Ovarian Dysfunction in Endometriosis-Associated and Unexplained Infertility

D. J. CAHILL,[1,2] P. G. WARDLE,[1] L. A. MAILE,[1] C. R. HARLOW,[1] and M. G. R. HULL[1]

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Purpose: *The impact of endometriosis and unexplained infertility on follicular function and fertilization of oocytes in cycles totally unperturbed by exogenous gonadotrophins, when compared with controls with tubal damage, were examined.*

Methods: *In natural cycles, without any exogenous gonadotropins, endocrine and ultrasonographic studies of follicular maturation in 18 women with minor endometriosis (41 cycles), 15 women with unexplained infertility (31 cycles), and 34 women with tubal damage (88 cycles) were performed.*

Results: *The endometriosis group had a significantly longer follicular phase (median: 15, 13, and 13 days). Both endometriosis and unexplained infertility had significantly reduced LH concentrations in follicular fluid compared with tubal damage (median: 12.1, 11.5, and 15.9 IU/L, respectively). Endometriosis was associated with a significantly reduced fertilization rate compared with unexplained infertility or tubal damage (46, 65, and 69%, respectively).*

Conclusions: *These data show continuing evidence of ovulatory dysfunction leading to reduced fertilization rates in women with minor endometriosis.*

KEY WORDS: endometriosis; unexplained infertility; ovulatory dysfunction; in vitro fertilization; natural cycles.

INTRODUCTION

We have previously shown that minor endometriosis is associated with reduced estrogen secretion and dysfunction of the midcycle endogenous luteinizing hormone (LH) surge measured in serum and follicular fluid (1). There was also an apparent but not statistically conclusive reduction in fertilization rates, although given only a single oocyte each, cycle numbers were relatively small. Our studies of much larger numbers of superovulated cycles have shown consistent significant reduction in fertilization rates (2–4). In contrast, pregnancy rates were good because sufficient embryos were available for transfer due to superovulation. The need to define the causes of natural subfertility associated with minor endometriosis remains, and this paper extends our previous studies of unstimulated cycles.

PATIENTS AND METHODS

Women with infertility who had been fully investigated (see below) were recruited. The following infertility investigations were normal: midluteal serum progesterone, 30 nmol/L or greater; normal thyroid function; normal early follicular-phase follicle stimulating hormone (FSH) and LH concentrations; and a positive well-timed postcoital test (three or more motile sperm per high-power field approximately 12 hr after coitus); and their male partners all had normal seminal analysis results (5). All the women had been fully investigated by laparoscopy involving as complete examination of all peritoneal and ovarian surfaces as possible. In these, either no abnormality was found (15 women) or previously untreated minimal–mild endometriosis was diagnosed at laparoscopy (18 women) (based on visual appearance using the revised Americal Fertility Society Classification, without histologic confirmation) (6). A group of 34 women with tubal damage as an isolated cause of infertility was recruited as functional controls.

Each patient had at least two cycles studied with a view to in vitro fertilization without any exogenous stimulation. Follicular development was monitored from the ninth day of the cycle until oocyte recovery

[1] Division of Obstetrics and Gynaecology, Department of Hospital Medicine, University of Bristol, St. Michael's Hospital, Bristol BS2 8EG, England.
[2] To whom correspondence should be addressed.

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(or ovulation) by daily transvaginal ultrasonography and serum 17β-estradiol and LH measurements, using a capillary blood sampling technique (Autolet, Owen Mumford, UK). When the dominant follicle reached 14 mm or greater in diameter, the frequency of blood sampling for LH increased to every 4 hr (except at 0400) and this frequency was maintained until the time of oocyte recovery. The LH surge was considered to have begun when a measurement exceeded the mean of the three previous results by 180% (and continued to rise thereafter) (7). The time of onset of the LH surge was estimated by extrapolation of a line backward from the rising LH surge values to the mean baseline level. The follicle was aspirated 32 hr after the onset of the surge with a view to fertilization of the oocyte, storage of follicular fluid at −70°C for later hormone assay, and granulosa cell culture studies (as described previously) (8).

The following variables were measured or calculated for comparison between the study groups: duration of the follicular phase (from day 1 of menstruation to the day of oocyte recovery), mean follicular diameter on the day of oocyte recovery, serum estradiol concentration on the day of onset of the LH surge (day LHo), "area under the estradiol curve" (as an index of estradiol production from 3 days before to the day after the onset of the LH surge), peak LH concentration, "area under the LH curve" (as an index of total LH secretion from the onset of the LH surge up to the time of oocyte recovery 32 hr later), fertilization rate of the oocytes collected, and follicular fluid concentrations of estradiol, progesterone, FSH, and LH.

Transvaginal ultrasonography was done on day 5 of the woman's cycle and daily from day 9, using a Siemens Sonoline SI-250 with a 5-MHz transducer. Follicular diameter was calculated as the mean of three measurements in perpendicular planes (9).

Hormone concentrations in serum (estradiol, LH) and follicular fluid (estradiol, LH, progesterone, FSH) were assayed by a rapid fluoroimmunometric method using monoclonal antibodies (Delfia, Wallac, UK). Assay standards for FSH and LH were WHO second international reference preparation (IRP) 78/548 and first IRP 68/40, respectively.

Based on previous publications (2), a sample size of 44 oocytes in each group was required to show a 20% difference in fertilization rates with 80% power at the 5% significance level. The results were analyzed using the Mann-Whitney test for nonparametric data or the chi-square test for trends when appropriate. Medians and 95% confidence intervals were derived by the method of Altman (10).

RESULTS

There were 18 women with minor endometriosis who had 41 cycles studied, 15 women with unexplained infertility who had 31 cycles, and 34 women with tubal damage, acting as controls, who had 88 cycles. The patient characteristics summarized in Table I show no difference in age of the partner or duration of infertility, although tubal infertility appears to be more often secondary and associated with higher sperm counts. In the women with endometriosis, the median disease score was 7 (range, 2–14) (6).

