sperm motility.

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KEYWORDS: fertilization, hepatitis B virus, IVF, sperm motility

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Introduction

Human hepatitis B virus (HBV) is the major epidemiological agent of acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma. About 2 billion people worldwide have been infected with the virus and about 350 million live with chronic infection, constituting a pool allowing continuous viral transmission (Liaw and Chu, 2009). In Northern Europe, the prevalence is below 1% and men are affected twice as often as women (Honeck et al., 2006).

Infectivity of hepatitis B is due to its presence in most body fluids of infected persons: blood, semen, vaginal secretions and saliva (Heathcote et al., 1974; Jenison et al., 1987). As a consequence, HBV could be transmitted through sexual contact and partners should receive the vaccine before allowing condom-free sexual intercourse (Scott et al., 1980). HBV is treated when a liver disease is diagnosed but the infection itself even in the case of assisted reproduction technology is not an indication of therapy. When assisted reproduction technology is required, the objective is to give birth to a healthy baby without contaminating the partner or other patients.

It is now well known that bacterial and viral infections may be deleterious to human fertility (Collodel et al., 2005; Durazzo et al., 2006; Figura et al., 2002). Adverse effects of HBV on human sperm function (like reduced progressive and non-linear motility and cumulus-free hamster oocyte penetration) were recently reported (Zhou et al., 2009). Moreover, HBV can integrate human sperm DNA and have mutagenic effects; as a consequence, it was postulated that HBV could possibly be transmitted vertically to the next generation via the germ line (Ali et al., 2006; Huang et al., 2003; Wang et al., 2003).

HBV-infected patients might also have altered sperm parameters (Huret et al., 1986; Moretti et al., 2008; Vicari et al., 2006). But these reports concerning the in-vivo effects of HBV on sperm quality are not yet conclusive, especially on IVF outcomes (Lam et al., 2010; Pirwany et al., 2004).

The objective of this retrospective case-control study is to investigate the in-vivo effects of HBV on sperm parameters and also on fertilization rates, embryo quality and pregnancy rates after standard IVF.

Materials and methods

Population

A 1:2 matched case-control study was conducted using data from the study centre's records, including information about the infection history. Cases were couples in which male partners were hepatitis B surface antigen (HBsAg) positive and who attended the assisted reproduction centre of Bichat-Claude Bernard University Hospital between January 2005 and March 2008 for an IVF cycle ($n = 32$). Their partners either had a resolved HBV infection or were vaccinated. A response was considered to be protective when the vaccine elicited the formation of antibodies to HBsAg (anti-HBs) at a concentration equal to or greater than 10 mIU/ml as determined by immune enzymatic assay.

Two controls were randomly selected for each case among couples attending the study centre in the same period but who did not have any history of viral infection ($n = 64$). Controls and cases were matched by age (women within 2 years; men younger or older than 40 years old) and number of motile spermatozoa on the day of oocyte retrieval (less or more than 24.25 million which corresponds to the median value of this variable's distribution in the cases group). Only the first two IVF attempts were considered. Cases and controls with no oocyte on the day of retrieval, no spermatozoa available on that day or men with genetic abnormalities were excluded.

HBV-infected patients came from West Africa ($n = 18$), Central America ($n = 5$), Asia ($n = 3$), India ($n = 1$), Mediterranean region ($n = 3$) or Eastern Europe ($n = 2$). Twenty five patients had a viral load between log 1.92 and 7.14/ml; in seven patients it was undetectable. None of the patients had cirrhosis or were infected by hepatitis delta. Only one patient had received an antiviral treatment before assisted reproduction.

Semen analysis

Semen samples were collected at the laboratory by masturbation into a sterile container, after 3—5 days of sexual abstinence. They were kept for 30 min at 37°C for liquefaction before being analysed according to the World Health Organization (WHO) guidelines (WHO, 1992). The volume of the ejaculate was measured by aspiration into a graduated pipette. Semen pH was read onto a pH paper (range pH 6.1—10.0). Consistency was graded normal, low or high. Sperm motility was evaluated by examination of a 10 μl drop of semen covered with a 22 mm × 22 mm coverslip under ×400 phase-contrast magnification at 37°C. Motility was graded as follows: (i) rapid progressive motility; (ii) slow progressive motility; (iii) non-progressive motility; and (iv) no motility according to WHO guidelines. Sperm vitality was assessed on a smear after staining with eosin—nigrosin. Spermatozoa and non-sperm cell (NSC) concentrations were assessed in duplicate with a haemocytometer on formalin-diluted 1/10—1/40 specimens. Sperm morphology was evaluated according to the modified David's classification (Auger et al., 2001) on Shorr-stained smears under ×1000 magnification. The values for motility, vitality and normal morphology were expressed as percentages. Semen bacterial analysis was carried out for all men. It included investigation of the usual bacteria, mycoplasms and chlamydiae.

