The follicular endocrine environment in stimulated cycles of women with endometriosis: steroid levels and embryo quality

Antonio Pellicer, M.D.,* Diana Valbuena, M.D.,* Celia Bauset, M.D.,* Carmela Albert, Ph.D.,* Fernando Bonilla-Musoles, M.D.,*† José Remohí, M.D.,* † and Carlos Simón, M.D.*

Instituto Valenciano de Infertilidad and Valencia University School of Medicine, Valencia, Spain

Objective: To assess the endocrine milieu in follicles of stimulated cycles comparing women with and without endometriosis. Steroids were measured in follicular fluid (FF) and in vitro culture of granulosa-luteal cells, and this status was related to the quality of the embryos obtained after IVF.

Design: Case-control study.

Setting: IVF program at the Instituto Valenciano de Infertilidad.

Patient(s): Twenty-four women with laparoscopically documented endometriosis and 26 controls undergoing IVF.

Intervention(s): Individual follicular aspiration, oocyte isolation, FF storage, and preparation of luteinized granulosa cells for culture; oocyte insemination and embryo cleavage in standard IVF.

Main Outcome Measure(s): Serum (day of ovum pickup) and FF measurements of estradiol, progesterone, testosterone, and androstenedione. Secretion of progesterone was measured in the cell-conditioned medium. Results were compared between patients with endometriosis and controls, as well as between oocytes that yielded embryos of different quality.

Result(s): Levels of progesterone in the FF increased with the severity of the disease, whereas testosterone accumulation in the FF decreased with the severity of the disease. An increase in progesterone accumulation in vitro was observed in basal and hCG-induced granulosa cell cultures. No difference was observed in terms of embryo quality, and no steroid marker was able to identify follicles with oocytes that displayed embryos of good or bad quality under the inverted microscope.

Conclusion(s): The data show differences in the steroidogenesis of follicles from stimulated women with and without endometriosis. These changes indicate good endocrine health but are not predictive of embryo quality. (Fertil Steril 1998; 69:1135–41. ©1998 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, follicular fluid, serum, granulosa cells, steroids, estradiol, progesterone, androstenedione, testosterone, embryo quality

Endometriosis is a common and enigmatic disease that has been associated unequivocally with infertility (1), although the specific mechanism(s) for such a relation remains a matter of controversy. The development of IVF-ET has provided a new diagnostic and therapeutic approach for endometriosis during the last decade. However, the results of IVF for these patients are controversial. Several investigators have recently reported that the outcome of IVF was poorer for patients with endometriosis than for patients with other causes of infertility (2–7), whereas other investigators have reported favorable results with IVF in patients with endometriosis (8, 9). Among the investigators who reported poor results, there is a consensus that implantation rates were lower in women with endometriosis, although decreased fertilization rates were found in only some studies (6, 7). Whether reduced implantation is due to embryo quality, the endometrial environment, or both is also controversial.

Brizek et al. (10) analyzed the aberrant morphologic development of human embryos using video documentation and described a statistically significant increase in several nuclear and cytoplasmic events in patients with endometriosis as compared with controls. Similarly,
we evaluated embryo development in vitro in women with and without endometriosis who underwent IVF and embryo replacement 72 hours after oocyte retrieval. We observed a significantly reduced number of blastomeres in embryos from patients with endometriosis as compared with controls, as well as an increased incidence of arrested embryos in vitro (3).

We also analyzed patients undergoing oocyte donation (2). In this study, we found that when the results of oocyte donation were classified according to the nature of the oocytes donated, patients who received embryos derived from endometriotic ovaries showed a significantly reduced ability to implant as compared with other groups of patients who received embryos from women without endometriosis (2). These findings suggest that infertility in patients with endometriosis may be related to alterations within the oocyte, which, in turn, result in embryos with a lower ability to implant.

