Preliminary Communication

IN-VITRO FERTILISATION OF OOCYTES FROM WOMEN WITH SERUM ANTISPERM ANTIBODIES

JOHN L. YOVICH JAMES D. STANGER
DAVID KAY BARRY BOETTCHER

Department of Obstetrics and Gynaecology, University of Western Australia, King Edward Memorial Hospital, Subiaco, Perth, Western Australia; PIVET Laboratory, Cambridge Hospital, Leederville, Perth; and Department of Biological Sciences, University of Newcastle, Shortland, New South Wales

Summary Five women whose infertility was believed to be related primarily to their serum antisperm antibodies underwent in-vitro fertilisation and embryo transfer. Follicle growth was stimulated with clomiphene citrate, sometimes combined with human menopausal gonadotropin, and ovulation was triggered off with human chorionic gonadotropin. 20 oocytes were collected from the five patients, and 15 of these were fertilised in the presence of donor serum. All embryos developed to morphologically normal 2-cell and 4-cell embryos. All five women proceeded to embryo transfer, and two became pregnant.

INTRODUCTION

ANTISPERM antibody activity in the serum or cervical mucus of the female is thought to account for the failure of conception in 7–17% of infertile couples.[1] Treatments such as condom therapy, abstinence, and steroid suppression therapy have been applied with generally poor results.[2] Where cervical hostility due to antisperm antibodies exists without humoral immunosurveillance, some success has been achieved with intrauterine insemination,[2] but not when serum antisperm antibody levels are high. It is therefore of interest to know whether oocytes from patients with antisperm antibody activity are capable of fertilisation. We present here the results of in-vitro fertilisation of oocytes from five patients with antisperm antibody activity in their serum.

MATERIALS AND METHODS

Patients.—Five women with antisperm antibody levels in their serum were admitted into the in-vitro fertilisation and embryo transfer (IVF/ET) programme at PIVET Laboratory, Perth.[3] The four who had otherwise normal reproductive profiles had spent varying periods of time undergoing conventional treatment for infertility due to the antibodies; the fifth had not been treated because she also had bilateral occlusive tubal disease. Her sperm antibody levels had been measured during the work-up phase because of negative postcoital tests. Two patients were admitted for a second attempt 3–5 months after the initial aspiration. As with all IVF patients, the follicles were stimulated with 150 mg clomiphene daily from days 2–6 of the cycle; two patients were also given human menopausal gonadotropin (hMG; 'Pergonal', Serono, Italy) 3 ampoules daily on days 6, 7, and 8. All patients were monitored for follicle development by ultrasonography, cervical scores, and serum hormonal profile (17β oestradiol, progesterone, and luteinising hormone). Ovulation was triggered off by 5000 units of human chorionic gonadotropin (hCG) administered intramuscularly when the diameter of the leading follicle was 1·8 cm or greater and the average oestrogen production per large follicle was about 1500 pmol/l.

Oocyte collection and embryo culture.—The follicle was aspirated 35 h after the administration of hCG. The oocytes were collected in modified 'Tyrodes T6' medium[4] containing 30 units of heparin per ml and 15% donor serum previously de-activated by heating at 37°C for 30 min. The oocytes were washed three times and incubated in the same medium containing 15% donor serum without heparin for 2–3 h before the addition of spermatozoa. Donor, rather than maternal, serum was used because these patients possessed serum antisperm antibodies. Spermatozoa were added at a concentration of 1×10^5 to 3×10^5 motile spermatozoa/ml 38–40 h after the hCG injection. The gametes were incubated overnight in an environment of 5% CO_2, 5% O_2 and 90% N_2 at 37°C and examined for evidence of fertilisation 16–20 h after the addition of spermatozoa. (If fertilisation had to be confirmed, repeat insemination would have been done with freshly collected semen, but this was not required for any of the five cases.) The oocytes were washed free of cumulus and coronal cells by the use of a finely drawn micropipette, and fertilisation was confirmed by the presence of two pronuclei and usually two polar bodies in the perivitelline space. The embryos were then transferred to 1 ml of fresh medium containing 15% donor serum and cultured for a further 24 h before being returned to the uterus by cervical transfer when the embryos were generally at the four-cell stage.

Antibody studies.—Antisperm antibody activity was assessed by the methods of Rose et al.[5] The antibodies reported were sperm agglutination antibodies as assessed by the gelatin agglutination test (GAT) and tray slide agglutination test (TSAT) and immobilising antibodies as assessed by the complement-dependent sperm immobilisation test (SIT).[6] In the two patients who conceived serum collected at the time of ovum aspiration was also assessed for anti-spermatozoal antibody activity. The remaining three women were known to have raised antibody levels during the preceding 2 months.

