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Original Research

Hepatitis B Infection Negatively Affects the Outcome of Fresh IVF/ICSI Cycles

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Abstract

Background: The study sought to determine whether hepatitis B infection in females and males plays a role in the outcome of the IVF/ICSI cycle. **Methods**: We performed a retrospective analysis of data collected from IVF/ICSI cycles carried out from January 2011 to December 2019 at the University Medical Centre Ljubljana. The data was analyzed using Pearson's chi-square test, Fisher's exact test, and Kruskal-Wallis test in order to determine the differences between the groups. **Results**: This study included 117 cycles with a past female hepatitis B infection (HF group), 91 cycles with couples with a past male hepatitis B infection (HM group), and 10,216 cycles with no past male or female hepatitis B infection (control group). There was no difference in sperm concentration, but total sperm motility was significantly higher in the HF and control groups compared to the HM group ($p = 0.008$ and $p < 0.001$). Also, sperm morphology was significantly higher in the control group, compared to both, the HM group ($p < 0.001$) and the HF group ($p = 0.004$). Furthermore, the rate of fertilized oocytes per number of retrieved oocytes was significantly higher in the HF ($p < 0.001$) and control groups ($p = 0.003$) compared to the HM group, but on the contrary, the rate of immature oocytes was lower in the HM ($p = 0.009$) and control groups ($p = 0.001$) compared to the HF group. The number of obtained embryos, blastocyst, and embryo utilization rates were similar between the groups, although the rate of cryopreserved embryos was higher in the HF ($p = 0.007$) and the HM groups ($p = 0.027$) compared to the control group. No significant difference was observed in the pregnancy and live birth rate per embryo transfer, while a trend towards a lower birth rate per aspiration was observed in the HM and in HF groups, which can be explained with a significantly higher miscarriage rate observed in the HM ($p < 0.001$) and HF groups ($p = 0.042$) compared to the control group. Gestational age was similar for all groups, although we observed a strong trend towards a higher birth weight of singletons in the HF group ($p = 0.043$) compared to the control group. **Conclusions**: The results indicate that hepatitis B infection could have a detrimental influence on sperm motility and morphology and cycle outcome, especially in terms of a higher miscarriage rate.

Keywords: *in vitro* fertilization; hepatitis B; pregnancy; miscarriage

1. Introduction

Hepatitis B virus (HBV) infection is a health problem prevalent in Southern Europe, Africa, Asia, and Latin America. It is a viral infection that affects the liver and can manifest in both acute and chronic disease. Chronic hepatitis in female patients is manifested as fatigue, weakness, irritability, dizziness; less frequent symptoms are nausea and vomiting. One of the possible signs of infection isendocrinopathy, which probably explains why women experience irregular menstrual cycles with heavy menstruations that were then replaced with short infrequent menstrual cycles, or even with the absence of a menstrual cycle. There seems to be a tendency toward changes in reproductive organs such as ovarian cysts and hysteromioma, diffuse mastopathy, an adhesive process in the lower pelvis, and even endometriosis [1]. So these changes alone, during the infectious phase of HBV, raise questions if this could present any long-term consequences to fertility. Since we know that HBV has also been found in ovaries [2], where it can be present in the ovum and granulosa cells [3,4], and testes [5], the answer is affirmative. It was revealed that HBV in semen can impair sperm concentration, morphology, viability, and motility [6,7]. It can integrate into human spermatozoa chromosomes, where it increases chromosomal instability, and can be vertically transmitted to its offspring [8,9]. Furthermore, it has been shown that abnormal IL-17 and IL-18 expression, induced by oxidative stress during HBV infection can be causing male infertility [10].