Stimulation protocols

Pituitary suppression was generally obtained by a gonadotrophin-releasing agonist (GnRH-a; triptorelin acetate, decapeptyl; Ipsen, France) administered subcutaneously at a dose of 0.1 mg/day, beginning on day 21 of a spontaneous menstrual cycle and for 10—14 days, until complete suppression of the pituitary activity (long protocol) or 1—3 days before rFSH introduction (short protocol). The initial dose of recombinant FSH (Gonal F; Merck, France; or Puregon; Shering-Plough, France) or human menopausal gonadotrophin (Menopur; Ferring, France) varied between 150 and 300 IU/day. For GnRH antagonist cycles, a GnRH antagonist

(ganirelix, Orgalutran, 0.25 mg/day, Shering-Plough, France; or Cetrotide, Merck, 0.25 mg/day, France) was administered when the leading follicle had reached 14 mm in its greatest diameter and until the day of human chorionic gonadotrophin. Duration and doses applied to each patient followed standard criterion of follicular growth and maturation assessed by ultrasound and serum oestradiol. A single dose of human chorionic gonadotrophin recombinant (0.5 ml Ovitrelle 250), Merck) was administered when at least three follicles had reached 17 mm. Oocyte retrieval was performed 36—38 h later by transvaginal ultrasound-guided needle aspiration. Luteal phase supplementation was started following oocyte retrieval using 400 mg/day of vaginal progesterone (Utrogestan, Besins Laboratory, Montrouge, France) until the pregnancy test (12 days after the embryo transfer) and, eventually, during the first 10 weeks of pregnancy if the test was positive.

IVF procedure

Semen samples were collected at the laboratory by masturbation into a sterile container, after 3—5 days of sexual abstinence. Freshly ejaculated semen was prepared on a two-layer gradient (90/45, v/v) (PureSperm; Origio, Limonest, France) according to the WHO guidelines (WHO, 1992). Final pellets were washed once, resuspended in Universal IVF Medium (Origio) and kept at 37°C. Insemination using the prepared spermatozoa was performed 2 h after oocyte retrieval in Universal IVF Medium (Origio). Fertilization was assessed 16—20 h after sperm insemination. Embryos were then cultured in ISM1 medium up to day 3 and, when possible, in ISM2 medium from day 3 to day 5 (Origio). Embryos were checked for early cleavage 25 h after sperm insemination. Day-2 embryos were observed 40—44 h after sperm insemination. The fertilization rate was defined as the proportion of oocytes with two pronuclei (2PN). One to three good-quality embryos were transferred to the uterine cavity 2, 3 or 5 days after the oocyte retrieval. All transfers were carried out under ultrasound guidance, using a soft catheter (Elliocath, Prince Medical, Ercuis, France). Supernumerary embryos were frozen for subsequent transfer. Noci et al. (2005) and Gardner et al. (2000) embryo classifications were used for embryo and blastocyst grading.

IVF outcome measures

Patient characteristics recorded were: patients' ages; type, duration and infertility aetiology; ovarian reserve evaluation (cycle day-3 FSH and anti-Müllerian hormone concentrations; antral follicle count); and sperm parameters. Data on treatment cycles that were recorded included: type and dose of gonadotrophin; oestradiol concentration on the day of human chorionic gonadotrophin; number of oocytes retrieved; and semen parameters on the day of oocyte retrieval. The fertilization rate, the number of good-quality embryos (type A and B), implantation and pregnancy rates were also recorded and compared between the two groups. The primary outcome was the fertilization rate which was categorized as low fertilization rate (LFR) ($\leq$33% of the oocytes fertilized, which is equivalent to the 10th percentile of the variable distribution) or high fertilization rate (HFR) (>33% of the oocytes fertilized). Clinical pregnancy was diagnosed by a gestational sac with fetal heart beat at 7 weeks of gestation. The implantation rate was defined as the number of gestational sacs per embryo transferred.

