Journal of Assisted Reproduction and Genetics, Vol. 21, No. 5, May 2004 (© 2004)

Assisted Reproductive Technology

Reproductive Performance of Couples Discordant for Hepatitis B and C Following IVF Treatment

Imran R. Pirwany,[1,2] Simon Phillips,[1] Simon Kelly,[1] William Buckett,[1] and Seang Lin Tan[1]

Submitted December 31, 2003; accepted March 17, 2004

***Purpose*:** To examine the reproductive performance of hepatitis B (HBV) and C (HCV) discordant couples following IVF-ET.

***Methods*:** A retrospective cohort study of 25 IVF-ET cycles in HBV and HCV discordant couples was performed. Thirteen patients in the study cohort were discordant for HBV (10 males and 3 females), and 12 (9 males and 3 females) for HCV. Twenty-seven consecutive age matched patients comprised the control group. All patients underwent controlled ovarian hyperstimulation using the long downregulation protocol followed by IVF or ICSI.

***Results*:** Patients in the three groups (HBV, HCV, and controls) had similar ages, and day 3 FSH concentrations. Despite comparable response to COH, and similar fertilization, and cleavage rates in the three groups, couples discordant for HBV or HCV had significantly poorer implantation and pregnancy rates (7.7%, 0% respectively) compared with controls (41%).

***Conclusions*:** Despite comparable response to COH, HBV and HCV positive discordant couples, have significantly lower implantation and pregnancy rates compared with age-matched controls.

KEY WORDS: Controlled ovarian stimulation; fertilization; hepatitis; hepatitis B; hepatitis C; in vitro fertilization; pregnancy.

INTRODUCTION

Hepatitis B (HBV) and C (HBC) are among the most common serious viral infections in humans. It is estimated that more than two billion people worldwide have been exposed to the HBV virus (1). In the USA, 4.9 and 1.8% of the population are believed to be infected with HBV and HCV, with approximately 80,000 and 35,000 new infections being reported each year respectively (2).

Except in acute cases or exacerbations of the disease, pregnancy is not contraindicated in subjects with HBV or HCV infections. Pregnancy does not appear to influence the course of the disease nor does the disease have an adverse effect on pregnancy outcome (3). Given the acceptability of a spontaneous pregnancy in patients chronically infected with HBV or HCV, there is no ethically sound reason for declining IVF treatment in these patients (4). Knowledge of the serostatus of the couple however, allows firstly for immunoprophylactic measures to be taken to reduce the risk of transmission to the partner, fetus, or newborn baby, and secondly, for precautions to be taken against cross-contamination during sample handing and embryo cryostorage. Furthermore, it enables couples to make an informed decision regarding pursuance of treatment (5). For the welfare of any child born as a result of ART in mind, the European Society of Human Reproduction and Embryology (ESHRE) recommends the screening of both the partners for HBV and HCV prior to commencing treatment (6).

In case of HBV affecting the male, the female partner can be immunized, thereby reducing the risk of

[1] Department of Obstetrics and Gynecology, McGill Reproductive Center, McGill University, Montreal, Canada.
[2] To whom correspondence should be addressed at Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Canada H3A 1A1; e-mail: imran.pirwany@mail.mcgill.ca.

1058-0468/04/0500-0157/0 © 2004 Plenum Publishing Corporation

transmission. Although HCV viral RNA has been detected in the semen, the role of sexual, perinatal, and other possible nonparenteral routes of HCV transmission is still unclear (7–9), but from the available evidence this risk appears to be minimal (10). Indeed, even in the presence of acute infection, semen viremia is extremely low as evaluated by PCR (11–14), suggesting a low risk of sexual transmission. Thus IVF is not contraindicated in these couples (15,16). Moreover, the value of routine detection of HCV viral RNA in seminal fluid and culture medium is questionable (17), and the results must be interpreted with caution, as seminal amplification inhibitors may render the results of PCR unreliable (11,18). Notwithstanding the possibility of sexual transmission, a recent report has drawn attention to the possibility of nosocomial transmission of HCV during ancillary procedures for ART (19), indicating the need for exercising special precaution in specimen handing in the embryology laboratory.