Infertility is defined as a couple trying to unsuccessfully conceive for at least a year. When all other treatment options are exhausted and the couple is planned to undergo *in vitro* fertilization (IVF), they are tested for sexually transmitted diseases, including HBV. If one or even both partners are HBV seropositive, there are several pretreatment recommendations. Firstly, female partners are recommended to get vaccinated if the male partner is infected with HBV, and the IVF is initiated after she develops immunity; secondly, they should undergo IVF treatment when the viral load is low [11]. Despite following all these recommendations, it is not clear whether such infection plays a role on the outcome of the IVF or ICSI (intracytoplasmic sperm injection) cycle. Some studies have

indicated that such condition does not significantly influence the outcome of the IVF/ICSI cycle [12–14], while on the other hand, some studies have shown that these patients could have significantly impaired outcomes [15,16]. The reason for such differences in outcomes may be in a low number of included patients in some studies, which were mostly single institutional. For example, the study by Cito *et al*. [12] had 134 infertile couples included, where 66 couples had HBsAg-seropositive men and 68 were controls; the study by Bu *et al*. [13] had 277 couples, where 20 men were HBC seropositive; the study by Lubis *et al*. [15] had 101 included patients, with 17 males in the HBV group and 84 in the control group. On the other hand, studies by Wang *et al*. [14] and Lin *et al*. [16] had a higher number of included patients. To be exact, there were 681 couples included in the study by Wang *et al*. [14], where there were 227 couples with male HBV and 504 included patients in the study by Lin *et al*. [16] with 199 seronegative, and 305 seropositive. While this topic is still open to questions and without final conclusions, we can offer a data analysis with a higher number of included patients compared to most aforementioned studies We carried out a retrospective analysis of data collected in the last ten years from IVF/ICSI cycles performed at our IVF centre. We compared the outcomes of cycles of the control group, with outcomes where women or men previously had a hepatitis B infection in terms of sperm quality, oocytes, embryos, pregnancy and live birth rate, and birthweight.

2. Materials and Methods

We performed a retrospective analysis of IVF/ICSI cycles of couples where females (HF group- 117 cycles) or males (HM group- 91 cycles) had serological reactivity to HBsAg or anti-HBc and compared the data to the control group (10,216 cycles) where both partners were seronegative for HBV. All three groups included patients treated at the clinic in the time period from January 2011 to December 2019 that underwent controlled ovarian stimulation protocols and had fresh semen sample used for oocyte fertilization. What is more, this analysis excluded couples who had spontaneous cycles, used vitrified/warmed oocytes, used cryopreserved ejaculated semen, used TESE (testicular sperm extraction) derived spermatozoa for fertilization, and who underwent PGT (preimplantation genetic testing).

Ovarian stimulation protocols and laboratory protocols were performed as described in details previously [17]. Data were analyzed to determine the differences between the groups using statistical program IBM SPSS Statistics version 21 (IBM, Armonk, NY, 10504-1722, USA).

The differences in percentages between the groups were analysed using Pearson's chi-square test and Fisher's exact test, while other non-parametric data were analysed using Kruskal-Wallis test (nonparametric one-way ANOVA on ranks). The normality of data was analysed using the Shapiro-Wilk test. A $p < 0.05$ value was considered statistically significant.

According to Slovene legislation the study did not have to be approved by the Slovenian National Medical Ethics Committee, as it was a register-based study where all participants signed individual personal approval and permission before undergoing the treatment (Personal Data Protection Act, Official Gazette of the Republic of Slovenia No 94/07, 2004). Additionally, as per Slovenian legislation, healthcare providers are obligated to collect the data about assisted reproduction procedures and monitor the success rates (Healthcare Databases Act, Official Gazette of the Republic of Slovenia No 65/00, 2000; No 47/15, 2015; 31/18, 2018).

3. Results

Sperm quality characteristics are presented in Table 1, general information on the cycles performed in Table 2, and the outcome of cycles in Table 3.

There was no difference in sperm concentration, but total sperm motility and sperm morphology were statistically significantly different when comparing all groups together (HF group *vs.* HM group *vs.* control group; 56.5 $\pm$ 24.6 *vs.* 44.9 $\pm$ 28.5 *vs.* 56.6 $\pm$ 24.6, $p < 0.001$; 12.8 $\pm$ 10.8 *vs.* 11.5 $\pm$ 11.8 *vs.* 17.1 $\pm$ 13.3, $p < 0.001$ respectively). A post hoc analysis of the differences between individual groups revealed that the total sperm motility was significantly higher in the HF and control group compared to the HM group ($p = 0.008$ and $p < 0.001$). The difference in morphology arose from the significantly higher sperm morphology in the control group, compared to both, the HM group ($p < 0.001$) and the HF group ($p = 0.004$).

