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Endometriosis

Physiopathological Aspects of Corpus Luteum Defect in Infertile Patients with Mild/Minimal Endometriosis

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***Purpose*:** We describe a physiopathological model to the luteal insufficiency of infertile patients with mild/minimal endometriosis with normal hormone measurements in the early follicular phase.

***Methods*:** We designed a case-control study with 24 patients, 14 fertile with in-phase endometrium (control group) and 10 infertile with mild/minimal endometriosis and luteal insufficiency (study group). The histologic dating of endometrium was performed during cycle days 23–25 and serum TSH, FSH, LH, prolactin, and estradiol levels were measured during the early follicular phase (cycle day 3). Progesterone serum levels were measured in three different occasions during the luteal phase.

***Results*:** Patients with out-of-phase endometrium have lower estradiol levels ($P = 0.031$) and decreased progesterone secretion ($P = 0.012$) during the late luteal phase. Serum prolactin, TSH, FSH, and LH levels were similar between the groups ($P > 0.05$).

***Conclusions*:** The physiopathology of luteal phase defect in infertile patients with mild/minimal endometriosis is associated with a small and large luteal cells dysfunction, characterized by abnormal follicular phase (lower estradiol serum levels) and lower progesterone LH-dependent secretion.

KEY WORDS: Endometriosis; endometrial biopsy; infertility; luteal phase defect; progesterone.

INTRODUCTION

Luteal phase insufficiency was first described in 1949, by Jones (1). After that several studies and papers showed various physiopathological mechanisms and endocrinological dysfunction as a main cause of this entity.

The main factor associated with this finding is a decreased progesterone (P) secretion associated with an altered endometrial maturation. This abnormality could be a consequence of an altered GnRH secretion, small luteal cell defect or a large luteal cell defect (2).

The small and large human luteal cells have similar steroidogenic characteristics than follicular theca and granulosa cells, respectively (3).

Small luteal cells are LH-responsive and produce the initial progesterone and estradiol secretion during the first days after ovulation. Otherwise, the large luteal cells are not LH-receptive and produce the basal P secretion during the luteal phase (2,3).

Patients with endometriosis have a higher prevalence of luteal phase defect detected by endometrial biopsies or P measurements. However, these findings can be explained by hyperprolactinemia (4).

Others (5–7) demonstrated that this particular group of infertile patients with endometriosis have

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a pituitary–ovarian dysfunction, with altered LH secretion and decreased steroidogenesis, that can cause their infertility.

We decided to investigate a selected group of infertile patients with mild/minimal endometriosis and with out-of-phase endometrial biopsies, to elucidate the type and the physiopathology of their luteal phase defect.

METHODS

Design

We did a case-control study with 24 patients from the Gynecological clinic of Hospital de Clínicas de Porto Alegre. The control group was formed by 14 fertile volunteer patients with in-phase endometrium, and the study group by 10 infertile patients with mild/minimal endometriosis and out-of-phase endometrium.

We considered out-of-phase endometrium according to the criteria of Noyes (8) and a discrepancy of more than 2 days from the next menstrual data and the day of endometrial biopsy.

Patients

Twenty-four patients were selected to participate in this study, between January 1999 and January 2000. The study group (Group 1) was formed by 10 infertile patients in whom, during the infertility investigation, the out-of-phase endometrial biopsy and the presence of mild/minimal endometriosis were the only clinical or laboratory findings.

The control group (Group 2) was formed by 14 fertile volunteer women (without endometriosis) with in-phase endometrial biopsy that underwent laparoscopy for tubal ligation (endometriosis was excluded during this procedure).

All patients had 1) regular menstrual cycles (28 ± 2 day interval), 2) normal prolactin and thyroid hormone values, 3) neither a medical history of hormonal disease nor were taking any hormonal medication, and 4) normal infertility investigation (study group).

The laparoscopy was carried out by the same investigator (JSCF) and staged according to the American Society for Reproductive Medicine classification (9).

The research project was approved by the institutional ethical committee and all patients signed an informed consent form.

Measurements

Baseline Hormonal Serum Levels. On the early follicular phase (cycle day 3), we collected a blood sample to measure FSH, LH, TSH, estradiol, and prolactin levels (pool).

All samples were centrifugated at 2500 rpm for separation of plasma, which was frozen at -20°C for later analysis. Hormones were analyzed using chemiluminescence kits (Immulite Ltd., USA). The largest inter- and intrakit variation was 5.45 and 13.3%, respectively, for PRL; 15 and 16% for estradiol; 17.5 and 13.8% for TSH; 6.1 and 6.5% for FSH; 6.5 and 5.3% for LH.

