Hepatitis B virus in oocytes and embryos: pregnancy outcomes and children's health

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Objective: To investigate whether the presence of hepatitis B virus (HBV) in oocytes and embryos affects pregnancy outcomes for in vitro fertilization and embryo transfer (ET) as well as is related to the vertical transmission of HBV to children.
Design: Retrospective cohort study.
Setting: A university-affiliated fertility center.
Patient(s): This study included 167 couples with at least 1 hepatitis B surface antigen–seropositive partner. These couples underwent in vitro fertilization–ET, and the discarded oocytes and embryos had been tested for HBV. Couples with HBV-positive oocytes or embryos were categorized as the positive group, whereas those couples with HBV-negative oocytes and embryos served as the negative group.
Intervention(s): None.
Main Outcome Measure(s): Pregnancy outcomes and the rate of children's HBV infection.
Result(s): The pregnancy outcomes of fresh and frozen ETs were not associated with the presence of HBV in the oocytes and embryos. Of the 106 infants born, 1 child whose mother tested positive for hepatitis B surface antigen but had negative oocytes and embryos was infected with HBV. Additionally, 26.09% of children who had been administered passive immunization and active vaccinations did not reach protective levels of anti-HBV antibodies (hepatitis B surface antibodies) and became nonresponders. The negative rate of children's hepatitis B surface antibody was associated with the presence of HBV in oocytes and embryos (odds ratio, 3.01; 95% confidence interval, 1.04–9.25).
Conclusion(s): The presence of HBV in oocytes and embryos did not affect pregnancy outcomes or result in the vertical transmission of HBV to the offspring of HBV carriers. Follow-up is needed for HBV-vaccinated children with an HBV-infected parent. Booster vaccinations are necessary for continued protection. (F S Rep® 2024;5:272–8. ©2024 by American Society for Reproductive Medicine.)
Key words: HBV, oocyte, embryo, vertical transmission, offspring

Hepatitis B virus (HBV) infection is a global health problem. According to the World Health Organization, there are 296 million chronic HBV carriers worldwide, including 65 million women of childbearing age (1). More than 820,000 people die from HBV-related complications per year worldwide (1). Annually, there are nearly 2 million new infections in children aged <5 years because of mother-to-child transmission and horizontal transmission in early life (2). China is a particularly highly endemic area for HBV. Approximately 60% of the population has a history of HBV infection, and 9.8% is chronically infected with HBV in China (Centers for Disease Control and Prevention, 2007). Hepatitis B virus affects 3.1%–10.0% of reproductive-age women in China (3). The chronic hepatitis B guidelines of China suggest that infants born to hepatitis B surface antigen (HBsAg)-positive mothers should be administered passive immunization with hepatitis B and at least 3 active HBsAg vaccinations (4). Despite timely passive and active vaccination, 5%–10% of HBV transmission cases could not be prevented by immunization (5–7). Moreover, perinatally acquired infections cause an estimated 21% of HBV-related deaths worldwide (8).

Hepatitis B virus deoxyribonucleic acid (DNA) and ribonucleic acid have been found in spermatozoa (9–11), oocytes (12–14), follicular fluid (15), and embryos (12–14). These data provide direct evidence supporting the

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hypothesis that germ cells may act as vectors for vertical transmission. The immunoprophylaxis failure may be related to germ cell transmission. However, there are limited data about the effect of HBV in human oocytes and embryos on pregnancy outcomes and children's health after vaccination.

We have detected HBV DNA in the nuclei of discarded human oocytes and embryos in our previous study. Hepatitis B virus DNA was present in 9.6% of oocytes and 14.4% of embryos, as well as couples in which the female, male, or both partners were HBsAg-positive had similar positive rates for embryos (13). The aim of this study was to investigate whether the presence of HBV in discarded oocytes and embryos affects pregnancy outcomes for in vitro fertilization (IVF) and embryo transfer (ET) as well as is related to the vertical transmission of HBV to children.