The mean age of women in the HF, HM, and control group was similar, as was the mean number of retrieved oocytes per cycle (Table 2).

Significant difference was observed in the rate of fertilized oocytes per number of retrieved oocytes (54.6% *vs.* 46.4% *vs.* 51.9%, per number of retrieved oocytes respectively, $p = 0.003$), rate of immature oocytes (12.5% *vs.* 17.0% *vs.* 16.4%, respectively $p = 0.004$), rate of degenerated oocytes per number of retrieved oocytes (11.2% *vs.* 12.8% *vs.* 9.2%, respectively, $p < 0.001$), and rate of cryopreserved embryos (27.7% *vs.* 27.8% *vs* 22.7%, respectively, $p = 0.002$), between the HF, HM, and control group respectively.

The post hoc analysis revealed that there was a statistically different rate of fertilized oocytes (per number of retrieved oocytes) (54.6% *vs.* 46.4%, $p < 0.001$), and immature oocytes (12.5% *vs.* 17.0%, $p = 0.009$) between the first two groups, HF and HM, respectively. When comparing the HM and control group we observed a statistically lower rate of fertilized oocytes (per number of retrieved oocytes) (46.4% *vs.* 51.9%, $p = 0.003$), but a statistically higher rate of degenerated oocytes (% per number of retrieved oocytes) (12.8% *vs.* 9.2%, $p = 0.001$) and rate of cryopreserved

Table 1. The results of basic sperm quality assessment of samples included into study. Statistically significant differences are marked with an asterisk (p value < 0.05).

	Hepatitis female group (HF)	Hepatitis male group (HM)	Control group (CG)	p value
Sperm concentration ($\times 10^6$/mL $\pm$ SD)	60.1 $\pm$ 37.5	53.5 $\pm$ 38.9	61.3 $\pm$ 42.2	0.309
Total motility (% $\pm$ SD)	56.5 $\pm$ 24.6	44.9 $\pm$ 28.5	56.6 $\pm$ 24.6	<0.001* (HF *vs.* HM = 0.008*; HM *vs.* CG <0.001*; HF *vs.* CG = 1.000)
Sperm morphology (% $\pm$ SD)	12.8 $\pm$ 10.8	11.5 $\pm$ 11.8	17.1 $\pm$ 13.3	<0.001* (HF *vs.* HM = 1.000; HM *vs.* CG = 0.000*; HF *vs.* CG = 0.004*)

SD, standard deviation; *, statistically significant difference.

Table 2. The outcome of the *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles in terms of oocytes and embryos. Statistically significant differences are marked with an asterisk (p value < 0.05).

