The use of enzyme-linked immunosorbent assay for detection of Mycoplasma hominis antibodies in infertile women serum samples

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BACKGROUND: Besides Chlamydiae trachomatis and Mycoplasma genitalium, Mycoplasma hominis may also cause infertility due to damage of the Fallopian tubes. Therefore serum samples from infertile women were analyzed for antibodies to M. hominis. METHODS: Sera from 304 infertile women were investigated for seropositivity to M. hominis by immunoblotting and a developed ELISA. Women were classified into groups based on the type of infertility: infertile due to lack of passage in Fallopian tubes (TFI, tubal factor infertility), an infertile male partner (MFI, male factor infertility) and unexplained infertility (UFI, unexplained factor infertility). Three M. hominis isolates were used in the immunoblotting analysis and clear differences in patient immunoprofiles were observed between two isolates. For the ELISA we used a mixture of Triton X-114 extracted membrane proteins from those two M. hominis isolates as antigen. RESULTS: Ninety-seven sera (32%) were seropositive to M. hominis when tested by the ELISA. There was a significant correlation between TFI and seropositivity to M. hominis (P = 0.0015, OR = 2.21, CI = 1.35–3.61). We compared the seropositivity of 304 patients to M. hominis with the presence of antibodies against two other bacteria Chlamydiae trachomatis and Mycoplasma genitalium and there was no statistical correlation between those bacteria and M. hominis. CONCLUSION: Our results indicate that M. hominis may be an independent predictor of TFI.

Key words: antibodies/ELISA/immunoblot/membrane proteins/Mycoplasma hominis

Introduction

The trivial name ‘mycoplasma’ is often used to denote any species in the Mollicutes class. M. hominis is spherical in shape, bounded only by a membrane which is exposed directly to the environment without any other protective structure. The presence of a tip structure characteristic for Mycoplasma genitalium and Mycoplasma pneumoniae is not found in M. hominis, and the mechanism of attachment to the host cell membrane is not known. M. hominis is a non-fermentating bacterium which uses arginine as a major source of energy. It requires a complex medium for growth in vitro. The first isolation from human Bartholin’s gland abscess was reported in 1937 (Dienes and Edsall, 1937). Direct isolation from Fallopian tubes of women with acute salpingitis in 1970 suggested that this species can cause tubal infections (Mardh and Westrom, 1970). The study was followed by infection of in vitro organ culture using sections from Fallopian tubes, which after overnight incubation with M. hominis showed swollen cilia of epithelial cells, especially in the distal part (Mardh et al., 1976).

M. hominis is an opportunistic pathogen and a common inhabitant of the female lower genital tract and it is believed to be sexually transmitted (Elshibly et al., 1996). It has been isolated from cases of post-partum fever and sepsis. However, the most common infection is that of the genito-urinary tract. In women with bacterial vaginosis (BV), M. hominis is frequently found (at least in 2/3 of women with BV, compared with 10% of healthy women) (Rosenstein et al., 1996). Isolation from cervix, endometrium and Fallopian tubes of women with salpingitis associated M. hominis with development of salpingitis and pelvic inflammatory disease (PID) (Mardh and Westrom, 1970). By use of a microimmunofluorescence technique it was shown that antibodies to M. hominis were found three times more often in infertile women who had PID than in healthy controls (Moller et al., 1985). It is therefore believed that M. hominis has the potential to cause...
infertility as a result of tubal diseases (Taylor-Robinson, 1996). The exact mechanism of pathogenesis of this species, however, has not been revealed.

Serological tests are not easy to develop due to the significant heterogeneity and antigenic variation among different *M. hominis* isolates (Andersen et al., 1987). The study revealed that different isolates had only between 41 and 72% of their proteins in common. In later studies (Christiansen et al., 1990; Olson et al., 1991) it was specified that these differences were found in membrane proteins and less frequently in cytoplasmic proteins. The membrane proteins are believed to be most immunogenic and are referred to as surface antigens.

In this study we show that past infections with *M. hominis* can have a relation to human reproductive failure. The measurement of antibody response was performed with ELISA. We show that a mixture of membrane proteins from two different *M. hominis* isolates is preferred as antigen rather than membrane proteins from just one isolate, due to the significant heterogeneity among *M. hominis* isolates.