Progesterone Measurements. The progesterone blood samples were collected on days 17 (early luteal), 20 (midluteal), and 23 (late luteal) of the menstrual cycle related to the first day of menses and considering that all patients had regular menses (28 ± 2 days interval).

The plasma was also frozen at -20°C for later assays. We used an Immulite kit with the largest inter- and intrakit variation of 13%.

Endometrial Biopsy. We performed an endometrial biopsy during days 23–25 of the menstrual cycle using a Novak curette. The material was analyzed according to Noyes's criteria and we considered out-of-phase the endometrium with more than 2-day lag from the biopsy day and the first day of the next menstrual period.

All biopsies were analyzed by the same pathologist.

Statistical Analysis. We compared the P and baseline hormonal serum levels between the groups using a Mann–Whitney U test as the variables showed a nonnormal distribution.

The P secretion was analyzed also by the Friedman test for repeated measures to compare the differences between P assays.

The significance level was 5% and the power calculation for this sample size was 80%.

RESULTS

The groups were not different regarding age, body mass index (BMI), or FSH, LH, TSH, or prolactin levels on the early follicular phase. However, patients with in-phase endometrium had higher serum estradiol levels than patients with endometriosis and out-of-phase endometrial biopsies ($P = 0.031$) (Table I).

Friedman test for repeated measures demonstrated that the P serum levels modify during the luteal phase ($P = 0.0001$) only in patients with in-phase

Table I. Clinical Characteristics and Serum Hormonal Levels

	Fertile and in-phase endometrium ($n = 14$)	Endometriosis and out-of-phase endometrium ($n = 10$)	Statistics P
Age (years)	34 (26–41)	32 (28–39)	0.371
BMI (Kg/m^2)	24 (21–26)	24 (22–28)	0.212
FSH (mIU/mL)	3.70 (1.90–6.90)	4.30 (2.20–8.90)	0.096
LH (mIU/mL)	2.65 (0.94–8.00)	2.60 (0.70–7.40)	0.886
Estradiol (pg/mL)	97.00 (35.00–117.00)	44.00 (29.00–94.18)	0.031
TSH (μIU/mL)	1.50 (0.50–3.60)	0.80 (0.31–3.57)	0.201
Prolactin (ng/mL)	6.00 (0.62–19.30)	10.20 (4.30–18.20)	0.625

Note. Values are expressed as medians and range.

endometrial biopsy. Otherwise, in patients with out-of-phase endometrium, this variation was not observed ($P = 0.122$) (Fig. 1).

Infertile patients with out-of-phase endometrial biopsies demonstrated significantly decreased progesterone serum level in the late luteal phase of the menstrual cycle compared to the control group ($P = 0.012$) (Fig. 1).

However, the total P secretion (sum of the three P measurements) of Group 1 (median: 33.70 ng/mL; range: 20.80–84.50) was not different than that of Group 2 (median: 39.70 ng/mL; range: 30.30–57.10) ($P = 0.147$).

DISCUSSION

We demonstrated that mild/minimal endometriosis and out-of-phase endometrial biopsies are associated with a lower P secretion on the late luteal phase and decreased serum estradiol levels in the beginning of the follicular phase. Moreover, the increased P secretion that normally takes place during the luteal phase was not shown.

The gold-standard test to evaluate the luteal phase is the integrated P that measures the total P output over the luteal phase. However, this test is impractical and the cutoff level was not defined (10). The clinical utility of a single P assay is disputed, because of its important variability during the cycle and even during the day (11). The utilization of three P assays is employed by some authors, because of its accuracy and higher sensitivity (10).

The endometrial biopsy was the first test for the diagnosis and characterization of luteal insufficiency (1). Noyes (8) defined the anatomopathological findings to evaluate the endometrial tissue. Since then, a lot of criticism and papers tried to elucidate and validate the endometrial biopsy utility. We decided to use the next menstrual cycle as the clinical criteria for luteal phase diagnosis because, confirming Jordan *et al.* (10), it demonstrated that this parameter is more

