EPIDEMIOLOGY AND ECOLOGY

The prevalence of *Chlamydia trachomatis* in fresh tissue specimens from patients with ectopic pregnancy or tubal factor infertility as determined by PCR and in-situ hybridisation

RACHEL E. L. BARLOW, IAN D. COOKE*, OLUSEGUN ODUKOYA*, MARK K. HEATLEY†, JULIAN JENKINS‡, GORDON NARAYANSingH§, SAMUEL S. RAMSEWA§ and ADRIAN ELEY

Division of Genomic Medicine, University of Sheffield Medical School, Floor F, Sheffield S10 2RX, 
*Department of Obstetrics and Gynaecology, Division of Surgical and Anaesthetic Sciences, University of Sheffield Medical School, Sheffield, †Department of Pathology, Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, ‡Division of Obstetrics and Gynaecology, Department of Hospital Medicine, University of Bristol, Bristol, UK and §Department of Clinical Surgical Sciences, University of the West Indies, St Augustine, Trinidad

The prevalence of chlamydial DNA determined by PCR and in-situ hybridisation (ISH) in fresh tissue specimens (endometrium, fallopian tube and ovary) was investigated in 33 women presenting with ectopic pregnancy (EP), 14 women with tubal factor infertility (TFI) and 50 control patients from the UK and the West Indies. In the UK EP group, chlamydial DNA was detected by PCR in 56% of patients; similar results were found in the Trinidad EP group (67%). In the TFI group, chlamydial DNA was detected in (71%) of patients by PCR. The detection of *Chlamydia trachomatis* DNA by ISH was highest in the TFI group (43%). Women presenting with EP and TFI showed evidence of previous or current genital *C. trachomatis* infection, underlining the importance of this micro-organism in the development of these conditions. Importantly, chlamydial DNA could be detected in DNA preparations from the endometrium, fallopian tube and ovary of EP and TFI patients at the time of surgery.

Introduction

*Chlamydia trachomatis* is a common bacterial cause of sexually transmitted disease world-wide and is responsible for high levels of morbidity. One of the major problems associated with genital chlamydial infection is that it may be asymptomatic. Ascending infections, especially in women, may result in detrimental sequelae in the upper genital tract such as tubal damage with subsequent tubal factor infertility (TFI) and ectopic pregnancy (EP).

Over the last few decades, there has been a rise in the incidence of EP in several European countries and the USA [1–4]. As well as *C. trachomatis*, there are other microbial causes of EP [5] and this has resulted in many investigations to determine aetiologiical agents. In a recent French study, it was suggested that 43% of ectopic pregnancies are caused by *C. trachomatis*, confirming it as the most common microbial cause [5].

Infertility is an important public health problem and in 10–20% of infertile couples from North America it has been shown to be caused by tubal disease [6]. The precise mechanism by which chlamydial infection results in tubal damage is still unclear despite many studies. As with chlamydial investigations in women who have had EP, the vast majority of TFI studies have been serological, with different methodological approaches ranging from the micro-immunofluorescence (MIF) test to heat-shock protein-60 (HSP-60) enzyme immunoassay (EIA) [7–18]. However, there are concerns that serological methods lack specificity.

Other diagnostic techniques have been applied to the detection of *C. trachomatis* in women with reproductive abnormalities such as cell culture, antigen detection, in-situ hybridisation (ISH) and PCR. By cell culture, the recovery of chlamydia from the endometrium and fallopian tubes has been poor with results ranging from 0 to 15% [10, 19]. Immunoperoxidase...
staining with a monoclonal antibody that recognises a species-specific determinant on the major outer-membrane protein of C. trachomatis has been used, but has been found to be less sensitive than ISH [14,20]. In contrast, ISH has confirmed the presence of chlamydial DNA in TFI patients, supporting the hypothesis that chlamydial DNA can persist in tissue specimens when the organism cannot otherwise be detected [20]. Despite the fact that PCR offers high sensitivity in levels of detection, there have been few studies where it has been applied to similar specimens [11,21]. The purpose of this study was to investigate fresh tissue specimens (endometrium, ovary, fallopian tube) from patients diagnosed with EP or TFI for the presence of C. trachomatis DNA by means of PCR and ISH.