Materials and methods

Selection and cultivation of *M. hominis* isolates

Three different *M. hominis* isolates were selected for immunoblotting assay: 132, 4195 and 7488. Two isolates: 132 and 4195, were used in the ELISA. BEa medium was used for culture of microorganisms (Freundt et al., 1979). *Mycoplasma hominis* cultures (1.7 ml) were incubated in 120 ml BEa medium for up to 72 h and when BEa medium changed colour from orange to pink (due to reduction of phenol red by the arginine hydrolysis), the microorganisms were harvested by centrifugation at 20 000 g for 30 min. The pellets were washed twice in phosphate-buffered saline (PBS) and stored at −70 °C prior to use.

Patient serum sample collection

The study was a part of the project approved by the local Scientific Ethical Committee (ref. no: VF 20010161). Serum samples were obtained from 304 women undergoing IVF treatment during the years 1997–2000. For detailed description of patient material see Clausen et al. (2001). All women were examined by hysterosalpingography (HSG) and laparoscopy. The patient blood samples were taken when they began the first in vitro fertilization (IVF) treatment.

The women were classified according to the cause of infertility into three diagnostic groups: (i) 132 women with tubal factor infertility (TFI), (ii) 64 in the male factor infertility group (MFI), and (iii) 108 in the unexplained infertility group (UFI). As normal material we used 31 serum samples obtained from healthy female blood donors collected at Skejby Hospital, Aarhus, Denmark. However, no information of the tubal status of the blood donors was available.

SDS–PAGE and immunoblotting

The pellets of each *M. hominis* isolate were suspended in SDS sample buffer (62.5 mM, 10% v/v glycerol, 2.3% v/v SDS, 5% v/v β-mercaptoethanol, 0.05% w/v bromphenol blue), heated to 100 °C for 2 min and separated by SDS–PAGE (7.5% SDS polyacrylamide gel with a 5% stacking gel). Proteins were electrotransferred to nitrocellulose membranes (Schleicher & Schull, Dassel, Germany). The molecular weight marker was cut out of the membrane and stained with Amido Schwartz. The membranes were blocked overnight with blocking buffer (20 mM Tris-base, 150 mM NaCl, 3% gelatine) at 4 °C. The immunostaining was performed as described previously (Birkelund and Andersen, 1988).

Preparation of antigen and ELISA

Pellets obtained from harvesting 120 ml of culture were suspended each in 800 µl of Tris–HCl (50 mM Tris–Cl pH 8.0, 0.15 M NaCl) and sonicated for 10 s by ultrasound using the Ultrasonic Processor Vibra Cell VCX 600 Watt (Sonnics & Materials, Newtown, CT) set at amplitude 20%. After sonication the concentration of proteins was adjusted to 1 mg/ml and Triton X-114 (TX-114) was added up to the final concentration of 1%. The *M. hominis* protein solutions with Triton X-114 were kept for 1 h on ice. To remove insoluble proteins the solution was centrifuged for 15 min at 20 000 g and 4 °C. For separation of aqueous and detergent phases the procedure of Bordier (1981) was applied. The protein content of each phase was analyzed by SDS–PAGE protein separation on 12.5% acrylamide gels and stained with Coomassie Blue.

Coating of 96-well microtitre ELISA trays (Maxisorb, Nunc, Roskilde, Denmark) was done with 60 µl of antigen per well. Plates were incubated overnight at 4 °C. To block the antigen, the plates were incubated for 1 h with 75 µl of 15% FCS (fetal calf serum in PBS). Serum samples were diluted 1:50 in antibody buffer (medac, Hamburg, Germany), tested in duplicate wells and incubated for 1 h at 37 °C. Fifty microlitres of a mixture of goat anti-human IgG (Jackson Immunoresearch Laboratories, Inc., West Grove, USA) horseradish peroxidase labelled (dilution 1:40000) and unlabelled (0.26 µg/ml) was used as a secondary antibody. Plates were developed with 50 µl tetramethyl benzidine (medac, Hamburg, Germany) for 30 min at 37 °C and the reaction was stopped with 100 µl of 1 N HCl. ELISA trays were washed between each incubation with PBS + 0.05% Tween-20. Then the plates were read by Sunrise-reader (Tecan, Salzburg, Austria) at a 450 nm wavelength with a reference length of 620 nm.

Statistical analysis

SPSS-software was used for analysis of data. Receiver-operating characteristics (ROC) curves were used to analyse correlation between tubal factor infertility and seropositivity to *M. hominis*. Multiple logistic regression analysis was used to analyse the connection between seropositivity to *M. hominis* and a list of variables such as: indicators for each of three patient groups, patients’ age, age squared and seropositivity to *M. genitalium* and *C. trachomatis*. The statistical model was reduced by backward selection, where insignificant variables are removed one after another.

