The safety of intracytoplasmic sperm injection in men with hepatitis B

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Abstract

Introduction: In this study, we aimed to evaluate the safety of using different sources of sperm when male partners were infected with hepatitis B virus (HBV).

Material and methods: A total of 338 couples receiving their first intracytoplasmic sperm injection (ICSI) cycle at the Department of Reproduction, Ren Ji Hospital, between 2007 and 2012 were enrolled if the female partner tested negative for HBV DNA, HBsAg, HBeAg, HBeAb and HBcAb. Couples were divided into HBV active infection (group B), convalescent infection (group C) and controls (group A). Subgroups were divided by source of sperm: ejaculated sperm and testicular sperm aspiration/percutaneous epididymal sperm aspiration (TESA/PESA) sperm.

Results: When using ejaculated sperm for ICSI, two pronuclear (2PN) fertilization rate, implantation rate, clinical pregnant rate, early miscarriage rate and live birth rate showed no significant difference between the three groups. However, in the three TESA/PESA groups, the early miscarriage rate was highest in men with active HBV infection (B2) (23.1%, $p = 0.035$). The 2PN fertilization rate and CPR were also lower in the active infection group (76.7%, 26.3%) than the convalescent infection (82.9%, 36.2%) and control group (78.2%, 50%), but the difference was not statistically significant. No malformed infant was found in any of these groups.

Conclusions: When men have an active HBV infection, using TESA/PESA sperm may cause lower fertilization, a high miscarriage rate and a lower live birth rate, which indicates that HBV active infection may cause adverse effects on ICSI reproductive performance when using testicular or epididymal aspirated sperm.

Key words: hepatitis B virus, intracytoplasmic sperm injection, male infertility.

Introduction

Hepatitis B virus (HBV) infection is a serious public health problem in China. Although a minority of individuals infected by HBV will develop a persistent infection [1], a total of roughly 200 million Chinese people suffer from chronic hepatitis B infection, about 8% of the country's total population [2].

As the number of couples seeking *in vitro* fertilization-embryo transfer (IVF-ET) increases year on year, the impact on IVF/intracytoplasmic sperm injection (ICSI) outcome is of concern if one or both members of a couple is affected by HBV. Although HBV concentration can be decreased during *ex vivo* semen processing, HBV integrated into host cells cannot be eliminated [3]. Hepatitis B virus is confirmed to integrate into the chromosome of sperm, which causes reduced motility, increased apoptosis and necrosis [4–6] and mediates a mutagenic effect on sperm chromosomes, inducing chromosome aberrations [7] and resulting in reduced male fertility.

One study in 2004 found that men who tested positive for serum HBV DNA were associated with low implantation and pregnancy rates but a similar fertilization rate in IVF cycles [4]. However, it was also reported that couples undergoing IVF-ET in which the male tested positive for HBV DNA had an increased pregnancy rate [8].

The latest results showed that HBsAg-seropositive men had similar IVF outcomes to the control group, but decreased rates of implantation and clinical pregnancy were found in ICSI cycles.

Intracytoplasmic sperm injection has two sources of sperm: ejaculated sperm and TESA/PESA sperm. Moreover, HBV infection has two stages: the active and convalescent stage, in which virus concentration in peripheral blood is different. These two factors may cause variable results of ICSI. So our retrospective cohort study attempts to evaluate the impact on the outcome of ICSI when men are in two HBV infection stages diagnosed by five HBV markers and discuss their feasibility of ICSI.

Material and methods

Subjects

All couples receiving their first ICSI cycles in the Department of Reproduction, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, between 2007 and 2012 were enrolled in a retrospective study. All patients with chromosomal abnormalities or acute or chronic infectious diseases aside from HBV were excluded, as were any couples in which the female partner tested positive for serum HBsAg, HBeAg, HBeAb or HBcAb, determined with diagnostic ELISA kits (Roche, USA), or HBV-DNA, determined by FQ-PCR with a cut-off of 10^3 copies/ml.