Materials and methods

Specimens

Specimens were collected from Sheffield (Jessop Hospital for Women), Bristol (St Michael's Hospital) and Trinidad (Mount Hope Women's Hospital, St Augustine and the General Hospital, Port of Spain). Three study groups were examined: women presenting with EP (n = 33), women undergoing surgery for TFI (n = 14) and control patients (n = 50). The controls from Sheffield and Bristol were patients undergoing hysterectomy or laparoscopic sterilisation; controls from Trinidad were patients undergoing sterilisation. Both control groups were considered to be of normal fertility potential.

Three tissue samples were collected from each patient in the study: an endometrial sample, a sample of the fallopian tube (ampulla, isthmus or fimbriae) and an ovarian core biopsy. Each set of samples was collected in duplicate and coded blind, one set was frozen and the other fixed immediately in buffered formalin 10%. Unfortunately, in some instances a complete set of tissue specimens was unavailable for investigation. The code for each patient was not broken until all the tests were completed. The study had Ethics Committee approval at all three sites.

DNA extraction

Tissue samples were thawed and homogenised with a sterile pestle and mortar in 1.0 ml of lysis buffer containing proteinase K (Sigma) 500 μg/ml, 10% KCl PCR buffer (Bioline) and Triton X-100 (BioRad) 1% v/v. For decontamination, pestles and mortars were soaked in 0.1 M HCl overnight before autoclaving. Appropriate controls were used to check for material carryover. The cellular suspension was incubated at 37°C overnight, boiled for 10 min, and then centrifuged at 10000 g for 2 min to sediment tissue debris. The resulting supernatant was subjected to phenol-chloroform, chloroform-isoamyl alcohol DNA extraction followed by ethanol precipitation with 3 M sodium acetate (pH 4.8) and glycogen at ~80°C overnight. The DNA was pelleted by centrifugation at 10000 g for 30 min at 4°C, washed twice with ice-cold ethanol 70%, air dried and finally resuspended in 100 μl of sterile water. Appropriate extraction controls were set up throughout the procedure to detect contamination between samples and from equipment or chemicals and the environment. Tissue homogenisation, DNA extraction, pre- and post-PCR manipulations were completed in separate rooms.

PCR detection of human genomic DNA

The presence of human genomic DNA was determined with primers PC03 [21] and PC06 [22] specific for the human β-globin gene. The PCR was performed on 10 μl of the extracted DNA sample in a final reaction mix of 50 μl. The final reaction mix contained 2.5 mM MgCl₂, 1× KCl PCR buffer, 200 μM of dNTP, 50 pmol of each primer and Taq DNA polymerase 1 U. The PCR consisted of 40 cycles of amplification, comprising denaturation at 94°C for 1 min, primer annealing at 55°C for 40 s and primer extension at 72°C for 40 s. Before the PCR assay, the tubes were incubated at 94°C for 4 min and after PCR at 72°C for 8 min. The expected PCR product of 326-bp was visualised on an agarose 0.8% gel after ethidium bromide staining. Human placental DNA (Sigma) was used as a positive control and sterile purified water was used as a negative control.

PCR detection of C. trachomatis DNA

Samples found to be positive for human genomic DNA were tested for the presence of C. trachomatis DNA with primers T1 and T2 aimed at the 7.5-kb plasmid [23]. The PCR (with the GeneAmp Carry Over Prevention System, Perkin Elmer, UK) was performed on 10 μl of the extracted DNA sample in a final reaction mix of 50 μl. The final reaction mix contained 2.5 mM MgCl₂, 1× KCl PCR buffer, 200 μM of dATP, dCTP and dGTP, 400 μM dUTP, 100 pmol of each primer, AmpEraser Ultra-N-Glycosylase (UNG) 0.5 U and Taq DNA polymerase 1 U. The PCR consisted of 40 cycles of amplification, comprising denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s and primer extension at 72°C for 30 s. Before PCR, the tubes were incubated at 28°C for 10 min, followed by 10 min at 95°C. After PCR, the tubes were held at 72°C and chloroform was added to denature any residual UNG. The expected product, a 517-bp amplicon, was analysed with an agarose 0.8% gel by standard protocols and transferred to a nylon membrane for Southern blot analysis [24]. PCR product confirmation and the detection of weak positive results was achieved with a 3’ fluorescein-labelled oligonucleotide (5’CGCG AgC gCT AgA ggC Cgg TCT ATT TAT gAT 3’) specific for an internal region of the PCR product [23]. The probe was labelled and detected with the ECL 3’ Oligo Labelling and Detection System (Amersham)
Pharmacia Biotech, St Albans, UK) according to the manufacturer's instructions. LGV1 DNA, extracted from tissue culture, was used as a positive control, sterile purified water was used as a negative control.