Results

Immunoblotting

304 patient sera were investigated for the presence of antibodies against three different *M. hominis* isolates (132, 4195, 7488) by immunoblotting. Positive reaction was characterized depending upon the strength of the protein band intensity. A scoring system: ‘−’ for negative serum, ‘1+’ for slightly positive reaction with patient serum, ‘2+’ for medium strength reaction and ‘3+’ for strongest reactions, was used for classification of results. Sera were defined as positive when immunoblot with at least one isolate showed ‘2+’ or ‘3+’ reaction. One isolate could produce similar (Figure 1A, lanes 10 and 11) or different (Figure 1A, lane 12)
immunoreaction profiles when reacted with different patient sera. In some cases, one serum sample could react with one and not the other isolate (Figure 1B, serum 1). Results of serum sample reacting similarly with the three isolates are seen in Figure 1B, serum 2. Most differences were observed between reactions with isolate 132 and 4195 (Figure 1B, sera 1–3). Based on these results, the isolate 7488 was excluded from further analysis by ELISA.

Eighty-four (27.6%) patient sera were classified as positive. Among positive patient sera, 51 (40%) belonged to the TFI group, 16 (23%) to the MFI group and 17 (16%) to the UFI group.

**Triton X-114 phase separation of proteins**

The partition of proteins of the *M. hominis* isolates 132 and 4195 was performed in the detergent Triton X-114, which is used to solubilize membrane proteins from whole cells. The partition resulted in three different protein phases: the insoluble protein phase, which was collected and removed; the aqueous phase containing hydrophilic, mostly cytoplasmic proteins; and the detergent phase containing hydrophobic mostly integral membrane proteins with an amphophilic nature, which are believed to be most immunogenic and species specific. After each separation, detergent, aqueous and total protein phases were compared by SDS–PAGE electrophoresis. The protein profiles of isolates 132 and 4195 were similar but small differences were noticed (Figure 2). When comparing the detergent and aqueous phase of the same isolate, elimination of many cytoplasmic proteins can be seen. Only the detergent phase was used as antigen in ELISA.

**Development of ELISA test and optimization**

Based on the immunoblotting results we selected 18 serum samples, referred to as standard sera, to optimize the ELISA. The selected samples were either negative for both isolates, slightly to medium positive for one or both isolates; or medium to strong positive for one or both isolates. We tested three different dilutions (1:375, 1:250, 1:200) of the antigen from each isolate separately with these sera to find the best ELISA for detection of *Mycoplasma hominis*. 

![Figure 1. Examples of immunoblotting reactions.](https://academic.oup.com/humrep/article-abstract/20/5/1277/2356798)
antigen concentration for ELISA. A dilution of 1:200 was found to be optimal for both isolates when tested separately. The OD$_{450}$ values of the negative samples lay between 0 and 0.2, and the OD$_{450}$ values for the positive samples reached up to 2.2 (Figure 3). Thus, we selected the antigen dilution 1:200 for performing analysis on 304 samples. While comparing OD$_{450}$ values obtained with 18 standard serum samples tested with the two isolates separately, we saw in some cases differences in the OD$_{450}$ values with serum from the same patient (Figure 4). Some would not have been counted as positive if only analyzed with antigen from one isolate (Figure 4, serum 6). Therefore a mixture of the two isolates was used at 1:1 of the 1:200 dilutions.

To study reproducibility of the assay the 18 standard serum samples were tested by ELISA on two different days, using TX-114 soluble protein from the selected isolates as antigen. Fresh dilutions of antigen and antibodies were used in each assay. The OD$_{450}$ values of standard sera in both tests are presented in Figure 5. The differences in OD$_{450}$ values were <20%. There was no significant difference among the means of both tests when analyzed by a two-tailed t-test ($P = 0.277$).

**Determination of the cut-off value for the ELISA test**

To determine the cut-off value for analysis of ELISA results we compared the OD$_{450}$ values of the 18 selected standard serum samples to the immunoblotting results (Table I). There...
was a good relation between the most negative ‘2’ immunoblots and the lowest OD450 values in the ELISA running from 0.03 to 0.41. The most positive samples indicated with ‘3+’ all had the high OD450 values placed in the range between 1.42 and 1.89. Among the negative immunoblots with the slight reaction to the patient sera, indicated with ‘1+’, we found one high OD450 that was in the positive samples range: 1.3, whereas in the medium positive immunoblots ‘2+’ one OD450 value was equal to 0.31, which is in the range of the most negative samples. Therefore, the two described samples were subtracted from the statistical analysis. We placed the cut-off value at the OD450 equal to 1.2, which was calculated from the mean OD450 of the negative samples plus three standard deviations of those samples.

