A randomized controlled trial of prophylactic antibiotics (co-amoxiclav) prior to embryo transfer

N.Brook¹, Y.Khalaf¹³, A.Coomarasamy¹, J.Edgeworth² and P.Braude¹

¹Assisted Conception Unit and ²Department of Microbiology, Guy’s and St Thomas’s Hospital Foundation Trust, London, UK
³To whom correspondence should be addressed at: Assisted Conception Unit, Guy’s Hospital, 4th Floor, Thomas Guy House, London SE1 9RT, UK. E-mail: yakoub.khalaf@kcl.ac.uk

BACKGROUND: Bacterial contamination of the transfer catheter during embryo transfer is associated with poor clinical outcomes. Antibiotics at the time of embryo transfer may improve outcomes. We evaluated the effect of co-amoxiclav on the rates of bacterial contamination of transfer catheters and clinical pregnancy. METHODS: On the day of oocyte collection, 350 patients were randomized, with sequentially numbered opaque-sealed envelopes containing treatment allocation assigned randomly by computer, to receive co-amoxiclav on the day before and the day of embryo transfer, or no antibiotics. Following transfer, the catheter tips were cultured and assessed to identify the organism(s) isolated and to quantify the level of the contamination. Couples were followed for 8 weeks to determine whether they had achieved clinical pregnancy. Outcome assessors were blinded to the treatment allocation, and the analysis was by intention to treat. RESULTS: Antibiotics significantly reduced catheter contamination rates (49.4 versus 62.3%, RR = 0.79, 95% CI: 0.64, 0.97, \( P \) = 0.03). There was no difference detected in clinical pregnancy rates between the two groups (36.0 versus 35.5%, \( P \) = 0.83) although there was a significant (\( P \) = 0.03) association between the level of bacterial contamination and clinical pregnancy rates. CONCLUSIONS: Co-amoxiclav reduces catheter contamination, but this is not translated into better clinically relevant outcomes such as clinical pregnancy rates. Our findings do not support the routine use of antibiotics at embryo transfer.

Key words: antibiotics/catheter contamination rates/clinical pregnancy rates/embryo transfer/randomised controlled trials

Introduction

Pelvic infection as a result of IVF/assisted reproduction techniques (ARTs) is uncommon (Sowerby and Parsons, 2004), despite the invasive nature of the transvaginal oocyte collection, embryo transfer and the risk of infection from the micro-organisms that make up the normal vaginal flora (El-Shawarby et al., 2004).

The impact of genital bacterial contamination on the outcome of an ART cycle was first suggested in 1978 (Czernobilsky, 1978). Studies have shown that bacterial contamination of the transfer catheter has a significant negative impact on the outcome of the cycle (Fanchin et al., 1990; Egbase et al., 1996; Salim et al., 2002). Persistent cervical sterility cannot be achieved with the routine use of vaginal antiseptics at the time of oocyte retrieval or embryo transfer, and there is evidence that vaginal antiseptics can have a negative impact on the quality of the oocytes collected and the embryos available for transfer (van Os et al., 1992). Currently, there is a paucity of evidence about the effects of antibiotic prophylaxis on ART cycle outcomes (Egbase et al., 1999; Moore et al., 2000; Peikrishvili et al., 2004).

This trial evaluates the effect of co-amoxiclav on the rates of bacterial contamination of catheters following embryo transfer, as well as ART cycle outcome. We also examined the prognostic value of the level of bacterial contamination on the pregnancy outcome.

Methods

Participants

Between April 2004 and March 2005, we studied 350 consecutive patients undergoing a transvaginal oocyte retrieval and embryo transfer cycle as part of their IVF, ICSI or PGD treatment. Each woman participated only once in the study. We excluded patients who had any contraindication to antibiotic treatment, were not intending to undergo embryo transfer (oncology patients and oocyte donors), had had previous pelvic infection or had a risk of pelvic infection that required i.v. antibiotic prophylaxis at the time of transvaginal oocyte retrieval.

Clinical indications for IVF-embryo transfer were male factor (51.4%), unexplained (26.3%), tubal abnormalities (9.1%), those requiring PGD for inherited genetic disease (6.3%), endometriosis (3.4%) and polycystic ovarian disease leading to anovulation (1.1%). None of the women included had clinical evidence of vaginitis or cervicitis. semen cultures were not performed routinely for male partners.