In-situ hybridisation

The ISH employed was a non-isotopic method based on the Amersham Pharmacia Biotech DNA Colour Kit. The DNA probe was homologous to part of the endogenous 7.5-kb plasmid of *C. trachomatis*. The double-stranded 320-bp DNA probe was produced by nested PCR; primary PCR was with the Claas *et al*, primers [21] and secondary PCR with in-house primers internal to the primary 517-bp PCR product with *Pfu* DNA polymerase (Stratagene). The probe was finally purified through a QIAquick PCR Purification Column (Qiagen) to remove primers, *Pfu* and dNTPs. A 326-bp human β-globin-specific PCR probe [21, 22] was generated in a similar manner and used as a positive ISH control. Each probe was diluted in sterile purified water to a final concentration of 25 ng/ml, denatured by boiling for 10 min, snap-cooled on ice and then labelled with nonamer primers and fluorescein-11-dUTP. The final fluorescein-labelled probes were stored in the dark at −20°C. Sections of tissue (5 μm) mounted on aminopropyltriethoxysilanes (APES)-coated slides were de-waxed in xylene for 15 min. The sections were then re-hydrated through graded ethanol (2 × 100%, 1 × 95%, 2 × 70%) and washed in PBS for 5 min. The sections were then digested for 15 min with pepsin (Sigma) 500 μg/ml in 0.2 M HCl at 37°C, rinsed in sterile water to prevent over-digestion and immersed in acetic acid 20% v/v for 15 s to remove any endogenous alkaline phosphatase activity. The sections were washed gently for 5 min in PBS, dehydrated through graded ethanol and left to dry in air.

The *C. trachomatis* probe was boiled for 5 min, snap-cooled on ice for 10 min and diluted to 400 ng/ml in a 1:1 hybridisation solution (hybridisation buffer supplied: de-ionised formamide, Sigma). The sections were then heated to 95°C for 10 min and 65 μl of hybridisation mix plus appropriate probe were placed over each section with ISH Easi Seal (Hybaid). The human β-globin-specific probe was diluted to 100 ng/ml in the same manner. The slides were then left to hybridise overnight at 42°C. The Easi Seal was removed and the slides were subjected to conditions of increased stringency (1 × SSC, SDS 0.1% w/v for 2 × 3 min followed by 0.5 × SSC, SDS 0.1% w/v for 2 × 10 min at 42°C) to remove non-specifically bound probe. The slides were then washed for 5 min in TBS (100 mM Tris-HCl, 400 mM NaCl, final pH 7.5) to remove any residual SDS, blocked for 1 h in TBS containing blocking agent 0.5% w/v (as supplied) at 37°C, rinsed in TBS for 1 min and then drained. The anti-fluorescein alkaline phosphatase conjugate (×1000) was diluted in TBS containing BSA fraction.

V 0.5% w/v to 1 in 500, c. 100 μl were placed over each section and the slides were incubated at 37°C for 1 h in a moist chamber. The slides were then washed in TBS for 3 × 5 min and left to drain. Finally, 150 μl of substrate – nitroblue tetrazolium (NBT) 45 μl, 5-bromo-4-chloro-3-indolyl phosphate (BCIP) 35 μl in detection buffer 10 ml containing 100 mM Tris-HCl, 100 mM NaCl (pH 9.5) and 50 mM MgCl₂ was placed over each section and the slides were left to develop in the dark for 4 h. Tap water was used to stop the reaction and the sections were counterstained with either methyl green 1% or eosin 1% before mounting. A positive signal was observed as a blue/purple/black precipitate within a cell as viewed by light microscopy.

