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UNEXPLAINED INFERTILITY AND ITS TREATMENT WITH INTERMITTENT GnRH APPLICATION

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Summary—Spontaneous LH fluctuations were estimated in patients with unexplained infertility. This suggested that irregular LH bursts and a slightly decreased frequency are present in these individuals. Administration of sex steroids suppressed LH serum levels and no LH pulses were noted thereafter. Intermittent GnRH application by means of an automatic pump (Zyklomat) induced regular LH fluctuations: follicular maturation was enhanced using 20 μg GnRH per pulse than with 10 μg; the former dose however appeared to be supra-physiological. Three pregnancies occurred in these patients who had not conceived following previous treatment with clomiphene and human menopausal gonadotropins.

INTRODUCTION

The short-term secretion patterns of LH and FSH throughout the menstrual cycle have been well characterized [1, 2]. In menstrual disorders such as amenorrhea pulses of LH may be absent or present [3, 4, 1]. More knowledge of the neuroendocrine control of the menstrual cycle [5] and the pathophysiology of hypothalamic ovarian failure [6] may have initiated detailed studies dealing with pulsatile LH secretion patterns; in particular in patients with menstrual dysfunction other than hypogonadotropic hypogonadism [7, 6, 8, 9]. The results of these investigations suggest a pronounced irregular hypothalamic LHRH discharge in patients with corpus luteum insufficiency in the presence [4] or absence [8] of hyperprolactinemia. In this paper the results of intermittent GnRH application to patients with unexplained infertility are reported.

MATERIALS AND METHODS

Patients with unexplained infertility ($n = 11$) lasting for 2–4 years entered the study. All subjects had a laparoscopy and chromopertubation as well as a hysteroscopy. Patients with tubal dysfunction and endometriosis were excluded. Serum prolactin (PRL) levels were normal (< 12 ng/ml); so were serum testosterone (≤ 0.5 ng/ml) and dehydroepiandrosterone (DHEA-S, < 3 μg/ml) levels. Thyroid function was within normal limits as judged from a thyroid stimulating hormone (TSH) stimulation test using a bolus of 400 μg thyrotropin releasing hormone, (TRH, basal TSH < 3, TSH after TRH < 18 μU/ml). All patients had been previously treated with clomiphene (for 5 days with 50–100 mg per day) for 7–13 cycles and with HMG for 3–6 cycles, but no pregnancy occurred. Male partners of these women had either normal sperm counts or only a slightly decreased number of spermatozoa ($> 30 \times 10^6$/ml); motility was $> 50\%$.

After a therapy free interval of approx 3 months the patients were subjected to a control cycle. In 4 of the subjects blood samples were taken in 15 min intervals for a period of 4 h during the mid-follicular phase of the menstrual cycle to evaluate the frequency of spontaneous LH spikes. An LH pulse was defined as a net increase of LH of at least 4 mU/ml descending to baseline levels over at least two continuously declining LH serum concentrations.

In all patients sonographic measurements of follicular diameters were carried out daily from day 8 up to day 18 of the menstrual cycle. Blood samples were taken when the leading follicles reached a diameter of ≥ 16 mm to determine serum estradiol (E_2) levels. Approximately 1 week later between day 18 and 24 of the cycle another blood sample was taken to measure mid-luteal E_2 and progesterone (Prog) serum concentrations. In the treatment cycles GnRH application was usually stopped by this time and 5000 IU of hCG were given intramuscularly to support corpus luteum function.

From day 14 of the following menstrual cycle patients were treated for 10 days with 40 μg of ethinyl-estradiol plus 4 mg chlormadinon-acetate. The day following the last administration of sex steroids in those 4 patients pulsatile LH release was re-evaluated as described above. All patients were subjected to the intermittent GnRH treatment from day 3 of the withdrawal bleeding using the Zyklomat infusion pump and set, respectively (Ferring Pharmaceuticals, Kiel, Germany). Twenty μg of GnRH were given at 90 min intervals via an indwelling venous catheter placed in the forearm. Sonographic folliculometry and blood sampling were carried out as pointed out above. In some of the women ($n = 4$) LH-pulses were reevaluated while being on the

Table 1. Cycle characteristics and hormonal parameters of patients with unexplained infertility before ($n = 11$) and during intermittent GnRH application using either 20 ($n = 11$) or 10 μg ($n = 10$) GnRH per pulse

	Controls ($n = 7$)	Before and during intermittent application of		
			20 μg	10 μg GnRH
Duration (days) of				
follicular phase	13 ± 1.5	15 ± 1.5	10 ± 2.5	12 ± 1.5
Luteal	14.1 ± 1.4*	12.5 ± 1.8	13.2 ± 2.4	23.3 ± 1.6
Follicular diameter (mm)	23 ± 1.0	17.4 ± 1.2	18.7 ± 2.1	19.5 ± 1.9
Pre-ovulatory E_2 (pg/ml)	410 ± 125	330 ± 95	407 ± 156	384 ± 117
Mid-luteal prog (ng/ml)	14.7 ± 2.1	10.8 ± 2.5	12.3 ± 1.7	12.8 ± 1.8
Conceptions	$n = 7$	$n = 0$	$n = 1$	$n = 2$

*From [13].
The data are compared to those obtained during conception cycle.

