Conventional dose intravenous pulsatile GnRH therapy does not induce ovulation in polycystic ovarian disease

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Abstract. The value of pulsatile GnRH therapy for induction of ovulation in patients with polycystic ovarian disease remains unclear. Intravenous pulsatile GnRH therapy was administered to a defined group of 5 patients with polycystic ovarian disease; all were infertile, had an LH:FSH ratio of >2:1 on two or more occasions, and had multiple cysts on ovarian ultrasonography. All had failed to respond to clomiphene citrate. The 5 patients received increasing doses of GnRH (3–40 µg/pulse) continuously for up to 6 weeks. The response was evaluated by serial hormone levels and ovarian ultrasonography. During nine treatment periods no patient ovulated, and in only one did the LH:FSH ratio revert to normal. Four patients have subsequently had wedge resection of the ovaries and in each case the diagnosis of polycystic ovarian disease was confirmed. Pulsatile GnRH therapy was of no value in the management of this group of infertile patients with strictly defined polycystic ovarian disease.

Since it was first demonstrated that the administration of pulsatile GnRH could induce ovulation in patients with hypotalamic amenorrhea, its value in the treatment of this condition has been established (Leyendecker & Wildt 1983; Hurley et al. 1984; Mason et al. 1984).

The cause of polycystic ovarian disease (PCOD) is still unknown. Circulating LH concentrations are inappropriately elevated and serum FSH levels relatively low (Yen et al. 1979). The high serum LH concentration is maintained by exagge-
Patients and Methods

Five subjects, all of whom wished to conceive, were recruited from the Infertility Clinic at the Royal Maternity Hospital, Belfast. The duration of infertility ranged from 1 to 6 years; four of the five had primary infertility. Two of the five were hirsute. Three of the patients had secondary amenorrhea and two had cycles from 2 to 4 months length. Therapy with clomiphene citrate had failed to induce ovulation in all five. All patients had normal secondary sexual characteristics; 3 patients who could be satisfactorily examined had palpably enlarged ovaries. The remaining 2 patients were obese. In all 5 patients ovarian ultrasonography revealed bilaterally enlarged ovaries containing many follicles of 5 mm or less in diameter.

Each patient had an LH:FSH ratio > 2:1 on two or more occasions. No blood sample was included which had been taken within 14 days of the next menstrual period. All had serum levels of dehydroepiandrosterone sulphate (DHEA-S) and testosterone at or above the upper limit of normal, while serum androstenedione levels were elevated (Table 1). Anterior pituitary function was normal in all cases, as demonstrated by response to a combined insulin-induced hypoglycemic test and TRH test. The male partners all had normal semen analysis.

Material and Methods

Synthetic GnRH (HRF, Ayerst, Watford) with 2% benzyl alcohol as diluent was used. The GnRH was administered by a syringe pump (MS27, Graseby Dynamics, Andover), attached aseptically to a forearm vein. Pulses were given every 90 min and the dose per pulse varied from 5 to 40 µg.

A basal blood sample was obtained for gonadotropins, androgens and estradiol estimation. Subsequent blood sampling at approximately 3-day intervals were performed at the same time of day in each patient as well as an ovarian ultrasonograph. If there was evidence of inflammation or bleeding, the infusion site was changed.

All patients gave informed consent to the study, which was approved by the Queen’s University of Belfast Medical Ethics Committee.

Hormonal estimations

Serum testosterone (Wheeler & Luther 1983), serum androstenedione (Goodall et al. 1979), serum DHEA-S (Smith et al. 1975), serum LH and FSH (Groom 1977) were measured by standard radioimmunoassay techniques. Serum estradiol was measured by radioimmunoassay, using reagents purchased from Steranti Research Ltd, St. Albans, Herts. Serum progesterone was measured by a direct 125I radioimmunoassay (Radcliffe et al. 1982). Ovarian ultrasonography was performed using an Hitachi EUB-25M real-time ultrasonic scanner fitted with a 3.5 mHz linear array transducer (Sonotron UK Ltd).

