Antibiotic treatment based on seminal cultures from asymptomatic male partners in in-vitro fertilization is unnecessary and may be detrimental


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We questioned the policy of routine microbiological culture of semen prior to in-vitro fertilization (IVF) with a view to prescribing antibiotics to reduce the risk of introducing seminal infection into the embryo culture system. An initial retrospective study examined semen microbiology reports of 449 couples undergoing IVF or gamete intra-Fallopian transfer (GIFT). In semen samples taking \( \geq 1 \) days to reach the microbiology laboratory compared with same-day delivery there was increased frequency of significant culture of enterococci (27 versus 15%, \( P < 0.01 \)). In samples taking \( \geq 2 \) days there was increased frequency of significant culture of Gram-negative bacilli (31 versus 12%, \( P < 0.01 \)) and of overall culture of other potentially pathogenic organisms (26 versus 14%, \( P < 0.01 \)). We questioned diagnostic accuracy and relevance. Therefore, in a prospective study, semen and high vaginal swabs obtained on the day of oocyte collection were cultured from 100 couples having IVF or GIFT, of whom 52 male partners had been treated with antibiotics following positive pre-IVF semen culture. The presence of bacteria in semen samples used only for IVF (\( n = 90 \)) did not reduce fertilization rates nor lead to infection of the embryo culture system. However, there was an increased incidence of significant culture of vaginal Gram-negative bacilli in patients with treated partners compared with untreated partners [15/52 (29%) versus 5/48 (10%), \( P < 0.05 \)]. Thus antibiotic therapy in the male partner may increase the likelihood of inoculation of antibiotic-resistant pathogenic bacteria from the vagina into the embryo culture system during vaginal oocyte collection. In asymptomatic patients, microbiological screening of semen samples prior to IVF treatment and subsequent treatment with antibiotic therapy in those with positive cultures appears to be unnecessary and may be detrimental to IVF outcome.

Key words: antibiotics/bacteriology/infection/in-vitro fertilization/semen

Introduction

Bacteria present in semen can adversely affect seminal characteristics such as progressive motility, morphology and hyper-osmolar swelling (Gopalkrishnan et al., 1994). The presence of *Escherichia coli* in semen has been shown to depress significantly the motility and viability of human spermatozoa *in vitro*, resulting in clumping and agglutination (Teague et al., 1971). Improvement in seminal quality and fertility in men with clinically suspected bacterial infection of the reproductive tract has been reported following treatment with antibiotics (Murdia et al., 1978). However, pathogenic aerobes and anaerobes can be commonly cultured from semen samples in an infertile population (Eggert-Kruse et al., 1987). Chronic prostatitis is a common condition, histological evidence of which is present in up to 22% of men under 40 years of age (Chandiok, 1994). In many cases the condition is asymptomatic, and the benefit to natural conception of treatment according to positive semen bacteriology remains uncertain. Conversely, with in-vitro fertilization (IVF) treatment there may be a risk of contamination of the embryo culture system with organisms which, although not necessarily pathogenic to the man, are regarded as potentially pathogenic should they enter an embryo culture system. Although this may be largely prevented by sperm separation techniques and antibiotics added to the culture medium (Wong et al., 1986), unexpected fertilization failure can occur due to seminal microbial contamination in spite of favourable sperm function and preparation (Rowlands et al., 1994). This experience (Rowlands et al., 1994) led us to employ antibiotic therapy routinely before IVF in asymptomatic men with potentially pathogenic bacteria cultured from their semen. However, this policy did not lead to the anticipated reduction of infection rates of the embryo culture system but paradoxically appeared to increase infection rates. One possible explanation could be that antibiotics administered to the male are known to be concentrated to varying degrees in the prostate and excreted into the seminal plasma (Berger et al., 1990; Cooper et al., 1990). It may be postulated that, during intercourse, antibiotics introduced through the ejaculate into the vagina may adversely affect the vaginal bacterial flora and encourage relative overgrowth of potentially pathogenic organisms. These may be inoculated into the embryo culture system during oocyte recovery performed vaginally. To test this hypothesis, the vaginal bacterial flora on the day of oocyte recovery was compared between patients whose partner had or had not been treated with antibiotics prior to the oocyte collection. In addition, a retrospective study was performed to establish the incidence of positive seminal bacterial culture in relation to time delay between sample production and culture for the patients attending our IVF clinic.