Statistical analysis

All statistical analyses were performed using SAS version 9.1.3 (SAS Institute Cary, NC, USA). Results were reported as mean ± standard deviation for quantitative variables or number (percentage) for categorical variables. Student's *t*-test and chi-squared test were used to compare various outcome measures as appropriate between cases and controls.

Odds ratios (OR) were calculated for the fertilization and pregnancy rates. Adjusted OR and 95% confidence intervals (CI) for pregnancy or LFR were calculated for HBV infection, controlling for women's and men's ages, ovarian reserve, duration of ovarian stimulation, sperm motility and number of mature oocytes in a stepwise model selection. To account for the matched case-control design, the crude and adjusted OR and the respective 95% CI were calculated using conditional logistic regression with the SAS PHREG procedure based on the Cox proportional hazards model (Cox, 1975). A *P*-value less than 0.05 was considered as statistically significant.

This study was approved by the study centre's institutional review board (CEERB) number 09—046.

Results

Tables 1 and 2 summarize the baseline characteristics and the IVF procedures and outcomes in selected patients. There were no differences in the patients' characteristics (age, ovarian reserve, infertility factors and cycle ranks) between the two groups (**Table 1**). However, sperm motility before sperm selection was significantly reduced in HBV-positive patients (36.3 ± 11.6 versus 45.3 ± 14.4, $P = 0.003$).

There were no significant differences concerning the cycle characteristics. The overall fertilization rate was comparable between groups (55.1% versus 65.6%), but LFR was observed more frequently in the HBV group than in controls (34.4% versus 15.6%, $P = 0.036$) (**Table 2**). Total fertilization failure was observed in 9.4% and 4.7% of HBV and control cycles, respectively, a difference not statistically significant. The number of cycles without embryo transfer was similar between cases and controls (15.6% versus 10.9%). The reasons were complete fertilization failure ($n = 6$), poor embryo quality ($n = 4$), ovarian hyperstimulation syndrome ($n = 1$) and contamination of a culture well ($n = 1$). No differences were observed regarding the embryo quality and the number of frozen embryos.

Table 3 summarizes the outcomes of the IVF cycles in the two groups. Implantation rates (13.5% versus 20.0%) and clinical pregnancy rates per cycle (18.8% versus 31.3%) and per transfer (23.1% versus 35.1%) were comparable between the two groups.

Table 1 Clinical characteristics.

	HBV group (n = 32)	*Control group (n = 64)*
Woman's age (years)[a]	32.0 ± 5.2	32.0 ± 5.0
Man's age (years)[a]	34.7 ± 5.0	35.3 ± 6.3
Basal semen parameters		
Concentration ($\times 10^6$/ml)	90.3 ± 70.3	88.8 ± 81.6
Progressive motility (a + b)[b]	36.3 ± 11.6	45.3 ± 14.4
Normal morphology (%)	37.6 ± 14.9	35.8 ± 12.8
Vitality (%)	65.6 ± 10.1	68.9 ± 15.1
No. of progressive spermatozoa on the day of oocyte retrieval ($\times 10^6$)[a]	36.9 ± 33.4	38.6 ± 28.8
Infertility factor		
Female	11 (34.4)	37(57.8)
Male	6 (18.8)	4 (6.3)
Mixed	8 (25.0)	10 (15.6)
Unexplained	7 (21.9)	13 (20.3)
Day-3 FSH (IU/l)	6.2 ± 1.9	6.9 ± 1.7
Day-3 AMH (ng/ml)	4.9 ± 4.7	4.5 ± 5.9
First cycle rank[a]	28 (87.5)	51 (79.7)

Values are mean ± SD or *n* (%).
[a]Matched variables.
[b]Significantly different, $P = 0.003$. AMH-anti-Mullerian hormone. HBV = hepatitis B virus.

Table 2 Ovarian stimulation and IVF results.

	HBV group (n = 32)	*Control group (n = 64)*
Duration of ovarian stimulation (days)	10.5 ± 2.3	11.0 ± 1.7
Total dose of gonadotrophins administered (IU)	2051.2 ± 747.6	2333.0 ± 732.0
Peak oestradiol concentration (pg/ml)	2257.9 ± 1586.1	2359.2 ± 1065.3
No. of oocytes retrieved	10.3 ± 5.2	9.8 ± 4.1
Fertilization rate (%)	55.1 ± 29.9	65.6 ± 28.8
Cycles with low fertilization rate[a]	11 (34.4)	10 (15.6)
No. of embryos on day 2 or 3	4.9 ± 3.3	5.9 ± 3.2
Cycles without embryo transfer	5 (15.6)	7 (10.9)
No. of frozen embryos	1.4 ± 2.6	0.7 ± 1.3
No. of embryos transferred	1.8 ± 1.3	2.1 ± 1.6

Values are mean ± SD or *n* (%).
[a]Significantly different, $P = 0.036$. HBV = hepatitis B virus.

