The immunobead test as a sperm antibody detector

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Summary

The occurrence of sperm antibodies on the surface of sperm can be demonstrated by several methods. A study to evaluate the immunobead (IB) test as a laboratory procedure during the detection of sperm antibodies among infertile males showed an overall prevalence of 6.8% and 5.8% for IgG and IgA respectively — convincing evidence that the IB test can be employed as a sperm antibody detector.

Sperm agglutination and immobilisation in the semen of infertile males have been widely used as diagnostic evidence for sperm auto-immunity. These results do not necessarily indicate that sperm in the reproductive tract are coated with antibodies. It is further well recognised that the titre in semen is generally lower than in serum. Sometimes it is higher and we may then conclude that there is local production of the antibody. In most cases, however, the antibody appears in seminal plasma by transudation from the blood. Friberg found that most of the antibody in seminal plasma was of the IgA class but that some was IgG. The concentrations of these immunoglobulins are approximately 1% of those in the serum.

However, antibody can be shown to be attached to sperm cells without auto-agglutination in the semen. The occurrence of sperm antibodies on the surface of sperm can be demonstrated by several methods. One of these methods, devised by Clarke et al.,1 is a rosette-type assay using immunobeads (IBs) for the detection of IgG, IgA and IgM antibodies on sperm surfaces. The principal aim of this study was: (i) to evaluate the IB test as a laboratory tool during the detection of sperm antibodies among infertile males; and (ii) to determine the prevalence of sperm antibodies as determined by the IgG and IgA-IB tests.

Subjects and methods

Included in the study were 126 consecutive men who visited the laboratory for fertility investigations. All patients were subjected to a full semen analysis according to World Health Organisation criteria. As well as the semen analysis all patients were screened for spermatozoal antibodies using the IB technique for IgG and IgA.

A 4% solution of human serum albumin (HSA) was prepared. From a commercial 25% solution of HSA (NYBCEN, New York) 2.0 ml was transferred to a container and 10.5 ml of normal saline was added to give a 4% solution of HSA.

Rabbit anti-human IBs (Bio-Rad Laboratories, Richmond, California), namely IgG (γ-chain) and IgA (α-chain), were used in testing. A volume of 5.0 ml of Baker’s buffer was added to the 50 mg of IBs in each bottle to resuspend them. From each suspension 1.0 ml was taken and washed three times with 4% HSA at 2 300 rpm for 5 minutes. Each of the final pellets was resuspended in 2.0 ml of 4% HSA to give a 5 mg/ml suspension of IBs. There were thus two reagents to use in the testing, namely IgG-IB and IgA-IB.

The semen samples used in the study were provided by normal healthy donors with sperm counts ranging from 21 x 10⁶/ml to 211 x 10⁶/ml and a motility of at least 50%. The count was eventually adjusted to 25 x 10⁶/ml. The most important parameter concerning the donor semen was the forward progression rate, which had to be at least 2+ on a scale of 1–3. Estimation of forward progression varies from one laboratory to the next, but we used the following scale: 0 = no movement; 1 = vibrations only; 2+ = occasional slight forward movement; 2 = erratic movements; 2+ = slow directly forward movements; 3 = fast directly forward movements.

The liquefied semen was washed three times with 4% HSA at 2 300 rpm for 5 minutes. The final pellet was resuspended in the amount of 4% HSA needed for the test. Sperm was then counted with a Makler Counting Chamber (Sefi Instruments, Haifa, Israel), and if necessary the count was adjusted to the final level of 25 x 10⁶/ml. The IgG/IgA-IB test results were evaluated according to the following scoring system: (i) doubly positively — if in a test only occasionally a mixed agglutinate involving a motile spermatozoon with an adhering IB was seen; (ii) positive — with the percentage of spermatozoa involved in mixed agglutinates expressed in multiples of 10, ranging from <10% (but more than 0.001% positive) to >90% (but less than almost 100%) of the motile spermatozoa attached to IBs; and (iii) strongly positive — if 100% or almost 100% of the motile spermatozoa were attached to the IBs.

In order to obtain an unbiased sample of patients, we included in this study only those patients having their first semen analysis within the stipulated period of the study. This criterion effectively eliminated any man previously diagnosed as having sperm auto-immunity or those suffering from low-motility sperm and oligozoosperma.

Results

Twenty-four semen samples (19%) were not suitable for antibody testing because of severe oligozoosperma and/or low motility values. With the IgG-IB test 95 of the 102 tested samples (93%) were negative and 7 (6.8%) positive. The IgA-IB test on the other hand showed 98 (96%) to be negative while 6 (5.8%) were positive.

Discussion

The extent to which sperm penetration into cervical mucus is impaired is found to correlate closely with the proportion of spermatozoa with surface-bound immunoglobulins. During a previous study11 we found good correlation between the results...
of the IB and sperm cervical mucus contact (SCMC) tests. There is reliable evidence that IgA-class sperm-specific antibodies are the main mediators of poor sperm penetration of mid-cycle cervical mucus associated with strong shaking reaction during SCMC testing. This phenomenon stresses the importance of sperm surface immunoglobulin detector tests. In this study the IgA and IgG-IB tests revealed a prevalence of 5-7% among consecutive male patients. The results compare excellently with other reports and we are convinced that the IB test can be employed as a sperm antibody detector. We compared the results of the IgG mixed antiglobulin reaction (MAR) with those of the IgG and IgA-IB tests (E. Pretorius, D. R. Franken, S. Shulman, L. Stevens — unpublished data). During that study we regarded the IB test as a specific and reliable test for the detection of sperm antibodies.

REFERENCES

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'n Kort kritiese beskouing

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The correct measurement of various blood pressures in clinical practice is of obvious importance. A method by which the frequency response and damping of various transducers and catheters can be determined is discussed and reference is made to values obtained in the validation of certain catheters.

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