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Induction of ovulation with chronic intermittent (pulsatile) administration of Gn-RH in women with hypothalamic amenorrhoea

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Summary. The physiological and pathophysiological basis of hypothalamic amenorrhoea are reviewed as well as the clinical results of chronic intermittent (pulsatile) administration of Gn-RH in the treatment of infertility. Hypothalamic amenorrhoea is considered to be the result of a deficient hypothalamic secretion of Gn-RH. By pulsatile administration of Gn-RH, which is a pre-requisite of normal pituitary gonadotrophic function, deficient endogenous Gn-RH is replaced. If an adequate dose of Gn-RH is provided, which takes into account the degree of impairment of hypothalamic function in the individual case, follicular maturation, ovulation and corpus luteum formation are achieved in nearly every treatment cycle. Although dependent also on factors other than the treated dysfunction, a high conception rate is achieved.

Introduction

Chronic intermittent (pulsatile) administration of Gn-RH as a new mode of treatment of infertility in women with hypothalamic amenorrhoea is based upon the following new physiological and pathophysiological findings. There is indirect evidence that Gn-RH is secreted by the hypothalamus in a pulsatile fashion with an average frequency of one pulse every 90–120 min in ovariectomized rhesus monkeys, hypogonadal women and during the proliferative and periovulatory phases of the human menstrual cycle (Dierschke, Bhattacharya, Atkinson & Knobil, 1970; Yen, Tsai, Naftolin, Vandenberg & Ajabor, 1972). Direct evidence for this secretory pattern is provided by measurement of immunoreactive Gn-RH in the portal stalk effluent of the rhesus monkey (Carmel, Araki & Ferin, 1976).

The physiological significance of the pulsatile pattern of Gn-RH secretion did not become apparent until recently, when it was shown that pulsatile, but not continuous, administration of Gn-RH was able to maintain pituitary gonadotrophic function in rhesus monkeys, in which endogenous Gn-RH secretion had been abolished by lesions in the medio-basal hypothalamus (Belchetz, Plant, Nakai, Keogh & Knobil, 1978; Knobil, 1980). The requirement of a pulsatile stimulation with Gn-RH by the pituitary gonadotrophs may explain why administration of long-acting analogues of the decapeptide are relatively unsuccessful in the treatment of secondary amenorrhoea (Katzorke, Popping, von der Ohe & Tauber, 1980) and may even depress pituitary gonadotrophic function in normal women (Dericks-Tan, Hammer & Taubert, 1977; Bergquist, Nillius & Wide, 1979).

The endocrine regulation of the menstrual cycles of primates appears to be fundamentally different from that of the oestrous cycle of the rat. In the rat the rostral part of the hypothalamus

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seems to be essential in the mediation of chronobiological signals and positive feedback reactions, but the assumption of such a 'cyclic centre' appears no longer to be justified for the primate (Nakai, Plant, Hess, Keogh & Knobil, 1978) in which the function of the hypothalamus in the regulation of the menstrual cycle appears to be 'permissive' only. This concept was based upon the observation that in female rhesus monkeys with hypothalamic lesions, which abolish endogenous Gn-RH secretion, the pulsatile administration of an unvarying amount of Gn-RH at a physiological frequency induced menstrual cycles which were not different from spontaneous ones (Knobil, Plant, Wildt, Belchetz & Marshall, 1980).

Hypothalamic amenorrhoea is considered as a defect of hypothalamic Gn-RH secretion (Leyendecker, 1979). Severe hypothalamic amenorrhoea can be compared with the functional state of the rhesus monkey following destruction of the arcuate nucleus of the medio-basal hypothalamus. In women with severe hypothalamic amenorrhoea, pulsatile administration of Gn-RH at a frequency of 90 min resulted in follicular maturation, ovulation and corpus luteum formation, thus showing that the concept of the permissive function of the hypothalamus developed in the rhesus monkey could be extended to the human female (Leyendecker, 1979; Leyendecker, Struve & Plotz, 1980a). These results also indicate that pulsatile administration of Gn-RH could be used as a new mode of treatment of infertility in patients with hypothalamic amenorrhoea (Leyendecker, Wildt & Hansmann, 1980b). Since its first introduction, pulsatile administration of Gn-RH has been applied to patients with different forms of hypothalamic amenorrhoea, using different protocols of treatment (Crowley & McArthur, 1980; Keogh, Mallal, Giles & Evans, 1981; Schoemaker, Simons, van Osnabrugge, Lugtenburg & van Kessel, 1981). It will also be used as a tool of physiological and pathophysiological research. In this paper a concept of the pathophysiology of hypothalamic amenorrhoea is presented, a classification of the hypothalamic impairment is proposed and the results of pulsatile administration of Gn-RH are described.