	Hepatitis female group(HF)	Hepatitis male group (HM)	Control group (CG)	p value
Number of cycles	117	91	10,216	
Number of couples	56	40	5398	
Female mean age ($\pm$ SD)	33.8 $\pm$ 4.1	34.4 $\pm$ 4.3	34.8 $\pm$ 4.6	0.136
Number of retrieved oocytes (mean number per cycle $\pm$ SD)	991 (8.5 $\pm$ 6.4)	741 (8.1 $\pm$ 6.1)	83,017 (8.1 $\pm$ 6.2)	0.715
Rate of fertilized oocytes (per number of retrieved oocytes)	54.6%	46.4%	51.9%	0.003* (HF *vs.* HM <0.001; HM *vs.* CG = 0.003; HF *vs.* CG = 0.089)
Number and rate of immature oocytes (%)	124 (12.5%)	126 (17.0%)	13,610 (16.4%)	0.004* (HF *vs.* HM = 0.009; HM *vs.* CG = 0.655; HF *vs.* CG = 0.001)
Number and rate of degenerated oocytes (% per number of retrieved oocytes)	111 (11.2%)	95 (12.8%)	7669 (9.2%)	<0.001* (HF *vs.* HM = 0.303; HM *vs.* CG = 0.001; HF *vs.* CG = 0.034)
Number and rate of polyploidies (% per number of retrieved oocytes)	39 (3.9%)	22 (3.0%)	3791 (4.6%)	0.075
Number of normal zygotes	541	344	43,069	
Number and proportion of embryos (% per zygotes)	527 (97.4%)	338 (98.3%)	41,885 (97.3%)	0.511
Mean number of embryos per cycle (mean number $\pm$ SD)	4.5 $\pm$ 4.3	3.7 $\pm$ 3.4	4.1 $\pm$ 3.6	0.291
Number of embryos cultured until day 5/6	492	300	33,369	
Number and rate of blastocysts (% per embryos cultured until day 5/6)	202 (41.1%)	129 (43.0%)	15,279 (45.8%)	0.072
Number and rate of embryo utilization (transferred plus frozen embryos)	281 (53.3%)	193 (57.1%)	22,260 (53.1%)	0.348
Number of cycles with at least one blastocyst	65 (55.6%)	42 (46.2%)	5403 (52.9%)	0.370
Number and rate of cryopreserved embryos (% of all embryos)	146 (27.7%)	94 (27.8%)	9527 (22.7%)	0.002* (HF *vs.* HM = 0.973; HM *vs.* CG = 0.027; HF *vs.* CG = 0.007)
Number and proportion of cycles with embryo cryopreservation	44 (37.6%)	27 (29.7%)	3065 (30.0%)	0.203
Number and proportion of cycles with freezing without ET	7 (6.0%)	8 (8.8%)	427 (4.2%)	0.061
Number and proportion of cycles without freezing/without ET	16 (13.7%)	14 (15.4%)	1310 (12.8%)	0.741

SD, standard deviation; ET, embryo transfer; *, statistically significant difference.

Table 3. Clinical outcome of IVF/ICSI cycles. Statistically significant differences are marked with an asterisk (p value < 0.05).

	Hepatitis female group (HF)	Hepatitis male group (HM)	Control group (CG)	p-values
Number of all fresh ETs	94	69	8463	
Mean number of transferred embryos (± SD)	1.4 ± 0.5	1.4 ± 0.5	1.5 ± 0.5	0.221
Number of pregnancies (% per ET)	32 (34.0%)	23 (33.3%)	2768 (32.7%)	0.957
Number of pregnancies (% per oocyte aspiration)	27.4%	25.3%	27.1%	0.925
Live births (% per ET)	19 (20.2%)	10 (14.5%)	2001 (23.6%)	0.152
Live births (% per aspiration)	16.2%	11.0%	19.6%	0.080
Miscarriages	12 (+ 1EU) (37.5%)	13 (56.5%)	690 (24.9%)	<0.001* (HF *vs.* HM = 0.244; HM *vs.* CG <0.001*; HF *vs.* CG = 0.042*)
Gestational age (all births)	38.8 ± 4.1	37.8 ± 4.3	38.4 ± 2.7	0.330
Gestational age for singletons	38.8 ± 4.1	39.7 ± 1.5	38.8 ± 2.4	0.395
Birth weight of singletons (g)	3592.4 ± 511.4	3300.7 ± 373.9	3220 ± 639.2	0.050* (HF *vs.* HM = 0.700; HM *vs.* CG = 1.000; HF *vs.* CG = 0.043*)
Twins	0	3 (30.0%)	232 (11.6%)	0.126

SD, standard deviation; ET, embryo transfer; *, statistically significant difference.

embryos (22.7% *vs.* 27.8%, $p = 0.027$) in the HM group. When comparing the HF group to the control we discovered a statistically higher rate of immature oocytes (%) (16.4% *vs.* 12.5%, $p = 0.001$), and a significantly lower rate of cryopreserved embryos (27.7% *vs.* 22.7%, $p = 0.007$) in the control group.