RESULTS

The clinical histories of the five patients are given in table I. One patient had low levels of agglutinating serum antibodies whilst the other four had high and persisting levels of both agglutinating and immobilising serum antibodies. The results of oocyte collection, fertilisation, and embryo development are summarised in table II. The number of oocytes obtained from the five patients varied considerably

TABLE I—PATIENT HISTORY

Patient	Duration of infertility (yr)	Known duration of antisperm antibody activity (mo)	Most recent assay			Previous treatment		
			GAT	SIT	TSAT	Condom	Steroid	AIH
1	4	14	1/40	7	17/100	+	–	+
2	5	14	1/20	10	52/100	+	+	+
3	7	15	1/160	11	27/100	+	+	+
4	4	16	Neg.	4	Neg.	+	+	+
5	5	1	Neg.	Neg.	52/100	–	–	–

GAT is expressed as the highest dilution showing macroagglutination; SIT is the proportionate percentage of immotile sperms in the presence of test serum compared with control; TSAT is expressed as the number of microagglutinates per 100 motile sperms.

TABLE II—IVF DETAILS

Patient	Number of oocytes (number mature)	Number of fertilised ova (number mature)	Number developing	Stage at transfer	Outcome
1 (a)	4 (4)	3 (3)	3	3×2 cell	Nil
(b)	5 (5)	5 (5)	5	5×4 cell	Nil
2	8	5 (5)	5	5×4 cell	Preg
3 (a)	5 (5)	4 (4)	4	4×4 cell	Nil
(b)	4 (4)	4 (4)	4	4×4 cell	Preg
4	3 (2)	2 (2)	2	2×4 cell	Nil
5	3 (3)	3 (3)	3	3×4 cell	Nil

and related partly to the nature of follicle stimulation. In patients 1 (attempt b), 2, and 3, in whom follicle stimulation was augmented by hMG administration, the number of developed follicles, and hence the number of oocytes obtained, were considerably increased.

In patients 1, 3, 4, and 5, whose average follicle diameter was greater than 1·8 cm, fertilisation was achieved in 21 of the 24 oocytes (87%; table II). In patient 2, whose average follicle diameter was less than 1·8 cm, 8 oocytes were obtained. However, only 5 showed a fully expanded cumulus matrix; the remaining 3 possessed a smaller, compacted cumulus matrix which is associated with follicle immaturity. All 5 mature oocytes fertilised, whereas the other three showed no evidence of fertilisation despite an extra 24 hours' incubation. All the zygotes developed to either morphologically normal 2-cell or 4-cell embryos by 44 h post insemination. Spermatozoa retained their motility over the 20 hours' incubation and all cumulus masses were fully dispersed at this time, except the three small cumulus masses considered immature. Two patients became pregnant, as diagnosed by rising β hCG levels and the detection of a fetal heart beat within an intrauterine gestational sac on ultrasonography.

The two women who became pregnant had serum GAT titres of 40 and SIT titres of 16.

DISCUSSION

Most of the mature oocytes obtained from the follicles of patients exhibiting high levels of serum antisperm antibody activity were fertilised. The cumulus masses had first been washed free of follicular fluid. All the embryos developed into morphologically normal 2 or 4 cell embryos. Two implanted and are developing normally. The implication of these findings is that IVF/ET should be considered as a means of treatment in those patients whose infertility relates to antisperm antibodies.

Although follicle fluid contains gammaglobulins at levels of 50–80% of serum activity, antisperm antibody activity in follicle fluid has not been reported.[6,7] Nevertheless, in recent years reports have suggested that antisera raised against spermatozoa inhibit fertilisation in vitro. An early report[8] indicated that pre-incubation of spermatozoa with antisera raised against either ejaculated or epididymal rabbit spermatozoa inhibited in-vitro fertilisation of rabbit ova. Later work[9] suggested that rabbit antihamster sperm antibodies did not inhibit the acrosome reaction and motility of hamster spermatozoa, but inhibited cumulus dispersion and sperm binding to the zona pellucida as well as egg fusion. The site of antibody binding may be to molecules found on the surface of either the plasma membrane or the inner acrosomal membrane, and the process may involve the recognition of complementary units on either the plasma membrane of the oocyte or the zona pellucida.[10] Antibodies directed against the sperm head are primarily responsible for the inhibition of in-vitro fertilisation of zona-free hamster oocytes by human sperm.[11] Further studies to assess antibody activity in uncontaminated follicle fluid aspirates are planned, but the results presented here suggest that if any intrafollicular antibody activity was present, it was sufficiently diluted by the normal procedures involved in IVF to levels that did not inhibit fertilisation. Furthermore, sperm characteristics associated with fertilisation, such as motility or hyaluronidase-dependent cumulus dispersal, were not affected. The high rate of fertilisation suggests that oocytes previously bathed in antisperm antibodies are not permanently affected by this exposure.