Materials and methods

The first study entailed a retrospective analysis of seminal bacteriological findings in all couples for IVF and gamete intra-Fallopian...
transfer (GIFT) treatment in the University of Bristol Centre for Reproductive Medicine during 1994. Patients using donor spermatozoa were excluded. Male partners routinely provided a semen sample into sterile dry containers for microbiological culture within 1 week of the woman commencing gonadotrophin therapy. Men were routinely asked to cleanse the hands and genital area with soap and water and then to rinse off the soap with clean water prior to producing their semen samples. Some produced their samples at home and posted them to the microbiology laboratory by first class post. Others produced their sample at the hospital so that it reached the laboratory the same day. Information recorded included the number of days for the sample to reach the microbiology laboratory from production, organisms cultured, significant positive bacterial growths and bacterial sensitivities. Complete microbiological records were available for 449 patients.

In the second study, 100 consecutive patients undergoing treatment by IVF (n = 90) or GIFT accompanied by IVF and embryo storage (n = 10) were recruited. Patients with sperm dysfunction defined by abnormal penetration of normal cervical mucus and/or abnormal sperm migration and motility in artificial medium, irrespective of seminal sperm counts, were included although cycles involving donor spermatozoa were excluded. None of the couples studied had symptoms of genital tract infection during the treatment cycle. All female patients routinely underwent serological screening for Chlamydia trachomatis at least 3 months prior to IVF treatment and if there was evidence of active or recent infection, denoted by a positive complement fixation test, both partners were treated with ofloxacin (Tarivid; Hoechst UK Ltd, Hounslow, UK), 400 mg daily for 5 days. Of the sexually transmitted diseases, direct culture for Neisseria gonorrhoea but not C. trachomatis and Mycoplasma hominis was routinely performed during the prospective study. The man sent a semen sample, produced into a sterile dry container, through the post for microbiological culture at the commencement of the woman’s gonadotrophin therapy. According to local policy, appropriate antibiotic therapy was prescribed if a significant growth of potential pathogens or skin flora occurred. According to sensitivities, patients (n = 52) received either ciprofloxacin (Ciproxin; Baypharm, Newbury, UK), 500 mg twice daily, or co-amoxiclav (Augmentin; Beecham, Welwyn Garden City, UK), 750 mg three times daily for at least 7 days up to and including the day of oocyte collection, so that the man had received antibiotic therapy within 4 h of production of his semen sample on the day of oocyte recovery. In two cases, erythromycin and cephradine (Veloset; Squibb, Hounslow, UK) were prescribed at a dosage of 500 mg four times daily due to a patient history of drug allergies or specific bacterial sensitivity. Patients in whom an insignificant growth of potential pathogens or skin flora was obtained did not receive antibiotics (n = 48) but were asked to pay particular attention to personal hygiene at the time of semen sample production. All patients were asked to avoid sexual intercourse and/or ejaculation for 3 days prior to expected oocyte recovery.

At the time of oocyte recovery, a cotton swab was used to sample the posterior vaginal fornix via a sterile speculum, the sample being sent to the laboratory in Amies transport medium containing charcoal (Bibby Sterillin Ltd., Stone, UK). A sample of semen produced into a sterile dry container on the day of oocyte collection was transported the same day to the microbiology laboratory. High vaginal swabs, semen and, when appropriate, the embryo culture medium were cultured on selective media comprising nalidixic acid–colistin blood agar and cystine–lactose–electrolyte–deficient (CLED) agar incubated in air at 37°C for 18 h, modified New York City agar incubated in 5% CO₂ at 37°C for 40 h, and neomycin blood agar incubated anaerobically in an atmosphere containing 10% hydrogen, 10% CO₂ and 80% nitrogen at 37°C for 40 h. In addition, high vaginal swab specimens were inoculated onto gardnerella-selective medium (Unipath Ltd., Basingstoke, UK) incubated in 5% CO₂ at 37°C for 40 h, and semen samples were inoculated onto blood agar which was then incubated in 5% CO₂ at 37°C for 18 h. Organisms growing on these media in numbers regarded as significant (>100 colonies per plate) were identified using standard microbiological techniques and tested for antibiotic susceptibility using the disc diffusion test. When isolated from more than one site, Gram-negative bacilli growing on CLED medium were further identified to the species level using the API 20E system (bioMérieux UK Ltd., Basingstoke, UK).

Fisher’s exact and χ² tests were used throughout for statistical analysis of the data as appropriate.