This was in agreement with a study of 31 serum samples from healthy female blood donors. We used the OD450 values from those patients to calculate the mean OD450. Three samples were excluded from the calculations due to the high OD450 values, which were in the range of the most positive samples (Figure 6). Again the cut-off value was placed at OD450 value equal to 1.2, calculated as described above. Thus both sets of serum samples gave the same cut-off values.

Specificity evaluation

Cross-reactivity to M. genitalium, M. pneumoniae and Ureaplasma urealyticum was examined. As primary antibody we used rabbit pAbs generated against whole cells of the different Mycoplasma species (dilution 1:2 000). As secondary antibody we used goat anti-rabbit IgG (Jackson Immunoresearch Laboratories) labelled with horseradish peroxidase (dilution 1:20000). The OD450 values were as follows: 0.012 for M. genitalium, 0.017 for M. pneumoniae and 0.269 for U. urealyticum. Thus, there was no cross-reactivity to M. hominis antigen.

Results of the ELISA test

Sera from 304 infertile women and from 31 healthy female blood donors were analyzed by the ELISA, using the mixture of detergent phases from two M. hominis isolates as antigen for coating ELISA plates. The OD450 values for patients belonging to each of the groups were analyzed and are shown in Figure 8. In all groups the largest number patients were in the negative range from 0 to 0.19. In the TFI group (Figure 7A) a generally greater frequency of the samples with the high OD450 values was seen, especially in the range between 1.4 and 2.19. A distribution most similar to the TFI group was observed in the UFI group (Figure 7B), where several high OD450 values were present. The least number of high OD450 values was seen in the MFI and blood donor groups (Figure 7C and D). For analysis of the results we transformed measurement data into binominal data (0, negative; 1, positive) according to the cut-off OD450 value. Of the 304 infertile women tested, 97 (32%) were positive by the assay. Fifty-five of them belonged to TFI group (which corresponds to 42% of the total number of patients in the TFI group), 15 (22%) belonged to MFI and 27 (25%) to the UFI group. The last two groups had markedly lower seropositivity to M. hominis when compared with the TFI group. We joined the two
groups (UFI + MFI = women with normal tuba) which resulted in 172 patients, out of which 42 (24.4%) were positive in the ELISA test.

Among 31 healthy women blood donors used as a negative control we found three (9.7%) patients that were positive in the ELISA.

Comparison of results from ELISA and immunoblotting

The results obtained from ELISA and immunoblotting were compared (Table II). In the ELISA, 97 patients were positive to *M. hominis*, while 84 patients were positive in immunoblotting. Sixty-nine (22.6%) patients were positive by both tests, 15 (4.9%) were positive in immunoblotting but not ELISA test, and 28 (9.2%) were positive only in ELISA.

Table II. The comparison of results obtained in two different serological tests: ELISA and immunoblotting

<table>
<thead>
<tr>
<th>ELISA results / immunoblotting results</th>
<th>+/+</th>
<th>−/+</th>
<th>+/−</th>
<th>−/−</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (%)</td>
<td>69 (22.7)</td>
<td>15 (4.9)</td>
<td>28 (9.2)</td>
<td>192 (63.1)</td>
<td>304</td>
</tr>
<tr>
<td>No. of ELISA positive patients</td>
<td>69</td>
<td>15</td>
<td></td>
<td></td>
<td>97</td>
</tr>
<tr>
<td>No. of immunoblotting positive patients</td>
<td>69</td>
<td>28</td>
<td></td>
<td></td>
<td>84</td>
</tr>
</tbody>
</table>

Comparison of seropositivity of patients to *M. hominis*, *M. genitalium* and *C. trachomatis*

In the previous study (Claussen et al., 2001) the same 304 patient sera were tested for seropositivity to two other genital microorganisms, *M. genitalium* and *C. trachomatis*. Both species are associated with development of TFI. The presence of antibodies against *M. genitalium* was tested by immunoblotting and the presence of *C. trachomatis*-specific antibodies was measured by the commercial pELISA (medac). The results of all tests were then combined and compared. Distribution of the forty *M. genitalium* positive sera in the different infertility groups showed that: 4 belonged to MFI, 29 to TFI and 7 to the UFI group. 106 patient sera were positive for presence of antibodies against *C. trachomatis* when tested in pELISA. The majority of sera, 75 (56.8%), belonged to the TFI, 11 (17.2%) to the MFI and 20 (18.5%) to the UFI group.

