Immunology of infertility

Current Research in Obstetrics and Gynaecology

Discussed by Mr Geoffrey Chamberlain and Mr W F Hendry, St Bartholomew's Hospital.

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<Opening titles>

<Mr Chamberlain and Mr Hendry seated for discussion with a display board for diagrams placed between them>

<Chamberlain to camera>

Hello and welcome to another edition of our series in contemporary research in obstetrics and gynaecology. This is being brought to you jointly by the University of London Audio-Visual Centre and the Blair Bell Research Society. Today we're going to discuss the difficult problem of the immunology of infertility and we have to lead us in this Mr Bill Hendry, my colleague and a consultant in male fertility at the combined fertility clinic at Chelsea Hospital for Women. Bill.

<Hendry to camera>

Thank you, Geoffrey. Sperms are antigenically different, both from the man who makes them and from the female that receives them. This is because the genetic material that we receive from our mother and our father is altered before the gametes are produced. As a result, the sperms normally have to be shielded from the bodies immune defence mechanisms. It's been known since about 1900 that injection of sperms into experimental animals causes production of antibodies and if the sperms are mixed with the appropriate adjuvant, the reaction can be so severe as to stop all sperm production in the male animal.

Now, it's been suspected for many years that antisperm antibodies could be a cause of infertility, but there have been many problems, both with the tests that have been performed, with their correlation with each other and with their correlation with the patient's fertility. Today, we're going to look at the various tests that are available to see how they correlate with each other and try and decide how far they are responsible for development of infertility in a barren couple.

<Hendry narrates over diagram illustrating method of production of antibodies>

Now, the first illustration that we have here shows a schematic representation of the method of production of the antibodies. Here we have the testicle; the sperm production here is normally shielded from the immune cell, which has been represented here. If the antigen is allowed to escape – for example, after inflammatory disease or following vasectomy, which is a good example – circulating antibodies may be found in the blood. Here they are usually in the form of IgG. Now, sometimes but not always, antibodies may also be present in the seminal plasma where they are chiefly in the form of IgA. It is thought that sometimes the immune response can be cellular in nature and that this can depress further sperm production, but this is by no means certain. And the possibility has been raised that sometimes antisperm antibodies may cause immune complex diseases. It is also

possible that the female may form antibodies against sperms that she received, but obviously the female's exposure to the sperms will be far more intermittent than the man who is constantly exposed to them being produced in the testicle.

Now, one of the snags has always been that the various tests to determine the presence or absence of antibodies have correlated poorly with each other and with the patient's fertility, so let's first of all look at the various tests that are available.

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<Hendry briefly to camera and then over table listing antibody tests in investigation for fertility>

This shows, on the left side, the names of the people that have invented the various tests; here are some abbreviations that have been agreed by the World Health Authority for their terminology, and here are the materials that the tests are suitable for testing. The first group of tests, agglutination tests, depend on the action of the patient's serum on normal donor sperms. In the presence of a positive result, they cause the sperms to clump together or agglutinate. Kibrick, Franklin-Dukes, the Friberg tests, which have been abbreviated to gelatine agglutination, tube-slide agglutination and the tray-agglutination tests. You can see that these microscopic tests, as well as being suitable for testing serum, are also suitable for testing seminal plasma and cervical mucus, and we will see later that this is very important.

Quite different is the immobilisation test of Isojima. This depends on the serum which is used with complement added to normal donor sperms, which stops the motion completely. Isojima's test, which is abbreviated as shown <*SIT(I)*>, only suitable for testing serum at the moment. Now, the immunofluorescent test has caused a lot of trouble. It depends on taking serum, mixing it with normal donor sperms and then testing it for attached IgG or IgA with fluorescent antibodies. It has a very high proportion of false positives, it has caused a lot of confusion in clinical practice and we have, therefore, said it is only suitable for research.

A new test, which is particularly valuable, or may be, is the mixed antiglobulin reaction of Jager and Kremer, which is done in the same that the Coombs' test is done to detect sensitised red cells. This is used to detect sensitised sperms by mixing them with red cells to see whether they inter-react.

<Hendry to camera and then over table showing types of agglutination in different agglutination tests>

Now, part of the confusion between the different tests has been caused by the fact that there are different types of antibodies and the different types of antibodies have different effects. For example, some antibodies cause the sperms to clump together head to head, H-H. Others cause the sperms to clump together tail to tail, and some even cause them to clump together tail tip to tail tip. Men, by and large, form tail-agglutinating antibodies, whereas women, by and large, produce head-agglutinating antibodies. And you can see that the different tests have different sensitivities for the different sorts of antibodies. So naturally, men with tail-agglutinating antibodies are best tested by the Kibrick or GAT test, whereas women, who produce head-agglutinating antibodies, are best tested by the tray-slide or the tray-agglutination, the tube-slide or the tray-agglutination tests, as you see here.

