Effect of progestogen therapy on follicular development, related hormone concentrations and fertilization in vitro in unstimulated cycles and unexplained and endometriosis-associated infertility

D.J. Cahill, P.G. Wardle, C.R. Harlow and M.G.R. Hull

University of Bristol Department of Obstetrics and Gynaecology, St Michael’s Hospital, Southwell Street, Bristol BS2 8EG, UK

1To whom correspondence should be addressed at: Department of Obstetrics and Gynaecology, Princess Anne Wing, Royal United Hospital, Combe Park, Bath BA1 3NG, UK

Evidence of pituitary–ovarian dysfunction in unexplained and endometriosis-associated infertility has been reported previously. Hormone-suppressive therapy is often used in an attempt to improve fertility, although benefits have not been proven. Our study examines the effect of progestogen (medroxyprogesterone acetate) treatment on women with endometriosis-associated and unexplained infertility, compared with women with tubal damage as functional controls. Pre-ovulatory follicular size and serum and follicular fluid hormone concentrations were measured, and oocyte collection and in-vitro fertilization were attempted, in natural cycles totally unperturbed by exogenous gonadotrophins, for two cycles before and two cycles following treatment with medroxyprogesterone acetate for 2 months. In the endometriosis and unexplained infertility groups, compared with the tubal group, the treatment led to significant reductions in the integrated luteinizing hormone (LH) values (483 versus 664, 559 versus 762 and 864 versus 820 notional IU/l respectively). There were no changes in serum oestradiol or follicular fluid oestradiol, progesterone, follicle stimulating hormone or LH concentrations after treatment. The results suggest that progestogen therapy has no beneficial effect on the pituitary–ovarian dysfunction which contributes to endometriosis-associated and unexplained infertility.

Key words: endometriosis/follicular fluid endocrinology/pituitary–ovarian dysfunction/progestogen therapy/unexplained infertility

Introduction

Hormone-suppressive therapy has been commonly used to try to improve subsequent fertility in women with minor endometriosis, but without any proven benefit. An early uncontrolled study using danazol found an increase in pregnancy rates after treatment (Dmowski et al., 1986). However, no benefit has been found in prospective controlled studies. No difference in the cumulative conception rates was found when women who had no other infertility factors apart from endometriosis were randomized to receive danazol, medroxyprogesterone acetate (MPA) or no treatment (Hull et al., 1987; Bayer et al., 1988). Two placebo-controlled studies also failed to show any improvement in the cumulative conception rates with treatment using gestrinone, danazol or MPA (Thomas and Cooke, 1987; Telimaa, 1988). Controlled studies with gonadotrophin hormone-releasing agonists have failed to show any improvement in fertility (Fedele et al., 1992).

The effect of hormone-suppressive therapy on the fertilization rate of oocytes in vitro has been studied with differing conclusions. In a preliminary uncontrolled study, Wardle et al. (1986a) demonstrated an apparent improvement in the fertilization rates after danazol or gestrinone therapy for minor endometriosis. In contrast, Mahmood (1991) showed no change in the fertilization rate after treatment with danazol for 6 months, but the study was limited to only 10 patients (and 10 cycles) in each group.

Recently we have demonstrated evidence of a pituitary–ovarian dysfunction in women with endometriosis (Cahill et al., 1995). Specifically, a reduced amplitude of the pre-ovulatory luteinizing hormone (LH) surge was demonstrated by serum measurements taken every 4 h and reduced concentrations of LH in follicular fluid at oocyte recovery. In addition, we found similar impairments in women with prolonged unexplained infertility, though to a lesser extent. Therefore this study examines whether that observed pituitary–ovarian dysfunction might be altered after hormone-suppressive therapy using the progestogen, MPA. Treatment was limited to 2 months because of empirical evidence suggesting that this period is sufficient to maximally suppress the activity of endometriosis (Thomas, 1991; Brosens, 1993). Women with tubal infertility were used as controls and were presumed to be otherwise functionally normal. They were also treated with progestogen because there is reported evidence of an apparent benefit suggested by subsequently increased follicular phase serum oestradiol concentrations (Petsos et al., 1985).

Materials and methods

Women with infertility were recruited who had been fully investigated (see below), including laparoscopy. No abnormality was found in nine patients with infertility of >3 years’ duration (unexplained infertility). Minor endometriosis [minimal or mild by revised American Fertility Society (AFS) scoring; AFS, 1985] was the only abnormality in 10 women, and they had received no previous treatment. A group of 16 women with tubal damage as a result of previous infection (i.e. not caused by endometriosis) as an isolated cause of infertility were recruited as functional controls. All other infertility investigations were normal: mid-luteal serum progesterone concentration >30 nmol/l, normal thyroid function, normal early follicular phase follicle stimulating hormone (FSH) and LH concentrations, a positive well-timed post-
coital test (at least three progressively motile spermatozoa per high power field ~12 h after coitus) and their male partners all had a normal seminal analysis (World Health Organization, 1992). Ethical approval was obtained for this study from the local ethics committee.