Patients were divided into two groups according to the male partner's HBV serostatus, which was assessed within the year preceding IVF commencement: group B (active infection) – HBV DNA seropositive; and group C (convalescent infection) – HBsAg, HBeAb and HBcAb positive and HBV DNA negative. We matched the female age, date of ICSI, basal FSH, ICSI protocol, and number of embryos transferred and found 121 couples as the control group (group A): male negative for HBsAg, HBeAg, HBeAb and HBcAb and HBV-DNA. Then according to different sources of sperm, we divided these three groups into the ejaculated group (A1, B1, C1) and TESA/PESA sperm (A2, B2, C2)

Controlled ovarian hyperstimulation protocol

Ovulation was induced in all patients by either the long or short protocol. The long protocol following the regular long protocol [9] involved pituitary down-regulation from the 7th day after ovulation by subcutaneous injection of 0.05 mg triptorelin (Ferring GmbH, Kiel, Germany), and measurement of serum luteinizing hormone (LH) and estradiol (E_2) levels and ultrasound on the 3rd day of the next menstrual cycle. When LH levels $\leq$ 5 IU/l, E_2 levels fell below 50 pg/ml and no follicle greater than 10 mm in diameter was observed by ultrasound, we began injecting rFSH (Gonal F, Merk Serono Switzerland). On the second or third day of the next menstrual cycle we injected 0.1 mg triptorelin and 150–300 IU rFSH. The ovarian response was monitored by ultrasound and serum E_2 concentrations. When at least two dominant follicles reached 18 mm in diameter, an ovulatory dose of 5000–10000 IU hCG (Livzon Bio-chemical Pharmaceutical Co., Zhuhai, China) was given and oocyte retrieval was performed within 34–36 h. Intracytoplasmic sperm injection was carried out four to 6 h later [10]. One to three embryos were transferred 2 to 3 days after retrieval. All transferred embryos were composed of four to five cells on day two, and seven or more cells on day three. The blastomeres were of equal size and less than 20% fragmentation and no multinucleation was detected [11]. Luteal support was provided from the 2nd day of retrieval by IM (60 mg/day) or vaginal (90 mg/day) progesterone (Crinone Merk-Serono, Switzerland) combined with 10 mg of oral dydrogesterone (Abbott, Holland).

Sperm acquisition

Sperm was collected on the day the wife retrieved the oocytes. There were two sources:

– Fresh ejaculated sperm was obtained on the oocyte retrieval day when men were oligozoospermic or asthenozoospermic.
– Percutaneous epididymal sperm aspiration (PESA) and testicular sperm aspiration (TESA) sperm was executed when men were azoospermic on the day of oocyte retrieval. Testicular sperm aspiration was performed in patients with non-obstructive azoospermia which was previously diagnosed.

Outcomes measured

Pregnancy was detected by urine or serum test 14 days after embryo transfer. If positive, a vaginal ultrasound scan of the pelvis was performed 2 weeks later to assess the implantation site, the number and viability of gestation. Childbirth was confirmed by follow-up mail or telephone call. Preterm birth was defined as gestational age ≥ 28 weeks and < 37 weeks.

Statistical analysis

Statistical analyses were performed using SPSS, version 18.0 (SPSS Inc, Chicago, IL, USA). One-way ANOVA was applied for continuous variables and all categorical data were analyzed using χ^2 tests. *P*-value < 0.05 was considered statistically significant.

Results

Between 2007 and 2012, our reproductive department carried out a total of 7978 IVF/ICSI cycles from which 338 couples were enrolled in this study. Group A included 121 couples, group B 92 and group C 125. There were no significant differences in female or male age, basal FSH, dose of Gn, serum E_2, endometrium thickness on the day of hCG or number of embryos transferred (Table I).

Comparison of ICSI outcome using different sources of sperm in two HBV infection groups

We compared among the 3 ejaculated groups and 3 TESA/PESA groups. In the 3 ejaculated groups, 2PN fertilization rate, clinical pregnant rate, early miscarriage rate and live birth rate showed no significant differences. However, we observed that the 2PN fertilization rate was lower in the active infection group (B2) using TESA/PESA sperm (76.7%, $p = 0.06$) compared to the convalescent infection group (C2) or control group (A2). Meanwhile, the early miscarriage rate in B2 was 23.1%, the highest among A2, B2 and C2 groups, $p = 0.035$. Consistently, CPR was lower in B2 than A2 and C2 (27.3%, 50%, 36.2%, respectively, $p = 0.12$).

