AJRI 1994:32:38–42
Printed in the United States of America - all rights reserved.

American Journal of Reproductive Immunology
ISSN 8755-8920

The Frequency of Antisperm Antibodies in the Cervical Mucus of Women With Poor Postcoital Tests and Their Effect on Pregnancy Rates

J.H. CHECK, A. BOLLENDORF, D. KATSOFF, AND J. KOZAK

The Frequency of Antisperm Antibodies in the Cervical Mucus of Women With Poor Postcoital Tests and Their Effect on Pregnancy Rates. Check JH, Bollendorf A, Katsoff D, Kozak J. AJRI. 1994; 32:38–42 © Munksgaard

PROBLEM: To determine the impact of the presence of antisperm antibodies (ASAs) in the cervical mucus of female partners in couples with unexplained poor postcoital tests (PCT). Furthermore, the efficacy of intrauterine insemination (IUI) in these same patients was determined by pregnancy rates (PRs).

METHOD: Pregnancy rates following IUI in patients with infertility and poor postcoital tests, whether the cervical mucus was positive or negative for ASAs, were evaluated.

RESULTS: The 6-month PRs were similar in the ASA negative (40.5%) versus the positive (42.4%) group.

CONCLUSIONS: It appears that the antifertility effect of ASA may be mainly the immobilization of sperm in the cervical mucus, and thus, performing IUI may effectively correct the problem.

Key words:
Antisperm antibodies, intrauterine insemination, unexplained poor postcoital test

J.H. CHECK
A. BOLLENDORF
D. KATSOFF
J. KOZAK
The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/ University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Camden, New Jersey

Submitted September 10, 1993; accepted December 1, 1993.

Address reprint requests to Jerome H. Check, M.D., 7447 Old York Road, Melrose Park, PA 19126.

INTRODUCTION

An unexplained poor postcoital test (PCT), defined as apparently good quality cervical mucus and normal appearing sperm without progressive forward motion (PFM) 8–12 h after intercourse, may be caused by a variety of situations. The possibility exists that (a) the mucus may not be of sufficient quality, despite appearing normal, (b) the sperm, although appearing normal, may possibly have antisperm antibodies (ASAs) in either the seminal fluid or on the sperm surface, and (c) there may be ASAs in the cervical mucus. We previously reported that ASAs in cervical mucus occurred in only 5 of 5,000 (0.1%) cases of unexplained poor PCTs.[1] Our findings are consistent with the report of Jager et al., where a 0.2% incidence was demonstrated.[2]

Kremer et al. reported ASAs in cervical mucus as the cause of a poor PCT in only 1 of 52 couples (2%); they did not mention the criteria for selection for the study, but it probably included females with poor quality mucus.[3] Previously, we found an incidence of 30 of 5,000 (0.6%) having an

unexplained poor PCT, but the mucus was considered to be of good quality.[1]

There have been reports of the successful use of intrauterine insemination (IUI) for non-immunological cervical factor.[4,5] However, because an adverse effect of ASAs has been demonstrated clearly in connection with in vitro fertilization (IVF), the possibility exists that when ASAs are present in cervical mucus they may also be present in follicular fluid and may impair fertilization, even if spermatozoa reach the oocyte.[6–8]

This study was initiated to reassess the incidence of ASAs in cervical mucus as the cause of the unexplained poor PCT. Furthermore, with the improvement in IUI techniques (sperm treatment and timing), the study would compare pregnancy rates (PRs) in those patients with unexplained poor PCTs grouped into positive versus negative for ASAs in cervical mucus.

MATERIALS AND METHODS

Patient Selection

All women registering for infertility at the Pennsylvania out-patient infertility center for the Division of Reproductive Endocrinology and Infertility of the Robert Wood Johnson Medical School at Camden, from January 1, 1986 to December 31, 1990, were prospectively enlisted into the study if there was a poor PCT, despite the presence of apparently normal sperm and cervical mucus. A minimum requirement for enlistment was a hysterosalpingogram or laparoscopy demonstrating bilateral tubal patency.

Definition of Normal Semen and Mucus

World Health Organization (WHO) criteria from 1986 were used to determine normal sperm for the semen parameters as follows: sperm count $\geq 20 \times 10^6$/ml, motility ≥ 50% with forward progression, and >50% normal forms.[9] For the male partner to be enlisted in the study, all normal criterion had to be met. Also, the semen analysis had to be negative for ASAs (<20%) using the direct immunobead test (IBT).[10] A poor PCT was defined as sperm without rapid progressive motility in normal cervical mucus.[9] Normal cervical mucus was based on the Moghissi grading system and a minimum score of 10 was considered normal.[11] The score was derived from the volume of cervical mucus and four variables that described its appearance and characteristics. These variables are consistency, ferning, spinnbarkeit, and cellularity. Each is given a score from 0 to 3. The scores were added, and the maximum was 15. A score greater than 10 was considered to represent good cervical mucus.[11] The PCTs were standardized 8–12 h following intercourse at the time of peak follicular maturation, as previously described.[12]

Indirect IBT for Cervical Mucus

Each mucus sample was evaluated for ASAs using the indirect IBT.[13] The mucus was first liquefied using a mixture of 2mg/ml bromelain in 5% BSA/BWW and then heat inactivated at 56°C for 20 min. The mucus was centrifuged to remove any cells, and the supernatant containing the heat-inactivated cervical mucus was mixed with donor sperm negative for ASAs and then incubated for 30 min at 37°C. The donor sperm was washed three times with 0.5% BSA/BWW. The washed sperm was then mixed with IgG beads on one slide and IgA beads on the other slide. Sperm having ≥50% bead binding were considered positive, 20–49% binding weakly positive, and <20% negative.