Table 3 Implantation and pregnancy rates.

	HBV group (n = 32)	*Control group (n = 64)*
Implantation rate (%)	13.5	20.0
Clinical pregnancy rate/cycle	6 (18.8)	20 (31.3)
Clinical pregnancy rate/transfer	6 (23.1)	20 (35.1)
Live birth rate/cycle	5 (15.6)	15 (23.4)

Values are *n* (%). There were no statistically significant differences between the two groups. HBV = hepatitis B virus.

Table 4 Relationship between low fertilization rate, pregnancy and hepatitis B virus infection in a conditional logistic regression model for matched-pairs data.

	Crude model		*Adjusted model*[a]	
	OR (95% CI)	*P-value*	*OR (95% CI)*	*P-value*
Low fertilization rate (%)	3.7 (1.12—12.12)	0.03	5.1 (1.03—96.23)	0.04
Clinical pregnancy rate/cycle (%)	0.47 (0.14—1.54)	NS	0.39 (0.10—1.53)	NS

[a]Adjusted for woman's age, number of mature oocytes and number of motile spermatozoa on the day of insemination. HBV = hepatitis B virus. NS = not statistically significant.

After adjustment for potential confounding factors, male HBV infection was still associated with an increased risk of LFR (adjusted OR = 5.1; 95% CI 1.03—96.23; **Table 4**).

Discussion

This study showed that HBV-infected men have spermatozoa with decreased motility before preparation of the semen. This anomaly was previously observed in a clinical study including 15 HBsAg-positive patients: two of them were azoospermic and 12 had a moderate but significantly higher asthenozoospermia than patients from the control group. This impaired motility was associated with increased necrosis or apoptosis of the spermatozoa (Moretti et al., 2008). In this study, the sperm vitality was comparable between groups and cannot explain the decreased motility. One hypothesis is that, as suggested by in-vivo observations, HBV binding to the glycoprotein receptor ASGP-R might reduce human sperm motility by the loss of sperm mitochondrial membrane potential (Zhou et al., 2009). Zhou et al. (2009) suggested that the higher the viral load is, the greater the sperm motility impairment. But even if the risk of detecting some virus in the semen is increased when the plasma viral load is high (Jenison et al., 1987), it is also possible to detect the presence of virus in semen in the absence of an important viral replication in the plasma (Davison et al., 1987). No correlation was found between in-vivo plasma viral load and sperm motility before selection (data not shown), which is being currently re-evaluated according to the dosage of the seminal and plasma viral loads on the day of IVF.

This study also demonstrates that couples, in which male partners have a chronic infection with HBV, have a significantly higher risk of a LFR after IVF, which led to a slight decrease in the total number of embryos. As far as is known, this is the first clinical study that shows the adverse effect of male HBV infection on fertilization, thus confirming previous results obtained *in vivo* (Zhou et al., 2009). Nevertheless, no significant decrease in the pregnancy rates was observed, probably because the LFR did not significantly reduce the number of good-quality embryos available for transfer.

Pirwany et al. (2004) studied IVF or intracytoplasmic sperm injection (ICSI) outcomes in HBsAg-positive men and found no difference in fertilization and cleavage rates between cases and controls: 13 hepatitis B-infected couples (including males and females) were matched with 12 hepatitis C-positive couples and 27 controls. In a larger comparative study (102 HBV couples in which only the male partner was infected versus 204 control couples), Zhao et al. (2007) reported that chronic HBV infection did not affect the outcome of IVF treatment. More recently, Lam et al. (2010) found an improvement of pregnancy and implantation rates after IVF or ICSI when the female partner had HBV, but not when the male partner (n = 14) was infected.