Neonatal birth weight did not have statistical significance and there was no malformed infants in the active infected group (B1 and B2) and convalescent group (C1 and C2). Table II shows the mean weight ± SD of neonatal birth weight.

Discussion

Hepatitis B virus is a double-stranded DNA virus belonging to the Hepadnaviridae family which is confirmed to penetrate the blood-testis barrier, enter the male germ line and integrate into their genome and hence increase the instability of sperm chromosomes [4]. Moretti *et al.* suggested that HBV infection could create extensive hereditary effects by altering genetic constituents and/or inducing chromosome aberrations, as well as the possibility of the vertical transmission of HBV via the germ line to the next generation [4].

Huang *et al.* also found that sperm of the subjects with HBV infection appeared to be able to penetrate into zona-free hamster oocytes and to develop to the first-cleavage metaphase [12, 13]. Among the analyzable metaphase spreads, chromosome aberrations observed included numerical anomaly (aneuploidy), gap, ring chromosome, tri-

Table I. Clinical characteristics of enrolled patients

Parameter	Control (A) (*N* = 121)		Active infection (B) (*N* = 92)		Convalescent infection (C) (*N* = 125)		*P*-value[a]
	Ejaculated (A1) *N* = 77	TESA/ PESA (A2) *N* = 44	Ejaculated (B1) *N* = 59	TESA/ PESA (B2) *N* = 33	Ejaculated (C1) *N* = 78	TESA/ PESA (C2) *N* = 47	
Female age [years]	30.6 ±3.8	29.6 ±3.0	30.7 ±4.1	28.9 ±5.1	31.1 ±4.1	30.3 ±4.7	0.12
Male age [years]	32.7 ±5.1	31.8 ±4.7	32.4 ±5.2	31.6 ±5.5	32.9 ±5.1	33.0 ±5.3	0.71
Basal FSH [IU/l]	6.7 ±1.8	7.1 ±1.9	7.4 ±2.2	6.5 ±1.5	7.0 ±2.4	7.1 ±2.3	0.44
Dosage of Gn [IU]	1491.8 ±315.5	1532.7 ±377.6	1479.4 ±370.6	1532.1 ±533.1	1544.8 ±445.9	1434.0 ±472.5	0.74
E2 level on HCG day [pg/ml]	3486.9 ±1676.9	3644.5 ±1748.6	3486.8 ±1809.2	3665.3 ±1734.6	3583.5 ±1969.4	3470.1 ±1937.3	0.99
Thickness of endometrium on HCG day [mm]	9.9 ±1.6	9.9 ±1.8	10.0 ±1.8	10.0 ±2.3	10.2 ±1.9	10.0 ±1.5	0.94
Retrieved oocytes	12.8 ±6.9	11.5 ±5.0	11.7 ±7.5	13.7 ±7.2	12.4 ±6.7	11.7 ±5.3	0.63
Embryo transferred	2.1 ±0.2	2.0 ±0.2	2.1 ±0.4	2.1 ±0.3	2.1 ±0.4	2.0 ±0.4	0.90

[a]*One-way ANOVA. P-value < 0.05 was considered statistically significant.*

Table II. Intracytoplasmic sperm injection outcomes and neonatal birth condition in three groups

Source of sperm	Ejaculated sperm				TESA/PESA sperm			
	A1 (*N* = 77)	B1 (*N* =59)	C1 (*N* =78)	*P*-value	A2 (*N* = 44)	B2 (*N* = 33)	C2 (*N* = 47)	*P*-value
2PN fertilization rate[b]	80.6% (654/811)	80.1% (446/557)	84.1% (665/791)	0.10	78.2% (333/426)	76.7% (286/373)	82.9% (401/484)	0.06
Implantation rate[b]	28.3% (45/159)	32.8% (39/122)	23.0% (38/165)	0.23	31.1% (28/90)	26.1% (18/69)	25.3% (24/95)	0.64
Clinical pregnant rate[b]	44.2% (34/77)	50.8% (30/59)	38.5% (30/78)	0.35	50% (22/44)	39.4% (13/33)	42.6% (20/47)	0.62
Early miscarriage rate[b]	8.8% (3/34)	0% (0/30)	6.7% (2/30)	0.27	0	23.1% (3/13)[d]	5.0% (1/20)	0.035
Live birth rate[b,c]	36.4% (28/77)	49.2% (29/59)	35.9% (28/78)	0.22	50% (22/44)	27.3% (9/33)	36.2% (17/47)	0.12
Preterm delivery rate[b]	0	17.2% (5/29)	0	0.006	4.5% (1/22)	11.1% (1/9)	5.9% (1/17)	0.79
Birth weight [g][a]	3084.5 ±615.6	3170.7 ±569.4	3075.2 ±552.9	0.79	3248.2 ±603.0	3086.1 ±385.1	3045.0 ±448.7	0.45
No. of malformed infants	0	0	0		0	0	0	