An oocyte of reduced quality may be the consequence of altered folliculogenesis. Considerable evidence in natural cycles supports this concept in patients with endometriosis. First, a reduction occurs in the peak serum estradiol concentration and size of the dominant follicle in women with endometriosis compared with controls (11), and the follicular phase is longer in patients with endometriosis (12, 13). In addition, impaired LH secretion has been described in these patients (13). Second, recent studies have shown that aromatase activity and accumulation of progesterone in vitro are impaired in cells derived from patients with mild endometriosis as compared with controls (7). This altered function may explain the reduced oocyte quality and the subsequent altered performance of the resulting embryos.

Because our previous observations were made in IVF-stimulated cycles (2, 3), we designed a prospective study to analyze the endocrine milieu (in terms of steroid levels) in women with endometriosis who were undergoing IVF. We compared this hormonal status with the quality of the embryos obtained to gain knowledge about the mechanism(s) associated with infertility in patients with endometriosis. The observations made in vivo induced the planning of experiments in vitro to clarify ovarian steroidogenesis in patients with endometriosis.

**MATERIALS AND METHODS**

**Steroid Levels In Vivo**

**Patients**

The study group included 24 women with primary infertility who had endometriosis diagnosed at laparoscopy or laparotomy, with an interval of <1 year between the diagnosis and IVF. They were classified according to the revised American Fertility Society score (14) and were placed in two groups for the purposes of the study: those having stage I–II endometriosis (n = 5) and those having stage III–IV (n = 19). Their ages ranged from 24 to 33 years (mean ± SEM, 30.8 ± 0.8 years), and the duration of infertility ranged from 1 to 13 years (mean ± SEM, 2.9 ± 0.9 years). All had regular menstrual cycles. The partners had normal semen analyses.

A control group consisted of 26 women who were also undergoing IVF because of tubal infertility. All women in the control group had had a diagnostic laparoscopy during the last year before the IVF cycle to rule out any degree of endometriosis. Their ages ranged from 26 to 37 years (mean ± SEM, 30.9 ± 0.7 years), and the duration of infertility ranged from 1.5 to 14 years (mean ± SEM, 4.7 ± 0.8 years). They also had regular menses and their partners had normal semen samples.

**Stimulation Protocol**

The protocol for ovarian stimulation was started by pituitary desensitization with daily subcutaneous administration of 1 mg of leuprolide acetate (Procrin; Abbott S.A., Madrid, Spain) beginning in the luteal phase of the menstrual cycle. Serum estradiol levels of <60 pg/mL (220 pmol/L) and negative vaginal ultrasonographic scans were used to define ovarian quiescence. On days 1 and 2 of ovarian stimulation, 2 ampules per day of hMG (Pergonal; Serono Laboratories, Madrid, Spain) were administered together with two ampules of high-purity FSH (Neo-Fertinorm; Serono). On days 3, 4, and 5 of ovarian stimulation, 1 ampule per day of hMG plus 1 ampule per day of FSH were administered to each patient. Beginning on day 6, hMG and FSH were administered on an individual basis according to serum estradiol levels and transvaginal ovarian ultrasound scans.

The criteria for hCG administration (10,000 IU, Profasi; Serono) were the presence of two or more follicles of >1.9 cm in greatest diameter and serum estradiol levels of >800 pg/mL (2,940 pmol/L). Injections of leuprolide acetate, FSH, and hMG were discontinued on the day of hCG administration. Oocyte retrieval was scheduled 36–38 hours after hCG injection. The luteal phase was supported with vaginal administration of 400 mg/d of micronized progesterone (Progellik; Laboratorios Effik, Madrid, Spain).