When comparing HF, HM, and control groups together for ETs, pregnancies, live births, miscarriages, gestational age, birth weight, and the number of twins, we have observed that there was a statistically significant difference in miscarriages and birth weight of singletons. There was a statistically higher rate of miscarriages in the HM group compared to the control group (56.5% *vs.* 24.9%, $p < 0.001$), as was for the HF group compared to the control (37.5% *vs.* 24.9%, $p = 0.042$). The birth weight of singletons (g) was statistically higher in the HF group compared to the control group (3592.4 $\pm$ 511.4 *vs.* 3220 $\pm$ 639.2, $p = 0.043$).

4. Discussion

In recent years we treated many infertile couples that were infected with HBV at some point in their lives before undergoing IVF treatment. Due to safety precautions, only couples with cleared HBV infection can be included in such treatment. Despite having recovered, the data from the literature suggests that the infection could leave long-term consequences, which can manifest in impaired fertility or can impact the outcome of IVF/ICSI cycles, and even neonatal outcomes. Such negative effect was confirmed with our analysis. Our data show that HBV seropositive males have lower total motility and lower sperm morphology compared to the HF and control groups, although on average, motility and sperm morphology were still in the range of normal values according to the WHO (World Health Organization) manual [18]. Additionally, there was a higher rate of miscarriages in the HM and HF groups compared to controls. When we compared the birth weight of singletons we discovered that the birth weight of singletons in the HF group is much higher compared to the HM and control groups, the lowest being in the control group.

Similar to our findings, Karamolahi *et al.* [7] found that HBV-infected men had decreased sperm morphology (3.23% $\pm$ 3.27% *vs.* 4.51% $\pm$ 3.15%), lower sperm count ($100.95 \times 10^6 \pm 118.59 \times 10^6$ *vs.* $166.27 \times 10^6 \pm 151.25 \times 10^6$), and progressive sperm motility (30.97% $\pm$ 25.88% *vs.* 40.87% $\pm$ 23.37%) compared to the control group. In addition to these impaired sperm quality parameters, it was suggested that HBV infection also negatively affects semen volume (3.02 mL $\pm$ 1.07 mL *vs.* 2.61 mL $\pm$ 1.04 mL; infertile male patients without hepatitis B virus infection *vs.* infertile male patients with hepatitis B virus infection) and increased seminal malondialdehyde concentration [10]. Furthermore, Han *et al.* [19] indicated that sperm chromatin structure fragmented when exposed to the HBV surface protein (HBs), sperm viability lowered, and sperm fertilizing capacity declined with increasing concentrations of HBs. When determining the status of sperm membrane integrity when exposed to HBs, Kang *et al.* [20] concluded that there was a rise in reactive oxygen species (ROS), lipid peroxidation, activation of caspases, and DNA fragmentation, which translated to a loss of integrity in sperm membrane, increased apoptosis, and sperm dysfunction. This could explain our observation of a lower fertilization rate in the HM group. A similar effect was previously observed by Lubis *et al.* [15], who had 17 couples in a study group of patients comparable to ours, even though we included 91 couples, and also by Shi *et al.* [21] who included 136 couples, and Zhou *et al.* [22], who included 457 couples for comparison. Nevertheless, some caution is needed in the interpretation of the results, because the study by Lubis *et al.* [15] indicates that the number of fertilized oocytes was lower in the male HBV seropositive group (5.58 $\pm$ 3.58 *vs.* 7.85 $\pm$ 3.97), however, the rate of fertilization was similar as in the control group (74.54 $\pm$ 24.79 *vs.* 76.69 $\pm$ 17.21). Some studies had a similar number of included patients compared to ours and did not show any negative effect on fertilization rates from this perspective [12,14]. A negative effect on the outcome of IVF/ICSI cycles was to some extent observed in the aforementioned studies observing impaired fertilization [15,21,22]. It was found that the implantation rate (OR: 0.57, 95% CI: 0.48–0.99, $p = 0.044$) [22] and clinical pregnancy rates ((OR: 0.66, 95% CI: 0.45–0.95, $p = 0.036$) [22], (23.52% *vs.* 51%; $p = 0.037$) [15]) can be significantly lower in a group of couples with hepatitis seropositive males. On the contrary, despite impaired fertilization (80.2% *vs.* 82.8%, $p < 0.05$), Shi *et al.* [21] did not observe a negative impact on the clinical pregnancy rate (58.1% *vs.* 53.7%; HBV male seropositive vs. negative matched control), although based on their other results they suggested that such infection is an important risk factor for infertility. No differences in implantation rates, clinical pregnancy rate, miscarriage rate, or live birth rates were observed in some other studies [12–14,23], even though an impaired quality of semen was observed in some studies (a trend toward significantly lower progressive motility (35.0% *vs.* 55.0%; $p < 0.05$) in male seropositive group [12], significantly lower sperm viability (74.1 $\pm$ 13.7 *vs.* 77.0 $\pm$ 12.8, $p < 0.01$), and significantly decreased sperm motility in HBV positive men in comparison to the control group (32.5 $\pm$ 14.6 *vs.* 35.5 $\pm$ 12.9, $p < 0.05$) [14], significantly reduced sperm motility in the male HBV group (36.3 $\pm$ 11.6% *vs.* 45.3 $\pm$ 14.4%, $p = 0.003$) [23]).