Given the paucity of available data on the reproductive performance of couple testing seropositive to HBV and HCV on initial screening, we undertook the present study to examine the IVF performance of serodiscordant couples. We wished to investigate the response to COH, fertilization rates, cleavage rates as well as embryo quality in these patients. Implantation and pregnancy rates were secondary study end points.

MATERIALS AND METHODS

All patients who screened positive for HBV or HCV, undergoing their first IVF cycle at McGill Reproductive Center between 1998–2002 were entered into the study. Consecutive age matched patients undergoing their first IVF cycle at the same time as subjects were selected for comparison as controls.

Screening for hepatitis B and C was performed as a part of routine workup of all patients undergoing infertility treatment at the McGill Reproductive Center. AxSYM® (Abbot Diagnostics, IL) version 2, a microparticle enzyme immunoassay was used for the qualitative detection of Hepatitis B Surface Antigen (HbsAg) in the serum. Reactive samples were confirmed by neutralization procedures utilizing human anti-HB. AxSYM® (Abbot Diagnostics, IL) version 3 was used for the qualitative detection of antibodies to hepatitis C virus (anti-HCV) in the serum. The assay was designed to detect antibodies to putative structural and nonstructural proteins of the HCV genome. Samples that repeatedly tested positive were confirmed by Monolisa® anti-HCV enzyme immunoassay (BioRad, France).

All patients underwent a long downregulation protocol following pretreatment with the oral contraceptive pill (OCP). The OCP (Marvelon®, Organon Canada Ltd) was administered for a minimum of 14 days with the onset of the preceding menses. Pituitary suppression was achieved with buserelin (Suprafact®, Aventis Pharma Inc, Quebec, Canada) in a dose of 500 μg per day. Pituitary downregulation was confirmed by estradiol (E_2) concentration $<$150 pmol/L and endometrial thickness $<$5 mm, and the absence of any ovarian cysts. Gonadotropin stimulation was commenced upon confirmation of pituitary quiescence with a concomitant reduction in the dose of buserelin to 200 μg/day. Recombinant follicle stimulating hormone (FSH), Gonal-F® (Serono Canada Inc, Ontario Canada) or Puregon® (Organon Canada Ltd, Ontario Canada) were started as a dose on the basis of woman's age, antral follicle count, basal FSH concentrations, and intraovarian stromal blood flow (20). Human chorionic gonadotropin (hCG) (Profasi®, Serono Canada Ltd), 10,000 IU was administered subcutaneously when at least three follicles $\geq$18 mm in diameter were observed. Oocyte retrieval was performed 36 h after hCG administration by transvaginal ultrasonography guided needle aspiration under intravenous sedation. After retrieval, oocytes were collected into Vitrolife® IVF media (Vitrolife, Goteberg, Sweden) and fertilized by conventional insemination or ICSI depending on whether there was severe male factor infertility. Fertilization was confirmed 16–18 h after insemination by the presence of two pronuclei; embryo assessment was made as previously described (21) and embryo transfer was performed on day 2/3 or 5/6 depending on the number and quality of the embryos available (22). The number of embryos transferred depended on the age and history of the individual patient, but generally patients less than 35 years of age received two embryos, up to 39 years of age received three embryos and patients over 40 years of age received four embryos. Progesterone (50 mg/day IM) in oil was used for luteal support. The same protocol was followed for both groups of patients. Hepatitis positive patients' embryos were cultured in a separate incubator and the semen was processed in a separate centrifuge to minimize the risk of cross-contamination. Pregnancy was diagnosed by serum βhCG estimation 14 days after embryo transfer. Clinical pregnancy was confirmed by transvaginal ultrasound examination 4 weeks after the positive pregnancy test.

The data were not normally distributed, and Mann–Whitney *U* test was used to test the null hypothesis of there being no significant difference between the control and the hepatitis groups. Chi-square test was used to study the difference in proportions between the groups. The data for HBV and HCV groups were analyzed separately and together. Given that the results in both groups were broadly similar, the results are presented collectively in the text.