**Study Design**

During oocyte collection, each follicle was aspirated separately and the follicular fluid (FF) was collected into a sterile plastic tube by electronic suction. The volume of FF was recorded and the oocyte was isolated from the aspirate. When the oocyte was not initially visualized, the follicle was flushed with Ham’s F-10 medium until recovery. Only follicles in which an oocyte was clearly identified were used for the study. After isolation of the oocyte, each FF aspirate was immediately centrifuged (1,500 × g), and the supernatant was aliquoted and stored.
at -80°C until required for analysis. Supernatants of FF in which red blood cells constituted >2-3% of the total volume were excluded. At the time of oocyte recovery, a blood sample was taken; the serum was isolated and properly stored as for the FF.

The isolated oocytes were placed in 20-μL droplets of IVF medium (Medicult, Copenhagen, Denmark) under mineral oil, inseminated, and cultured for 20-24 hours. Thereafter, the cytoplasm was checked under the inverted microscope for the presence of normal fertilization, and the zygotes were further incubated in new medium for an additional 24 hours. On the day of transfer (approximately 48 hours after retrieval), the number of blastomeres and the degree of fragmentation of each embryo were recorded (15). Each individual oocyte was followed from retrieval to replacement, and the events of the IVF laboratory were compared with the biochemistry of the serum and FF.

In subsequent experiments, granulosa-luteal cells from seven women with stage III-IV endometriosis and seven controls were isolated and cultured as described later.

**Progesterone Secretion In Vitro**

**Hormones, Drugs, and Reagents**

Human chorionic gonadotropin (hCG Lepori, 2,500 IU/mL; Farma Lepori, SA, Barcelona, Spain) was diluted in M-199 medium (BioMérieux, Charbonnières les Bains, France) supplemented with fetal bovine serum (FBS; GIBCO BRL, Glasgow, Scotland, UK) and L-glutamine (GIBCO BRL). Gentamicin and fungizone (GIBCO BRL) were added as antibiotics to the culture medium. Percoll and trypan blue were purchased from Sigma Chemical Co. (St. Louis, MO).

**Cell Preparation and Culture**

All visible follicles in women included in the present study were harvested by ultrasonography-guided vaginal aspiration. As stated earlier, four follicles in each patient were aspirated in individual plastic tubes to avoid mixture of different follicular aspirates and to obtain accurate FF measurements. Oocytes were identified under the dissecting microscope and isolated for further insemination and culture. The remaining follicular contents were centrifuged at 1,500 rpm for 10 minutes, and the supernatants were decanted.

Cells from all follicles in each woman were combined, washed twice in 2 mL of M-199 medium supplemented with 20% FBS, and centrifuged at 1,500 rpm for 10 minutes. Subsequently, cells were layered onto 5 mL of 50% Percoll columns and centrifuged at 500 rpm for 30 minutes to pellet the red cells. A purified granulosa-luteal cell preparation was aspirated from the interface, washed, resuspended, and counted in a hemocytometer. Viability in all cases was >95% as assessed by trypan blue staining.

All cell cultures were performed in duplicate at a density of 20,000 cells/well in tissue culture dishes (Nunclon; Nunc Delta, Kamstrup, Denmark) using a shaking waterbath. Cells were incubated in 150 μL of M-199 medium supplemented with 10% FBS in an atmosphere of 5% CO₂ in air at 37°C. Cells were cultured in the absence or presence of 1 IU/mL of hCG. Cultures were maintained for 3 hours, and the conditioned media were collected in aliquots and stored at -20°C for subsequent analysis.

**Hormone Measurements**

Serum and FF levels of estradiol, progesterone, testosterone, and androstenedione were measured with the use of commercially available RIA kits (BioMérieux). The accumulation of progesterone in conditioned medium from the granulosa cell cultures was also measured using these kits. The interassay and intrasay coefficients of variation were as follows: for estradiol at a concentration of <40 pg/mL, 2.8% and 4.3%, respectively; for progesterone at a concentration of 0.6 ng/mL, 9% and <10%, respectively; for testosterone at a concentration of 0.2 ng/mL, 5.4% and 3.6%, respectively; and for androstenedione at a concentration of 0.4 ng/mL, 12% and 3.1%, respectively.