Furthermore, male hepatitis virus B serostatus was not correlated with gestational age at delivery, whereas female hepatitis B virus serostatus was [16], since it decreased gestational age at delivery. Nevertheless, similarly to observed results in studies focusing mainly on hepatitis B seropositive males, conflicting results were also presented in studies with only hepatitis B seropositive females. To illustrate, it was suggested that IVF/ICSI cycle outcomes in terms of the

oocyte, embryos, implantation rates, and live births rates are similar between female seropositive and seronegative groups [24–26]. On the contrary, it was observed that the implantation rate (35.7% *vs.* 38.7%; $p = 0.013$) is significantly lower in the female HBV seropositive group, although this does not lead to a difference in clinical pregnancy rate, miscarriage rate, live birth rate, neonatal outcomes, and pregnancy complications [27]. Interestingly, one study suggested that the pregnancy rate (53.3% *vs.* 24.2% per cycle with embryo transfer) and live birth rate (43.3% *vs*. 18.4%) is even higher in the HBV seropositive group as in controls [28]. According to our data, there is no difference in the pregnancy rate, live birth rate, and gestational age at delivery. However, we have observed a higher miscarriage rate (37.5% *vs.* 24.9%, $p = 0.042$), and different neonatal outcomes in terms of singleton birth weight, which was higher in the female HBV seropositive group (3592.4 g $\pm$ 511.4 g *vs.* 3220 g $\pm$ 639.2 g; $p = 0.043$) compared to controls. In cases of higher miscarriage rate (56.5%) in the male HBV seropositive group, it can be suggested that this is due to impaired semen quality, while there is no obvious reason for a higher miscarriage rate in the female HBV seropositive group. Observed vertical transmission of HBV from the ovum to the embryo could explain the phenomenon [29–31], or from sperm to embryo [30,31], however, this is just an assumption. Interestingly, the rate of HBV-positive embryos seems to be similar regardless of whether female, male, or both parents are HBV seropositive. In patients with high serum levels of HBV DNA the rate of HBV-positive oocytes and embryos seems to be significantly higher and the same is true for oocytes derived from women, whose mothers were also HBV positive [31]. The higher rate of HBV positive oocytes in patients with higher serum levels of HBV DNA go hand in hand with the suggestion that viral replication in follicles is stimulated during IVF/ICSI cycle and in these cases the levels of HBV DNA can be even higher in follicular fluid than in serum from the same patients [32]. All these data indicate that current approaches in preventing vertical transmission of HBV should be more effective.

5. Conclusions

The results of this retrospective analysis do not suggest that IVF/ICSI cycle treatment for patients infected with HBV should be adjusted. It has shown that HBV infection can have a negative effect on the quality of semen and fertilization rate, but it does not negatively affect the blastocyst rate, pregnancy, and live birth rate which is the main goal of an IVF treatment. However, results have shown an increased miscarriage rate in both, male and female HBV seropositive groups. However, we should still use sperm-washing techniques and use closed-system vitrification devices to prevent cryopreserved material from coming into direct contact with liquid nitrogen.

