

Sperm dysfunction in partners of infertile patients with minimal or mild endometriosis

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Summary: A review of the literature does not show that minimal or mild endometriosis unquestionably causes infertility although its association is known; the cause only becomes apparent as the disease progresses. In these patients therefore, prolonged infertility for other reasons may have exacerbated or predisposed a tendency in them to develop the disease. Sperm dysfunction in their partners may have been one factor. Sperm penetration assays using mid-cycle (estrus) bovine cervical mucus were studied in 22 men whose wives were confirmed to have minimal or mild endometriosis. All men had normal semen analyses. For controls, 16 men of proved fertility were used. Whilst all but one (94%) of the fertile men showed adequate penetration (>30 mm), only 59% of the men in the study group achieved this penetration ($p < 0.05$). These findings provide evidence that minimal or mild endometriosis may not be the primary cause of infertility in patients in whom the diagnosis is made.

INTRODUCTION

Endometriosis has been reported to be 19 times more common in infertile women when compared to fertile controls (¹). This implies that it causes infertility. The reasons in moderate or severe disease can be explained. Dense tubal adhesions, endometriomata and obliteration of the posterior cul-de-sac all prevent oocyte transport; occasionally the tubes are blocked. But the reasons for infertility in minimal or mild disease remain obscure. These patients have as their only abnormality, small foci of endometriosis with little or no distortion of pelvic anatomy. This has led workers to believe that there are subtle rea-

sons not being detected by conventional means. A review of the literature however (^{2, 3}) has shown that the reasons for infertility in these patients remain obscure. Furthermore, treatment of these patients either medically or by surgery did not improve pregnancy rates over expectant management (^{4, 5, 6, 7}).

In these patients therefore, the endometriosis may have been only a coincidental finding and infertility for other reasons has predisposed or exacerbated a tendency in them to develop the disease.

One probable cause for their infertility might be sperm dysfunction in their partners. This study was done to confirm this hypothesis.

Sperm - mucus penetration reflects a characteristic related to fertility and both in vivo and in vitro tests have evolved to study this function. The slide (Kurzkrook and Miller) (test (⁸)) and the capillary tube (Kremer) test (⁹) are often used. However these tests do not differentiate between

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sperm or mucus function; a negative test does not discern whether it is due to a defect in the sperm, mucus or both.

Substitute substances such as mid-cycle (estrus) bovine cervical mucus have been shown to be good alternatives to evaluate sperm function as the only variable^(10, 11, 12).

It is rheologically similar to mid-cycle human cervical mucus. Their glycoproteins are similar in sugar and amino acid content; their viscoelastic properties are similar; and the ferning patterns are almost identical. Furthermore, human spermatozoa penetrate both bovine and human cervical mucus in a similarly concerted, unidirectional manner and with identical flagellar motion⁽¹²⁾. There is good correlation in penetration rates when the two are compared^(13, 14).

MATERIALS AND METHODS

Recruitment for the study was from couples attending the Infertility Clinic, Aberdeen Royal Infirmary. Male partners whose wives were confirmed to have minimal (Stage I) or mild (Stage II) endometriosis (Revised American Fertility Society Classification)⁽¹⁵⁾ within 18 months of the study were either interviewed or informed by post about the study. Of the 46 couples approached, 22 agreed to take part in the study. The couples received explanation about the procedure and their verbal consent obtained.

The following were the criteria for inclusion of the couples in the study:

- 1) Infertility for more than 2 years.
- 2) The female partner being less than 35 years old.
- 3) A history of the couple having coitus at least 2 to 3 times a week.
- 4) A history of regular menstrual cycles in the female partner with mid-luteal estimations of serum progesterone to confirm ovulation.
- 5) Confirmation of the female partner having Stage I or II endometriosis at laparoscopy. All laparoscopies were performed using a standard two portal entry technique.
- 6) Seminal analysis in the male partners showed a sperm concentration of more than 20 million per ml with a motile fraction of more than 40% with forward progressive motility.

Post coital tests were not undertaken on the couples because of their doubtful significance^(16, 17).

For controls, semen samples were obtained from men of proven fertility, having fathered at least one child recently. These included:

- 1) Regular donors to the Artificial Insemination by Donor Programme (AID) organized by the Department of Obstetrics and Gynaecology, Aberdeen Royal Infirmary.

- 2) Husbands of patients requesting laparoscopic sterilization.

The men were counselled about the procedure and their verbal consent obtained.

Preparation of semen samples

Semen samples from both the test group and controls were collected by masturbation after abstinence from intercourse for at least 2 days. Once in the laboratory, the semen was allowed to liquefy and its volume, sperm concentration and motility determined. In addition, any abnormal viscosity or agglutination was noted.

Preparation of bovine cervical mucus

Estrus bovine cervical mucus was obtained commercially, frozen in flat sealed capillary tubes (Penetrak, Serono). Each tube had a score mark for easy breaking. The tubes were stored at -18°C till used. When required the tubes were thawed for about 15 minutes in an upright position at room temperature with the score marks at the upper end.

The test was performed within 2 hours of collecting the semen samples. After liquefaction, 200 μl of semen sample was placed in a conical flask. The capillary tubes were snapped at the score mark and the open end placed in the flask containing the semen sample. After 90 minutes the tube was removed, wiped clean of any excess semen and placed on a graduated microscope slide with its open end at the zero mark.

To assess the degree of penetration into the cervical mucus a light microscope with a phase contrast a x200 magnification was used. By referring to the distance provided on the graduated slide, two measurements were determined:

- 1) The number of spermatozoa within one field view at the 20 mm mark of the capillary tube. (The mean of 3 separate viewing fields was calculated).