MATERIALS AND METHODS

This retrospective study was conducted from December 1, 2011, to December 31, 2020, at the Women's Hospital, School of Medicine, Zhejiang University. Totally, 122 couples with an HBsAg-seropositive woman, 27 couples with an HBsAg-seropositive man, and 18 couples with both HBsAg-seropositive partners whose discarded unfertilized oocytes (including the germinal vesicle, metaphase I, and metaphase II stages after failure to fertilize, 250) and poor-quality or polyspermy embryos (578) during assisted reproductive technology (ART) had been tested for HBV DNA using fluorescence in situ hybridization were included (13). According to the tested results, couples with at least 1 HBV-positive oocyte or embryo were categorized as the positive group, whereas those without HBV-positive oocytes and embryos served as the negative group. The ART treatment of patients with infertility was performed between June 1, 2006, and November 30, 2009. Synthetic serum substitute (Irvine Scientific, Santa Ana, CA) was used for embryo culture. The fertilization method (IVF or intracytoplasmic sperm injection [ICSI]) was determined according to the semen parameters. Sperm were prepared using the routine density gradient centrifugation or swim-up method. The gamete and embryos were conducted in a separate area for couples who had a seropositive HBsAg, and the frozen embryos were stored in separate tanks.

Data on the ART procedures and offspring were retrieved from the database, including age, HBV serostatus, duration of infertility, body mass index (BMI), type of infertility (primary or secondary), cause(s) of infertility, ovarian reserve assessment (cycle day 3 follicle-stimulating hormone and estradiol [E_2] serum levels), and the number of previous cycles. The outcomes were derived from controlled ovarian stimulation, including the duration and total dose of gonadotropin treatment, peak E_2 level, number of oocytes retrieved, fertilization method (IVF or ICSI), number of fertilized oocytes, number of embryos transferred, pregnancy rate, live birth rate, gestational age, pregnancy outcome of frozen ET (FET) from the same stimulation cycle, and the HBV test results of the offspring. The serum HBV markers were tested when the children were aged 4–6 years. The coding region for the major polypeptide of HBsAg is preceded by an in-phase open reading frame termed presurface (preS), and the large protein coded by the preS/S region has a further N-terminal extension termed preS1 (16, 17). preS1 is similar to hepatitis B e antigen (HBeAg); it is also an important marker of HBV replication (18, 19). If HBsAg was seropositive, preS1 was further examined at our center. The results of preS1 were also collected.

Outcome Measures

The outcomes of the IVF-ET cycles were assessed using the serum β-human chorionic gonadotropin test, performed 2 weeks after ET. If the test result was positive, a vaginal ultrasound scan of the pelvis was performed 3 weeks later to assess the site, number, and viability of the gestation. The primary outcome measures of the study were clinical pregnancy rate (the presence of a gestational sac on transvaginal ultrasound per cycle with ET), implantation rate (the number of gestational sacs per embryo transferred), pregnancy loss (the loss of a clinical pregnancy before gestational week 28), and live birth (the delivery of at least 1 live infant after at least a 28-week gestation).

Data related to offspring vaccination were obtained by trained interviewers using a standardized, structured questionnaire by telephone or face-to-face interviews. The serum HBV markers, including HBsAg and its antibody (hepatitis B surface antibody [HBsAb]), HBeAg and its antibody, as well as the antibody against the hepatitis B c antigen (HBcAg), were tested using quantitative enzyme immunoassays. Humoral immunity from the vaccination was defined as an HBsAb titer level of >10 mIU/mL in those children who were negative for HBsAg. Vaccinations, both passive (HBV hyperimmunoglobulin [HBIG] within 2 hours after birth) and active (HBV vaccine, ≥ 3 shots), were offered to all newborns.

Ethical Approval

The present study was conducted according to the Declaration of Helsinki for Medical Research involving Human Subjects. The project was approved by the Ethics Committee of the Women's Hospital, School of Medicine, Zhejiang University (approval number, IRB-20210029-R).

Statistical Analysis

The clinical pregnancy rate of IVF-ET was set at 40% (20). To detect a difference of 20% in the rates of clinical pregnancy as being statistically significant, the sample size of 79 subjects in each group would be needed to provide a power of 80% at a level of significance 0.05 between the negative and positive groups.