Author Contributions

PP, TF, and MS designed the study, PP and TF collected and analyzed the data, PP and MS wrote the manuscript, NJ and EVB advised in interpreting the results and edited the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. MS is serving as one of the Editorial Board members and Guest editors of this journal. We declare that MS had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to CHCK.

References

[1] Kurmanova AM, Kurmanova GM, Lokshin VN. Reproductive dysfunctions in viral hepatitis. Gynecological Endocrinology. 2016; 32: 37–40.

[2] Kong Y, Ye F, Jin Y, Shi J, Qiu H, Lin S. Hepatitis b virus expression and replication in ovum and the influencing factors. Saudi Journal of Gastroenterology. 2016; 22: 215–219.

[3] Ye F, Yue Y, Li S, Chen T, Bai G, Liu M, *et al*. Presence of HBsAg, HBcAg, and HBVDNA in ovary and ovum of the patients with chronic hepatitis B virus infection. American Journal of Obstetrics and Gynecology. 2006; 194: 387–392.

[4] Jin Y, Ye F, Shi J, Qiu H, Zhao Y, Lin S, *et al*. Hepatitis B virus infection and replication in primary cultured human granulosa cells. Archives of Virology. 2011; 156: 1–7.

[5] Liu W, Han R, Wu H, Han D. Viral threat to male fertility. Andrologia. 2018; 50: e13140.

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

[7] Karamolahi S, Yazdi RS, Zangeneh M, Makiani MJ, Farhoodi B, Gilani MAS. Impact of hepatitis B virus and hepatitis C virus infection on sperm parameters of infertile men. International Journal of Reproductive Biomedicine. 2019; 17: 551–556.

[8] Huang J, Huang T, Qiu H, Fang X, Zhuang T, Liu H, *et al*. Effects of hepatitis B virus infection on human sperm chromosomes. World Journal of Gastroenterology. 2003; 9: 736–740.

[9] Ali BA, Huang T, Salem H, Xie Q. 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. 2006; 8: 273–279.