- 2) The greatest distance penetrated by the sperm up into the tube, i.e. the distance travelled by the vanguard sperm.

The penetration depth of the vanguard sperm was arbitrarily divided into 2 penetration zones based on the distance travelled by the vanguard sperm after 90 minutes.

- 1) Less than or equal to 30 mm, indicating reduced penetration.
- 2) Greater than 30 mm indicating normal penetration (11).

Statistical Analysis

Where appropriate, statistical analysis was done either by Chi-squared analysis or the Pearsons Correlation Coefficient using the Stata Statistical package (Computing Resource Center, Los Angeles, California).

RESULTS

A difference was observed in the penetration rates between the two groups. All but 1 (94%) of the fertile donors had penetration rates of more than 30 mm. On the other hand, despite having normal seminal assays, only 59% of the test group achieved this penetration ($P < 0.05$).

Reliability of test procedure.

- 1) Correlation between penetration and concentration of motile sperm.

In order to evaluate the influence of the motile sperm density in the semen sample on the penetration rates, a scattergram was plotted for the two variables. This would indicate if the test could discriminate for sperm function and not be solely a function of the motile sperm density. A Pearson's correlation coefficient of $r = 0.53$ was obtained.

- 2) Within-assay coefficient of variation (CV).

Repeated analysis of the mucus penetration tests of the same semen sample was done in 3 subjects. A mean CV of 5.2% (range 2.5 - 6.9%) was obtained.

- 3) Inter-assay coefficient of variation.

A valuable indicator of the precision of the mucus penetration test would be the consistency of results obtained for individual patients at separate times. This was done in 2 patients, each assay being done 3 weeks apart. A mean CV of 13.8% was obtained.

DISCUSSION

The bovine mucus penetration test used in this study appears to give reliable and repeatable estimates of human sperm function in an in vitro situation. Firstly, the depth of penetration discriminated for sperm function - it was not solely a function of the motile sperm density in the semen sample. The correlation coefficient ($r = 0.53$) between motile sperm density and the depth of penetration was not outstanding. Although this correlation was significant ($p = < 0.001$), in statistical terms, it indicated that only 28% (r^2) of the penetration depth was accounted for by the motile sperm density.

An intra-assay coefficient of variation of 5.2% for the test was acceptable. Similarly, an inter-assay coefficient of variation of 13.8% was encouraging. It compares favourably, for example, with that of the zona-free hamster egg penetration test which has an inter-assay coefficient of variation of 12 to 12.5% (18, 19).

It has been argued that, in addition to the depth of penetration of the vanguard sperm, other criteria should be included in evaluating sperm penetration tests; the density of sperm (number per high power field) at the second or third centimeter has been suggested (20); this would indicate that sufficiently large numbers of sperm would have progressed to higher parts of the genital tract. This criterion was evaluated in the study. Firstly, it showed good correlation ($r = 0.7$; $p = < 0.001$) with the depth of penetration, that is, a high density of sperm were seen at 20 mm in those samples which showed good penetration; this made the criteria redundant. Secondly, a wide inter-assay coefficient of variation (47%) was seen in repeated samples of the same patient. This made meaningful comparison difficult.

From the analysis it appears that there are intrinsic differences in the penetrating

capacity of sperm from normal fertile men as opposed to those from some partners of patients with minimal or mild endometriosis. Whilst 94% of the fertile men had penetration rates of more than 30 mm, only 59% of the men in the latter group achieved this penetration ($p=0.05$). This difference has been shown to have prognostic significance. Using bovine mucus penetration tests, Alexander⁽¹¹⁾, reported a difference in penetration rates between men whose wives had achieved pregnancy and those who had not; no obvious female factors were present in either group. Of the 9 men who showed good penetration rates, 6 of the wives conceived in the subsequent 6 months. On the other hand, of the 18 couples in which the husbands had poor penetration rates, only 4 of the wives conceived within the subsequent 6 months.

In this study, the lower limit of normality for penetration has been arbitrarily fixed at 30 mm. However, this figure only represents the lower limit of the range for normal fertile men. It does not indicate that conception was impossible with lower penetration rates. It might be anticipated however, that it would be associated with some difficulty in achieving conception.

The results of this study suggests that sperm dysfunction in their partners may be the cause of the infertility in patients with minimal or mild endometriosis. The hypothesis may therefore be advanced that endometriosis developed in these subset of infertile women because of the infertility; it would not otherwise have developed if they had conceived and had the benefit of the protective effect of pregnancy^(21, 22). Pepperell and McBain⁽²³⁾ relaparoscoped their infertile patients who had previously had normal pelvic findings and who had failed to conceive after a two year period. They found that in 20% of them, endometriosis had developed.

They concluded that it was unlikely that the diagnosis was missed at the first laparoscopy and that prolonged infertility predisposed this group of women to develop the disease over the two years. However, the reasons why some infertile women develop endometriosis and others do not remains an enigma. Familial or genetic susceptibility has been suggested^(24, 25).

Further evidence implicating a male factor for the infertility in these patients comes from Escudero *et al.*⁽²⁶⁾. They compared 40 patients with minimal endometriosis who had timed coital exposure with their husbands (who were presumed to be fertile) to 21 comparable patients who had been treated by artificial insemination with donor semen. Those patients who had artificial insemination not only had significantly higher pregnancy rates but had a monthly probability of pregnancy of 0.201 which, could be considered normal.

In conclusion, the findings of this study suggest that the presence of endometriosis in a subset of infertile patients may be the result, and not the cause, of their infertility, the cause being a defect in their partners. It follows therefore, as has been shown by others^(4, 5, 6, 7) that the treatment of these patients will not necessarily improve pregnancy rates.

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