In-vitro fertilization was undertaken in two unstimulated cycles before and two cycles following the administration of MPA 50 mg daily for 2 months. Follicular development was monitored by daily transvaginal ultrasonography from day 9 of the cycle until oocyte recovery (or ovulation), measuring the dominant follicle in three perpendicular planes to calculate the mean diameter. Serum 17β-oestradiol and LH concentrations were initially measured once daily on capillary samples (collected using an Autolet; Owen Mumford, Oxford, UK). When the dominant follicle reached >14 mm in diameter, the number of samples was increased to one sample every 4 h (08:00-24:00 h, but not at 04:00 h). The time of onset of the LH surge was estimated by extrapolation backwards of the plot of rising LH surge values until it reached the mean baseline values. Follicular size and serum oestradiol concentrations were monitored until oocyte recovery was undertaken 32 h after the calculated onset of the LH surge. The area under the curve (AUC) for pre-ovulatory serum oestradiol, calculated by integration of the daily measurements for the 3 days before the surge to the day of oocyte recovery (over 5 days in total) and expressed as notional pmol/L, was taken as an index of late follicular phase granulosa cell function. The AUC for serum LH, calculated by integration of the 4-hourly measurements from 36 h before to 4 h before oocyte recovery (32 h in total), expressed as notional IU/L, was taken as an index of pre-ovulatory pituitary production and release of LH. Blood-free follicular fluid was collected at the time of oocyte recovery, and stored at ~70°C until assayed at the end of the study. Measurements of oestradiol and LH in the serum, and oestradiol, LH, progesterone and FSH in the follicular fluid, were undertaken using monoclonal antibodies in a fluorometric assay (Delfia; Wallac, UK) and WHO standards for human pituitary FSH (2nd IRP 78/548) and LH (1st IRP 68/40). Laboratory methods for sperm preparation, oocyte and embryo culture, and embryo transfer were performed as described previously (Wardle et al., 1986b).

The endpoints studied before and after treatment were: duration of the follicular phase, follicular growth rate (from 3 days before to the day of the LH surge), peak follicular diameter, pre-ovulatory serum oestradiol and LH concentrations, follicular fluid hormone concentrations and fertilization rates in vitro of the oocytes collected. Hormonal data were compared if available paired before and after treatment.
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Table III. Pre-ovulatory follicular fluid hormone measurements in unstimulated cycles before and after treatment with medroxyprogesterone acetate according to the diagnostic classification of infertility (median and 95% confidence interval)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tubal (control)</th>
<th>Infertility group</th>
<th>Endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>No. of cycles (patients)</td>
<td>16 (8)</td>
<td>16 (8)</td>
<td>14 (7)</td>
</tr>
<tr>
<td>Oestradiol concentration (μmol/l)</td>
<td>5.9 (3.6-6.9)</td>
<td>5.3 (3.1-8.4)</td>
<td>3.5 (1.2-10.9)</td>
</tr>
<tr>
<td>Progesterone concentration (μmol/l)</td>
<td>63.5 (35.5-91.0)</td>
<td>63.3 (27.0-92.2)</td>
<td>73.0 (22.9-333.0)</td>
</tr>
<tr>
<td>Follicle stimulating hormone</td>
<td>4.1 (3.1-5.4)</td>
<td>4.4 (2.3-5.9)</td>
<td>5.2 (3.7-6.6)</td>
</tr>
<tr>
<td>concentration (IU/l)</td>
<td>19.7 (14.8-29.2)</td>
<td>16.3 (10.2-26.2)</td>
<td>9.8 (6.2-16.3)</td>
</tr>
<tr>
<td>Luteinizing hormone concentration</td>
<td>8.9 (5.5-15.9)</td>
<td>7.4 (5.5-19.8)</td>
<td>12.1 (6.0-28.2)</td>
</tr>
<tr>
<td>(IU/l)</td>
<td>38.9 (22.4-109.7)</td>
<td>93.3 (32.0-357.0)</td>
<td>49.7 (33.8-425.0)</td>
</tr>
</tbody>
</table>

There were no significant differences.

treatment, but fertilization rates were analysed in all cycles started, using Wilcoxon's signed-rank sum test on paired non-parametric data or Fisher's exact test, as appropriate. The numbers recruited for this study were based on fertilization data in previous published work on stimulated cycles (Wardle et al., 1986a). Based on this, 44 oocytes in each group were required to achieve significant differences in the fertilization rates before treatment, with a power of 0.2 and a significant difference of 0.05.

Results
The median duration of infertility (and range) for the tubal (control), unexplained and endometriosis groups was 6 (1.5–12.0), 4 (3–7) and 5 (2–7) years respectively. The pre-ovulatory follicle size and serum hormone measurements are summarized in Table I. There were no differences observed after MPA administration in the endometriosis and unexplained infertility groups in serum oestradiol concentrations (peak values and integrated AUC) and peak LH concentrations during the pre-ovulatory surge. Significant differences were observed in the AUC for LH in the endometriosis and unexplained infertility groups after treatment.