Timing of IUI

The timing of IUI was based on pelvic sonography along with serum estradiol (E_2), progesterone (P), and luteinizing hormone (LH) levels.[14] E_2 was measured by solid-phase radioimmunoassay (RIA; Diagnostic Products Corporation, Los Angeles, CA). Semi-automated enhanced luminescence assay (Amersham Corporation, Arlington Heights, IL) was used to measure P. A double-antibody RIA assay (Amersham Corporation) was used to measure LH levels.[14] Daily sera E_2, P, and LH levels were assayed once a follicle with an average diameter of 17 mm was present. The IUI was performed 36–40 h after detection of the LH surge (defined as >50 mIU/ml) unless the serum P was ≥0.9 ng/ml. If this occurred, the IUI was done 12–20 h later. If after a repeat sonogram a clear follicle of approximately the same size was present, the IUI was performed again 12 h later, when possible. There were no cycles where purposeful ovarian hyperstimulation was performed. Only natural cycles were included in this study.

Preparation of Sperm for IUI

The sperm preparation for IUI was a discontinuous Percoll density gradient procedure.[15] The semen was diluted 1:1 with 0.5% BSA Hank's medium. The layers were 1.0 ml of 80%, 70%, 60%, and 50% isotonic Percoll. Up to 2 ml of diluted semen was layered on top, then centrifuged for 20 min at 500*g*.

TABLE I. Correlation of Pregnancy Rates According to ASA Results

ASA	Total patients	No. pregnant	% Pregnant
Negative (< 20% IBT)	74	30	40.5
Positive (≥ 50% IBT)	14	6	42.4

The top semen layer was removed and discarded. Approximately 0.75 ml of the bottom 80% Percoll layer was washed two times with medium and resuspended to 0.2 ml for IUI.

RESULTS

Ninety-one patients were found to have an unexplained poor PCT during the 5 years of the study. The incidence of positive-ASA cervical mucus (≥50% indirect IBT) was 15.3% (14 of 91). The 6-month PRs, as seen in Table I, were very similar in the negative (40.5%) versus positive (42.4%) group. There were three patients with ASA levels of 20–49%, who were not included in either group; however, none of those three conceived. If those three were added to the positive-ASA group, the PR would drop to 6 of 17 (35.3%).

Chi-square analysis showed no significant differences in PRs between the groups ($P = 0.8$). A life table analysis (Table II) is presented for the cumulative probability of pregnancy for the groups.

All positive and weakly positive cervical mucus had sperm-head binding. For IgG in nonconceivers, the mean percent of directed antibody was 43.4% for head and 16.3% for tail, whereas for IgA the mean percent of directed antibody was 97.6% for head compared with 42.3% for tail. For IgG the comparison of the six conceiving patients for the mean percent of directed antibody was 31.6% for head and 18.7% for tail. The corresponding head-directed and tail-directed IgA levels in conceivers was 98.8% and 23.5%, respectively.

There was no statistical difference in motile sperm density (count ($\times 10^6$/ml) × % motility/100) between the conceivers (52.4 ± 38.8 [$\times 10^6$/ml]) and the nonconceivers (52.2 ± 46.2 [$\times 10^6$/ml]), $P < .05$.

DISCUSSION

Previous studies have demonstrated no direct correlation between ASAs in serum, mucus, and uterine

TABLE II. Life Table Analysis

No. of cycles	No. of patients	Pregnancies achieved	Lost to follow-up	Patient months of treatment	Pregnancy rate/month	Cumulative probability of pregnancy
Positive ASA ≥ 50% IBT						
1	14	1	0	13.50	0.07	0.07
2	13	0	0	13.00	0.00	0.07
3	13	2	0	12.00	0.17	0.23
4	11	0	6	8.00	0.00	0.23
5	5	1	0	4.50	0.22	0.40
6	4	2	0	3.00	0.67	0.80
Negative ASA <20% IBT						
1	74	2	0	73.00	0.03	0.03
2	72	4	0	70.00	0.06	0.08
3	68	3	5	64.00	0.05	0.13
4	60	8	6	53.00	0.15	0.26
5	46	5	4	41.50	0.12	0.35
6	37	8	0	33.00	0.24	0.51

and peritoneal fluids.[16] However, the incidence of positive-ASA peritoneal fluid levels is more likely to be present in women who have positive ASA in the cervical mucus than those who do not.[16]

The failure to demonstrate a lower PR in women with poor PCTs and positive ASA in cervical mucus versus those negative for ASAs suggests that one cannot explain the conception failures as an adverse effect of these antibodies on the fertilization process or as impairing progression of the sperm to the fallopian tubes. The possibility does exist, however, that some unknown male factor leads to the poor PCT in those patients negative for ASAs, but with unexplained poor PCTs; thus, one might still have different reasons to explain the IUI failures. Therefore, the theory that some cases fail to conceive because of ASAs in their cervical mucus has not been disproven and may be secondary to follicular fluid antibodies that impair fertilization; but the data cannot support this hypothesis either.