The outcomes of interest in this trial were the rates of bacterial contamination on the embryo transfer catheter tip, and the success of...
ART was defined as the presence of a clinical pregnancy (a gestational sac with cardiac activity on ultrasound scan).

The participants’ database was maintained by the trial co-ordinator blinded to the results of the microbiological analysis. The Guy’s and St Thomas’s Hospital Ethics Committee and Research and Development Department (EC04/033) approved the trial.

**Protocols for controlled ovarian stimulation**

Each patient underwent a standardized controlled ovarian stimulation (COS) for IVF, ICSI, PGD or oocyte recipient treatment cycles starting with buserelin (Suprefact, Hoechst UK Ltd, Hounslow, UK) in the mid-luteal phase. Complete pituitary desensitization was confirmed by transvaginal ultrasound to verify that the endometrial thickness was <5 mm and the absence of ovarian cysts.

Ovarian stimulation was achieved using recombinant FSH injections (Gonal-F, Serono Ltd, Middlesex, UK; Puregon, Organon Ltd, Cambridge, UK) or urinary-derived HMG (Menopur, Ferring Pharmaceuticals Ltd, Berkshire, UK). These protocols remained standard throughout the course of the trial (stimulation dosages ranged from 150 to 450 IU/day). HCG (Pergynl, Organon Ltd; Ovitreel, Serono Ltd) was administered when at least three follicles reached ≥18 mm in diameter. Oocytes were retrieved 34–38 h after the injection by transvaginal ultrasound guidance. All accessible follicles were aspirated.

Randomization was achieved through computer-generated numbers, and information on treatment allocations was sealed in opaque envelopes that were opened sequentially. A third party not involved in the trial produced the randomization codes and the sealed envelopes. Following confirmation of suitability for the trial and having obtained consent, the clinician performing the transvaginal oocyte retrieval allocated the patients randomly either to the antibiotic or to the no-antibiotic arm using the opaque pre-sealed envelopes. The patients allocated to the antibiotics group received 1.5 g of co-amoxiclav tablets (Augmentin, GlaxoSmithKline PLC, Mddx, UK), 750 mg the night before the transfer and 750 mg 2 h before the transfer. No placebo tablets were used for this trial.

Transcervical embryo transfer was performed on day 2 (n = 149), day 3 (n = 152) or occasionally day 4 (n = 21) after oocyte retrieval. The luteal phase was supported with daily micronized progesterone 400 μg pessaries (Cyclogest, Shire Pharmaceuticals PLC, Hampshire, UK) or 50 mg injections (Gestone Ferring Pharmaceuticals Ltd) starting on the day of oocyte retrieval.

**Cervical samples**

A disposable sterile cusco speculum was inserted into the vagina, and the vagina and cervix were cleaned with normal saline. Antiseptic solution was not used. Vaginal and cervical secretions were removed using sterile cotton swabs and buds. All embryos were transferred using the same catheters (Edwards–Wallace embryo replacement catheter, Smiths Medical International Ltd, Kent, UK), using a non-touch sterile replacement technique (sterile disposable drapes, speculum and non-latex gloves). Contact between the transfer catheter, the vaginal walls and ecto-cervix was avoided. Embryos were transferred to the mid-cavity of the uterus under ultrasound guidance.

Following withdrawal of the catheter, and confirmation that the embryos had been transferred, the embryologist cut off the distal 2 cm of the catheter tip using sterile scissors. Each individual tip was then rolled using sterile forceps on to a 5% horse blood agar plate. It was then placed in brain/heart infusion broth (BHI solution contains beef heart infusion solids 17.5 g/l, protease peptone 10.0 g/l, glucose 2.0 g/l, sodium chloride 5.0 g/l, disodium phosphate 2.5 g/l. It is used to optimize bacterial growth from specimens by providing a nutrient-rich environment). The plates and broth solution were incubated aerobically at 37°C for 48 h. The embryologist performing the embryo transfer was blinded to the randomization assignment of the trial.

A single microbiologist (J.E.), blinded to the randomization, performed the microbiological assessment of the plates. Bacteria were identified by standard laboratory techniques and quantified using a semi-quantitative four-point grading system for gram-positive organisms: the absence of growth after 48 h [no growth (NG)], <10 bacterial colonies (+), >10 bacterial colonies (+++) and semi-confluent or confluent growth (++++) on the blood agar plate. Positive BHI cultures were plated out and the bacteria identified then graded as (+). Gram-negative bacterial contamination was not quantified.