RESULTS

Twenty-five patients comprised the study cohort. Thirteen subjects tested positive for HBV; in 10 patients the male partner was affected, and in 3 the female partner was affected. Twelve patients were seropositive for HCV. In nine subjects, the male partner was affected, while in three the female tested positive for the virus. All patients tested negative for HIV, and had undergone a detailed assessment of their liver function by the hepatologist prior to commencing IVF treatment. Most patients (10/12) in the HCV group had biochemical evidence of mild chronic hepatitis, but none had moderate or severe chronic hepatitis. No patient had received antiviral treatment prior to commencing the IVF cycle. Patients were matched by diagnoses, and there were no differences in the causes of infertility in the two groups. The semen analysis was not significantly different between the two hepatitis groups and between the hepatitis group and controls.

The data in each of the groups were analyzed together and separately. There were no significant difference in age and basal FSH concentrations between the two hepatitis groups and controls (Table I). The response to COH was similar in all groups as shown by total gonadotropin dosage requirement, the maximal E_2 on day of hCG, and the number of oocytes retireved. In the control group 22 patients had IVF, while 5 patients had ICSI. In the hepatitis group as a whole, 17 patients had IVF, 5 had ICSI, while 3 patients had rescue ICSI following failed fertilization.

The fertilization and cleavage rates and the number of embros transferred were similar in both hepatitis groups and between the hepatitis group and controls. In the control group 11 clinical pregnancies were observed on ultrasound scan; in 7 patients a single gestation sac was seen, while 4 patients had multiple pregnancies and 1 patient had a triplet pregnancy. One pregnancy was recorded in the HBV group where a single gestation sac was observed on ultrasound scan. These differences in pregnancy rates were highly significant ($\chi^2 = 7.52$; $p = 0.0061$).

DISCUSSION

To the best of our knowledge, this is the first study that has examined the reproductive performance of patients discordant for HBV and HCV following IVF treatment. The results of this study suggest that these patients have suboptimal outcomes with regard to pregnancy and implantation rates when compared with age and diagnosis matched controls. To the extent that our conclusions are on the basis of a small number of patients, the results of the present study should be considered preliminary. The reasons for the disparate pregnancy rates between groups are not immediately obvious. It is possible that the results could be attributable to the small sample size and to type 1 error. However, given the significant difference in pregnancy rates in the two groups, retrospective power analysis reveals that the present study had a 91.5% power to detect a significant difference between the two groups with 5% alpha.

Despite the demonstration of low risk of cross contamination and of nosocomial transmission during

Table I. Baseline and Reproductive Response Parameters of Hepatitis B, C, and Control Subjects

Variable	HBV ($n = 13$)	HCV ($n = 12$)	Controls ($n = 27$)
Age (years)	36.4 (34.3–38.6)	34.3 (32.5–36.1)	35.3 (33.9–36.7)
FSH (IU/L)	6.27 (5.02–7.53)	7.26 (5.56–8.96)	6.92 (6.16–7.7)
FSH dose (IU)	3133 (2642–3623)	3635 (2718–4552)	3140 (2660–3620)
Max E_2 (pmol/L)	6230 (3353–9108)	5931 (3190–8672)	7079 (5717–8440)
Oocytes retrieved (*n*)	9.84 (5.8–13.8)	8.0 (5.41–10.6)	12.07 (9.3–14.8)
Viable oocytes	9.54 (5.7–13.4)	7.83 (5.1–10.1)	11.74 (9.0–14.4)
Fertilization	6.0 (3.5–8.5)	5.0 (2.5–7.6)	7.9 (5.9–9.9)
Fertilized (%)	63.3 (54.1–80.4)	63.9 (46.3–81.5)	75.86 (68.3–90.0)
Cleaved (%)	95.7 (89.3–100)	99.4 (97.9–100)	95.5 (91.1–100)
CES	28.9 (17.3–40.6)	34.5 (24.0–45.0)	35.0 (28.8–41.3)
Embryos transferred	2.6 (2–3.5)	2.91 (2.5–3.3)	2.6 (2.33–2.92)
Clinical pregnancies	1	0	11 (41%)

IVF or ICSI techniques from HCV+ couples (17,19), the possibility for transmission merits special precautions in handing potentially infective material in the laboratory. This includes handling the samples separately in the laboratory from other HCV-couples, and using separate incubators for embryo culture. It is possible that these extra precautions and handling techniques could have contributed to the dissimilar results observed in implantation and pregnancy rates between hepatitis groups and controls. Notably though, all the hepatitis samples were handled in exactly the same way and by the same personnel as control subjects, and the same incubator was used for embryo culture that is routinely used in all our IVF and ICSI cases. Furthermore the cleaning procedure and the techniques for handling all the specimens were similar in both groups of patients (3).