Results

As seen in Table I there was no significant difference in case mix between patients in the first and second studies.

The results of the first study are summarized in Table II. The frequency of significant enterococcal growth from semen was significantly increased when the sample took ≥1 day to reach the laboratory (P < 0.01). The frequency of significant culture of Gram-negative bacilli was significantly increased when the sample took ≥2 days to reach the laboratory (P < 0.01). Actual numbers of significant isolates of Gram-negative bacilli organisms for <1, 1 and ≥2 days respectively were as follows: coliforms, n = 18, 15 and 19; proteus, n = 2, 2 and 2; pseudomonas, n = 0, 0 and 1; non-lactose fermenting Gram-negative bacilli not further specified, n = 4, 6 and 11. Notable culture of other organisms was significantly greater when the sample took ≥2 days to reach the laboratory (P < 0.01). Actual numbers of significant isolates of other organisms for <1, 1 and ≥2 days respectively were as follows: mixed anaerobes, n = 5, 1 and 2; non-haemolytic streptococci, n = 2, 0 and 1; β-haemolytic streptococci, n = 6, 4 and 5; mixed streptococci, n = 1, 1 and 1; coagulate-negative staphylococci, n = 10, 5 and 12; mixed faecal flora, n = 0, 1 and 0; bacillus, n = 0, 0 and 1; diphtheroids, n = 4, 4 and 1; gardnerella, n = 1, 0 and 1 and Candida albicans n = 0, 0 and 2. There was no significant difference in culture positive rates of skin flora (Staphylococcus epidermidis, micrococci and Corynebacterium) in relation to length of time taken to reach the laboratory. There were significantly fewer samples with no significant growth (P < 0.01) when samples took ≥2 days to reach the laboratory, and when samples took ≥2 days
Table II. Frequency of bacteria and yeast cultured in significant numbers (≥100 colonies per plate) from semen as related to the interval between sample production and laboratory culture. Values in parentheses are percentages

<table>
<thead>
<tr>
<th>Delay to culturing (days)</th>
<th>n</th>
<th>Gram-negative bacilli</th>
<th>Enterococci</th>
<th>Skin flora</th>
<th>Others</th>
<th>No significant growth</th>
<th>One organism in sample</th>
<th>&gt;1 organism in sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>201</td>
<td>24 (12)</td>
<td>30 (15)</td>
<td>31 (15)</td>
<td>29 (14)</td>
<td>113 (56)</td>
<td>66 (33)</td>
<td>22 (11)</td>
</tr>
<tr>
<td>1</td>
<td>143</td>
<td>23 (16)</td>
<td>38 (27)*</td>
<td>19 (13)</td>
<td>17 (12)</td>
<td>67 (47)</td>
<td>56 (39)</td>
<td>20 (14)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>105</td>
<td>33 (31)*</td>
<td>35 (33)</td>
<td>12 (11)</td>
<td>27 (26)*</td>
<td>30 (28)*</td>
<td>50 (48)</td>
<td>25 (24)</td>
</tr>
</tbody>
</table>

n = number of semen samples.

*Significantly higher than the corresponding value for <1 day delay to culture (P < 0.01, 0.05 respectively).

**Significantly lower than the corresponding value for <1 day delay to culture (P < 0.01).

Table III. In-vitro fertilization (IVF) rates and frequency of diagnosis of sperm dysfunction related to significant bacterial culture from semen in 90 consecutive IVF treatment cycles. Values in parentheses are percentages unless otherwise indicated

<table>
<thead>
<tr>
<th>n</th>
<th>Potential pathogens</th>
<th>Skin flora</th>
<th>No significant growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>90</td>
<td>10 (11)</td>
<td>14 (16)</td>
</tr>
<tr>
<td>Sperm dysfunction</td>
<td>24</td>
<td>2 (8)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Normal spermatozoa</td>
<td>66</td>
<td>8 (12)</td>
<td>11 (17)</td>
</tr>
<tr>
<td>% fertilization rate</td>
<td>60 (68/114)</td>
<td>60 (58/97)</td>
<td>55 (364/656)</td>
</tr>
</tbody>
</table>

(fertilized /total oocytes)

Table IV. Significant bacterial and yeast culture from high vaginal swabs (HVS) taken at time of oocyte collection as related to antibiotic treatment of the male partner. Values in parentheses are percentages

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Antibiotic treated</th>
<th>Untreated</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>48</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>19 (37)</td>
<td>17 (35)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>13 (29)</td>
<td>5 (10)</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>4 (8)</td>
<td>0</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Total other organisms 25</td>
<td>31</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Two organisms on HVS 11</td>
<td>5</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

*Fisher’s exact test was used. NS = not significant.

there were significantly more samples where more than one organism was cultured from the sample (P < 0.05). There was no significant difference in the culture rate of potentially pathogenic organisms from semen between those patients with sperm dysfunction (n = 114) and other diagnostic groups (n = 335): 59/114 (52%) versus 180/335 (54%), irrespective of the time from production to culture.