Results

The 5 patients underwent a total of nine treatment periods, the duration of continuous treatment ranging from 28 to 52 days (Table 2). The starting dose used for 3 patients was 5 µg of GnRH per pulse, but if there was no serum estradiol rise or ultrasound evidence of follicular development by day 7–10, the dose was increased.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Day</th>
<th>LH (U/l)</th>
<th>FSH (U/l)</th>
<th>DHEA-S (µmol/l)</th>
<th>Androstenedione (nmol/l)</th>
<th>Testosterone (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>1</td>
<td>13.0</td>
<td>3.9</td>
<td>4.5</td>
<td>15.0</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>17.0</td>
<td>4.3</td>
<td>5.0</td>
<td>17.5</td>
<td>7.7</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>1</td>
<td>16.2</td>
<td>4.2</td>
<td>8.4</td>
<td>15.5</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47</td>
<td>3.7</td>
<td>5.1</td>
<td>6.1</td>
<td>9.8</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>1</td>
<td>15.0</td>
<td>5.4</td>
<td>9.4</td>
<td>10.1</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39</td>
<td>31.0</td>
<td>5.7</td>
<td>10.4</td>
<td>13.1</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>1</td>
<td>16.0</td>
<td>5.1</td>
<td>4.5</td>
<td>17.5</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51</td>
<td>17.0</td>
<td>6.8</td>
<td>4.5</td>
<td>15.9</td>
<td>4.5</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>1</td>
<td>17.5</td>
<td>7.5</td>
<td>12.9</td>
<td>12.7</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29</td>
<td>5.6</td>
<td>4.8</td>
<td>12.7</td>
<td>14.5</td>
<td>3.6</td>
</tr>
</tbody>
</table>
Results of pulsatile GnRH therapy. In each patient, GnRH was given continuously throughout the total period. In the column marked 'Duration of treatment period' day 1 was dated either from the initial day of treatment or subsequently from the first day of any vaginal bleeding. Serum estradiol estimations and ovarian ultrasonography were performed at 3-day intervals. Pulses were given at 90-min intervals.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total duration of therapy (days)</th>
<th>Duration of treatment period (days)</th>
<th>Minimum/maximum dose per pulse (µg)</th>
<th>Maximum estradiol (pmol/l)</th>
<th>Maximum progesterone (nmol/l)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>15</td>
<td>15/15</td>
<td>1350</td>
<td>&lt; 3.0</td>
<td>Bilateral ovarian cysts 4 cm developed by day 10.</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>25</td>
<td>15/20</td>
<td>530</td>
<td>&lt; 3.0</td>
<td>Cysts persisted.</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>39</td>
<td>5/40</td>
<td>334</td>
<td>&lt; 3.0</td>
<td>Patient bled before progesterone assayed.</td>
</tr>
<tr>
<td>4</td>
<td>51</td>
<td>40</td>
<td>5/20</td>
<td>820</td>
<td>&lt; 3.0</td>
<td>Follicular cyst 3.2 cm developed by day 21. Anovulatory cervical mucus.</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>18</td>
<td>10/20</td>
<td>233</td>
<td>&lt; 3.0</td>
<td>Developed bilateral ovarian cysts 3 and 4 cm in diameter by day 11 of this second treatment period. Bleeding continued for 14 days. Treated with norethisterone.</td>
</tr>
</tbody>
</table>

* NM = not measured.

by 5 µg. This was repeated weekly until a maximum of 20 µg per pulse was reached. In 2 patients the starting dose was 10 and 15 µg per pulse, respectively. In patients No. 3 and 4, a maximum dose of 40 µg per pulse was reached without effect. During the first 10 days of treatment a rise in LH and estradiol occurred, but this was not statistically significant (paired Student's t-test). There was no change in serum FSH. For the purpose of this paper, each treatment period is defined as having lasted from day 1 until the onset of vaginal bleeding. In no patient was there biochemical or ultrasonic evidence of ovulation.

Patient No. 1 developed asymptomatic bilateral ovarian cysts approximately 4 cm in diameter by day 10 (at dose of 15 µg per pulse), and these persisted until treatment was discontinued on day 40. Thereafter, the cysts gradually reduced in size. Patient No. 4 also developed asymptomatic bilateral ovarian cysts, 3 and 4 cm in diameter, by the last day of her treatment. These 2 patients developed prolonged episodes of vaginal bleeding during GnRH therapy, and in patient No. 4 norethisterone was used to stop the bleeding at the time the pump was discontinued. There were no other complications with therapy and all patients managed the pump without difficulty.

During six of the nine treatment periods the serum estradiol levels altered very little, as exemplified by patient No. 3 (Fig. 1), whose serum estradiol ranged from 89 to 334 pmol/l. In three others (patients No. 1, 2, 4 – see Fig. 1 for patient No. 2 as a representative sample), a rise in estradiol level to 1350, 1247 and 820 pmol/l, respectively, occurred within 7 days of commencing therapy, but there was no ultrasound evidence of a dominant follicle developing and the ovaries maintained their polycystic appearances. Serum progesterone levels were measured at each visit after day 18 (unless the patient bled vaginally prior to this) and on no occasion was the progesterone level consistent with ovulation, > 30 nmol/l (Hull et al. 1982; Abdulla et al. 1983). Only in patient No. 2 did serum progesterone level exceed 3.0 nmol/l. During the second period of treatment in this patient, a serum estradiol level of 1247 pmol/l was achieved by day 20 (Fig. 1). Ultrasound examination revealed a persisting folli...
P.V. bleeding

\[ \text{Patient 2} \]

\[ \text{Patient 3} \]

Days of Treatment

![Graph showing serum estradiol levels during pulsatile GnRH therapy. P = progesterone level. ↑ denotes dose (µg) per pulse. Patient No. 2 received 47 days' treatment, doses per pulse ranging from 5–20 µg. She had 6 days vaginal bleeding during therapy and bled again on day 47. During the second period of therapy a follicle was seen in the right ovary, reaching a maximum diameter of 32 mm. Peak estradiol two days after the follicle was first noted was 1247 pmol/l. However, serial progesterone estimations taken one week later were low (peak 12.4 nmol/l). Vaginal bleeding occurred 10 days after the maximum follicular diameter was noted. Patient No. 3 received 39 days' continuous GnRH therapy, in doses per pulse ranging from 5–40 µg. She had no vaginal bleeding. Serum estradiol levels did not change appreciably and serum progesterone levels remained low. P.V. bleeding = vaginal bleeding.]