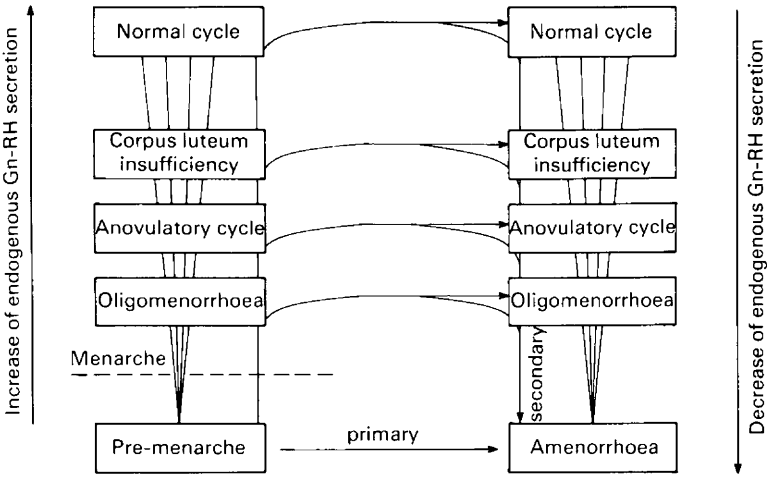
Pathophysiology

The relationship between psyche and ovarian function is well known. Terms such as stress, psychogenic, situational or 'Notstands-' amenorrhoea have been used to describe this phenomenon. The term hypothalamic amenorrhoea was initially coined by Klinefelter, Albright & Griswold (1943) to characterize amenorrhoea of suprapituitary origin. Probably due to some cases described in their publication, this term, however, was later confined to psychogenic amenorrhoea (Reifenstein, 1946; Quigley, Sheehan, Casper & Yen, 1980). In this paper, the term hypothalamic amenorrhoea is used in its broader original sense. The view has been proposed that the common mechanism of this dysfunction is a lack of adequate stimulation of the pituitary by Gn-RH (Leyendecker, 1979; Leyendecker, Wildt & Plotz, 1981). Consequently, idiopathic hypothalamic amenorrhoea, anorexia nervosa, lesions of the pituitary stalk and the hypothalamus as well as Kallmann's syndrome are included and regarded as different forms of hypothalamic amenorrhoea. Since the introduction of pulsatile administration of Gn-RH, it has been possible to demonstrate the potential normal function of the pituitary–ovarian axis by inducing normal menstrual cycles in these patients.

Amenorrhoea as a symptom merely reflects a level of hypothalamic impairment within the pathophysiological entity of hypothalamic ovarian failure. Depending upon the reduction of hypothalamic Gn-RH secretion, clinical pictures such as corpus luteum insufficiency, anovulatory cycles, oligomenorrhoea and, finally, amenorrhoea may occur. This view is supported by the induction of anovulatory cycles and corpus luteum insufficiency when an insufficient dose of Gn-RH is used in the treatment of hypothalamic amenorrhoea by pulsatile administration of Gn-RH (see below). Furthermore, history and observation of patients suffering from secondary hypothalamic amenorrhoea often reveal that corpus luteum insufficiency and anovulatory cycles are transitory stages during the development of and recovery from amenorrhoea. Results from

studies on pulsatile administration of Gn-RH to prepubertal female rhesus monkeys suggest that the gradual start of ovarian function during puberty may largely depend on a gradual onset of an adequate hypothalamic release of Gn-RH (Wildt, Marshall & Knobil, 1980). After menarche, in the human female, oligomenorrhoea, anovulatory cycles and luteal-phase defects are observed before normal menstrual cycles occur indicating full 'functional maturation' of the control system in the brain.

Text-figure 1 is a schematic representation of this notion. Primary hypothalamic amenorrhoea results if the hypothalamic secretion of Gn-RH never reaches a degree which causes menarche in the developing girl. Examples of this group include idiopathic hypothalamic amenorrhoea, Kallmann's syndrome and primary amenorrhoea in association with a craniopharyngeoma. Sometimes psychogenic factors as well may be involved in the pathogenesis of primary hypothalamic amenorrhoea.



Text-fig. 1. Schematic representation of the pathophysiological continuum of hypothalamic ovarian failure and of the development of primary and secondary hypothalamic amenorrhoea on the basis of deficient Gn-RH secretion. (Modified from Leyendecker *et al.*, 1981.)

According to this notion, secondary hypothalamic amenorrhoea is the result of the reduction of a pre-existing secretion of Gn-RH by the hypothalamus and therefore, functionally, a relapse of the pituitary–ovarian axis into the pre-menarchal state. Frequently, women presenting with secondary hypothalamic amenorrhoea have never had a previous 'normal' cycle. Thus, secondary hypothalamic amenorrhoea may develop from all functional states (Text-fig. 1). The development of secondary hypothalamic amenorrhoea is often associated with more or less overt psychogenic or emotional stress (Fries & Nillius, 1973). Sometimes a temporary weight loss is the only indication of this stress (Warren *et al.*, 1975). In women with previously normal menstrual cycles and an overt association between stress and the development of secondary hypothalamic amenorrhoea there is a great likelihood of spontaneous improvement of ovarian function as soon as the psychogenic disturbance is no longer present (Kaufmann, 1951).

The pathophysiological continuum of hypothalamic ovarian failure depending on different degrees of hypothalamic impairment extends into amenorrhoea as well. The functional state of the pituitary–ovarian axis, and thus of the hypothalamus in patients with hypothalamic amenorrhoea, can easily be assessed by the application of progestagen, clomiphene and Gn-RH tests.