**Statistical Analysis**

Data were expressed as means ± SEM. Student's t-test and the χ² test were used to discriminate between groups. When patients with endometriosis were divided into two additional subgroups, analysis of variance (ANOVA) was used to differentiate between them and controls. Tukey's, Bonferroni's, and Scheffé’s tests were used to distinguish among groups when ANOVA showed statistically significant differences. P<0.05 was defined as statistical significance. The statistical analysis was performed with use of the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL).

**RESULTS**

**Serum and FF Steroid Levels In Vivo**

Table 1 shows data from the IVF cycles in the controls and the patients with endometriosis. We were unable to find any difference among groups with regard to the number of days they were under the effects of GnRH agonist and the days necessary to reach an optimal response. Similarly, the diameter of the follicles evaluated and the FF volume were similar among the groups. This table also shows the number of oocytes retrieved, fertilization rates, number of embryos transferred, and implantation rates. There were no statistically significant differences among the groups in this small series of patients.

Serum steroid levels are shown in Table 2. There were no differences among the groups with regard to serum levels of estradiol, progesterone, testosterone, or androstenedione or in the ratios of estradiol to progesterone, estradiol to testosterone, or estradiol to androstenedione on the day of ovum pickup. Table 3 shows the concentrations of the same ste-
In vitro fertilization outcome of control patients versus patients with endometriosis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 19)</th>
<th>Endometriosis (n = 17)</th>
<th>I–II (n = 5)</th>
<th>III–IV (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of days of GnRH-agonist</td>
<td>20.5 ± 1.2</td>
<td>17.5 ± 1.1</td>
<td>20.2 ± 1.6</td>
<td>19.5 ± 1.2</td>
</tr>
<tr>
<td>No. of days of stimulation</td>
<td>24.4 ± 1.2</td>
<td>26.8 ± 2.3</td>
<td>26.7 ± 1.1</td>
<td>26.7 ± 1.0</td>
</tr>
<tr>
<td>Follicular diameter (cm)</td>
<td>19.1 ± 0.3</td>
<td>19.9 ± 0.4</td>
<td>19.2 ± 0.2</td>
<td>19.5 ± 0.3</td>
</tr>
<tr>
<td>Follicular volume (mL)</td>
<td>3.5 ± 0.2</td>
<td>3.5 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>No. of oocytes</td>
<td>12.1 ± 1.3</td>
<td>12.8 ± 2.4</td>
<td>11.2 ± 1.5</td>
<td>11.6 ± 1.2</td>
</tr>
<tr>
<td>No. of oocytes fertilized</td>
<td>5.5 ± 0.9</td>
<td>6.8 ± 2.2</td>
<td>5.4 ± 1.1</td>
<td>5.8 ± 1.1</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>3.7 ± 0.4</td>
<td>3.4 ± 0.7</td>
<td>3.7 ± 0.4</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>6/38 (15.8%)</td>
<td>8/26 (30.8%)</td>
<td>8/31 (25.8%)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Values are means ± SEM unless otherwise indicated. No statistically significant differences among these groups were observed.

Serum steroid levels in control patients versus patients with endometriosis.

