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Observations on the use of purified follicle-stimulating hormone in the treatment of luteal phase defects

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We treated 18 infertile patients affected by histologically confirmed luteal phase deficiency with 75 IU of purified follicle-stimulating hormone (FSH) daily during the first 5 days of the cycle. Patients who were not pregnant after the first cycle of treatment underwent a second cycle. In the second cycle the daily doses of purified FSH were doubled if luteal phase deficiency had persisted during the first cycle. During the two cycles before treatment and during treatment, patients underwent an endometrial biopsy 1–3 days before the expected onset of menses. An assessment of progesterone serum concentrations was also performed on days 8, 6 and 4 before the expected onset of menses. Treatment was administered in a total of 33 cycles resulting in 30 ovulatory cycles. Six pregnancies were achieved. Among non-conception ovulatory cycles, 13 presented delayed endometrial dating and 11 normal endometrium. The mean ± SD of the sum of the three progesterone determinations was 14.7 ± 1.4 ng/ml in pretreatment cycles, 14.6 ± 1.6 ng/ml in cycles with normalization of endometrial dating, 14.8 ± 1.7 ng/ml in cycles with persistence of luteal phase deficiency and 30.4 ± 3.0 ng/ml in conception cycles ($P < 0.05$ versus other groups). We conclude that purified FSH, if effective in the treatment of luteal phase deficiency, does not act through an increase in progesterone concentrations.

Key words: endometrium/FSH/luteal phase defect

Introduction

In 1980, Stouffer and Hodgen were able to induce a decrease in luteal phase progesterone production in rhesus monkeys by selectively lowering FSH concentrations during the follicular phase, through the administration of porcine follicular fluid. In these monkeys, luteal phase deficiency could be successfully treated by FSH-rich human menopausal gonadotrophin (HMG) administration on cycle days 1–4 (diZerega and Hodgen, 1981). These data suggested that a deficit of folliculogenesis might lead to a defective luteal phase. Indeed, in women with luteal phase deficiency, reduced FSH concentrations and FSH/luteinizing hormone (LH) ratio during the follicular phase have been described by some authors (Strott *et al.*, 1970; Sherman and Korenman, 1974; Cook *et al.*, 1983) and denied by others (Rotten *et al.*, 1988; Soules *et al.*, 1989a).

These studies prompted a few authors to investigate the use of gonadotrophin administration in the follicular phase in the treatment of luteal phase deficiency (Huang *et al.*, 1984; Minassian *et al.*, 1988; Balasch *et al.*, 1990). The results of these studies, although contradictory, appear to indicate a good therapeutic effect of gonadotrophins on luteal phase deficiency.

In this study, we report on the treatment of a small group of patients with histologically diagnosed luteal phase deficiency with purified FSH, in an attempt to improve the evaluation of the effects of this therapy.

Materials and methods

A total of 18 infertile patients with luteal phase deficiency were treated for a total of 33 cycles with purified FSH (Metrodin; Serono, Rome, Italy; one ampoule contained 75 IU of FSH and <0.11 IU of LH).

The patients were selected from those referred to the Department of Obstetrics and Gynecology at the University 'Federico II' of Naples, Italy. All patients gave their informed consent. The study had received the approval of the ethics committee of our Medical School. Diagnosis of luteal phase deficiency had been established by endometrial biopsies performed in two consecutive cycles showing a lag of ≥3 days in endometrial dating behind that expected, as determined retrospectively by the subsequent onset of menses, according to Noyes *et al.* (1950). All patients were otherwise healthy and other causes of infertility had been previously ruled out. The mean age for the 18 selected patients was 28.1 ± 5.2 years. Mean infertility duration was 3.2 ± 0.9 years.

Endometrial biopsies were performed in pretreatment and treatment cycles 1–3 days before the onset of menses (as expected based on the day of ovulation), and were always preceded by a serum β-human chorionic gonadotrophin (HCG) test, so as to avoid wasting a possible pregnancy.

Endometrial biopsies were read by one gynaecologist and were evaluated according to the criteria of Noyes *et al.* (1950). Progesterone serum concentrations were determined in pretreatment and treatment cycles, and were assessed 8, 6 and 4 days before the expected onset of menses according to the criteria of Abraham *et al.* (1974).