The rate of ovum penetration and embryo development would appear to be more a function of oocyte maturity than of immunological activity even though the pattern and duration of immune reactions differed amongst the five patients (table I). Also, the two patients who became pregnant had antisperm antibody activity at the time of IVF/ET.

Infertility due to immunological reasons remains a poorly characterised area possibly because of difficulty in relating agglutination titres to infertility, but the results from this small study of five patients indicate the potential of in-vitro fertilisation as a means of treating infertility attributable to sperm antibodies in the female.

Correspondence should be addressed to J. L. Y. 1/442 Barker Rd, Subiaco, Perth, Western Australia 6008.

REFERENCES

1. Jones WR. Assessing immunity to sperm. *Clinical Reprod Fertil* 1982; **1:** 3–12
2. Jones WR. Immunology of infertility. *Clin Obstet Gynaecol* 1981; **8:** 611–38.
3. Yovich J, Puzey A, De'Atta R, Roberts R, Reid S, Grauaug A. In vitro fertilisation pregnancy with early progestogen support. *Lancet* 1982; ii: 378–79.
4. Whittington D. Culture of mouse ova. *J Reprod Fertil* 1971; **14** (supp): 7–21.
5. Rose N, Hjort T, Rumke P, et al. Techniques for the detection of iso- and auto-antibodies to human spermatozoa. *Clin Exp Immunol* 1976; **23:** 175–99.
6. Edwards RG. Follicular fluid. *J Repod Fertil* 1974; **37:** 189–19.
7. Velazquez A, Reyes A, Changay J, Rosado A. Amino acid and protein concentrations of human follicular fluid. *Fertil Steril* 1977; **28:** 96–100.
8. Russo T, Metz CB. Inhibition of fertilisation in vitro by treatment of rabbit spermatozoa with univalent iso-antibody. *J Reprod Fertil* 1974; **28:** 211–15.
9. Tzartos SJ. Inhibition of in-vitro fertilisation of intact and denuded hamster eggs by univalent anti-sperm antibodies. *J Reprod Fertil* 1979; **55:** 447–55.
10. Yanagimachi R, Okada A, Tong K. Sperm auto-antibodies and fertilisation: II Effects of anti-guinea pig sperm auto-antibodies on sperm-ovum interactions. *Biol Reprod* 1981; **24:** 512–18.
11. Aitkens RJ, Rudak EA, Richardson DW, Dor J, Djahanbakhch O, Templeton AA. The influence of anti-zona and anti-sperm antibodies on sperm-egg interactions. *J Reprod Fertil* 1981; **62:** 597–606.

Reviews of Books

Health Surveys in Practice and in Potential

A Critical Review of their Scope and Methods. Ann Cartwright. Oxford: Oxford University Press, and London: King Edward's Hospital Fund. 1983. Pp 227. £8.50.

THIS interesting book originated from a request by the Social Science Research Council (now re-named the Economic and Social Research Council) to review the content and methods of surveys in the health and health-care field. No small request. Ann Cartwright is candid in her foreword, where she acknowledges the difficulty and scale of her task and how she had to be selective in her review. The result is a compact and pithy critique. It gives various examples of health surveys and is sufficiently comprehensive to include the main techniques and the problems that arise in such surveys. She approaches her task by considering the kinds of questions that health surveys have sought to answer and then discussing the various survey methods, some unusual and innovative, that have been used. The bulk of the book discusses surveys of health and sickness, the nature of disease, assessment of need, use of services, effects and side-effects of care, acceptability of services, and organisation of care. The book ends with chapters on various methodological issues, ethics, and use and potential of surveys. Over 300 articles and sources are cited, and 50 or more studies.

This is not a textbook on how to do field surveys; there are a number of these already. Nor is it a comprehensive review of health surveys such as that by Alderson and Dowie (*Health Surveys and Related Studies,* Pergamon Press, 1979). Where this book differs from other texts is in the combination of a critical discussion of specific surveys with that of the methodology employed.

Probably the most interesting chapter for the practitioner, and I hope also for potential commissioners of such surveys, is the last. It discusses, far too briefly in my view, the contribution that health surveys have made, and what their potential may be. Cartwright is a shade too optimistic in her assessment of the extent to which the