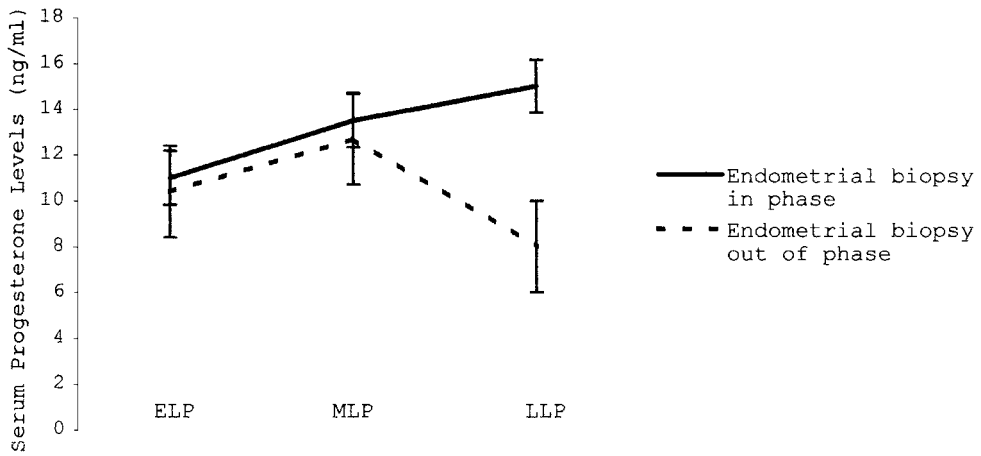

Fig. 1. Serum progesterone (ng/mL) levels (medians and SD) during the early luteal phase (ELP), midluteal phase (ELP), and late luteal phase (LLP). Patients with endometriosis and out of phase endometrium have decreased P secretion on late luteal phase ($P = 0.012$).

sensitive for the dating of endometrium in comparison to the midcycle events.

Even fertile patients may, transitorily, present an out-of-phase endometrial biopsy, and some authors believe that a minimum of two out-of-phase biopsies are necessary to establish the diagnosis of luteal phase defect (12,13).

However, the same authors that recommended the two-biopsies criteria utilized one endometrial sample as a luteal phase defect criteria (13).

The two endometrial samples on two different cycles raised, also, an important and unsolved question: what is the diagnosis when the biopsies are discordant? In addition, two endometrial biopsies, for a clinical proposal, are a painful and an expensive procedure.

We carefully selected a group of infertile patients with mild/minimal endometriosis and luteal insufficiency with normal hormone evaluation to investigate the type of luteal defect controlling for hyperprolactinemia and thyroid disorders as a confounding bias.

Several investigators have already studied the luteal phase in endometriotic patients and found discordant results (14–26). This is probably because different methods were used and the characterization of control and study groups were heterogeneous.

The definition of control group is crucial for an adequate comparison and appropriate conclusion.

If we compare our findings with others (25), they studied infertile patients with and without endometriosis. However, infertile patients may have, intrinsically, an ovulatory dysfunction and were not the most adequate control group.

Some investigators (27) emphasize that the precise cause–effect relationship between endometriosis and infertility remains controversial. We agree with those authors that endometriosis is an heterogeneous disease with different clinical presentations and laboratory findings.

The etiology of infertility in endometriotic patients is, sometimes, not so obvious, and may involve several physiopathological mechanisms. Moreover, we should view endometriosis not only as a syndrome with an important hormonal abnormality, but also as a particular immunologic and inflammatory response.

In addition, this is the first paper that defines a physiopathologic mechanism on a selected group of patients with mild/minimal endometriosis and out-of-phase endometrium. Moreover, we utilized fertile patients with in-phase endometrium as the control group to improve our statistics probability and power (Types I and II errors).

Probably, the corpus luteum of these patients (mild/minimal endometriosis and out-of-phase endometrium) have both small (theca) and large (granulosa) luteal cell dysfunction.

The granulosa cell abnormality is evidenced by lower estradiol serum levels during the early follicular phase. In fact, the inadequate exposure to estradiol during the follicular phase can induce an altered large luteal cell and, consequently, a luteal deficiency (28). Moreover, other investigators presented consistent results regarding the diminished steroidogenesis in infertile patients with endometriosis (7) and a midcycle dysfunction on the LH surge (6).

Hence, the theca cells will originate the small luteal cells that produce and secrete the LH-receptive P during the late luteal phase (2).

The small luteal cells produce the initial augmentation in P secretion during the early luteal phase, which is LH-independent. However, the decreased P secretion seen in the late luteal phase are associated with a lower P pulsatility, which is LH-responsive (29).

We showed that only the group of infertile patients with mild/minimal endometriosis presented lower serum P levels during the late luteal phase. Thus, this dysfunction is associated with an abnormal small luteal cell function.

Recently, we also demonstrated that altered prolactin secretion, lower estradiol serum levels, and luteal insufficiency are related to infertility and mild/minimal endometriosis (4,30). This particular group of patients with endometriosis seems to present a pituitary–ovulatory abnormality explored by others (5), and may cause the hormonal follicular dysfunction (luteal phase defect) observed in the present study.

In conclusion, patients with mild/minimal endometriosis presented a luteal dysfunction associated with large and small luteal cell abnormalities evidenced by lower estradiol serum levels during the early follicular phase, and a decreased P secretion on the late luteal phase.

A randomized trial to assess the improvement of Assisted Reproductive Technologies results in this group of patients with small luteal cell dysfunction, administering hCG to support the corpus luteum, is necessary to confirm our hypothesis.

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