The reverse transcriptase activity of the HBV viral DNA polymerase may explain the integration of HBV DNA in the spermatozoa at least during the acute phase of HBV infection (16). Although this raises the question of vertical transmission of HBV infection, its effect on semen parameters, particularly on sperm motility has not been examined. There is also no published evidence of the effect of chronic infection or of past infection on semen parameters. Thus the explanation for the poor implantation and pregnancy rates observed in this group of patients is not self evident. In contrast, HCV being a RNA virus lacks reverse transcriptase activity, making it is impossible for the viral RNA to integrate into the genome of the host (16). Although it is unlikely that sperm supports HCV replication, it has been suggested that it may interfere with sperm motility by passive adsorption to the cell membrane (23). Interestingly, in the present study, although the semen parameters were similar in controls and hepatitis patients, the three cases of failed fertilization occurred in the hepatitis group (two in the HBV group and one in the HBC patient), suggesting that impaired sperm binding in these patients may, in part be responsible for the poor results observed. It is also possible that viral infections per se may impair fertility by immunological, inflammatory, or direct toxic effects on spermatogenesis (24). It is tempting to speculate that these effects on sperm function may have contributed to the poor results observed in the HCV-positive group of patients. Indeed, results of one small study demonstrated the benefit of ICSI over IVF in HCV positive patients (17). It is possible that sperm washing may reduce the HCV viral load prior to insemination or ICSI (14) and may have a favorable effect on pregnancy rates in HCV+ subjects. This needs to be explored in the context of a larger prospective study. In contrast to the findings of the current study, in the only other published study that examined the percentage of apoptotic granulosa cells in six HCV+ and six HCV− controls (25) demonstrated no difference in pregnancy rates in the two groups of patients. However, neither ovarian response nor IVF outcomes were the end points of this study.

CONCLUSION

In conclusion, the present study has demonstrated a poor reproductive outcome in HBV and HCV patients undergoing ART. Despite the methodological shortcomings, the results indicate that these subjects have suboptimal pregnancy rates compared to age matched control subjects. This should be investigated in a larger prospective study.

REFERENCES

1. Lee WM: Hepatitis B virus infection. N Engl J Med 1997;337:1733–1745
2. Centers for Disease Control and Prevention: Disease Burden from Viral Hepatitis A, B, and C in the United States. 2002, Centers for Disease Control and Prevention, Atlanta, GA, U.S.A.
3. Mishra L, Seeff LB: Viral hepatitis, A though E, complicating pregnancy. Gastroenterol Clin North Am 1992;21:873–887
4. Steyaert SR, Leroux-Roels GG, Dhont M: Infections in IVF: Review and guidelines. Hum Reprod Update 2000;6:432–441.
5. Hart R, Khalaf Y, Lawson R, Bickerstaff H, Taylor A, Braude P: Screening for HIV, hepatitis B and C infection in a population seeking assisted reproduction in an inner London hospital. Br J Obstet Gynecol 2001;108:654–656
6. Van den EB: Investigation and treatment of infertile couples: ESHRE guidelines for good clinical and laboratory practice. European Society of Human Reproduction and Embryology. Hum Reprod 1995;10:1246–1271
7. Tedder RS, Gilson RJ, Briggs M, Loveday C, Cameron CH, Garson JA, Kelly GE, Weller IV: Hepatitis C virus: Evidence for sexual transmission. BMJ 1991;302:1299–1302
8. Kao JH, Hwang YT, Chen PJ, Yang PM, Lai MY, Wang TH, Chen DS: Transmission of hepatitis C virus between spouses: The important role of exposure duration. Am J Gastroenterol 1996;91:2087–2090
9. Alter MJ: Hepatitis C virus infection in the United States. J Hepatol 1999;31(Suppl 1):88–91
10. Rooney G, Gilson RJ: Sexual transmission of hepatitis C virus infection. Sex Transm Infect 1998;74:399–404
11. Levy R, Tardy JC, Bourlet T, Cordonier H, Mion F, Lornage J, Guerin JF: Transmission risk of hepatitis C virus in assisted reproductive techniques. Hum Reprod 2000;15:810–816
12. Passos EP, Silveira TR, Salazar CC, Facin AC, Souza CA, Guerin YL, Gratao AA, Cunha-Filho JS: Hepatitis C virus infection and assisted reproduction. Hum Reprod 2002;17:2085–2088