The data were analyzed using the Statistical Package for the Social Sciences for Windows, version 26.0. Continuous variables conforming to a normal distribution were characterized by the mean and standard deviation. Comparisons between the 2 groups were analyzed using the Student's *t*-test. Categorical variables were characterized using the proportion and analyzed with the χ^2 or Fisher's exact test. A P

TABLE 1

Baseline characteristics of patients with infertility on the basis of hepatitis B virus status of the discarded oocyte and embryo.

Group	Negative group (n = 91)	Positive group (n = 76)	*P* values
Female age (y)	31.45 ± 3.65	29.88 ± 3.47	.64
Duration of infertility (y)	4.45 ± 3.33	4.24 ± 3.10	.39
Basic FSH level (IU/L)	6.29 ± 1.77	6.14 ± 1.78	.50
Basic E_2 level (pmol/L)	119.62 ± 43.06	138.12 ± 86.81	.27
BMI (kg/m^2)	21.11 ± 4.93	20.44 ± 3.60	.07
Type of infertility	—	—	.85
Primary infertility	32.97% (27/86)	31.58% (23/74)	—
Secondary infertility	67.03% (61/91)	68.42% (52/76)	—
Cause of infertility	—	—	.28
Tubal	54.94% (50/91)	56.58% (43/76)	—
Male	14.29% (13/91)	23.69% (18/76)	—
Female and male	21.98% (20/91)	13.16% (10/76)	—
Others	8.79% (8/91)	6.58% (5/76)	—
No. of IVF/ICSI cycles	1.09 ± 0.33	1.19 ± 0.46	<.01

Note: Results are expressed as means ± standard deviations or percentages. BMI = body mass index; E_2 = estradiol; FSH = follicle-stimulating hormone; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization.

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value of <.05 was considered statistically significant. Regression analysis was performed to determine the importance of each individual variable in association with the pregnancy outcome. Regression analysis was performed to determine the associations between the presence of HBV DNA in oocytes/embryos and pregnancy outcomes while adjusting for the potential confounding factors female age, cause of infertility, BMI, number of treatment cycles, and couple's HBeAg and preS1 status.

RESULTS

Study Population and Patient Characteristics

Among the 167 couples, 76 had HBV-positive oocytes or embryos, whereas 91 had the negative oocytes and embryos. The patient characteristics are summarized in Table 1. There were no significant differences between the positive and negative groups in the baseline characteristics (i.e., female age, infertility duration, basic follicle-stimulating hormone and E_2 levels, BMI, as well as type and causes of infertility). However, the number of IVF/ICSI cycles in the positive group was significantly higher than that in the negative group (1.19 ± 0.46 vs. 1.09 ± 0.33, $P<.01$).

Influence of HBV in Oocytes and Embryos on Ovarian Stimulation and Pregnancy Outcomes of IVF-ET

No significant differences were found between the 2 groups with regard to the fertilization method, duration of stimulation, total gonadotropin dose, number of oocytes retrieved, or fertilization rate. Pregnancy outcomes for fresh ET, including the live birth, preterm, clinical pregnancy, implantation, and miscarriage rates, were not associated with the presence of HBV in the oocytes and embryos (Table 2). There were also no significant differences between the positive and negative groups for the pregnancy outcomes for FET (Table 3). Regression analysis was performed to test female age, cause of infertility, BMI, number of IVF or ICSI cycles, female or male HBeAg and preS1 status, as well as presence of HBV DNA in oocytes and embryos to determine the importance of each individual variable and its association with the outcome of IVF and ET cycles, namely, clinical pregnancy and live birth. After correction for confounding effects, the rates of clinical pregnancy and live birth were not associated with the presence of HBV DNA in oocytes/embryos. The adjusted odds ratios were 0.76 (95% confidence interval [CI], 0.37–1.56; $P>.05$) and 0.81 (95% CI, 0.39–1.69; $P>.05$), respectively.