Representative data from 2 patients (Nos. 2 and 3) showing the serum estradiol levels during pulsatile GnRH therapy. P = progesterone level. ↑ denotes dose (µg) per pulse. Patient No. 2 received 47 days' treatment, doses per pulse ranging from 5–20 µg. She had 6 days vaginal bleeding during therapy and bled again on day 47. During the second period of therapy a follicle was seen in the right ovary, reaching a maximum diameter of 32 mm. Peak estradiol two days after the follicle was first noted was 1247 pmol/l. However, serial progesterone estimations taken one week later were low (peak 12.4 nmol/l). Vaginal bleeding occurred 10 days after the maximum follicular diameter was noted. Patient No. 3 received 39 days' continuous GnRH therapy, in doses per pulse ranging from 5–40 µg. She had no vaginal bleeding. Serum estradiol levels did not change appreciably and serum progesterone levels remained low. P.V. bleeding = vaginal bleeding.

The LH:FSH ratio did not alter significantly during GnRH therapy in 4 of the 5 patients. Similarly, androgen levels did not become normal. In 4 patients, the testosterone levels had increased when treatment was completed (Table 1).

Four patients (patients No. 1–4) have had a bilateral ovarian wedge resection performed within several weeks of completing their GnRH therapy. In each case PCOD was confirmed. The ovaries were enlarged at operation and histological examination revealed thickening of the capsule and multiple follicular cysts.

Discussion

Current therapies for ovulation induction in PCOD are grossly inadequate. The present lack of precise understanding of the pathogenesis does not help in the search for new alternatives. In PCOD the LH:FSH ratio is abnormally high due to exaggerated pulsatile LH release (Rebar et al. 1976). It has been postulated that stimulation with
appropriate exogenous pulsatile GnRH administration could override the abnormality in PCOD (Burger et al. 1983; Ory et al. 1985). Results of previous studies are discussed in the introduction. In the study of Adams et al. (1985) ovulation was reported to have occurred in 40% of GnRH treatment cycles. The precise criteria for the diagnosis of PCOD in this series were not given. Multifollicular and polycystic ovaries were differentiated by ultrasonography. Polycystic ovaries were differentiated by ultrasonography. Polycystic ovarian disease was not precisely delineated by endocrine data. It is therefore possible that the 40% response rate to GnRH is in part due to less stringent diagnostic criteria in that series. In contrast to the reports already mentioned, there have been various statements that GnRH therapy is unsatisfactory for patients with PCOD (Leyendecker & Wildt 1983; Mason et al. 1984). We have studied the effect of GnRH therapy on the ultrasound appearance of the ovary, serum LH, FSH, estradiol and progesterone levels, the LH:FSH ratio and serum androgen levels. We gave GnRH by the more physiological intravenous route in order to avoid the possible criticism that disordered release of LH may occur when the subcutaneous route is used (Menon et al. 1984).

In none of our cases was ovulation achieved. In a number of cases peak estradiol values reached levels previously shown to be consistent with ovulation (Menon et al. 1984). However, in none of these cases did a dominant follicle emerge and in none was a peak progesterone of $>30$ nmol/l seen (range $<3.0–12.4$ nmol/l). In six of the seven treatment phases in which serum progesterone values were obtained, the level did not exceed 3 nmol/l. Of the remaining two, patient No. 4 bled throughout her second treatment period and so progesterone was not measured; patient No. 2 experienced bleeding 9 days after the estradiol peak, so that even if ovulation had occurred but had not been diagnosed, the luteal phase was inadequate.

Why did GnRH fail to induce ovulation in our patients with PCOD? There are a number of possible explanations. Firstly, the disturbed LH:FSH ratio seen in the condition may be a secondary rather than a primary phenomenon. Alteration of the GnRH input may therefore make no difference. Secondly, GnRH pulses at 90-min intervals may not be capable of resetting the disturbed frequency pattern of GnRH pulses seen in the

condition. Thirdly, the further rise in androgen levels noted in this study may lead to further inappropriate follicular maturation (McNatty 1981).

Since our work began it has been shown in one small series that iv GnRH therapy administered at a very low dosage was associated with apparent successful ovulation in 5 out of six 6 in 4 subjects (Ory et al. 1985). While it must be noted that 2 of their 4 patients had already had wedge resection of the ovary (the time gap before GnRH therapy not being stated), it is possible that very low doses of GnRH may be of value in PCOD as opposed to the lack of success when conventional doses such as we administered are used.

In the present study conventional iv doses of GnRH, given to patients with strictly defined PCOD, did not induce ovulation. Different therapeutic approaches require assessment, for example, long-acting GnRH analogue combined with Pergonal® therapy, pure FSH therapy or very low dose GnRH. Very carefully defined groups of patients with PCOD should be assessed in such studies.

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References


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