It is thought at present that the TAT test, being equally sensitive to the different sorts of antibodies and allowing microscopic examination of the agglutinated clumps, is probably the most applicable for general use today.

<Hendry to camera and then over table comparing titres of agglutination tests in men with those in women>

Now, men, being constantly exposed to the sperms as antigens, tend to get higher concentrations of antibodies in the serum. This is detected by testing the serum in serial dilution to find the titre, or dilution, at which the activity disappears. And here we see some examples. At titre, the serum has been diluted 1024 times before the activity disappears. Notice how much higher this is in men compared to the relatively low titres that may be expected in women. Notice also that the various tests do not

correlate terribly well with each other, which is, of course, what we would expect remembering that they are testing different sorts of antibodies. And in order to be sure of reaching the right diagnosis, we should perform a battery of tests in order to pick up the different types of antibodies.

<Hendry to camera and then over table of results showing correlation between GAT titre of normospermic men and resultant pregnancy>

Now, in deciding what is a significant titre of antibodies, it is necessary to study the natural behaviour of a man with antisperm antibodies when no treatment is given at all. Fortunately, Rumke in Holland followed 132 men with normal sperm counts for 10 years, gave them no treatment at all and waited to see what happened. As you can see, with a titre of less than 32, almost 50% produced pregnancies. Whereas, as the titre or dilution was increased so the number of pregnancies produced fell off. And as a result of this very careful study, a titre of 1 in 32 is generally taken as the lower limit of significance for antisperm antibody testing in men.

I regret to say that no such study has been done in women to the best of my knowledge and as a result, the significance of titres in women is far less clearly defined.

00:10:27:01

<Hendry narrates over table showing results of studies comparing GAT titre of fertile with infertile men>

Now, there have been several large studies of both fertile and infertile men and these have shown that one can find significant antisperm antibody levels in about 2% of fertile men and in about 13% of infertile men. Our own experience at Chelsea Hospital for Women, we found 8.5% positive in 591 infertile men and we believe that this was the primary cause of the infertility in these couples.

<Hendry narrates over table showing GAT and TSAT titres in females and males amongst infertile couples>

These figures are taken from Professor Schulman's work in New York. He is considered to be one of the leading experts in this field. You will see that his incidence of positive tests by the Kibrick method, or the GAT, for males is very similar to our own findings at Chelsea. Notice that men, as would be expected, have a lower incidence on Franklin-Dukes or TSAT testing. But look at the very high incidence of positive tests that he reports in women: 17.6% with this test <*indicates GAT*>, 14.9% with this test <*indicates TAST*>. And the situation is even further confused because these different tests correlate poorly with each other.

<Hendry to camera and then over table comparing GAT and TSAT tests in women>

So we find that in a series of women those that were positive with the GAT or Kibrick test were not necessarily positive with the TSAT test, and similarly those that were positive with TSAT test were not necessarily positive on the Kibrick test. Now, if we add all this up together, it turns out that no less than 28% of infertile women, investigated by Professor Shulman, gave a positive antisperm antibody test, and at the most optimistic or pessimistic appraisal of the situation, this is probably far too high a proportion of women to be significant. As a result the meaning of the results of these tests in female infertility work is much less clearly defined.

Because of the difficulty in interpreting the results of the tests, we need to have an objective measurement which will tell us whether the result of the antisperm antibody test means something or not. Fortunately, the very careful work done by Dr Kremer in Holland has provided us with an answer.

<Hendry narrates over illustration showing effects of cervical mucus on sperm in the presence of antisperm antibodies>

Sperms, which normally swim fairly freely in seminal plasma, with the antisperm antibodies may clump together a little, but they can maintain their normal motility. However in the presence of antisperm antibodies, either in the man or in the women, when they are put into cervical mucus, which they will normally penetrate with ease, in the presence of antibodies they become attached to glycoprotein molecules in the cervical mucus. They become attached and they are unable to move freely. As a result, they shake or jerk or wriggle and this can be clearly seen under the microscope as a positive shaking phenomenon. This used to be called cervical hostility. This is clearly a bad name because this phenomenon can be observed whether the female or the male has the antibodies and it is simply sensitised sperms meeting with an appropriate receptor in the cervical mucus.