Influence of HBV in Oocytes or Embryos on HBV Infection of Children

A total of 106 infants were born from 87 deliveries, including 19 sets of twins. No newborns exhibited defects at birth. Of the 106 children, 48 were born to couples with HBV-positive oocytes or embryos, 58 were born to couples with HBV-negative oocytes or embryos, 60 were born after fresh ET, and 46 were born after FET. All infants born to the HBsAg-positive couples were given HBIG within 2 hours after birth and 3 doses of HBV vaccine (at birth and then at 4 weeks and 6 months of age). After HBIG and HBV vaccine administration, 92 infants (86.79%) were observed at 4–6 years of age. Fourteen infants were lost to follow-up; 12 (11.32%) were lost because of the inability to locate information, and 2 (1.89%) refused to enroll in this study. One child (1.09%) was infected with HBV. The mother of this child was positive for HBsAg, HBeAg, HBcAg before and during pregnancy. The mother's serum and follicular fluid HBV DNA load values on the oocyte retrieval day were 2.48×10^7 copies/mL and 9.48×10^5 copies/mL, respectively. Her 6 unfertilized oocytes and 5 discarded embryos were HBV-negative. Twenty-four children (26.09%) did not achieve protective levels of HBsAb (10 mIU/mL) and became nonresponders; the rest were HBsAb positive. Compared with the negative group, the positive group showed a significantly higher rate of HBsAb-negative children (34.95% vs. 15.56%, $P<.05$). After adjusting for

TABLE 2

Comparison of cycle characteristics and pregnancy outcomes for transferred fresh embryos.

Group	Negative group (n = 91)	Positive group (n = 76)	*P* values
Fertilization method	—	—	.37
IVF	74.73% (68/91)	68.42% (52/76)	
ICSI	25.27% (23/91)	31.58% (24/76)	—
Dosage of Gn used (IU)	2,753.63 ± 985.14	2,303.60 ± 850.04	.29
Oocyte retrieved	16.60 ± 7.03	17.55 ± 7.73	.35
Duration of stimulation (d)	10.03 ± 1.60	9.50 ± 1.51	.96
No. of 2PN fertilized oocytes	11.08 ± 6.22	10.93 ± 6.62	.79
Peak E_2 level (pmol/L)	16,984.76 ± 9,172.47	16,936.76 ± 8,796.83	.96
No oocyte pickup or no embryo transfer	8.00% (8/91)	9.21% (7/76)	.78
Freeze-all embryos	7.69% (7/91)	5.26% (4/76)	.53
IVF fertilization rate	70.19% (756/1,077)	66.67% (602/903)	.09
ICSI fertilization rate	70.65% (219/310)	66.99% (207/309)	.33
No. of transferred embryos	2.11 ± 0.50	2.00 ± 0.58	.95
Implantation rate	23.08% (39/169)	25.37% (34/134)	.64
Clinical pregnancy rate	36.14% (30/83)	40.58% (28/69)	.56
Miscarriage rate	10.00% (3/30)	10.34% (3/29)	.69
Ectopic pregnancy	2.30% (1/30)	2.01% (1/29)	.94
Preterm rate	14.81% (4/27)	20.83% (5/24)	.57
Live birth rate	32.93% (27/83)	34.78% (24/69)	.81

Note: Results are expressed as means ± standard deviations or percentages. E_2 = estradiol; FSH = follicle-stimulating hormone; Gn = gonadotropin; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; 2PN = 2-pronuclear.

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TABLE 3

Comparison of pregnancy outcomes for frozen embryo transfer.

Group	Negative group (n = 89)	Positive group (n = 63)	*P* values
Implantation rate	14.04% (32/228)	15.43% (25/162)	.70
Clinical pregnancy rate	29.21% (26/89)	31.75% (20/63)	.56
Miscarriage rate	19.23% (5/26)	15.00% (3/20)	.71
Ectopic pregnancy	11.54% (3/26)	3.45% (1/29)	.25
Preterm rate	25.00% (5/20)	18.75% (3/16)	.65
Live birth rate	24.10% (20/83)	25.40% (16/63)	.86

Note: Results are expressed as percentages.

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the female or male HBeAg and PreS1 status, the increased risk of children's negative HBsAb was still positively correlated with the presence of HBV DNA in oocytes and embryos (odds ratio, 3.01; 95% CI, 1.04–9.25; $P<.05$).