Statistical analysis

The results for each study group were compared with the appropriate control group by the Fisher’s exact probability test (two-tailed). A p value < 0.05 was considered to be statistically significant. The Bonferroni correction was used for multiple comparisons.

Results

Control patients

Sheffield and Bristol. Of the 20 study control patients (mean age 40.5 years, range 33–57 years, SD 6.3), 20% (4 of 20) were positive by one or more of the methods used. Chlamydial DNA was detected in 15% of the patients by PCR/Southern blotting and in 5% of patients by ISH. PCR/Southern blotting detected chlamydial DNA in the fallopian tube samples of three patients and in the ovarian sample of one. ISH detected chlamydial DNA in the fallopian tube specimen of only one patient who was negative by PCR/Southern blotting at the same site.

Trinidad. Of the 30 patients sampled (mean age 31.7 years, range 22–44 years, SD 5.3), 40% (12 of 30) were positive by one or more of the methods employed. Chlamydial DNA was detected in 40% of the study controls by PCR/Southern blotting and in 12% by ISH. By PCR/Southern blotting, chlamydial DNA was detected in seven fallopian tube samples, nine ovarian samples and one endometrial sample. ISH detected chlamydial DNA in two fallopian tube specimens.

Ectopic pregnancy (EP)

Sheffield and Bristol. Of the nine EP patients (mean age 31.1 years, range 24–40 years, SD 5.5) enrolled in the study from the UK, five (56%) showed evidence of *C. trachomatis* infection by one or more of the tests used (Table 1). PCR/Southern blotting detected chlamydial DNA in 56% (p > 0.05) of the patients and ISH in 22% (p > 0.05). With PCR/Southern blotting, chlamydial DNA was detected in the fallopian tube samples of three patients and two patients were positive.
in the endometrium and ovary. For ISH, chlamydial DNA was located in the fallopian tube specimens of two patients and in the endometrium of one.

**Trinidad.** This group comprised a total of 24 patients (mean age 31.8 years, range 20–40 years, SD 5.1) with an overall chlamydial prevalence of 79% (19 of 24) by one or more of the methods used (Table 2). Within this group, PCR/Southern blotting detected chlamydial DNA in 67% (p > 0.05) of the patients and ISH in 38% (p > 0.05). By PCR/Southern blotting, *C. trachomatis* DNA was detected in the fallopian tube and ovarian samples of 11 patients and from the endometrium of four. Chlamydial DNA was localised by ISH in the fallopian tube of five and in the endometrium of two patients.

**Tubal factor infertility (TFI)**

**Sheffield and Bristol.** Samples were taken from 14 patients at the time of surgery (mean age 29.7 years, range 24–36 years, SD 3.7) and 79% (11 of 14) of the TFI group were positive by one or more of the tests used (Table 3). By PCR/Southern blotting, 71% (p < 0.02) were positive; seven patients had detectable chlamydial DNA in the endometrium, five in the

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>DNA detection</th>
<th>Clinical history</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>+ (OF)</td>
<td>NA</td>
</tr>
<tr>
<td>61</td>
<td>–</td>
<td>+ (F) History of PID</td>
</tr>
<tr>
<td>62</td>
<td>NA</td>
<td>Fimbrial blockage</td>
</tr>
<tr>
<td>63</td>
<td>NA</td>
<td>One abortion</td>
</tr>
<tr>
<td>64</td>
<td>+ (F)</td>
<td>History of PID</td>
</tr>
<tr>
<td>65</td>
<td>+ (OF)</td>
<td>One abortion, history of PID</td>
</tr>
<tr>
<td>66</td>
<td>–</td>
<td>Primary infertility</td>
</tr>
<tr>
<td>67</td>
<td>+ (F)</td>
<td>NA</td>
</tr>
<tr>
<td>68</td>
<td>–</td>
<td>+ (F) One previous EP</td>
</tr>
<tr>
<td>69</td>
<td>+ (O)</td>
<td>One abortion, PID and STD</td>
</tr>
<tr>
<td>70</td>
<td>–</td>
<td>+ (F) NA</td>
</tr>
<tr>
<td>71</td>
<td>–</td>
<td>+ (E) NA</td>
</tr>
<tr>
<td>72</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>73</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>74</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>75</td>
<td>+ (OF)</td>
<td>NA</td>
</tr>
<tr>
<td>76</td>
<td>+ (OF)</td>
<td>NA</td>
</tr>
<tr>
<td>77</td>
<td>NA</td>
<td>+ (EF) Two abortions</td>
</tr>
<tr>
<td>78</td>
<td>+ (OF)</td>
<td>NA History of PID</td>
</tr>
<tr>
<td>79</td>
<td>+ (O)</td>
<td>+ (F) NA</td>
</tr>
<tr>
<td>80</td>
<td>+ (OF)</td>
<td>NA</td>
</tr>
<tr>
<td>81</td>
<td>+ (EO)</td>
<td>History of PID</td>
</tr>
<tr>
<td>82</td>
<td>+ (OF)</td>
<td>NA</td>
</tr>
<tr>
<td>83</td>
<td>+ (EOF)</td>
<td>History of PID</td>
</tr>
</tbody>
</table>