The results of the second study are summarized in Tables III and IV. In the 90 cases undergoing IVF, the semen samples used for treatment which had no bacterial growth were compared with those where a significant growth of either skin flora or potentially pathogenic organisms were isolated (Table III). There was no significant difference between the groups with regard to number of patients with normal spermatozoa and sperm dysfunction. Significant potential pathogens isolated were as follows: enterococci (n = 3), coliforms (n = 2), non-lactose-fermenting Gram-negative bacilli not further specified (n = 1), coagulase-negative staphylococci (n = 3), mixed anaerobes (n = 1), α-haemolytic streptococci (n = 1) and non-haemolytic streptococci (n = 1). Two patients had two organisms isolated from the same semen sample, one with normal spermatozoa (enterococci/gram-negative bacilli) and one with sperm dysfunction (enterococci/skin flora). No significant difference was seen in fertilization rates per oocyte, nor in complete fertilization failure rates (two cases where potential pathogens were isolated from the semen and three cases with no significant growth) between groups, irrespective of male fertility diagnostic category. There was no significant difference in mean age of either partner between groups or the proportion of cases with sperm dysfunction.

Table IV shows that the most common bacteria potentially pathogenic to an embryo culture system which were cultured in significant numbers from the vagina on the day of oocyte collection were the Gram-negative bacilli. These were cultured significantly more often in patients whose partner had received antibiotic treatment prior to providing a semen sample (13 coliforms and two non-lactose-fermenting Gram-negative bacilli not further specified) compared with those who had not (five coliforms). There was no significant difference in isolation rate of other individual organisms regarded as vaginal commensals, but also potentially pathogenic to an embryo culture system, between antibiotic-treated and untreated groups. The organisms cultured in these groups were enterococci (n = 4 and 5), coagulase-negative staphylococci (n = 2 and 4), mixed anaerobes (n = 7 and 8), non-haemolytic streptococci (n = 1 and 1), mixed streptococci ((n = 0 and 1), α-haemolytic streptococci (1 and 0), β-haemolytic streptococci (n = 7 and 8), gardnerella (n = 1 and 0), Staphylococcus aureus (n = 0 and 1) and skin flora (n = 2 and 3) respectively. In four cases significant numbers of Candida albicans were cultured; this organism was regarded as both a vaginal pathogen and potentially pathogenic to the embryo culture system.

Discussion

This study confirms the high incidence of potentially pathogenic bacterial species which may be cultured from semen, the frequency of which increases with delay in the sample reaching the microbiology laboratory. The commonest organisms from seminal culture were the Gram-negative bacilli and enterococci. The limited duration of action of naturally
occuring bactericidal and bacteriostatic substances within the seminal and prostatic fluid may account for increasing detection with time. Mardh and Colleen (1975) showed that the growth of Gram-positive aerobic bacteria but not Gram-negative aerobic or anaerobic bacteria was inhibited by seminal fluid in vitro. In contrast, others have found variable spectra of inhibitory effects of prostatic and seminal fluid (Gupta et al., 1967; Rusk et al., 1973).