DISCUSSION

To our knowledge, the present study is the first to focus on the influence of HBV in oocyte/embryo on ART pregnancy outcomes. Our study showed that the presence of HBV in oocytes/embryos had no negative effects on the rates of live birth, implantation, and miscarriage. Although no previous studies have been conducted on the relationship between HBV infection of oocyte/embryo and ART outcomes, there has been controversy about the effect of couples' HBsAg seropositivity on pregnancy outcomes for ART. Wang et al. (21) and Farsimadan et al. (22) reported that HBV infection was not an independent contributor to pregnancy outcomes. Lee et al. (23) also found that the ongoing pregnancy and live birth rates for HBsAg-seropositive women did not differ significantly from those for HBsAg-seronegative women. Moreover, another study demonstrated that HBsAg-seropositive women had significantly lower fertilization and top-quality embryo rates than healthy controls; however, the clinical pregnancy rates did not differ significantly between the HBsAg-seropositive and HBsAg-seronegative groups (24). In contrast, Lam et al. (25) showed that couples with at least 1 HBV-seropositive partner had higher pregnancy and implantation rates in IVF-ET cycles than the control couples. In our study, the IVF fertilization rate in the positive group was lower than that in the negative group, although there was no significant difference. The higher incidence of male factor infertility in the positive group may be responsible for this. However, a larger sample size is needed to verify this in future studies. The increased number of IVF/ICSI cycles may have contributed to a higher incidence of male factor infertility and lower fertilization rate in the positive group. According to our results, the pregnancy outcomes may not be related to the HBV infection of oocytes and embryos.

In this retrospective study, all children received combined passive and active immunoprophylaxis. Only 1 child with an HBsAg-, HBeAg-, and HBcAg-positive mother developed chronic hepatitis. However, none of the children born to couples with HBV-positive oocytes or embryos remained chronically infected or became chronic carriers, despite the fact that the HBV DNA had been detected in spermatozoa, oocytes, and embryos (9–14). To date, only 1 study has been published on the effects of HBsAg in oocytes and embryos on vertical transmission to offspring on the basis of 12 infants (26). Our results are consistent with this study that showed that none of the children born to couples with HBV-positive oocytes and embryos remained chronically infected or became chronic carriers of HBV. The vertical transmission risk is one of the most important impacts of HBV infection on reproductive issues. It has been suggested that 5%–10% of failed prevention of HBV infection is related to its vertical transmission by germ cells (27). However, in the present study, the risk of parent-to-child HBV transmission did not increase when HBV DNA was detected in the discarded oocytes or embryos. Cell surface receptors may play a key role in the uptake of HBV virions, the identity of which remains unknown (28). Asialoglycoprotein receptor was speculated to be involved in the process of HBV infection (29, 30). On the one hand, the HBV carried by oocytes and spermatozoa could be introduced into embryos. On the other hand, the HBV in follicular fluid, granulosa cells, and seminal fluid could enter into the embryo during the procedure of fertilization. Then, the viruses replicate and express themselves in embryos, which are regulated by host genes (12–14, 31, 32). For ethical reasons, only discarded immature (germinal vesicle or metaphase I stage) or unfertilized (metaphase II stage) oocytes and poor-quality or polyspermy embryos (i.e., unsuitable for transfer or cryopreservation) were detected. The presence of HBV infection in parts of oocytes and embryos and no children with chronic HBV infection in the positive group suggest that HBV-infected oocytes or embryos did not successfully complete fertilization and implantation and develop into a fetus (33) or were aborted after implantation. Our study provided direct evidence that HBV in oocytes and embryos may not result in vertical transmission of HBV to the offspring of HBV carriers.

One child of immunoprophylaxis failure born to an HBeAg-positive mother had high HBV DNA levels. This result was consistent with a previous study that showed that the maternal HBV DNA levels of >7 log10 copies/mL were significantly correlated with perinatal HBV transmission (34). The parents of the child underwent IVF for tubal factor. No child born after ICSI was infected with HBV. Theoretically, routine semen processing could prevent introduction of the HBV into the oocyte in the case of ICSI (35). Although our previous study had shown that embryos from HBV-infected men or both partners infected with HBV in the ICSI group had higher rates of HBV positivity than these in the IVF group, the difference was not significant (13). The child's mother had been prescribed antiviral treatment before and during pregnancy. However, the mother did not take it regularly. The child routinely received the first dose of the vaccine at birth and the last dose at 6 months of age, and the child was observed at 6 years old. For serologic HBV markers not being detected before the first or after the last vaccination, it is impossible to conclude whether or not this individual is already infected in utero via placental infection or an infected germ cell because no method is currently used to detect HBV of the transferred embryos. The HBV infection of the child also possibly occurs during delivery and postnatally through close contact.