<table>
<thead>
<tr>
<th>Serum level</th>
<th>Control (n = 19)</th>
<th>I–II (n = 5)</th>
<th>III–IV (n = 12)</th>
<th>Total (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pg/mL)</td>
<td>1,053.9 ± 151.8</td>
<td>1,272.4 ± 487.8</td>
<td>505.7 ± 123.5</td>
<td>800.6 ± 218.1</td>
</tr>
<tr>
<td>Progesterone (pg/mL)</td>
<td>19.6 ± 6.8</td>
<td>5.8 ± 2.0 (5)</td>
<td>19.2 ± 5.6 (7)</td>
<td>13.6 ± 3.8 (12)</td>
</tr>
<tr>
<td>Testosterone (pg/mL)</td>
<td>22.6 ± 3.8</td>
<td>18.2 ± 6.8 (5)</td>
<td>25.6 ± 18.3 (9)</td>
<td>22.9 ± 11.7 (14)</td>
</tr>
<tr>
<td>Androstenedione (pg/mL)</td>
<td>33.2 ± 5.1 (10)</td>
<td>35.5 ± 0.8 (5)</td>
<td>30.6 ± 2.3 (8)</td>
<td>32.5 ± 1.6 (13)</td>
</tr>
<tr>
<td>Estradiol/progesterone</td>
<td>800.6 ± 218.1</td>
<td>8,818.5 ± 8,702.5</td>
<td>46.4 ± 15.3 (7)</td>
<td>3,701.5 ± 3,629.8 (12)</td>
</tr>
<tr>
<td>Estradiol/testosterone</td>
<td>38.2 ± 7.5 (10)</td>
<td>230.9 ± 147.3 (5)</td>
<td>169.8 ± 80.2 (7)</td>
<td>195.3 ± 73.6 (12)</td>
</tr>
<tr>
<td>Estradiol/androstenedione</td>
<td>38.2 ± 7.5 (10)</td>
<td>35.4 ± 13.3 (5)</td>
<td>16.7 ± 4.3 (8)</td>
<td>23.9 ± 6.0 (13)</td>
</tr>
</tbody>
</table>

Note: Values are means ± SEM (no. of individuals tested). No statistically significant differences among these groups were observed.

In Vitro Secretion of Progesterone by Cultured Human Granulosa Cells

The statistically significant differences found in FF levels of progesterone and testosterone prompted us to investigate the behavior of human granulosa cells in vitro. However, because granulosa cells lack P450c17 activity, only progesterone accumulation in vitro was tested. Figure 1 shows the secretion of progesterone in the absence and presence of hCG. Severe endometriosis was associated with increased secretion of progesterone in basal and hCG-induced conditions as compared with controls.
### Follicular Fluid Steroid Levels in Control Patients versus Patients with Endometriosis

<table>
<thead>
<tr>
<th>Steroid Level in FF</th>
<th>Control</th>
<th>I–II</th>
<th>III–IV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pg/mL)</td>
<td>13,329.3 ± 307.3 (43)</td>
<td>13,026.0 ± 1,301.6 (18)</td>
<td>12,412.8 ± 1,464.8 (25)</td>
<td>12,669.5 ± 1,001.1 (43)</td>
</tr>
<tr>
<td>Progesterone (pg/mL)</td>
<td>102.6 ± 2.6 (40)*</td>
<td>1,260.7 ± 432.8 (14)*</td>
<td>1,898.6 ± 389.9 (20)*</td>
<td>1,636.9 ± 291.3 (34)*</td>
</tr>
<tr>
<td>Testosterone (pg/mL)</td>
<td>129.5 ± 5.3 (61)†‡</td>
<td>112.1 ± 84.1 (18)</td>
<td>99.2 ± 77.1 (32)†</td>
<td>103.8 ± 80.4 (50)‡</td>
</tr>
<tr>
<td>Androstenedione (pg/mL)</td>
<td>36.6 ± 2.5 (54)</td>
<td>36.8 ± 0.7 (10)</td>
<td>35.5 ± 1.1 (19)</td>
<td>35.9 ± 0.8 (29)</td>
</tr>
<tr>
<td>Estradiol/progesterone</td>
<td>130.5 ± 1.2 (39)§</td>
<td>64.2 ± 15.2 (14)§</td>
<td>48.7 ± 13.1 (20)§</td>
<td>55.1 ± 9.8 (34)§</td>
</tr>
<tr>
<td>Estradiol/testosterone</td>
<td>118.8 ± 6.8 (43)‖</td>
<td>276.3 ± 120.3 (18)</td>
<td>352.3 ± 143.9 (24)</td>
<td>339.7 ± 96.1 (42)‖</td>
</tr>
<tr>
<td>Estradiol/androstenedione</td>
<td>429.6 ± 42.1 (40)</td>
<td>336.5 ± 50.5 (10)</td>
<td>427.1 ± 37.7 (18)</td>
<td>393.2 ± 30.8 (28)</td>
</tr>
</tbody>
</table>