The results of attempted oocyte recovery and in-vitro fertilization are given in Table II. There were no differences in the rates of cycles proceeding to oocyte recovery, with or without success, or to pregnancy. There was an apparent reduction in fertilization and cleavage rates in the endometriosis and unexplained groups, before or after treatment, as found previously in much larger studies of stimulated cycles (Mills et al., 1992), but the numbers were few and the differences were not significant in our study.

The results of follicular fluid hormone measurements are given in Table III. Comparison of the results before and after treatment within each diagnostic group showed no differences.

Discussion
We have shown previously that in women with endometriosis the capacity of the luteinized granulosa cells to produce proges-

terone in vitro is impaired (Cahill et al., 1994), and that in women with infertility associated with minor endometriosis, or with prolonged unexplained infertility, there is a reduction in the pre-ovulatory serum oestradiol concentrations and the amplitude of the LH surge (Cahill et al., 1995). However, the main aim of this study was to determine whether progestogen therapy might lead to an improvement in follicular function. The power of this study was reduced because we failed to achieve the required number of cycles in the pre-treatment group. Therefore we stress that our results must be accepted with caution. Nevertheless, appropriate paired statistical methods were applied to the pre- and post-treatment groups for analysis.

The results show no changes in the pre-ovulatory serum concentrations of oestradiol or LH (Table I) or in the follicular fluid concentrations of oestradiol, progesterone, testosterone, androstenedione, LH or FSH (Table III). Previously published reports (Cahill et al., 1995) have suggested differences between patients in the diagnostic groups examined in this study, but these differences were not seen in the current study. Nor was there any alteration in the oocyte fertilization rates, although numbers were too small to be conclusive (Table II). There was, however, a significant reduction in the amplitude of the LH surge in women with endometriosis and unexplained infertility after progestogen treatment, but no change in those with tubal infertility (Table I). It seems unlikely that the further attenuation of the LH surge seen after progestogen treatment represents a benefit because follicular growth and the intrafollicular environment remained unchanged and indistinguishable from the findings in women with tubal infertility.

The mid-cycle LH surge is critical in normal ovulation to achieve the resumption of meiotic activity in the oocyte, increased vascularization and blood flow to the follicle, luteinization of the granulosa cells, and breakdown of the follicle wall (Bomsel-Helmreich et al., 1979; Thibault and Levasseur, 1988). The abnormalities in LH release, which we have observed in women with endometriosis or prolonged unexplained infertility, may have a critical effect on their chances of natural conception.
This has been demonstrated by Cohen et al. (1993), who studied women undergoing donor insemination cycles and found that shorter LH surges or lower serum concentrations of LH during the surge reduced the likelihood of conception. The mechanism for this abnormality of pituitary–ovarian function is not clear. Serum gonadotrophin surge-attenuating factor (GnSAF) concentrations are high during the early follicular phase but fall during the pre-ovulatory phase, and evidence from a study of follicular fluids in ovulation induction cycles indicated that GnSAF is mainly produced by the smaller follicles (Fowler et al., 1994a). GnSAF has also been identified recently in the follicular fluid of the ovarian follicle in unstimulated cycles (Fowler et al., 1994b). It may be that the excessive release of GnSAF from the ovarian follicles of women with endometriosis could cause the impaired LH surge. Other possible mechanisms for the pathogenesis of endometriosis-associated subfertility include immunological disorders (Weed and Arquerbourg, 1980; Mathur et al., 1982), reducing the ability to remove endometrial debris shed into the peritoneal cavity at each menstruation (Blumencrutz et al., 1981). Endometriosis may worsen the situation by altering the peritoneal fluid environment, by producing heat-labile soluble tissue factors, thus decreasing fertilizing ability (Steinleitner et al., 1990), or by reducing sperm motility (Drudy et al., 1994). The effect of progesterone therapy on the LH surge in women with unexplained infertility was not expected, although we have reported previously abnormalities of the LH surge in women with unexplained infertility (Cahill et al., 1995).

Almost all previously published controlled studies of fertilization and pregnancy rates have failed to demonstrate any benefit of hormone-suppressive therapy on the infertility associated with endometriosis (Hull et al., 1987; Thomas and Cooke, 1987; Bayer et al., 1988; Telima, 1988; Fedele et al., 1992). Temporal suppression of the activity of minor endometriosis may be irrelevant. Indeed, it is even debatable whether minor endometriosis should be considered a disease (Wardle and Hull, 1993). If subtle pituitary–ovarian dysfunction is a common cause of endometriosis-associated and apparently unexplained infertility, our findings indicating a failure of progesterone therapy to correct the pituitary–ovarian disorder help to explain why there is no improvement in subsequent fertility.

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References