Zyklomat. In all subjects the basal body temperature was recorded and the day when the temperature rose by 3 to 10°C was designated as the first day of the luteal phase; the day before menstruation occurred was considered to be the last day of the luteal phase. Serum estradiol and LH were measured using commercially available radioimmunoassay kits supplied by INC Corporation, Minneapolis, U.S.A. Patients who did not conceive were subjected to another similar treatment schedule; however, only 10 μg of GnRH were used per pulse.

Data gathered from conception cycles served for comparison. Statistical analyses were carried out using Student's paired and unpaired t-test respectively.

RESULTS

Table 1 gives the duration of the follicular and of the luteal phase of patients before and after intermittent GnRH application. When compared to conception cycles the duration of the follicular phase was slightly extended in patients with unexplained infertility and the luteal phase was shortened if the function of a normal corpus luteum is considered to be approx 14 days [10]. Administration of GnRH resulted in enhanced follicular maturation which was more pronounced using 20 μg than 10 μg of GnRH per pulse; when the pre-treatment duration of follicular maturation was compared to that of conception cycles it was slightly retarded but significantly accelerated by both, 20 μg ($P < 0.01$) and 10 μg ($P < 0.05$) of GnRH per pulse. Sizes of follicles estimated by ultrasound were greatest in controls and significantly ($P < 0.01$) smaller in patients with unexplained infertility; GnRH treatment resulted in slight increases of the average diameter of periovulatory follicles (Table 1).

Pre-ovulatory serum E_2 concentrations were highest in the controls and lowest in untreated patients, but increased by GnRH application (Table 1).

The duration of the luteal phases was somewhat paralleled by mid-luteal serum progesterone level; low progesterone before therapy ($P < 0.01$, when compared to conception cycles) were associated with the shortest duration of corpus luteum function. The levels of progesterone during both periods were increased following GnRH treatment. The number of pregnancies following GnRH therapy is also indicated in Table 1.

Figure 1 shows a representative short term LH-fluctuation pattern of one of the 4 subjects in whom repeated blood samples were taken for a period of 4 h. Mid-follicular phase LH serum levels exhibit three irregular LH bursts; in the 4 subjects studied intervals of LH spikes varied between 30–140 min. During the 4 h period of observation 2–4 pulses were seen (mean 2.6 ± 1.2). Shortly after discontinuation of sex steroid application serum LH concentration was suppressed and lacked LH pulses. Intravenous GnRH delivery by means of the Zyklomat infusion pump resulted in regular elevations of serum LH.

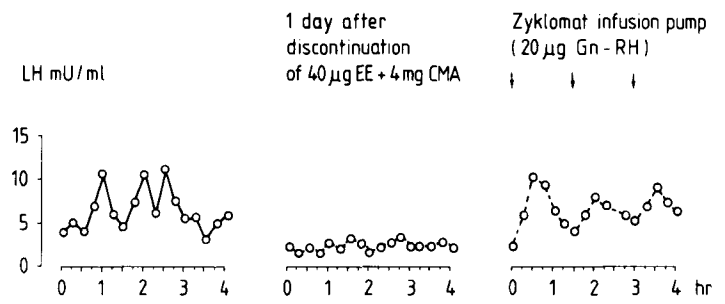

Fig. 1. Short serum fluctuations of serum LH during the follicular phase of the menstrual cycle of a patient with unexplained infertility (left panel). The middle panel shows serum LH levels of the same patients the day when sex steroids were discontinued. The right panel depicts LH pulses induced by exogenously given GnRH by the Zyklomat infusion pump.

DISCUSSION

The results of our study suggest that in patients with unexplained infertility irregular LH discharge from the pituitary gland may be the reason for inadequate follicular maturation and inadequate luteal function. A slightly decreased number of LH spikes may also be a detrimental factor. Clomiphene which has been widely used for successful treatment of corpus luteum insufficiency [11] has been shown to induce pulsatile LH secretion in patients with menstrual disorders previously lacking regular LH pulses [1]. In patients with unexplained infertility subjected to an *in vitro* fertilization program, wide variations of serum LH levels were also observed [9]. Burger and co-workers [7] reported that in patients with polycystic ovarian syndrome endogenous irregular LH secretion has been overcome by a high dose of GnRH delivered in 90 min intervals by an automatic infusion pump. In previous studies [12] we have shown that in patients with menstrual disorders not only LH serum levels, but also pituitary LH pools are decreased following administration of gestagens. These observations have been extended by these investigations; administration of sex steroids abolishes pulsatile LH release. Subsequent intermittent infusion of exogenous GnRH may result in physiological elevations of serum LH and in amelioration of follicular growth, corpus luteum function and finally in pregnancies which could not be achieved by other hormonal treatment procedures. The duration of the follicular phase of the menstrual cycle has been shown to last 13 days on average [10]; this is in line with our observations in conception cycles, while in patients with unexplained infertility follicular maturation is prolonged. Using 20 μg of GnRH per pulse the follicular phase lasted only 10 days suggesting that the dose of GnRH may be supra-physiological, while a dose of 10 μg per pulse appears to be more appropriate. Leyendecker and co-workers [14] have reported that with increasing doses of exogenous GnRH serum E_2 levels increase in patients with hypothalamic amenorrhea. In addition, the average length of the follicular phases during GnRH administration depends on the grade of hypothalamic derangement before treatment. From our studies it becomes obvious that in spontaneously menstruating infertile women there is practically no or little shortage of endogenous GnRH; thus only small doses of the decapeptide should be given for normal LH pulse generation. Pre-treatment with sex steroids may shorten the period which would be necessary for exogenous GnRH to overcome irregular endogenously secreted GnRH.

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