**Note:** Values are means ± SEM (no. of individual follicles studied).

* P<0.001 by ANOVA.
† P<0.01 by ANOVA plus Bonferroni’s correction and Scheffé’s test.
‡ P<0.009 by ANOVA plus Bonferroni’s correction and Scheffé’s test.
§ P<0.01 by ANOVA.
‖ P<0.04 by ANOVA plus Bonferroni’s correction and Scheffé’s test.

### DISCUSSION

Several lines of clinical evidence from IVF and oocyte donation programs have led to the hypothesis that follicular development may be impaired in patients with endometriosis, resulting in oocytes and embryos of lower quality. Lower fertilization rates found in some studies (6, 7) and lower implantation rates described in others (2–7) both point in the same direction. Our studies have shown lower implantation rates in ovum donation when the embryos were derived from women with endometriosis as compared with other donors, whereas patients with severe endometriosis receiving oocytes from healthy donors displayed normal implantation rates for ovum donation (2). These results provided the rationale to suspect reduced oocyte quality in patients with endometriosis. Detailed morphologic evaluation of embryos from women with endometriosis has added power to this hypothesis (3, 10).

A group of investigators from Bristol has worked for several years in this area. They initially found reduced fertilization in patients with endometriosis (16), and more recently they described lower LH levels in endometriosis cycles, suggesting pituitary-ovarian dysfunction as the cause of endometriosis-associated unexplained infertility (13). In addition, they have recently published studies using granulosa-luteal cells from women with mild endometriosis who were undergoing IVF, which showed reduced aromatase activity and impaired ability of these cells to secrete progesterone during a 3-hour culture as compared with controls (7). These

### Follicular Fluid Steroid Levels Classified According to Embryo Quality

<table>
<thead>
<tr>
<th>Steroid Level in FF</th>
<th>Control patients</th>
<th>Patients with endometriosis</th>
<th>Control patients</th>
<th>Patients with endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pg/mL)</td>
<td>13,367.7 ± 310.6 (10)</td>
<td>14,358.1 ± 1,095.8 (17)</td>
<td>13,819.9 ± 155.2 (9)</td>
<td>13,824.9 ± 2,409.0 (7)</td>
</tr>
<tr>
<td>Progesterone (pg/mL)</td>
<td>105.7 ± 2.2 (8)</td>
<td>1,603.5 ± 409.7 (16)*</td>
<td>96.2 ± 10.4 (10)*</td>
<td>1,673.3 ± 737.9 (7)</td>
</tr>
<tr>
<td>Testosterone (pg/mL)</td>
<td>130.6 ± 10.9 (10)</td>
<td>105.6 ± 15.0 (20)</td>
<td>148.4 ± 10.0 (16)</td>
<td>108.9 ± 20.7 (9)</td>
</tr>
<tr>
<td>Androstenedione (pg/mL)</td>
<td>51.2 ± 8.9 (10)†</td>
<td>35.7 ± 1.3 (13)</td>
<td>30.8 ± 3.4 (13)†</td>
<td>37.6 ± 0.6 (5)</td>
</tr>
<tr>
<td>Estradiol/progesterone</td>
<td>127.2 ± 0.7 (8)§</td>
<td>57.4 ± 14.7 (16)§</td>
<td>131.1 ± 2.9 (9)§</td>
<td>52.8 ± 20.6 (7)§</td>
</tr>
<tr>
<td>Estradiol/testosterone</td>
<td>109.6 ± 10.2 (10)</td>
<td>464.1 ± 210.8 (16)</td>
<td>113.1 ± 13.3 (9)</td>
<td>458.4 ± 295.4 (7)</td>
</tr>
<tr>
<td>Estradiol/androstenedione</td>
<td>303.5 ± 46.3 (8)</td>
<td>399.0 ± 36.1 (13)</td>
<td>464.6 ± 70.0 (9)</td>
<td>376.4 ± 78.7 (5)</td>
</tr>
</tbody>
</table>