[a]One-way ANOVA, [b]χ^2, [c]A1 – 3 late abortions, B1 – ectopic pregnancy, B2 – 1 ectopic pregnancy, C2 – 1 late abortion and 1 ectopic pregnancy, [d]3 early miscarriages occurred in women at age 30, 32 and 34.

radial, dicentric chromosome, pulverization, acentric fragment and deletion. They observed that 33 out of 233 (14.8%) analyzable sperm metaphase spreads in the hepatitis group contained chromosome aberrations. This incidence rate was significantly higher than that in the control group (4.3%, $p < 0.005$) [12]. Furthermore, they confirmed that HBV DNA was able to integrate into a human sperm chromosome by the discovery of 5 HBV DNA signals on different sperm chromosomes by FISH in a serum HBV-DNA positive person.

The overall influence of HBV-infected men on IVF outcome is controversial [6, 8] and not discussed in this paper. However, Zhou *et al.* found ICSI results to be adversely affected by a HBV-infected male partner [6]. In their study, the fertility rate, good embryo rate, implantation rate and PR (70.9%, 57.6%, 18.3% and 31.2%, respectively) in the seropositive HBsAg group were statistically significantly lower than in controls (74.0%, 60.4%, 24.2%, 39.3%).

Our results showed that HBV actively infected men using PESA/TESA for ICSI had a lower 2PN fertilization rate, live birth rate and high early miscarriage rate compared to the convalescent infection and control group. There was no difference in ICSI outcome between the convalescent and control group.

Some research has shown that the HBV infection rate of human sperm was much lower than that of liver cells, leading to a very low rate of male germ cell or embryo infection [2, 11], especially in the convalescent infection group. In this course of infection, HBV replication is in a stationary state and its body fluid concentration is lower, even to zero. When sperm are thoroughly washed, the density of HBV virus is also dramatically reduced. Qian *et al.* found a lower number of serum HBV DNA copies in sperm than in serum in 2 HBV carriers [2]. Also, these people had been in a convalescent stage for more than 1 year, which exceed the spermatogenic cycle. These studies may explain why no adverse effect was observed in the HBV convalescent infection group.

Since there are different serum HBV concentrations in active and convalescent HBV infection stage, we speculate that during the course of percutaneous epididymal and testicular aspiration, a high concentration of virus will be passed from blood into extracts. This may raise the opportunity for virus contacting with sperm and integrating into sperm. Also sperm may be introduced into oocytes when performing ICSI. The integrations of viral DNA into sperm chromosomes with multisite and nonspecific features can further increase the instability of sperm chromosomes. Even if the zygote could develop to cleavage metaphase, the ability of embryo development would be impaired and cause a high miscarriage rate, which implies

that HBV infection may affect the capacity for embryo development.

Miscarriage rates might be unaffected when sperm are collected by ejaculation due to different processing methods. In this study, density gradient centrifugation was used (different from the simple centrifugation used in TESA and PESA), which washed the semen more thoroughly. Moreover, the integration rate is low in ejaculated sperm (Huang *et al.* found that HBV DNA was only detected in one out of 9 men with chronic hepatitis B [12]).

In conclusion, the male partner with convalescent HBV infection did not appear to influence the outcome of ICSI. This should be noted when the husband in the active infection stage is undergoing ICSI using TESA/PESA sperm. For these patients we suggest that anti-virus is necessary before ICSI cycles. Studies of larger samples should be required for this group of infertile couples. Although our study followed up neonatal birth condition, it also remains unknown whether exogenous HBV gene fragments could have a long-term impact on human gene variation, which needs further exploration.

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Conflict of interest

The authors declare no conflict of interest.

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