When analysing women from the TFI group for presence of antibodies against *M. hominis*, *C. trachomatis* and *M. genitalium*, 23 patients were positive only to *M. hominis* (Table III), 14 were positive for both *M. genitalium* and *M. hominis*, and 28 had antibodies to *M. hominis* and *C. trachomatis*. There were 10 patients that had antibodies to all three genital bacteria. In summary, out of 132, 106 (80%) women were seropositive to at least one of the three genital agents: *M. hominis*, *M. genitalium* or *C. trachomatis* (Table III).
Factor infertility.

A significant correlation was found between the results of the ELISA test and tubal factor infertility. The reduction was accepted (women with normal tubes: 172, and women with TFI: 132). Where the aetiology was composed of only two groups: presence of antibodies against M. genitalium as a relative risk. In our statistical model we tested for a reduction in the measurement data by introducing the cut-off OD450 value. In our statistical model we tested for a reduction where the aetiology was composed of only two groups: women with normal tubes; 172, and women with TFI: 132. The reduction was accepted (P = 0.8177). We found significant correlation between tubal factor infertility and seropositivity to M. hominis (P = 0.0015, OR = 2.21, 95%CI = 1.35–3.61). TFI patients had a 2.13-fold higher risk of having antibodies against M. hominis compared with patients with normal tubes, when odds ratios were interpreted as a relative risk.

We did not find any significant correlation between presence of antibodies against M. genitalium, C. trachomatis and seropositivity to M. hominis, when statistically tested (P > 0.05).

Since M. genitalium and C. trachomatis are both correlated with development of TFI we analyzed whether M. hominis could be an independent predictor of TFI, by defining patients from the TFI group as a dependent variable and seropositivity to other species as independent variables in our statistical model. The presence of significant correlation to M. hominis with P = 0.006; OD = 2.1, 95%CI = 1.253–3.706, suggested that M. hominis can cause TFI independently of C. trachomatis and M. genitalium. Both M. genitalium and C. trachomatis were also significantly correlated to TFI as described by Clausen et al. (2001).

Discussion

Antigenicity of specific M. hominis proteins in the human immune response is not known, and the marked intraspecies heterogeneity may cause diagnostic problems. Various serological assays have been used, including ELISA (Brown et al., 1983, 1987; Liepmann et al., 1991; Lo et al., 2003). In the first ELISA attempts, whole-cell protein lysate was used as antigen but one-way cross-reactivity of rabbit antisera to other Mycoplasma species and M. hominis antigen was found (Brown et al., 1983). Cytoplasmic proteins are the products of the housekeeping genes and are similar in all living organisms, and therefore they may cause the cross-reactivity problem. To overcome this problem we removed the cytoplasmic proteins by detergent phase partitioning and used hydrophobic membrane proteins in our ELISA. Such proteins are mainly exposed on the surface of the bacterial cell and are therefore believed to be the targets for the human immune response. Additionally, there was no reaction with the polyclonal rabbit antibodies raised against M. pneumoniae, U. urealyticum and M. genitalium in the developed ELISA. This strongly indicates the lack of cross-reactivity in Triton X-114 prepared antigen.

When the whole cell lysate from single isolates was used as an antigen for ELISA, none of the M. hominis isolates could detect more than 87% of positive sera (Brown et al., 1987). Therefore, the use of antigen pools from different isolates was suggested for optimal antibody detection. These serological findings are in agreement with our observations.
When antigens from two different isolates were compared separately by ELISA some of the patients gave a positive response to only one of the isolates (Figure 4). Thus, a mixture of the two isolates 132 and 4195 was used in our serological study. In a recent study, where lipid-associated proteins (LAMPS) were used as antigen (Lo et al., 2003) only one isolate of M. hominis was suggested for ELISA, despite the existence of antigenic variation between different isolates. This is, however, in disagreement with our findings.