**Statistics**

Sample-size calculation to detect a 10% difference in bacterial contamination rates (60 versus 50%) with a type I error rate of 5% and type II error rate of 20% indicated the study required 350 patients, 175 into each arm. Interim analysis was performed after every 100 patients had been recruited, and the ‘stopping’ criteria included a significant increase or decrease in clinical pregnancy rates or a significant level of adverse patient incidents.

Statistical analyses were performed using Statview™ (JMP Software, USA) and STATA™ (TX, USA) statistical softwares. Measures of tendency used were means, and the measures of variability were standard errors. For non-parametric data, medians and ranges were used. When the data were distributed normally, the unpaired Student’s t-test and chi-square test were used. The Mann–Whitney U-test was applied if the distribution was not clearly defined. P < 0.05 was considered statistically significant. Analysis was by intention to treat.

To examine the relationship between the level of bacterial contamination and the clinical pregnancy rates, adjusted for various prognostic factors, we used logistic regression analysis.

**Results**

**Participant flow**

A total of 775 patients underwent transvaginal oocyte retrieval during the recruitment period of which 274 patients were not eligible for the trial (Figure 1). Of those not eligible, 215 required prophylactic i.v. antibiotics at the time of transvaginal oocyte retrieval due to a risk of pelvic infection. The others were excluded from the trial for the following reasons: allergic to penicillin (n = 55), on concurrent antibiotic treatment (n = 3) and oncology patient where all embryos frozen (n = 1).

Of the 501 eligible women, 114 declined to take part, and a further 37 were excluded as they had taken part in the trial in a previous cycle.

Of the remaining 350 women recruited to the trial, 178 received antibiotics, and 172 were in the control arm. Bacteriological catheter analysis was performed on 284 of 350 women (81%). The pregnancy data were available for all patients recruited (350). In the antibiotic group, analysis was performed on 154 catheters. Of the remainder, 12 catheters were discarded in error, 10 patients had failed fertilization and 2 had failed cleavage. In the control group, analysis was performed on 130 catheters. Of the remainder, 26 were discarded in error, 12 patients had failed fertilization, and 4 had failed cleavage.

No patient was withdrawn due to an adverse event.

**Outcomes**

Patient’s age, the duration of infertility, indications for assisted conception, the number of previous assisted conception cycles, ovarian reserve assessment (early-follicular-phase FSH levels),
Cultures were positive (the presence of colonies) in 157 of the 284 embryo transfers performed (55.3%). In the positive culture group where the number of colonies ranged from <10 bacterial colonies to semi-confluent or confluent growth, mixed gram-positive bacteria growth was the most common bacterial contaminant (Table III). (The baseline characteristics between the non-growth group and various groups of growth were comparable for prognostic factors such as age, duration of infertility, the number of previous cycles, FSH level and the cause of infertility.)

The severity of the gram-positive bacterial contamination affected the likelihood of a successful outcome. The heaviest contamination of the catheter tips with gram-positive bacteria was associated with a largest reduction in the clinical pregnancy rate. Catheters that showed no growth were associated with a clinical pregnancy rate of 47.2% compared with a rate of 15.8% in those women whose catheter had a semi-confluent/confluent growth of gram-positive bacteria (Table IV).

### Table II. The effect of antibiotics on assisted reproduction technique outcome

<table>
<thead>
<tr>
<th>Contamination</th>
<th>Co-amoxiclav group (%)</th>
<th>Control group (%)</th>
<th>Relative risk (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contamination of catheter tips</td>
<td>76/154 (49.4%)</td>
<td>81/130 (62.3%)</td>
<td>0.79 (0.64 – 0.97)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>64/178 (36.0%)</td>
<td>61/172 (35.5%)</td>
<td>1.01 (0.81 – 1.24)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