All patients received 75 IU of purified FSH daily for 5 days starting on the first day of the cycle. Patients who were not

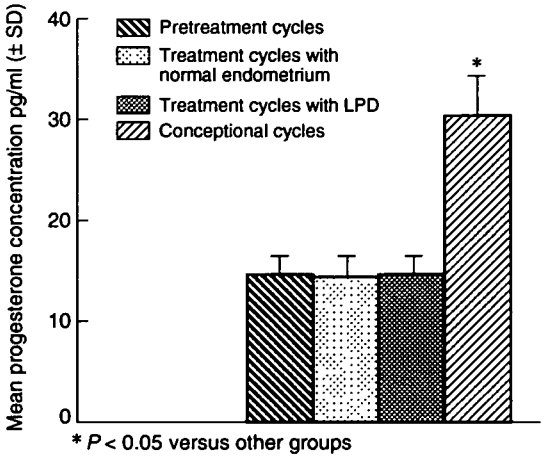

Figure 1. Mean of three progesterone determinations in pretreatment cycles (n = 18), treatment cycles with normalization of endometrial dating (n = 17), treatment cycles with persistence of luteal phase deficiency (LPD; n = 16) and treatment cycles resulting in pregnancy (n = 6).

pregnant after the first treatment cycle underwent a second cycle of treatment. In this second cycle, purified FSH daily doses were doubled if the defect of endometrial dating had persisted during the first cycle of treatment.

All cycles were monitored during the follicular phase by ultrasound pelvic examinations and serum oestradiol determinations performed on alternate days. FSH and LH concentrations were determined on cycle days 2–3 and 8–9 during both pretreatment and treatment cycles.

Data of anovulatory cycles were not included in the evaluation of mean hormonal concentrations.

Plasma hormonal concentrations were measured by commercially available radioimmunoassay kits. All results were expressed as means ± SD. A statistical evaluation of data was performed by analysis of variance followed by Scheffé's procedure for multiple comparison among means or by Student's t-test for paired data when appropriate.

Results

The mean endometrial dating defect during the two pretreatment cycles was 3.4 ± 0.4 days. All pretreatment cycles were ovulatory as shown by ultrasound examination and serum progesterone concentrations.

An overall summary of the study is presented in Table I. Treatment with purified FSH induced a normal endometrial pattern in a total of 17 out of 33 treatment cycles (51.5%; considering also the endometrium of conception cycles as normal) and in 11 out of 18 treated patients (61.1%). In all, 30 treated cycles were ovulatory and six out of 18 patients conceived, giving a cumulative pregnancy rate of 33.3% after two cycles. One miscarriage was observed. No multiple pregnancies were reported (Table I).

During the first cycle of treatment three patients conceived, one patient did not ovulate and endometrial biopsies were performed in the remaining 14 patients. Among the latter group, the defect of endometrial dating was still present in

Table I. Clinical outcome of treatment with different doses of purified follicle-stimulating hormone

Patient no.	First cycle 75 IU	Second cycle	
		75 IU	150 IU
1	P		
2	P		
3	P		
4	N	P	
5	N	P	
6	N	N	
7	N	N	
8	N	N	
9	N	N	
10	D		P
11	D		N
12	D		D
13	D		D
14	D		D
15	D		D
16	D		D
17	D		ANOV
18	ANOV		ANOV

P = pregnancy; N = normal endometrium; D = endometrial deficit; ANOV = anovulatory cycle.

eight cases (Table I). Among the three patients who conceived, one abortion was observed.

The 15 non-pregnant patients underwent a second cycle of purified FSH administration. During the second cycle of treatment, two pregnancies were achieved among the six patients who had presented a normal endometrium during the first cycle (and were therefore treated with 75 IU/day purified FSH). The endometrial biopsy was normal in the four non-pregnant patients. Among the nine patients treated with 150 IU/day purified FSH, one patient conceived, two cycles were anovulatory, and the endometrial biopsy was normal in one patient and out of phase in five (Table I).

The mean of the sum of the three progesterone determinations was 14.7 ± 1.4 ng/ml in pretreatment cycles, 14.6 ± 1.6 ng/ml in cycles with normalization of endometrial dating, 14.8 ± 1.7 ng/ml in cycles with persistence of defective endometrial dating and 30.4 ± 3.0 ng/ml in cycles resulting in pregnancy ($P < 0.05$ versus other groups; Figure 1).

The nine patients who were treated with 75 IU/day in the first cycle and 150 IU/day in the second cycle did not present any significant difference in serum progesterone concentrations in the two cycles.

The ultrasound monitoring of the follicular phase of pretreatment cycles showed an apparently normal follicular development in 10 patients with a mean pre-ovulatory follicle diameter of 20.5 ± 2.1 mm (range 18.0–24.1). A small pre-ovulatory follicle (<18 mm) on the day before rupture was evident in eight patients. A corpus luteum was identified in all cases.