The results given in Table II show that fertilization rates were reduced and that in women with endometriosis, the duration of the follicular phase was longer compared with women with tubal damage. The follicular fluid concentration of LH was reduced and there was no difference in follicular fluid estradiol, progesterone, or FSH concentrations. In the unexplained infertility group, compared with the tubal group, there was no difference in the duration of the follicular phase, follicular diameter, serum estradiol, LH surge values, or fertilization rates, but follicular fluid LH was significantly reduced, although other follicular fluid hormone levels were not different.

DISCUSSION

Previous endocrine studies have suggested abnormalities of pituitary or ovarian function in association with minimal–mild endometriosis (1,11,12), and the present findings of reduced LH levels in the preovula-

Table I. Background Details of the Infertile Patient Groups Studied (Medians, Ranges)

	Tubal	Unexplained	Endometriosis
Number	34	15	18
Woman's age (yr)	32 (26–39)	33 (27–39)	32 (29–38)
Partner's age (yr)	34.5 (24–45)	34 (31–41)	33.5 (29–46)
Infertility duration (yr)	6 (1.5–14)	5 (3–10)	5 (2–8)
Serum LH (IU/L)	4.3 (3.4–5.5)	4.4 (3.8–4.8)	4.2 (3.3–4.9)
Serum FSH (IU/L)	4.8 (4.2–6.1)	6.1 (3.3–6.5)	5.5 (4.6–6.5)
Nulligravid (%)	50	88	89
Partner's sperm concentration ($\times 10^6$/ml)	100* (68–152)	37 (23–140)	64* (45–81)

* $P < 0.05$.

Table II. Outcome of Cycles Started for IVF in Infertile Patient Group Studies, as Percentages and 95% Confidence Intervals (CIs), with Follicular-Phase Measurements and Serum and Follicular Fluid (FF) Measurements (as Medians and 95% CI)*

	Tubal	Unexplained	Endometriosis
Patients	34	15	18
IVF data			
Cycles commenced	88	31	41
Attempted oocyte recovery	64	27	34
Oocytes recovered as % (95% CI) attempts	59 92 (86–98)	20 74 (58–90)	28 82 (69–95)
Embryos as % (95% CI) oocytes	41 69 (57–81)[a]	13 65 (44–86)	13 46 (28–64)[a]
Pregnancies as % (95% CI) embryos	4 10 (9–19)	2 15 (0–30)	0 0
Follicular-phase measurements			
Duration of follicular phase (days)	13.0[b] (13.0–14.0)	13.0[c] (12.0–15.0)	15.0[b,c] (14.0–15.0)
Peak follicle diameter (mm)	20 (19.5–21)	21.2 (20–22.8)	20 (19–21)
Serum and FF estradiol measurements			
Day LHo serum estradiol (mol/L)	1062 (1008–1249)	1278 (1012–1492)	1062 (953–1318)
Area under estradiol curve (mol/L)	3083 (2758–3710)	3620 (2932–4382)	3181 (2951–722)
FF estradiol (mol/L)	5.5 (4.8–6.4)	5.7 (2.9–8.8)	4.5 (2.5–5.7)
Serum and FF LH measurements			
Peak LH serum (IU/L)	50 (43–56)	43 (34–60)	48 (41–63)
Area under LH curve (IU/L)	837 (768–940)	824 (630–1102)	726 (661–1096)
FF LH (IU/L)	15.9[d,e] (13.7–20.1)	11.5[d] (8.8–14.4)	12.1[e] (8.6–18.2)
FF progesterone and FSH			
Progesterone (mol/L)	62 (42–68)	46 (28–70)	56 (38–78)
FSH (IU/L)	4.7 (3.9–5.7)	4.5 (3.9–6.6)	4.6 (3.4–6.2)

* Significant differences: [a,d,e]$P < 0.05$; [b,c]$P < 0.005$.

tory follicle are consistent with those earlier reports. We have also previously demonstrated impaired granulosa cell steroidogenic capacity in either unstimulated or stimulated cycles (8) and reduced fertilization rates in stimulated cycles. The present study of unstimulated cycles is also of sufficient size to confirm a significant reduction in the fertilizing ability of the oocyte in women with minor endometriosis: 46%, compared with 69% in women with infertility acting as functional controls.

Some reported studies have shown no difference in fertilization rates between women with minor endometriosis and controls either with stimulation (13,14) or without stimulation, although too small a study (15). Our own studies using stimulation in strictly comparable groups have consistently shown reduced fertilization associated with endometriosis (2–4), and some others agree (16,17).

In the present study of unstimulated cycles, the sample size required to meet the designed statistical power was achieved only in the tubal reference group. Nevertheless, a statistically significant difference was achieved, although sample sizes were unequal: 59 oocytes in the tubal group and 28 in the endometriosis group. The power of the study, when corrected for this inequality, was 75% at the 5% significance level (10).

Although the previously observed significant reductions in serum LH values (1) were not confirmed in the present larger study, evidence remained of reduced incorporation of LH into the preovulatory follicle, indicated by the reduced follicular fluid concentration of LH. This finding raises the question of whether the follicular and oocyte dysfunction that we have demonstrated in association with minor endometriosis is primarily of pituitary or ovarian origin, as discussed in detail elsewhere (1,18). Whatever the basic cause,

impaired fertilizing ability of the oocyte could largely explain the subfertility associated with minor endometriosis.

Although the basic defect cannot be corrected by maximal stimulation using exogenous gonadotropins, assisted conception treatments are successful because the implanting ability of the embryos achieved in excess number is favorable.

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