---

**Table 2. Detection of *C. trachomatis* by PCR/Southern blotting and ISH in the Trinidad EP group**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>DNA detection</th>
<th>Clinical history</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>+ (F)</td>
<td>Two previous EPs, previous STD, PID and pelvic adhesions</td>
</tr>
<tr>
<td>52</td>
<td>–</td>
<td>One previous EP, previous PID</td>
</tr>
<tr>
<td>53</td>
<td>–</td>
<td>One previous EP, previous PID, 4 years infertility</td>
</tr>
<tr>
<td>54</td>
<td>+ (O)</td>
<td>NA</td>
</tr>
<tr>
<td>55</td>
<td>+ (F)</td>
<td>NA</td>
</tr>
<tr>
<td>56</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>57</td>
<td>+ (EF)</td>
<td>One previous EP, previous IVF</td>
</tr>
<tr>
<td>58</td>
<td>+ (EO)</td>
<td>NA</td>
</tr>
<tr>
<td>59</td>
<td>–</td>
<td>Perihepatitis, peri-ovarian adhesions</td>
</tr>
</tbody>
</table>

E, endometrium; O, ovary; F, fallopian tube; PID, pelvic inflammatory disease; STD, sexually transmitted disease; NA, not available.
falllopian tube and five in the ovary. ISH detected chlamydial DNA in 43% (p > 0.05) of the TFI patients, five of whom had *C. trachomatis* DNA in the fallopian tube whilst two patients were positive in the endometrium.

**Discussion**

In the last few years there have been several studies in which EP and TFI have been investigated with regard to *C. trachomatis* involvement. Methods of investigation have included serology and molecular methods such as PCR and ISH. However, in these previous studies fresh clinical material was rarely investigated. In the present study of 97 patients, fresh clinical material was examined by both PCR and ISH to determine the presence of chlamydial DNA.

Four UK study control patients had detectable chlamydial DNA by PCR or ISH, or both. Chlamydial DNA was not detected in the endometrium of any control study patients from this group but it was detected in the fallopian tubes and ovarian samples (PCR only) of four patients. From the results, ISH was not as sensitive as PCR, although one patient (no. 14) was positive only by ISH; this may be due to sampling error or loss of chlamydial DNA during the extraction procedure before PCR assay.

The relatively high combined prevalence (20%) may be accounted for by the high mean age (40.5 years) of the patients in the UK control group. However, the sample size is small and because there have been few similar studies on biopsy material, it is difficult to make comparisons. A recent study in the UK where women under 25 were screened for *C. trachomatis* by molecular methods on urine, gave a prevalence of *c.* 10% [25]. A criticism of the present study is that there was no simultaneous testing for *C. trachomatis* infection of the lower genital tract. This might have been helpful in understanding whether the disease manifestations were acute or chronic.

Of the 30 study control patients in Trinidad sampled for PCR, 12 (40%) were positive for chlamydial DNA. The detection of chlamydial DNA by ISH was 12%. In Martinique, Chout et al. [26] used cell culture methods to screen the urethra and cervix of 1411 prenatal patients and obtained a chlamydial isolation rate of 27%. More recently, Dowe et al. [27] reported a prevalence of between 35% and 55% in Jamaican women attending family planning, gynaecology or STD clinics.

