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DEBATE continued

Antisperm antibodies

Use of the mixed agglutination reaction (MAR) test using latex beads

A.Mahmoud and F.Comhaire[1]

Department of Internal Medicine, State University Hospital, 185 De Pintelaan, B-9000 Gent, Belgium

[1]To whom correspondence should be addressed

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The purpose of a debate contribution is to stir up controversy and discussion, and a recent paper (Helmerhorst *et al*., 1999) has done exactly that. However, aside from several criticisms already highlighted (Bronson, 1999), it seems that the viewpoint of Helmerhorst *et al*. (1999) is biased, among other things by the selection of references.

Both Helmerhorst *et al*. (1999) and Bronson (1999) fail to mention the mixed agglutination reaction (MAR) test using latex beads instead of red blood cells (SpermMar test; Fertipro, Beernem, Belgium); (Jager *et al*., 1978; Vermeulen and Comhaire, 1983). In contrast to the polyacrylamide beads used in the immunobead test (MacMillan and Baker, 1987), the latex particles have a uniform diameter of 2 μm. In the SpermMar test for immunoglobulin (Ig)G, the particles are coated with IgG, and they do attach to the region of the spermatozoa where the antisperm antibodies are located. This attachment is obtained by adding strong and highly specific anti-IgG to the mixture of fresh, untreated spermatozoa and latex beads (Rasanen *et al*., 1994). There are also SpermMar tests for IgA and IgM.

The MAR test using latex particles does not require preliminary separation of the spermatozoa, as against the Immunobead test that requires washing the spermatozoa free of seminal plasma. As a result, the former test is easier and faster, and it requires less volume of ejaculate (10–12 μl). It can also be applied in semen samples with a lower motile sperm concentration than the Immunobead test (Ackerman *et al*., 1988; Rasanen *et al*., 1994).

A comparison between the Immunobead and SpermMar tests has shown a high degree of agreement, but the former was less accurate than the latter (MacMillan and Baker, 1987; Ackerman *et al*., 1988; Hellstrom *et al*., 1989; Khoo *et al*., 1991; Raja *et al*., 1992; Andreou *et al*., 1995). Also, the SpermMar test for IgA detects only secretory IgA in semen, whereas the Immunobead test sometimes reacts with non-secretory IgA present in serum (Andreou *et al*., 1995).

The diagnostic accuracy of the direct (in semen) and indirect (in serum) SpermMar test has been assessed in comparison with several other tests. This comparison included the tray agglutination test (Kay and Boettcher, 1992; Paschke *et al*., 1994; Sedor and Hirsch, 1994), the tube slide agglutination test (Kay and Boettcher, 1992), the gelatin agglutination test (Kay and Boettcher, 1992), the sperm immobilization test (Kay and Boettcher, 1992), the adenosine triphosphate release cytotoxicity test (Hinting *et al*., 1988), the flow cytometry test using monoclonal antibodies (Nikolaeva *et al*., 1993; Rasanen *et al*., 1994), and the sperm–cervical mucus contact test (Paschke *et al*., 1994).

Furthermore, a large number of peer-reviewed publications originating from various reference laboratories confirm the clinical significance of the SpermMar test (Stedronska and Hendry, 1983; Bronson *et al*., 1984; Grobler *et al*., 1984; Scarselli *et al*., 1985; Meinertz, 1987; Comhaire *et al*., 1988; Hellstrom *et al*., 1989; Dondero *et al*., 1991; Khoo *et al*., 1991; Raja *et al*., 1992; Devine *et al*., 1993; Sinisi *et al*., 1993; Paschke *et al*., 1994; Sedor and Hirsch, 1994; Ombelet *et al*., 1997; Evans *et al*., 1998), but testing of seminal plasma or serum was found to be less relevant for fertility assessment (Eggert-Kruse *et al*., 1995; Rasanen *et al*., 1996).

In a study of 200 couples, men whose motile spermatozoa presented binding of particles were significantly more likely to be infertile [odds ratio (OR) 3.59; 95% confidence interval (CI) 1.13–11.41], and none of the fertile controls tested >40% positive in the SpermMar test for IgG ($\chi^2 = 6.78$, $P = 0.034$) (Comhaire *et al*., 1988).

We (Mahmoud *et al*., 1996) and others have recorded the spontaneous treatment-independent pregnancy rate being reduced if the male partner presents 40% or more positive reaction in the direct SpermMar test (1.7% per couple-cycle, or 10 pregnancies among 70 couples followed up during 601 couple-months). This confirms previous observations of a 10 year follow-up study of 264 couples by Rumke *et al*. (1974), indicating reduced fecundibility among men with normal sperm concentration and serum agglutinin titre >1/32 (OR = 0.23, CI = 0.096–0.527). In contrast, the presence of sperm agglutinins in serum had no influence on the pregnancy rate in cases with oligo- or azoospermia (OR = 1.33, CI = 0.23–7.61).

The diagnosis of immunological male infertility implies that treatment by assisted reproduction is indicated. It has been demonstrated convincingly that use of a condom (in case of female immunological infertility), antibiotics, testosterone, and immunosuppressive corticosteroids are useless and obsolete (for review, see Hinting and Mahmoud, 1996).

We have recommended intrauterine insemination (IUI), which must be performed 37–42 h after an injection of 10 000 IU of human chorionic gonadotrophin (HCG) in a cycle where 150 IU of human menopausal gonadotrophin (HMG) was

given on days 8 and 12, without clomiphene stimulation (Hinting and Mahmoud, 1996). The ejaculate must be collected in oocyte culture medium to which 3% of human serum albumin is added to act as an antioxidant. The sample must immediately be centrifuged over a density gradient column and resuspended in 0.25 ml of culture medium for intra uterine insemination.

As a result of this procedure, 12 pregnancies were attained in 36 couples inseminated during 139 cycles, with pregnancy rate of 8.6% per cycle. This pregnancy rate is significantly higher than the one of untreated couples (OR for number of couples attaining pregnancy 3.00, CI 1.14–7.86, OR for pregnancy rate per cycle 5.58, CI = 2.36–13.21) (Mahmoud *et al*., 1996). IUI was found particularly effective in cases with antisperm antibodies, independent of their Ig subclass and localization, when conventional sperm characteristics were better than the reference values suggested in the World Heath Organization (WHO) laboratory Manual (WHO, 1999). Cases with abnormal conventional sperm characteristics were less successful, and those with severe oligo- and/or astheno- and teratozoospermia should rather be referred for in-vitro fertilization (IVF)–intracytoplasmic sperm injection (ICSI) (Lahteenmaki, 1993; Ombelet *et al*., 1997).

Whereas some evidence-based medicine adepts may still feel uncertain about the diagnostic methods, the clinical importance, and the therapeutic approach of male immunological infertility, the majority of clinicians dealing with infertile couples are convinced of the success of the strategy outlined above. Their conviction is based on the outcome of cohort studies, on the calculation of effective cumulative pregnancy rates, and on the derived data regarding time to pregnancy (Mahmoud *et al*., 1996). In medicine in real life, this evidence is probably sufficient. Hence, it seems ethically unacceptable to consider future randomized prospective studies.

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