It would be interesting to further evaluate those patients with unexplained poor PCTs who fail to conceive despite IUI, by performing IVF; this future study would compare the fertilization rates and PRs in ASA-positive versus -negative patients.[17]

Furthermore, future studies will also prospectively compare PRs following IUI for poor PCTs where mucus is of good quality versus when quality is poor, to better determine if there is something more than mere impediment of sperm reaching the fallopian tubes for fertilization when there is an unexplained poor PCT.

Although our study did not include IVF, previous studies have. At least two studies found a reduced fertilization rate when serum of females who were positive for ASA was added to oocyte culture fluid.[7,8] The difference in our study is that the culture media uses BSA instead of maternal serum. The concentration of antibody that the sperm are exposed to in the petri dish may be much higher in the uterine cavity following IUI, and thus could decrease IVF pregnancy rates but not affect IUI PRs. Substituting BSA for maternal serum as the protein source should negate these poor IVF results. All of our positive serum samples had head-directed antibodies, so we cannot comment on the relative importance of head- versus tail-directed antibodies.

CONCLUSIONS

The presence of ASAs in cervical mucus is found only in a minority of women with unexplained poor PCTs. Treatment with IUI seems to be effective for those women, whether negative or positive for ASA.

REFERENCES

1. Check JH, Nowroozi K, Adelson HG, Bollendorf A, Chern R, Press M. An in vivo technique for screening immunologic factors in the etiology of the unexplained poor postcoital test. Int J Fertil. 1990; 35:215–221.
2. Jager S, Kremer J, de Wilde-Jansen IW. Are sperm immobilizing antibodies in cervical mucus an explanation for a poor postcoital test? Am J Reprod Immunol. 1984; 5:56–60.
3. Kremer J, Jager S. The significance of antisperm antibodies for sperm-cervical mucus interaction. Hum Reprod. 1992; 7:781–784.
4. Check JH, Chase JS, Spirito P. Efficacy of intrauterine insemination versus sexual relations versus intracervical insemination for treatment of cervical factor infertility. Am J Gynecol Health. 1991; 5:11–17.
5. Hull ME, Magyar DM, Vasquez JM, Hayes MF, Moghissi K. Experience with intrauterine insemination for cervical factor and oligospermia. Am J Obstet Gynecol. 1986; 154:1333–1338.
6. Clarke GW, Heleh C, Joh SH, Cauchi MN. Sperm antibodies, immunoglobulins, and complement in human follicular fluid. Am J Reprod Immunol. 1984; 5:179–181.
7. Clarke GN, Lopata A, Johnson WI. Effect of sperm antibodies in females on human in vitro fertilization. Fertil Steril. 1986; 46:435–441.
8. Mandelbaum SL, Diamond MP, DeCherney AH. Relationship of antisperm antibodies to oocyte fertilization in in vitro fertilization embryo transfer. Fertil Steril. 1987; 57:644–651.
9. World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interaction. Cambridge: Cambridge University Press, 1987.
10. Bronson R, Cooper G, Rosenfeld D. Sperm antibodies: Their role in infertility. Fertil Steril. 1984; 42:171–183.
11. Moghissi KS. Post-coital test: Physiologic basis, technique and interpretation. Fertil Steril. 1976; 24:117–129.
12. Check JH, Nowroozi K, Wu CH, Liss J, Dietterich C. The use of pelvic sonography and serum estradiol and progesterone assays in diagnosis and treatment of cervical factor. Infertility. 1986; 9:247–256.
13. Clarke GN, Stojanoff A, Cauchi MN, McBain JC, Speirs AL, Johnston WIH. Detection of antispermatozoal antibodies of IgA class in cervical mucus. Am J Reprod Immunol. 1984; 5:61–65.
14. Check JH, Peymer M, Jairaj P. The relationship of single daily periovulatory estradiol progesterone and luteinizing sera hor-

mone levels in natural cycles used for better prediction of timing of ovulation. Gynecol Obstet Invest. In press.

15. Iizuka R, Kuneko S, Kobanawa K, Koboyashi T. Washing and concentration of human semen by Percoll density gradients and its application to AIH. Arch Androl. 1988; 20:117–124.
16. Stern JE, Dixon PM, Manganiello PD, Brinck-Johnsen T. Antisperm antibodies in women: Variability in antibody levels in serum, mucus, and peritoneal fluid. Fertil Steril. 1992; 58:950–958.
17. Check JH, Nowroozi K, Chase J, Nazari A, Braithwaite C. Comparison of pregnancy rates following in vitro fertilization-embryo transfer between the donors and the recipients in a donor oocyte program. J Assist Reprod Genet. 1992; 9:248–250.