A comparison of the Trinidad and Sheffield/Bristol study control groups shows a different picture for the prevalence of *C. trachomatis*. From the data presented here, the high prevalence rate of *C. trachomatis* in the West Indies suggests a population with an increased rate of silent or asymptomatic chlamydial infection and the question of surveillance for *C. trachomatis* and possibly other sexually transmitted diseases needs to be addressed.

Within the UK EP group, chlamydial DNA was detected in all the sample types by PCR and in fallopian tube and endometrial samples by ISH, thus emphasising the importance of ascending infection in the development of tubal damage that can predispose to EP (Table 1). Lan et al. [21], in a retrospective study of 48 archived, paraffin-embedded salpingectomy specimens from 37 women with EP, detected chlamydial DNA (by PCR) in only one patient (3%). In the present study, chlamydial DNA was detected by PCR in 56% of the EP patients. This apparent difference in the detection rate of chlamydial DNA may be due to the use of archival material (where the DNA may have degraded) or the amount of archived material used for DNA extraction.

Of the 24 Trinidad EP patients (Table 2), samples for

**Table 3. Detection of *C. trachomatis* by PCR and ISH in the Sheffield and Bristol TFI group**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>DNA detection</th>
<th>Clinical history</th>
</tr>
</thead>
<tbody>
<tr>
<td>84</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>85</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>86</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>87</td>
<td>+ (F)</td>
<td>Primary infertility</td>
</tr>
<tr>
<td>88</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>89</td>
<td>+ (E)</td>
<td>Secondary infertility, two miscarriages</td>
</tr>
<tr>
<td>90</td>
<td>+ (EF)</td>
<td>Secondary infertility</td>
</tr>
<tr>
<td>91</td>
<td>+ (F)</td>
<td>Secondary infertility, one miscarriage</td>
</tr>
<tr>
<td>92</td>
<td>+ (O)</td>
<td>Secondary infertility</td>
</tr>
<tr>
<td>93</td>
<td>+ (EOF)</td>
<td>Primary infertility</td>
</tr>
<tr>
<td>94</td>
<td>+ (EOF)</td>
<td>Primary infertility, EP after tubal surgery</td>
</tr>
<tr>
<td>95</td>
<td>+ (EO)</td>
<td>Secondary infertility</td>
</tr>
<tr>
<td>96</td>
<td>+ (EO)</td>
<td>Secondary infertility</td>
</tr>
<tr>
<td>97</td>
<td>+ (E)</td>
<td>Secondary infertility, one miscarriage</td>
</tr>
</tbody>
</table>

E, endometrium; O, ovary; F, fallopian tube; NA, not available.
PCR were obtained from 21 patients and samples for ISH from 16 patients. The low number of samples for ISH was due to insufficient sample fixation before processing or inadequate histological processing, making the samples unsuitable for ISH due to loss of cellular architecture. Of the EP patients, 67% were positive for chlamydial DNA at one or more sites sampled by PCR and 38% were positive for chlamydial DNA at one or more sites by ISH. Again, even though more samples were available for PCR, ISH was less sensitive, although there were four patients who were ISH positive and PCR negative.

All the Trinidad EP patients with a recorded history of PID had evidence of having had a chlamydial infection, as did all but one patient (no. 63) who had had at least one induced abortion. This has implications in the management of chlamydial infection in Trinidad and may reflect inadequate treatment or diagnosis of chlamydial PID. With respect to induced abortion, the presence of *C. trachomatis* DNA may underline the need for a post-operative antibiotic regimen to prevent ascending infection and its sequelae.

It is interesting to note that Lan et al. [21], as previously discussed, failed to detect a large number of patients with chlamydial DNA at the time of EP (unlike this study), but chlamydial DNA was detected in cervical smears from up to 3 years before the reported EP. They suggested that the EP in these cases was a result of a late post-inflammatory response to an ascending infection resulting in tubeal damage. The presence of chlamydial DNA in a very high proportion of EP patients in the present study may indicate that tubal damage was the result of an ongoing infection or the persistence of *C. trachomatis* or chlamydial DNA.