<Hendry to camera and then over tables showing correlation between SCMC and TAT test, interspersed with talk to camera>

Now, this test, called the SCMC test, can then be correlated with the results of the antibody tests. Here you see the result of the SCMC test from very positive, through moderately positive, to negative. And here you see the results of serum testing on husband or wife. Note that a positive SCMC test indicating seminal plasma is at fault, by using donor sperms and cervical mucus, always correlated with antibodies in the husband. Whereas if the cervical mucus was at fault, this invariably associated with antibodies in the wife. And notice also, this correlated with antibodies in the seminal plasma and the cervical mucus.

Further studies by Kremer indicated that the positive SCMC test here was always associated with the presence of IgA in the genital secretions. And that in a small number of patients, there could be a positive serum test due to IgG but a negative IgA test in the seminal plasma, and in this case, the SCMC test was negative. It follows that the factor that is most important is the presence of IgA in the genital secretions; that this is where the effect is produced.

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<Hendry to camera and then over table showing results of treatments on men with Kibrick titre of more than 1:32>

Let us say that we have a man who has a Kibrick titre of more than 1 in 32. His SCMC test shows that his sperms are unable to move in the cervical mucous, what treatment can we give him? Well, we have treated 50 men at Chelsea Hospital for Women in one of three ways: prednisolone 5mg, 3 times a day for 2 to 12 months, average of about 6 months – 33 patients treated, 3 pregnancies produced; not very good. Methylprednisolone: 96 milligrams a day for 7 days, given to the man from day 21 to 28 of his wife's menstrual cycle, is the treatment recommended by Shulman, and we have had 2 pregnancies and 1 miscarriage in 20 couples treated. Halim suggested washing the sperms in a balanced buffered salt solution, resuspending them and artificially inseminating them; we've had 3 pregnancies in 30 couples treated for 1 to 13 inseminations – an awful lot of work for a very small return. At present the treatment is not satisfactory and further research is necessary.

<Hendry to camera and then over table showing antisperm antibody results after vasectomy>

Now, I want to conclude by considering the situation after vasectomy. We know that over 50% of men will develop antisperm antibodies following a vasectomy. What happens when we undo the vasectomy and will they be fertile? Fortunately, very careful work by Rumke in Holland has shown a clear difference between naturally infertile men, whose blood test being positive correlates very well with the presence of antibodies in the genital secretion as against men following a vasectomy, where a positive blood test is very much less often associated with antibodies in the seminal plasma. And this is why we can reverse vasectomy even in the presence of antibodies and expect reasonable fertility in most of the patients.

To sum up, if I can, this highly complex matter, I have no doubt that antisperm antibodies matter in infertile men. It's probably the cause of the infertility in about 10% of them. It doesn't matter so much following vasectomy for reasons that we

have just discussed. I think the situation in infertile women is highly confused and is desperately in need of further accurate research.

<Chamberlain and Hendry in discussion to camera>

<Chamberlain>

Thank you very much, Bill. That was a very good and clear account. It's the sort of subject which every time I hear it, you and other experts talk about it, I understand it, and then two hours later, I don't understand it <*laughs*>. Let me go through some points, if I may, that as you were talking arose to me here. Would you clear the air a little? Reports have been made about females who are virgins having antibodies there. I think it was Harrison, who's now in Ireland, isn't it, who was one? How could that occur if no sperm's been exposed to them?

<Hendry>

Yes, there are two ways that this can happen. You will remember I said that the immunofluorescent test will very often show a positive reaction with antibodies being deposited on the surface of sperms. Now, this is a nonspecific reaction. It is cross-reacting antigens, if you like, and this can be observed in 30% of the normal population, be they virgins, pregnant women, prostitutes or whatever. And it is almost certain that the immunofluorescent test for clinical purposes means nothing and you should put this from your mind. The other complicating factor in women is that they have in their serum a factor called a beta sperm agglutinin, which has been defined by an Australian called Boettcher. And this beta sperm agglutinin is not an antibody, it is a steroid of some sort and it causes false positive reactions in 2 or 3% of women who have not got antibodies at all, so you see that is …

<Chamberlain>

So it is really an error of measurement …

<Hendry off camera>

Exactly.

<Chamberlain>

… rather than a basic [unclear words]

<Hendry off camera>

A false positive, exactly.

<Chamberlain>

I'm glad you've cleared that, thank you. Is there an analogous situation in men? Men who have congenital azoospermia, have they been measured for antibodies?

<Hendry>

What a good question. You see, it is … a very good question. It is just possible, of course, a man with no sperms could have antibodies and the antibodies have wiped out his sperms.

<Chamberlain off camera>

Yes.

<Hendry>

I don't know is the answer, I …

<Chamberlain>

But if he has an anatomical problem which was, obviously, he's never had had sperm?