**Note:** Values are means ± SEM (no. of individual embryos studied).

* P<0.008.
† P<0.02.
‡ P<0.01.
§ P<0.02.
Secretion of progesterone in vitro in basal conditions and in response to 1 IU of hCG. Progesterone levels were determined after incubation of $10^3$ cells per mL for 3 hours. *$P<0.05$; †$P<0.01$.

A second approach to reconcile our findings with the literature involves the severity of the disease. Studies performed in natural and stimulated cycles were based on mild endometriosis (7, 13). It is interesting to note how some mediators of the gonadotropin response, such as cytokines, are expressed differently in peritoneal fluid according to the degree of endometriosis. In keeping with this concept, the ligand interleukin (IL)-1β is higher in mild endometriosis, whereas the antagonist of the system (IL-1 receptor antagonist) is enhanced in severe endometriosis (19). We know that there is an inverse relation between IL-1β mRNA levels and progesterone accumulation in human monocyte cultures (20).

Further, when granulosa-cell cultures were made, an enhancement of progesterone accumulation was observed in basal conditions and after hCG stimulation. Others have also shown increased progesterone accumulation in vitro in the presence of peritoneal fluid from patients with endometriosis (17), suggesting that the peritoneal fluid may contain factors that stimulate progesterone production and potentiate the response to hCG.

Other studies of natural (13, 18) and stimulated cycles (13) in women with mild endometriosis have failed to demonstrate any differences in steroid levels in the serum and FF between patients with endometriosis and controls.

The first approach to explain the differences found in natural and stimulated cycles in this study lies in hCG administration. In stimulated cycles, hCG may potentiate the accumulation of progesterone in FF and conditioned medium (17), resulting in significant differences between endometriosis samples and controls. Whether the effect of hCG is direct or through other substances also remains to be determined, but it may be linked to the second hypothesis raised to explain the differences between natural cycles and our present study, as described below.

The correlation between several proteins found in the FF and the outcome of IVF was investigated frequently in the 1980s (21), and it was learned that oocyte immaturity correlated with lower FF levels of progesterone and androstenedione than in mature oocytes (22). Fertilized oocytes came from follicles whose FF contained higher estradiol levels, and the best cleavage ratio in fertilized human oocytes was obtained from follicles with more estradiol and less testosterone. More recently, Andersen (23) reevaluated the correlation between FF steroid levels and IVF outcome.
in 23 patients in whom a single oocyte yielded a single pregnancy. The results showed that pregnancy was associated with follicles with a higher estradiol to testosterone ratio and higher FF volume and follicular diameter. Conversely, our results point in a different direction: Progesterone was higher in endometriosis (indicating mature oocytes), there was no difference in terms of follicular volume, and the ratio of estradiol to testosterone was more favorable in patients with endometriosis. Thus, the measurements do not explain our clinical data showing reduced embryo quality in patients with endometriosis (2, 3) based on the endocrine environment of the follicle in terms of steroid levels because they show good endocrine health in stimulated cycles (21–23).

In summary, our studies clearly show marked differences in the endocrine status (steroid levels in vivo and in vitro) of the follicle in stimulated cycles when women with endometriosis are compared with controls. These studies differ from other reports but were based on stimulated cycles in severe cases of endometriosis. Our measurements, however, indicate normal maturation of the oocytes and a positive ratio of estradiol to testosterone, which should correlate with good-quality embryos and do not explain the marked differences in embryo quality that we have described previously in patients with endometriosis.

References