The possible role of persistence in EP needs to be addressed by such methods as reverse transcriptase PCR (RT-PCR) to determine whether the DNA detected arises from actively replicating organisms. Recently, RT-PCR was used to analyse 10 patients with EP [28]. Interestingly, seven were positive for *C. trachomatis* by both PCR and RT-PCR, suggesting that viable metabolically active chlamydia were present in the fallopian tubes of the EP patients examined.

In the present study, chlamydial DNA detected by ISH in the fallopian tubes in both the UK and Trinidad EP groups was generally localised to the fallopian tube ciliated epithelium. Infection of the ciliated cells of the fallopian tubes may affect the movement of the fertilised ovum towards the endometrium, which could predispose to tubal implantation and hence ectopic pregnancy. These findings support the conclusion that a higher proportion of EP may be caused by a current *C. trachomatis* infection than was previously thought.

In the Sheffield and Bristol TFI group (Table 3), the detection of chlamydial DNA was high, with 71% of patients testing positive by PCR (p <0.02) and 43% by ISH (p > 0.1). Again, all sample types were found to be positive and only one patient (no. 84) was positive by ISH alone. In the fallopian tube, a positive ISH signal was found localised in the epithelium, unlike the findings of Patton et al. [14] where the signal was found in the mucosa/submucosa and in adhesions, suggesting a persistent chlamydial infection. This latter study found *C. trachomatis* DNA by radioactive ISH in 50% of patients with post-infectious tubal infertility, a detection rate comparable to the present study.

The importance of an ovarian sample that is positive by PCR and negative for chlamydial DNA by ISH is debatable. This discrepancy may be due to the relative insensitivity of ISH when compared with PCR and the relatively small amount of ovarian sample tested by ISH. Alternatively, the *C. trachomatis* DNA detected may have been a contaminant of the peritoneal cavity as described by Marana et al. [29], who cultured *C. trachomatis* from the peritoneal fluid of 2 of 34 infertile women. The question of possible chlamydial infection of the ovarian epithelia is intriguing; such an infection could potentially affect ovarian function. Recently, other workers have shown that 7 (36%) of 19 ovarian specimens tested positive for chlamydia antigen or DNA [30].

Of the 10 TFI PCR-positive patients, seven had positive endometrial samples, five positive ovarian samples and five positive fallopian tube samples. Two patients (nos. 53 and 54) had PCR-positive results for all three sample sites. Tubal occlusion resulting from hydrosalpinx and peritubal adhesion formation and tubal scarring are the underlying pathological changes associated with TFI following acute salpingitis. The majority of the TFI patients tested by ISH were found to be positive for chlamydial DNA in the fallopian tubes, located mainly in the ciliated epithelium (or the secretory epithelium if the ciliated epithelium was absent) and occasionally in the lamina propria of the mucosal folds and submucosa.

The results of the present study have shown that chlamydial DNA can be detected in a statistically significant proportion of TFI patients. For women presenting with EP, chlamydial DNA could be found in a larger number of patients when compared with the appropriate control group. The relationship between chlamydial PID and the development of EP and TFI is well established. However, there is controversy as to whether chronic sequelae following chlamydial infection are due to persistent latent infection or an autoimmune reaction consequent upon breakdown of tolerance to heat-shock protein-60 [31]. Nevertheless, if routine screening and the prompt diagnosis of chlamydial PID were implemented, a reduction in the incidence of post-infectious tubal damage that can predispose to the development of EP and TFI may be observed [32, 33].
This study was supported in part by a grant from WellBeing, London, UK (grant no. E1/95). A statement of informed consent was obtained from patients and ethics committee approval of the different insti-
tutions was obtained for human experimentation. We thank Kevin
Corbett, Ionascu Gallagher (Department of Pathology, University of
Sheffield) for the preparation of tissue samples for ISH and Dr Owen
Caul (Public Health Laboratory, Bristol) for a critical review of the
manuscript. We thank Dr Lee Ann Campbell and colleagues (University of
Washington, Seattle, USA) for demonstration of their
isotopic ISH methodology. This work was presented in part at the
FEMS Workshop "Human Chlamydial Infections", Izmir, Turkey (12–
16 May 1997; Abstract no. 1) and at the 176th Meeting of the
1998; Abstract no. 214).