13. Fiore RJ, Potenza D, Monno L, Appice A, DiStefano M, Giannelli A, LaGrasta L, Romanelli C, DiBari C, Pastore G: Detection of HCV RNA in serum and seminal fluid from HIV-1 co-infected intravenous drug addicts. J Med Virol 1995;46:364–367
14. Pasquier C, Daudin M, Righi L, Berges L, Thauvin L, Berrebi A, Massip P, Puel J, Bujan L, Izopet J: Sperm washing and virus nucleic acid detection to reduce HIV and hepatitis C virus transmission in serodiscordant couples wishing to have children. AIDS 2000;14:2093–2099
15. Duffaut M, Valla D: Assisted reproduction and hepatitis C virus infection. Contracept Fertil Sex 1997;25:534–537
16. Pawlotsky JM: Assisted reproduction and viral hepatitis: The virologist's viewpoint. Contracept Fertil Sex 1997;25:530–533
17. Cassuto NG, Sifer C, Feldmann G, Bouret D, Moret F, Benifla JL, Porcher R, Naouri M, Neuraz A, Alvarez S, Poncelet C, Madelenat P, Devaux A: A modified RT-PCR technique to screen for viral RNA in the semen of hepatitis C virus-positive men. Hum Reprod 2002;17:3153–3156
18. Debono E, Halfon P, Bourliere M, Gerolami-Santandrea V, Gastaldi M, Castellani P, Cartouzou G, Botta-Fridlund D, Cau P, Gauthier A: Absence of hepatitis C genome in semen of infected men by polymerase chain reaction, branched DNA and in situ hybridization. Liver 2000;20:257–261
19. Lesourd F, Izopet J, Mervan C, Payen JL, Sandres K, Monrozies X, Parinaud J: Transmissions of hepatitis C virus during the ancillary procedures for assisted conception. Hum Reprod 2000;15:1083–1085
20. Zaidi J, Barber J, Kyei-Mensah A, Bekir J, Campbell S, Tan SL: Relationship of ovarian stromal blood flow at the baseline ultrasound scan to subsequent follicular response in an in vitro fertilization program. Obstet Gynecol 1996;88:779–784
21. Dean NL, Phillips SJ, Buckett WM, Biljan MM, Tan SL: Impact of reducing the number of embryos transferred from three to two in women under the age of 35 who produced three or more high-quality embryos. Fertil Steril 2000;74:820–823
22. Staessen C, Nagy ZP, Liu J, Janssenswillen C, Camus M, Devroey P, Steirteghem AC: One year's experience with elective transfer of two good quality embryos in the human in vitro fertilization and intracytoplasmic sperm injection programmes. Hum Reprod 1995;10:3305–3312
23. Levy R, Bourlet T, Maertens A, Salle B, Lornage J, Laurent JL, Pozzetto B, Guerin JF: Pregnancy after safe IVF with hepatitis C virus RNA-positive sperm. Hum Reprod 2002;17:2650–2653
24. Keck C, Gerber-Schafer C, Clad A, Wilhelm C, Breckwoldt M: Seminal tract infections: Impact on male fertility and treatment options. Hum Reprod Update 1998;4:891–903
25. Sifer C, Benifal JL, Branger M, Devaux A, Brun-Vezinet F, Madelenat P, Feldmann G: Effects of hepatitis C virus on the apoptosis percentage of granulosa cells in vivo in women undergoing IVF: Preliminary results. Hum Reprod 2002;17:1773–1776