<Hendry>

So yes, we would …

<Chamberlain>

There can't be many of them.

<Hendry>

We would have to test, some Del Castillo's syndromes. I don't know is the answer. I would think that they probably would not have antibodies, but it would be a very interesting group.

00:20:38:24

<Chamberlain>

Yes well, to come back to the female again. In order that they should develop antibodies, does there have to be a breach in the epithelial lining? Does the sperm have to somehow, the protein, get into the bloodstream or can it be absorbed by broken down proteins? I don't quite understand.

<Hendry>

Well, it's, again you've put your finger on a very good question, it's not known why some women form antibodies and others do not. It is known that if you take sperms and put them in a syringe and inject them into somebody, they will then form antibodies and, indeed, this is being looked into as a method of contraception.

<Chamberlain off camera>

Yes.

<Hendry>

But why some women, who meet sperm through the normal channels, should form antibodies against them and others do not is not known. There is perhaps a hint that men who have prostatitis, inflammation in the prostate, are more likely to get antisperm antibodies than those who have not. And it may be inflammation in the genitalia may breach the normal defence mechanisms and allow the sperms to get through, but it is unknown.

<Chamberlain>

Would that contraception which you mentioned a moment ago by injecting sperm, would this be a fairly nonspecific contraception which would do for all future sperm, any man or just for that husband?

<Hendry>

No, it is thought that the antisperm antibodies are nonspecific, i.e. they act against all sperms. There is a group in Rome who disagree with this, who say that a woman specifically forms antibodies against only her husband's sperms, but these people are in the minority and the great authorities do not agree with this.

<Chamberlain>

That wouldn't happen to fit with the teachings of a certain group that come from Rome, would it? <*Laughs*> Can I go back to the female again here? You told us of – I think it was Shulman's work with the 28% of women having antibodies, and you thought this was too high. Now, do you think this is too high that this is again a fault

of measurement or it's a fault of interpretation and they have gotten there and we're not measuring the right thing?

<Hendry>

Yes, I think it's both really. You see, I think in women you expect a much lower titre anyway, so you're more likely to get false positives and also there has been a great lack of the very careful correlative studies that this is where we're so indebted to Kremer for insisting on careful correlation between the activity of the sperms in the cervical mucus, on the one hand, and the results of the antisperm antibody tests, on the other. And this is where I think Kremer has made such a valuable contribution. My feeling is that a positive antisperm antibody test should always be checked by observing the interaction between sperms and cervical mucus, checked if necessary with donor sperms and mucus before deciding it's significant in any couple.

<Chamberlain>

I think that was a terribly important point because so many departments merely take what you quite rightly castigated as cervical hostility as being something – oh, let's give the girl a bit of oestrogen, it's bound to be put right, and all you're saying is that there's a fault between sperm and mucus …

<Hendry>

Yes.

<Chamberlain>

…and we haven't, unless you do a cross-hostility test, you don't know this. Yes, that's a very good point. May I go back though to the low titres in the female of antibodies: do you think, do you feel as your gut feeling that a low titre in the woman is going to be as significant as a higher titre in the male? Or else if that isn't so, why do they have lower titres?

<Hendry>

Well, I think, I think they have lower titres because they are less constantly exposed to the antigen.

<Chamberlain>

And therefore would be less significant then, if it is ineffective?

<Hendry>

Very likely so, yes, I think likely so.

<Chamberlain>

And the people you, the group you mentioned who are perhaps injected sperm indirectly to get contraception, were they getting higher titres where they having a more direct approach from antigen?

<Hendry>

Do you know, I can't tell you that as a matter of fact.

<Chamberlain>

One would expect it, but again it is intermittent, of course, as you rightly say. Yes. Well, thank you very much, Bill, for taking us through this. It really is one of the harder subjects and it is a subject which is terribly important and it's only been opening up the last few years and it's going to open up a lot more as more people like you, who understand it, talk to more of us who don't understand it so much. Perhaps, we'll understand it better. There is going to be very soon an excellent book on this subject which is coming from the Institutes of Urology and the Institutes of

Obstetrics and Gynaecology of which Mr Hendry has been one of the editors. I'd recommend any of you who are interested in the subject, and who find it as perplexing as I do, get hold of this book. It is I believe, Bill, it is On the Immunology of Infertility is the title, is it?

<Hendry>

It's called Spermatozoa, Antibodies and Infertility.

<Chamberlain>

Thank you, Spermatozoa, Antibodies and Infertility.

<Hendry>

It'll be out any day now.

<Chamberlain>

Good, I think that'll be a first rate book to have and one which will certainly go into my library. Thank you very much.

<Hendry>

Thank you.

<End credits>