If the available data on some of the in-vivo effects of HBV on sperm function seem to be clear (Zhou et al., 2009), those on the consequences of HBV infection on the outcome of IVF still need to be clarified. It is known that HBV virus can be free in the seminal fluid but can also integrate into the genome of germ and somatic cells and then be mutagenic (Davison et al., 1987; Hadchouel et al., 1985; Huang et al., 2003; Lang, 1993). But this virus is not directly responsible for cell lesions; it induces an immune response that is presumed to be the main mechanism causing both liver damage and virus control (Liaw and Chu, 2009). Moreover, some viruses were also involved in the occurrence of premature chromosome condensation (Aula, 1973). The LFR observed in the current study after IVF cannot be explained by the decreased motility of unselected spermatozoa, the two groups being matched on the progressive motility of the selected spermatozoa on the day of oocyte retrieval. The potential role of other bacteria or leukocytes was also excluded, all bacterial analysis being controlled as negative before ovarian stimulation. A persistent inflammatory response induced by HBV may be deleterious to spermatozoa as well as the direct role of this virus on sperm nuclear decondensation; these two hypotheses have to be explored.

Most of the present study's patients came from Africa where genotype E is predominant, whereas those included in previous studies were Asian, and likely infected by genotypes B or C (Liaw and Chu, 2009). These differences could also explain this work's results. Further studies are required to confirm or exclude this genotype effect hypothesis.

Even if HBV integration into the genome is possible, no case of vertical transmission after ICSI has yet been reported. One publication reported vertical transmission of the HBV from Asian fathers to their children after natural conception (Wang et al., 2003). However, it is known that the prevalence of hepatitis B e antigen (HBeAg) is higher in Asian carriers, HBeAg-positive patients having usually high viral load (Liaw and Chu, 2009).

In conclusion, this study confirms that spermatozoa of men infected by HBV have a decreased progressive motility and shows that there is a higher risk of LFR after IVF. It seems adequate to propose to these couples, for the first

stimulation cycle, a mixed technique (half ICSI, half IVF). The effect of HBV on in-vitro development to blastocyst stage and on pregnancy rates, and the link between abnormal sperm parameters in HBV-infected men and the genotype and/or the viral load in blood and in semen, are currently under investigation.

References

Ali, B.A., Huang, T.H., Salem, H.H., Xie, Q.D., 2006. Expression of hepatitis B virus genes in early embryonic cells originated from hamster ova and human spermatozoa transfected with the complete viral genome. Asian Journal of Andrology 8 (3), 273–279.

Auger, J., Eustache, F., Andersen, A.G., Irvine, D.S., Jørgensen, N., Skakkebæk, N.E., Suominen, J., Toppari, J., Vierula, M., Jouannet, P., 2001. Sperm morphological defects related to environment, lifestyle and medical history of 1001 male partners of pregnant women from four European cities. Human Reproduction 16, 2710–2717.

Aula, P., 1973. premature chromosome condensation (PCC) in single cells and G-bands of PCC-chromatin. Hereditas 74 (1), 81–87.

Collodel, G., Baccetti, B., Capitani, S., Moretti, E., 2005. Necrosis in human spermatozoa I. Ultrastructural features and FISH study in semen from patients with uro-genital infections. Journal of Submicroscopic Cytology and Pathology 37 (1), 67–73.

Cox, D.R., 1975. Partial likelihood. Biometrika 62, 269–276.

Davison, F., Alexander, G.J., Trowbridge, R., Fagan, E.A., Williams, R., 1987. Detection of hepatitis B virus DNA in spermatozoa, urine, saliva and leucocytes, of chronic HBsAg carriers. A lack of relationship with serum markers of replication. Journal of Hepatology 4 (1), 37–44.

Durazzo, M., Premoli, A., Di Bisceglie, C., Bertagna, A., Faga, E., Biroli, G., Manieri, C., Bo, S., Pagano, G., 2006. Alterations of seminal and hormonal parameters: An extrahepatic manifestation of HCV infection? World Journal of Gastroenterology 12 (19), 3073–3076.

Figura, N., Piomboni, P., Ponzetto, A., Gambera, L., Lenzi, C., Vaira, D., Peris, C., Lotano, M.R., Gennari, L., Bianciardi, L., Renieri, T., Valensin, P.E., Capitani, S., Moretti, E., Colapinto, R., Baccetti, B., Gennari, C., 2002. Helicobacter pylori infection and infertility. European Journal of Gastroenterology and Hepatology 14 (6), 663–669.

Gardner, D.K., Lane, M., Stevens, J., Schlenker, T., Schoolcraft, W.B., 2000. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertility and Sterility 73 (6), 1155–1158.