### Table III. Type of organisms identified in patients who underwent embryo transfer

<table>
<thead>
<tr>
<th>% (proportion)</th>
<th>Study (n = 178)</th>
<th>Control (n = 172)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed growth of gram positive/negative</td>
<td>62.0 (98/157)</td>
<td>22.8 (36/157)</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>51.4 (8/157)</td>
<td>25.0 (4/157)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>5.1 (8/157)</td>
<td>2.5 (4/157)</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>0.6 (1/157)</td>
<td>0.6 (1/157)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>5.1 (8/157)</td>
<td>2.5 (4/157)</td>
</tr>
<tr>
<td>Streptococcus (Alpha haemolytic)</td>
<td>0.6 (1/157)</td>
<td>0.6 (1/157)</td>
</tr>
<tr>
<td>Candida</td>
<td>22.8 (36/157)</td>
<td>55.3 (85/157)</td>
</tr>
</tbody>
</table>

### Table IV. Effect of grade of bacterial contamination of the embryo transfer catheter on clinical pregnancy and implantation rates

<table>
<thead>
<tr>
<th>Level of contamination</th>
<th>Clinical pregnancy rate (%)</th>
<th>Crude (Unadjusted OR)</th>
<th>Adjusteda OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>60/126 (47.2%)</td>
<td>1b</td>
<td>1b</td>
</tr>
<tr>
<td>GP+</td>
<td>36/90 (40.0%)</td>
<td>0.73</td>
<td>0.61</td>
</tr>
<tr>
<td>GP++</td>
<td>12/37 (32.4%)</td>
<td>0.53</td>
<td>0.41</td>
</tr>
<tr>
<td>GN</td>
<td>3/19 (15.8%)</td>
<td>0.21</td>
<td>0.18</td>
</tr>
<tr>
<td>PGD</td>
<td>2/11 (18.2%)</td>
<td>0.22</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*Adjusted for female age, cause of infertility, duration of infertility, cycle number, basal FSH level, embryo transfer operator and type of assisted reproduction technique (IVF, ICSI or PGD). *Reference group for the comparisons.
Women in whom catheter tips were contaminated with gram-negative infection still could achieve a pregnancy (18.2%), but this was significantly reduced in comparison with the sterile catheter group (47.2%), $P < 0.05$ (Table IV).

Discussion

The principal findings of this study are the demonstration of a significant reduction in embryo transfer catheter contamination rates when antibiotics were administered prophylactically with no equivalent improvement in the clinical pregnancy rate. In addition, we showed pregnancy rates are highly dependent on the presence or absence of bacterial contamination and on the degree and type of this contamination.

It is difficult to explain why there is no improvement in ART outcome, although co-amoxiclav reduces bacterial contamination of the embryo transfer catheter. It is possible that antibiotic regime chosen was inappropriate in dose, duration, bacterial sensitivity and the route of administration in our population. We chose a drug that provided a broad spectrum of coverage, could be taken orally, had few side effects, is known to be highly effective against the endocervical/vaginal flora (Egbase et al., 1999; Salim et al., 2002) and is commonly used for presurgical prophylaxis (Wenzel, 1992; Ryan et al., 2004).

The aim of the regimen was to reduce the antibiotic burden on the patient and to decrease the morbidity associated with a substantial prolonged antibiotic course (Davey et al., 2005). The regimen would more likely achieve cervical sterility without the risk of overgrowth with antibiotic resistant organisms that are likely with the extended antibiotic courses used in other trials (Egbase et al., 1999; Moore et al., 2000). In our unit, we administer antibiotics to those women whom we judge to be at high risk of pelvic infection following oocyte retrieval, including those with previous pelvic infection, the history of endometriosis, hydrosalpinges or multiple ovarian punctures at the time of oocyte collection. We have found about a quarter of our ART population have one or more of the above risk factors and therefore receive treatment. Our study, therefore, excluded such women. However, we appreciate that the antibiotic use in other assisted conception units may be lower or higher, and the applicability of our findings to such units may be limited.

The inclusion of PGD patients in our trial merits a comment as most assisted conception units are unlikely to have a significant number of PGD patients in their workload, and the prognosis or outcome in PGD patients may be different to other ART groups. In our trial, there were 30 PGD patients, and they were found to have been allocated evenly with 16 receiving no treatment while 14 receiving antibiotics. Furthermore, pregnancy rates for the three groups (in both treatment arms)—ICSI 39.9% (75/188), IVF 45.6% (60/132) and PGD 36.7% (11/30)—were similar. We therefore feel that the inclusion of PGD patients does not compromise the validity of our study.