Among treatment cycles, 21 (63.6%) showed a single fully developed pre-ovulatory follicle, while in nine (27.3%) cycles two follicles reached the fully pre-ovulatory stage. Three (9.1%) cycles were anovulatory.

Peak serum oestradiol concentrations significantly increased during treatment cycles (424.2 ± 69.3 pg/ml) in comparison with pretreatment cycles (255.6 ± 46.6 pg/ml; $P < 0.05$). No

difference in peak oestradiol concentrations was detected among treatment cycles with normal and abnormal endometrial dating.

For cycles treated with 75 IU of purified FSH, the mean FSH/LH ratio on cycle days 2–3 was 1.18 ± 0.07 and 1.87 ± 0.19 in pretreatment and treatment cycles respectively ($P < 0.01$). For the same cycles, the mean FSH/LH ratio on cycle days 8–9 was 1.08 ± 0.06 and 1.68 ± 0.20 in pretreatment and treatment cycles respectively ($P < 0.01$).

For cycles treated with 150 IU of purified FSH, the mean FSH/LH ratio on cycle days 2–3 was 1.17 ± 0.08 and 1.94 ± 0.22 in pretreatment and treatment cycles respectively ($P < 0.01$). For the same cycles, the mean FSH/LH ratio on cycle days 8–9 was 1.10 ± 0.07 and 1.79 ± 0.27 in pretreatment and treatment cycles respectively ($P < 0.01$).

Discussion

Our results in terms of ovulatory rate, normalization of endometrial dating and pregnancy rate are in agreement with those reported by other authors (Huang *et al.*, 1984; Minassian *et al.*, 1988; Balasch *et al.*, 1990).

In their paper, Huang *et al.* (1984) observed that mean progesterone concentrations were significantly greater in the treatment cycles than in the pretreatment cycles, and in the cycles with normal endometrial dating than in the cycles with abnormal endometrial dating after treatment. On the contrary, Balasch *et al.* (1990) observed similar hormonal concentrations in control and treatment cycles and in cycles with normal and abnormal endometrial dating. These discrepancies may be the result of either the different methods used in the determination of serum luteal phase progesterone concentrations or the fact that while Huang *et al.* (1984) also included in the determination of progesterone concentrations during treatment values of pregnant patients, Balasch *et al.* (1990) did not include data of pregnant patients. Indeed, in their study Huang *et al.* (1984) reported significantly higher progesterone concentrations in conception cycles than in non-conception cycles.

In our study we separately considered progesterone concentrations in conception cycles, non-conception cycles with normal endometrium, non-conception cycles with abnormal endometrial dating and control cycles. It is evident that the normalization of endometrial dating in non-pregnant patients after treatment is not associated with a significant increase in progesterone concentrations, thus confirming the data reported by Balasch *et al.* (1990).

Indeed, various authors have reported repeatedly that the majority of cases of histologically documented luteal phase deficiency are associated with normal progesterone concentrations (Li and Cooke, 1991; American Fertility Society, 1991).

Our study is not a randomized prospective trial and therefore does not allow us to draw definite conclusions about the effectiveness of the purified FSH treatment in luteal phase deficiency. Indeed, pregnancies may reflect spontaneous conception rates independent of treatment (Karamardian and Grimes, 1992). Moreover, it is probable that similar results would have been achieved with the use of HMG.

Nevertheless, from our results we can suggest that purified FSH, if effective in the treatment of luteal phase deficiency, does not act through an increase in progesterone concentrations during the luteal phase.

In our patients, the mean FSH/LH ratio during the follicular phase in pretreatment cycles was higher than that reported by Cook *et al.* (1983). However, other authors (Rotten *et al.*, 1988; Soules *et al.*, 1989a,b) did not find a significant association between low FSH/LH ratio and luteal phase deficiency, suggesting that basal gonadotrophin measurements have limited predictive value on luteal phase characteristics. Therefore it would be tempting to speculate that in our study patients responsive to purified FSH administration might be suffering from a subclinical form of aberrant folliculogenesis, leading to a reduction of endometrial progesterone receptor synthesis and therefore to luteal phase deficiency. Indeed, a significant reduction in endometrial progesterone receptor has been reported in patients with luteal phase deficiency both in proliferative (Jacobs *et al.*, 1987) and secretory phase (Laatikainen *et al.*, 1983). On the contrary, patients unresponsive to purified FSH treatment may represent other pathophysiological forms of luteal phase deficiency unrelated to aberrant folliculogenesis, as suggested by Balasch *et al.* (1990).

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Erratum: Figure 1. The y-axis label should read: Mean progesterone concentration ng/ml (± SD).