Hadchouel, M., Scotto, J., Huret, J.L., Molinie, C., Villa, E., Degos, F., Brechot, C., 1985. Presence of HBV DNA in spermatozoa: a possible vertical transmission of HBV via the germ line. Journal of Medical Virology 16 (1), 61–66.

Heathcote, J., Cameron, C.H., Dane, D.S., 1974. Hepatitis-B antigen in saliva and semen. Lancet 1 (7847), 71–73.

Honeck, P., Weigel, M., Kwon, S.T., Alken, P., Bross, S., 2006. Assisted procreation in cases of hepatitis B, hepatitis C or human immunodeficiency virus infection of the male partner. Human Reproduction 21 (5), 1117–1121.

Huang, J.M., Huang, T.H., Qiu, H.Y., Fang, X.W., Zhuang, T.G., Liu, H.X., Wang, Y.H., Deng, L.Z., Qiu, J.W., 2003. Effects of hepatitis B virus infection on human sperm chromosomes. World Journal of Gastroenterology 9 (4), 736–740.

Huret, J.L., Jeulin, C., Hadchouel, M., Scotto, J., Molinie, C., 1986. Semen abnormalities in patients with viral hepatitis B. Archives of Andrology 17 (1), 99–100.

Jenison, S.A., Lemon, S.M., Baker, L.N., Newbold, J.E., 1987. Quantitative analysis of hepatitis B virus DNA in saliva and semen of chronically infected homosexual men. The Journal of Infectious Diseases 156 (2), 299–307.

Lam, P.O., Suen, S.H.M.R.C.O.G., Lao, T.T., Cheung, L.P., Leung, T.Y., Haines, C., 2010. Hepatitis B infection and outcomes of *in vitro* fertilization and embryo transfer treatment. Fertility Sterility 93 (2), 480–485.

Lang, Z.W., 1993. Distribution of hepatitis B virus in testicle tissue in patients with hepatitis B infection. Zhonghua Yi Xue Za Zhi 73 (6), 329-331–379.

Liaw, Y.F., Chu, C.M., 2009. Hepatitis B virus infection. Lancet 373 (9663), 582–592.

Moretti, E., Federico, M.G., Giannerini, V., Collodel, G., 2008. Sperm ultrastructure and meiotic segregation in a group of patients with chronic hepatitis B and C. Andrologia 40 (3), 173–178.

Noci, I., Fuzzi, B., Rizzo, R., Melchiorri, L., Criscuoli, L., Dabizzi, S., Biagiotti, R., Pellegrini, S., Menicucci, A., Baricordi, O.R., 2005. Embryonic soluble HLA-G as a marker of developmental potential in embryos. Human Reproduction 20 (1), 138–146.

Pirwany, I.R., Phillips, S., Kelly, S., Buckett, W., Tan, S.L., 2004. Reproductive performance of couples discordant for hepatitis B and C following IVF treatment. Journal of Assisted Reproduction and Genetics 21 (5), 157–161.

Scott, R.M., Snitbhan, R., Bancroft, W.H., Alter, H.J., Tingpalapong, M., 1980. Experimental transmission of hepatitis B virus by semen and saliva. The Journal of Infectious Diseases 142 (1), 67–71.

Vicari, E., Arcoria, D., Di Mauro, C., Noto, R., Noto, Z., La Vignera, S., 2006. Sperm output in patients with primary infertility and hepatitis B or C virus; negative influence of HBV infection during concomitant varicocele. Minerva Medica 97 (1), 65–77.

Wang, S., Peng, G., Li, M., Xiao, H., Jiang, P., Zeng, N., Wang, Z., 2003. Identification of hepatitis B virus vertical transmission from father to fetus by direct sequencing. Southeast Asian Journal of Tropical Medecine and Public Health 34 (1), 106–113.

World Health Organization (1992) WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. Cambridge University Press, Cambridge, UK.

Zhao, E.Y., Chen, S.L., Sun, L., Yin, M.N., Xiong, X.S., Song, J., et al., 2007. Influence of chronic HBV infection in the husband on the outcome of IVF-ET treatment. Nan Fang Yi Ke Da Xue Xue Bao 27 (12), 1827–1829.

Zhou, X.L., Sun, P.N., Huang, T.H., Xie, Q.D., Kang, X.J., Liu, L.M., 2009. Effects of hepatitis B virus S protein on human sperm function. Human Reproduction 24 (7), 1575–1583.

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