One mechanism by which co-amoxiclav may have failed to improve clinical pregnancy rates is through destruction of the cervical flora potentially allowing the bacterial remnants to activate an inflammatory response (Klebanoff et al., 2001). Any immune response can be expected to have a detrimental effect on embryo implantation. Co-amoxiclav could cause an alteration in the ratio of hydrogen peroxide-producing lactobacilli to other lactobacilli subtypes. A higher ratio of H$_2$O$_2$ lactobacilli has been shown to be more protective against potential pathogens. This could have a negative effect on the likelihood to success in any ART cycle (Moore et al., 2000). Antibiotics may themselves have a detrimental effect on the likelihood of embryo implantation. Even though there is no clear evidence to support this theory, the recently published PREMET study on the role of metronidazole in women at risk of pre-term labour showed that the antibiotic used prophylactically may result in a poorer obstetric outcome (Shennan et al., 2006). There may be a mechanism of action of these drugs that go beyond their function as anti-microbials.

Our bacterial catheter contamination rates were comparable with those shown in other studies (Egbase et al., 1999; Salim et al., 2002) in both treatment arms of the trial, but the drug regimen differed. However, the bacterial cultures showed marked differences between the predominant organisms identified when compared with the other studies. The proportion of patients with gram-negative catheter contamination in our group was only 5.7% similar to that identified in Egbase’s study group (1999) but much less than Salim’s group (2002) (64%) and demonstrated a higher proportion of mixed growth than Egbase et al. (1999) (20.7%) and Moore et al. (2000) (25%) (Table III).

This trial also uniquely identified endocervical contaminants at the time of embryo transfer from the catheter used for the transfer of the embryos. In comparison with other studies, no mock embryo transfers or endocervical swabs were used. To ensure optimal bacterial sensitivity and quantification, we processed the tip of the embryo transfer catheter, with direct plating and broth culture. We accept that the use of the embryo transfer catheter as the sampling instrument may not be as sensitive as a microbiological swab (Fanchin et al., 1990; Salim et al., 2002), but the transfer catheters do gain access to the endometrial cavity. We also accept that, as in other studies, the presence of penicillin in the embryo transfer media used to culture the embryos from which the catheter was loaded, even though of negligible volume, could have led to a relatively lower or more variable catheter contamination rate.

There are several pathophysiological mechanisms that can explain the reduction in the clinical pregnancy rate in women with embryo transfer catheter contamination. The impact of bacterial contamination may decrease the embryo’s capacity to implant due to the effects on both the embryo itself and the endometrium (Navot et al., 1989; Paulson et al., 1990; Lessey et al., 1992; Tabibzadeh and Babaknia et al., 1995; Egbase et al., 1999; Moore et al., 2000). The zona pellucida of the embryo, which has a barrier function against infection at the cleavage stage, is lost from the blastocyst before implantation exposing the embryo to the detrimental effects of the bacteria (Lavilla-Apelo, 1992). In the endometrium, any acute inflammatory response will generate cytokines, macrophages, prostaglandins and leukotrienes, which can have a delirious effect on embryo implantation (Spandorfer et al., 2001). It has been shown that Escherichia coli inoculated into rat uteri produce a purulent endometritis, destroying the potential for implantation by activating a massive inflammatory response (Nishikawa, 1985).
Subclinical endometritis is associated with lower pregnancy rates (Czernobilsky, 1978).

The severity and the type of bacterial contamination have previously been found to have a negative effect on the likelihood of conceiving (Salim et al., 2002). This was confirmed in our study that although in contrast to Salim’s study (2002) where no pregnancies resulted in the presence of gram-negative bacteria, two pregnancies occurred. The effect of severity and the type may be explained by the fact that there could be a more pronounced inflammatory response interrupting the potential for implantation affecting both the embryo and the endometrium.

Based on the findings in this study, prophylactic co-amoxiclav cannot be recommended during embryo transfer. However, it is reasonable to propose that the embryo transfer practitioner should try to ensure maximum catheter sterility to improve the clinical pregnancy rate.

Acknowledgements

The authors acknowledge the help of Nisha Nair, medical student and Eleanor Wharf, clinical embryologist, and other members of Guy’s and St Thomas’ Hospital ACU for their help in this study.

Conflict of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this study.

References


Submitted on March 9, 2006; resubmitted on April 6, 2006, May 22, 2006; accepted on June 7, 2006