The rationale and benefit of treating asymptomatic male partners with positive semen bacteriology remain unclear. Eggert-Kruse et al. (1988, 1992) concluded that microbial colonization of the male and female reproductive tract is of minor importance for sperm–mucus interaction and that the benefits of antibiotic treatment in asymptomatic couples is limited. In this study the presence of bacteria in IVF semen samples did not appear to show an adverse effect on fertilization rates. De Geyter et al. (1994) similarly found no significant effect on fertilization rates in a study of 281 couples undergoing IVF. Studies assessing treatment of positive semen bacteriology and fertilization rates in natural conception suggest controversial results and limited benefit, in particular failing to show the link between Gram-negative bacilli and infertility (Matthews et al., 1978; Bar-Chama et al., 1994). Naessens et al. (1986) found that no single aerobic or anaerobic organism could be related to abnormal semen samples. Eggert-Kruse et al. (1995), despite special screening measures for the detection of anaerobes in the semen that resulted in a 4-fold increase in detection over standard techniques, also found no significant difference in sperm parameters or ability to penetrate cervical mucus associated with their presence. Fowler and Mariano (1984) suggested that apparent prostatic infection may be due to bacterial contamination of the seminal fluid by urethral bacteria. Bacteria may contaminate the semen sample also from the genital skin and hands. Boucher et al. (1995) showed that bacterial contamination of the semen sample could be reduced following direct verbal counselling on how to avoid bacterial contamination. If the presence of bacteria in the semen is common and its subsequent antibiotic treatment shows no benefit in terms of improvement in sperm parameters, then there appears to be no logical reason for such treatment. Of concern are the potentially deleterious effects that certain antibiotics, such as nitrofurans, aminoglycosides, macrolides and sulphur drugs, may have on sperm function (Schlegel et al., 1991).

This study shows a significantly greater incidence of Gram-negative bacilli in the high vaginal swabs of patients whose asymptomatic partners had antibiotic treatment for positive semen culture prior to IVF. Ideally, to confirm that prior antibiotic treatment of the male caused this, rather than the fact that the female partners of these men had coexistent abnormal vaginal flora, a high vaginal swab prior to the partner’s antibiotic treatment should be compared with that obtained on the day of oocyte collection.

Of the commonly prescribed antibiotics in our unit, ciprofloxacin is known to be concentrated in the seminal fluid (Berger et al., 1990) and co-amoxycclav is concentrated in the prostate, though to lesser degree due to its poorer lipid solubility (Cooper et al., 1990). During sexual intercourse, antibiotics may thus be deposited in the vagina from the ejaculate. In a primate study model, the indigenous vaginal bacterial microflora was shown to be significantly disturbed by vaginal instillation of amoxycillin (Winberg et al., 1993). Following amoxycillin administration, in 17 of 21 cases spontaneous rectum to genital tract spread of Escherichia coli was observed, resulting in vaginal colonization which could persist for several months. This could occur after a single dose of amoxycillin, and the study demonstrated that amoxycillin could break down vaginal colonization resistance and so promote colonization with a different E. coli species (Winberg et al., 1993).

A possible route of bacterial entry into the embryo culture system is through vaginal contamination at the time of trans-vaginal oocyte collection. If there has been significant detrimental alteration in the vaginal bacterial flora as a result of the male partner’s antibiotic administration, this could increase the chance of pathogenic bacterial contamination of the embryo culture system. Further, in the second phase of our study, two of the 100 couples developed bacterial infection of the embryo culture system which was isolated as the same Gram-negative bacillus species (E. coli, with same API test profile) as that found in the high vaginal swab of the patient on the day of oocyte collection. The male partners in both cases had been treated with co-amoxycclav for positive bacterial growth in their pre-IVF semen, one for heavy growth of an enterococcus, the other for a heavy growth of skin flora, and both semen cultures on the day of oocyte collection were negative. This cautions against antibiotic treatment in asymptomatic male partners. If the male partner is given antibiotics for a coincidental infection during the IVF cycle, he should be advised to avoid intercourse or to use condoms. Of greater concern might be the effect on vaginal flora of antibiotic treatment for coincidental infection in the female prior to IVF treatment.

In our study we found no significant difference in the fertilization rates of patients according to the presence or absence of bacterial pathogens on skin flora on the day of oocyte collection, irrespective of whether the male partner had received prior antibiotic treatment. However, a possible detrimental effect on the vaginal microflora was demonstrated which may predispose to contamination of the embryo culture system. Since this study was completed, we have stopped the routine microbiological culture of semen prior to IVF in asymptomatic male patients. In a 4 month period covering 208 cycles of IVF treatment there has been no bacterial infection of the embryo culture system. It would therefore seem unnecessary and possibly unwise to screen and treat the semen of asymptomatic male partners undergoing IVF.

Acknowledgements

We are grateful to Dr Andrew Turner and the Department of Microbiology, Bristol Royal Infirmary, for the culture and reporting of samples in this study and to the nursing and laboratory staff of the Bristol University IVF Service for their help in the collection of samples for microbiological examination.

References

Seminal bacteriology prior to IVF


Received on November 28, 1995; accepted on March 14, 1996