Among the 92 children born to a HBsAg-positive parent in our study, the immunoprophylaxis failure (HBsAg-positive) rate was 1.09% (1/92), consistent with previous studies reporting infection rates of 2% (2/100) (36) and 4.32% (4/370) (37) for children born to HBsAg-positive mothers. Accordingly, we concluded that ART did not increase the risk of infecting offspring with HBV. Moreover, 26.09% (24/92) of the children who received combined passive and active immunoprophylaxis in this study were negative for HBsAb, and the chance of children without protective levels of HBsAb increased when HBV DNA was detected in the discarded oocytes or embryos. The high maternal serum HBV DNA levels are also an important factor in the infection of oocytes and embryos (13). Moreover, a person who is at high-risk of exposure to blood or bodily fluids of HBV infection is a risk factor for a lack of response to the vaccine (38). This may be the reason that children born from parents with HBV DNA-positive oocytes/embryos were more likely to be nonresponders to vaccination. Mother-to-child transmission accounts for the most HBV infections in children, even in countries with infant vaccination (2). The maternal HBsAg-positive status is an independent risk factor for vaccination-protected children to develop breakthrough HBV infections in adulthood (39). Up to one third of incident HBV infections can be attributed to horizontal transmission in early childhood because of child-to-child, household, or intrafamilial transmission. When HBV infection is acquired perinatally or in early childhood, it is also likely to lead to chronic infection (2). For the less developed immune system of younger children, vaccination in infancy results in less prolonged immunogenicity than vaccination in adolescence (40). The HBsAb levels will likely continue to decrease over time in those immunized at birth (41). In recent years, breakthrough HBV infections in adults vaccinated during infancy have been documented in different areas, especially among individuals born to HBsAg-positive mothers (42–44). Although some studies have reported that booster vaccinations are not necessary for immunologic memory in a healthy population (45, 46), the risk of infection increases when antibody titers are below the protective level (47), particularly for those children born to HBsAg-positive mothers (37). Then, active surveillance should be performed in infants of HBsAg-positive mothers, including regular screening for HBsAb (47). If the titer level is <10 mIU/mL, a booster dose is necessary (40). Although our study confirmed the efficiency of HBV vaccination and highlighted the need for postvaccination follow-up, particularly in at-risk categories, it is still necessary to calculate the potential usage of booster doses to prolong protection.

Our study has 3 key limitations. First, HBV infection of the offspring was assessed on a small number of subjects

and should be confirmed using a larger cohort. Second, the serologic data of the infected child were not obtained before the first or after the last vaccination. Thus, it was impossible to conclude whether she was infected in utero or subsequently. Finally, 13.21% of the children were lost in this study because of the long follow-up period. However, our findings offer reassurance that HBV in oocytes and embryos does not increase the risk of infection for the offspring.

CONCLUSION

The presence of HBV DNA in oocytes or embryos may not affect pregnancy outcomes or result in the vertical transmission of HBV to the offspring of HBV carriers. Follow-up is needed for HBV-vaccinated children with an HBV-infected parent. Booster vaccinations are necessary for continued protection.

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CRediT Authorship Contribution Statement

Xiaoling Hu: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Yingzhi Yang:** Visualization, Validation, Software, Project administration, Methodology, Data curation, Conceptualization. **Guofang Feng:** Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Formal analysis, Data curation. **Xiaoqian Zhou:** Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Data curation. **Minyue Tang:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Formal analysis, Data curation. **Huanmiao Yan:** Writing – review & editing, Visualization, Software, Methodology, Formal analysis, Data curation. **Miao Li:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Formal analysis, Data curation. **Aixia Liu:** Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Data curation. **Yimin Zhu:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Conceptualization.

Declaration of Interests

X.H. has nothing to disclose. Y.Y. has nothing to disclose. G.F. has nothing to disclose. X.Z. has nothing to disclose. M.T. has nothing to disclose. H.Y. has nothing to disclose. M.L. has nothing to disclose. A.L. has nothing to disclose. Y.Z. has nothing to disclose.

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