The isolates 132 and 4195 were selected based on the isolation origin (both isolated from vagina) genomic, and phylogenetic differences (Andersen et al., 1987; Ladefoged et al., 1996; Boesen et al., 1998). A split graph analysis of different housekeeping genes (Sogaard et al., 2002) revealed similarities and differences between M. hominis isolates where isolate 132 was very similar to isolate DC63 in all analyses, but different from isolate 4195. Moreover, the analysis of Triton X-114 protein profiles by SDS–PAGE showed differences between those two isolates (Figure 2). The reason of excluding from ELISA isolate 7488, which was used in the immunoblotting, was based on the similarities in immunoblotting profiles of isolates 7488 and 132, and also that no additional sera were ELISA positive when detergent phase proteins of 7488 were included. Type strain PG21 was not selected, since in many tests, including what was found by Sogaard et al. (2002), it reacted differently from other isolates.

Differences observed in the results of immunoblotting and ELISA may reflect differences in the type of antigen used. Failure of the immunoblotting to detect 28 patients positive by ELISA may be related to increased sensitivity in ELISA. The antigen used for immunoblotting, unlike ELISA antigen, consisted of both cytoplasmic and membrane antigens. A cross-reactivity problem with cytoplasmic proteins could be one of the reasons why 16 patients were found positive only by immunoblotting and not by ELISA. Differences between immunoblotting and ELISA results even when the same antigen was used were described by Baseeman et al. (2004), who suggested that the reason may involve different protein conditions used in the tests (reducing conditions in SDS–polyacrylamide gel electrophoresis are used in immunoblotting in contrast to nondenaturating conditions in the ELISA).

Statistical analysis of data from 304 infertile patients was performed using two tests, ROC curves (measurement data) and multiple logistic regression analysis (binominal data). To change data from measurement to binominal, a cut-off OD450 value was introduced. The estimation of the cut-off OD450 value can be performed by testing serum samples from the negative patients, calculating the mean OD450 value, plus three standard deviations. Mycoplasma hominis infections are mostly asymptomatic (none of the patients had any symptoms of PID when they visited the infertility clinics) and thus it was difficult to justify which patients were truly negative. Therefore, the use of both ELISA and immunoblotting was beneficial in estimating the negative patients. Among healthy blood donors expected to be less often positive to M. hominis, three had high IgG antibody titre, which could be explained by the retrospective character of the immunological assay. Antibodies may remain in the serum even when the patient does not harbour the microorganism anymore.

Both statistical methods showed correlation between presence of antibodies to M. hominis and tubal factor infertility (TFI).

The results of this study suggested that M. hominis is related to development of TFI, probably due to tubal infection and scarring. The earlier study (Mardh and Westrom, 1970) where M. hominis was isolated in pure culture from the Fallopian tubes in 12.9% of the patients with acute salpingitis (and who also had M. hominis in the cervix), supports our findings. The influence that M. hominis can have on the epithelial cells in Fallopian tubes was analyzed in an in vitro organ study, where a noticeable swelling occurred in the cilia of epithelial cells after an overnight incubation with M. hominis (Mardh et al., 1976). A study by Taylor-Robinson and Carney (1974) failed, however, to show damage or loss of ciliary activity in mycoplasm-infected Fallopian tube organ culture.

When the TFI patient group was tested as a dependent variable and seropositivity to different genital microorganisms as independent variables, all three bacteria were significantly correlated with tubal factor infertility.

There was no correlation between positivity to M. hominis and positivity to the other two genital microorganisms, M. genitalium and C. trachomatis, and 23 TFI patients were positive only for M. hominis. This indicates that M. hominis can be an independent factor of tubal factor infertility.

Interestingly, 80% of the TFI cases were associated to a past bacterial infection with one of the three genital agents: M. hominis, M. genitalium, C. trachomatis, since the patients were seropositive to at least one of the microbes. The majority of patients (75) were seropositive to C. trachomatis; however, 31 had antibodies to M. hominis and/or M. genitalium which may indicate that genital mycoplasmas are important tubal pathogens.

Ten TFI patients had antibodies to all three microorganisms. It is, however, not known whether the patients had infections with those bacteria at the same time (co-infections) or at different time periods. In the case of co-infection it is important to diagnose all infectious microbes because different antibiotics should be given for C. trachomatis, M. genitalium and M. hominis infections (Falk et al., 2003; Ngan et al., 2004).

Acknowledgements

We would like to thank Helle Hartvig for providing help with the statistical analysis. We are also grateful to Karin Sørensen for skilled laboratory practice and Lisbet Wellejus Pedersen for excellent linguistic assistance of this paper. This work was supported financially by the Vejle Amts Sundhedsvidenskabelige Forskningsfond (Journal nr.: 2-16-41-10-03).

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Submitted on November 10, 2004; resubmitted on December 17, 2004; accepted on January 11, 2005