References

1984 women with laparoscopically verified disease and 657 control women with normal laparoscopic results. Sex Transm
421–447.
6. Kosem S, Brunham RC. Fallopian tube obstruction as a sequel to Chlamydia trachomatis infection. J Inf Control
Biol 1996; 8: 584–590.
7. Moore DE, Foy HM, Daling JR et al. Increased frequency of serum antibodies to Chlamydia trachomatis in infertility due to
9. Chow JY, Yonkura ML, Richwald GA, Greenland S, Sweet RL, Schachter J. The association between Chlamydia trachoo-
serological and histologic correlates. J Infect Dis 1992; 165:
1076–1081.
11. Osler S, Persson K. Chlamydia antibodies and deoxyribonu-
57: 578–582.
12. Uminnian SS, Wu CH, Jungkind D, Gobal B, Filer RB, Glassner M. Chlamydia antibody, as determined with an
enzyme-linked immunosorbent assay, in tubal fertility factor.
protein and tubal infertility. J Infect Dis 1991; 165:
1236–1240.
women with post-infectious tubal infertility. Am J Obstet
15. Fredkam HM, Clad A, Herr AS, Wiedmann-Al-Ahmad M, Jung B. Immune response to Chlamydia trachomatis heat-
shock protein in infertile female patients and influence of
Chlamydia pneumoniae antibodies. Eur J Clin Microbiol Infect
16. Mehanna MTK, Rizk MA, Feewis NVM et al. Chlamydial
serology among patients with tubal factor infertility and ectopic
presence of serum antibody to the chlamydial heat shock protein (CHSP60) as a diagnostic test for tubal factor
diagnose upper genital tract Chlamydia trachomatis infections?
Studies on women with pelvic pain, with and without chlamydial
infant DNA in endometrial biopsy tissue. Sex Transm Dis 1998;
19. Shepard MK, Jones RB. Recovery of Chlamydia trachomatis from endometrial and Fallopian tube biopsies in women
BA, Wang S-P. Detection of Chlamydia trachomatis deoxy-
ribonucleic acid in women with tubal infertility. Fertil Steril
1993; 59: 45–50.
21. Lan J, van den Brule AIC, Hemrika DJ, et al. Detection of
diagnosis of sickle cell anemia. Science 1995; 230:
1350–1354.
23. Claas FH, Melchers WJ, de Bruijn BH et al. Detection of
Chlamydia trachomatis in clinical specimens by the polymerase
24. Sambrook J, Fritsch EF, Manietis T. Molecular cloning. A
laboratory manual, 2nd edn, vol. 2. Cold Spring Harbor, NY,
25. Tohn JM, Hannaia V, Tucker LJ. The future of chlamydia
Screening for Chlamydia trachomatis infection in pregnant women
27. Dowe G, Smilke M, King SD, Wynn H, Frederick J, Hylton-
Kong T. High prevalence of genital Chlamydia trachomatis
infection in women presenting in different clinical settings in
Jamaica: implications for control strategies. Sex Transm Infect
28. Gérard HC, Brangan PJ, Balsara GR, Heath C, Minassian SS,
Hudson AP. Viability of Chlamydia trachomatis in fallopian
tubes of patients with ectopic pregnancy. Fertil Steril 1998; 70:
945–948.
29. Marana R, Lucisano A, Leone F, Sanna A, Dell’Acqua G, Mancuso S. High prevalence of silent chlamydial colonization of
the tubal mucosa in infertile women. Fertil Steril 1990; 53:
354–356.
30. Toth M, Patton DL, Campbell LA et al. Detection of
chlamydial antigene material in ovarian, prostatic, ectopic
pregnancy and semen samples of culture-negative subjects. Am
31. LaVerda D, Kalayoglu MV, Byrne GL. Chlamydia heat shock
proteins and disease pathology: new paradigms for old
32. Egger M, Low N, Davey Smith G, Lindblom B, Herrmann B.
Screening for chlamydial infections and the risk of ectopic
pregnancy in a cohort in Sweden. ecological analysis. BMJ
33. Karmwenda F, Forslin L, Bodin L, Danielsson D. Epidemiology
of ectopic pregnancy during a 28 year period and the role of
pelvic inflammatory disease. Sex Transm Infect 2000; 76:
28–32.