[10] Qian L, Li Q, Li H. Effect of hepatitis B virus infection on sperm quality and oxidative stress state of the semen of infertile males. American Journal of Reproductive Immunology. 2016; 76: 183–185.
[11] Mocanu E, Drakeley A, Kupka MS, Lara-Molina EE, Le Clef N, Ombelet W, *et al*. ESHRE guideline: medically assisted reproduction in patients with a viral infection/disease. Human Reproduction Open. 2021; 2021: hoab037.
[12] Cito G, Coccia ME, Fucci R, Picone R, Cocci A, Sessa M, *et al*. Hepatitis B Surface Antigen Seropositive Men in Serodiscordant Couples: Effects on the Assisted Reproductive Outcomes. The World Journal of Men's Health. 2021; 39: 99.
[13] Bu Z, Kong H, Li J, Wang F, Guo Y, Su Y, *et al*. Effect of male hepatitis B virus infection on outcomes of *in vitro* fertilization and embryo transfer treatment: insights from couples undergoing oocyte donation. International Journal of Clinical and Experimental Medicine. 2014; 7: 1860–1866.
[14] Wang Z, Liu W, Zhang M, Wang M, Wu H, Lu M. Effect of Hepatitis B Virus Infection on Sperm Quality and Outcomes of Assisted Reproductive Techniques in Infertile Males. Frontiers in Medicine. 2021; 8:744350.
[15] Lubis HP, Halim B, Adenin I, Rusda M, Prasetiawan E. Hepatitis B virus infection on male partner has negative impact on in-vitro fertilization. IOP Conference Series: Earth and Environmental Science. 2018; 125: 012045.
[16] Lin S, Li R, Zheng X, Wang L, Ren X, Chen L, *et al*. Impact of hepatitis B virus carrier serostatus on neonatal outcomes after IVF-ET. International Journal of Clinical and Experimental Medicine. 2015; 8: 6206–6211.
[17] Stimpfel M, Jancar N, Vrtacnik-Bokal E. Collecting semen samples at home for IVF/ICSI does not negatively affect the outcome of the fresh cycle. Reproductive BioMedicine Online. 2021; 42: 391–399.
[18] Organization WH. WHO laboratory manual for the examination and processing of human semen. 6nd edn. World Health Organization: Geneva. 2021.
[19] Han T, Huang J, Gu J, Xie Q, Zhong Y, Huang T. Hepatitis B virus surface protein induces sperm dysfunction through the activation of a Bcl2/Bax signaling cascade triggering AIF/Endo G–mediated apoptosis. Andrology. 2021; 9: 944–955.
[20] Kang X, Xie Q, Zhou X, Li F, Huang J, Liu D, *et al*. Effects of hepatitis B virus S protein exposure on sperm membrane integrity and functions. PLoS ONE. 2012; 7: e33471.
[21] Shi L, Liu S, Zhao W, Zhou H, Ren W, Shi J. Hepatitis B virus infection reduces fertilization ability during *in vitro* fertilization and embryo transfer. Journal of Medical Virology. 2014; 86: 1099–1104.
[22] Zhou X, Hu X, Zhu Y, Qu F, Sun S, Qian Y. Comparison of semen quality and outcome of assisted reproductive techniques in Chinese men with and without hepatitis B. Asian Journal of Andrology. 2011; 13: 465–469.
[23] Oger P, Yazbeck C, Gervais A, Dorphin B, Gout C, Jacquesson L, *et al*. Adverse effects of hepatitis B virus on sperm motility and fertilization ability during IVF. Reproductive Biomedicine Online. 2011; 23: 207–212.
[24] Chen H, Ge H, Lv J, Wu X, Xi H, Huang J, *et al*. Chronic hepatitis B virus infection in women is not associated with IVF/ICSI outcomes. Archives of Gynecology and Obstetrics. 2014; 289: 213–217.
[25] Lee VCY, Ng EHY, Yeung WSB, Ho PC. Impact of positive hepatitis B surface antigen on the outcome of IVF treatment. Reproductive Biomedicine Online. 2010; 21: 712–717.
[26] Mak JSM, Leung MBW, Chung CHS, Chung JPW, Cheung LP, Lao TT, *et al*. Presence of Hepatitis B virus DNA in follicular fluid in female Hepatitis B carriers and outcome of IVF/ICSI treatment: a prospective observational study. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2019; 239: 11–15.
[27] Wang L, Li L, Huang C, Diao L, Lian R, Li Y, *et al*. Maternal chronic hepatitis B virus infection does not affect pregnancy outcomes in infertile patients receiving first *in vitro* fertilization treatment. Fertility and Sterility. 2019; 112: 250–257. e1.
[28] Lam PM, Suen SH, Lao TT, Cheung LP, Leung TY, Haines C. Hepatitis B infection and outcomes of *in vitro* fertilization and embryo transfer treatment. Fertility and Sterility. 2010; 93: 480–485.
[29] Ye F, Jin Y, Kong Y, Shi JZ, Qiu HT, Zhang X, *et al*. The presence of HBV mRNA in the fertilized *in vitro* embryo of HBV patients confirms vertical transmission of HBV via the ovum. Epidemiology and Infection. 2013; 141: 926–930.
[30] Nie R, Jin L, Zhang H, Xu B, Chen W, Zhu G. Presence of hepatitis B virus in oocytes and embryos: a risk of hepatitis B virus transmission during *in vitro* fertilization. Fertility and Sterility. 2011; 95: 1667–1671.
[31] Hu XL, Zhou XP, Qian YL, Wu GY, Ye YH, Zhu YM. The presence and expression of the hepatitis B virus in human oocytes and embryos. Human Reproduction. 2011; 26: 1860–1867.
[32] Mak JSM, Lao TT. Assisted reproduction in hepatitis carrier couples. Best Practice & Research Clinical Obstetrics & Gynaecology. 2020; 68: 103–108.