Table 1. Grading of hypothalamic amenorrhoea on the basis of progestagen, clomiphene and Gn-RH tests

Grade		Result of test
1		Clomiphene positive with bleeding following
	1a	Normal luteal phase
	1b	Insufficient luteal phase
	1c	Anovulatory cycle
2		Progestagen positive
		Clomiphene negative
3		Progestagen negative with pituitary response to 100 μg Gn-RH i.v.
	3a	'Adult response'
	3b	'Prepubertal response'
	3c	No response

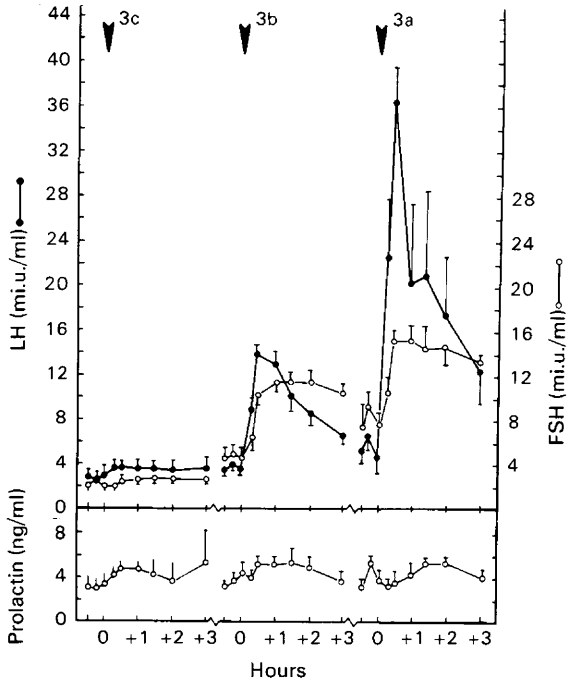
Concomitant measurements of basal serum LH, FSH and oestradiol levels and of the pulsatile pattern of LH are a precise reflection of hypothalamic activity in hypothalamic amenorrhoea and thus serve as tools for the assessment of the degree of severity of the condition (Table 1) (Leyendecker, 1979; Leyendecker *et al.*, 1981).

In hypothalamic amenorrhoea, a positive clomiphene test (bleeding) (100 mg/day for 5 days, started on the 5th day after a progestagen-induced withdrawal bleeding) indicates that there is only little impairment of hypothalamic function. Following the temporary elevation of serum gonadotrophins during clomiphene administration, the residual hypothalamic function is sufficient to promote further follicular maturation and eventually to cause ovulation. The positive test (bleeding) may therefore be sub-classified according to the occurrence of a normal luteal phase, an insufficient luteal phase or an anovulatory bleeding following the administration of the test dose of clomiphene, reflecting different degrees of hypothalamic impairment within the clomiphene-positive group (grades 1a–1c of hypothalamic amenorrhoea).

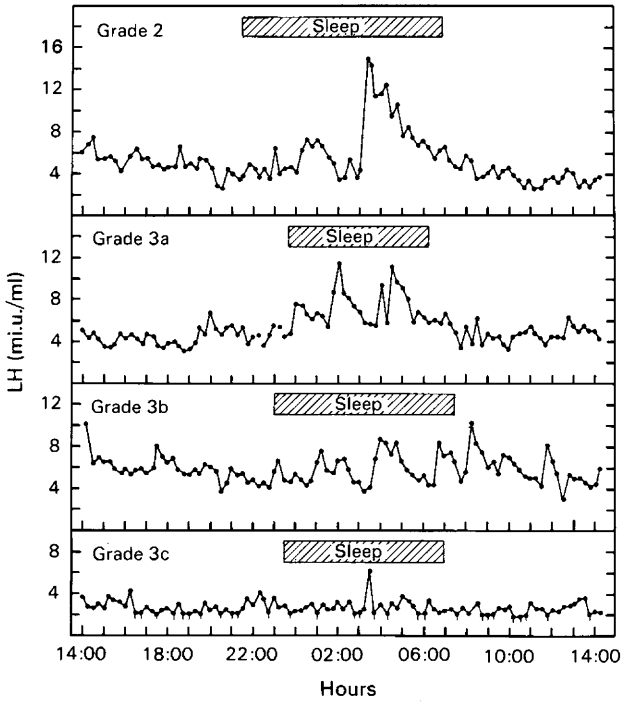
Some patients who are progestagen positive (bleeding following administration of 10 × 10 mg of medroxyprogesterone acetate) do not bleed after the administration of clomiphene. Patients displaying these results are suffering from an intermediate grade (grade 2) of hypothalamic amenorrhoea because of the severity of hypothalamic impairment.

In severe hypothalamic Gn-RH deficiency there is little or no stimulation of the pituitary gonadotrophs and thus in turn little follicular stimulation. Due to the low serum oestradiol levels in these patients the endometrium is not sufficiently proliferated to allow a secretory transformation and withdrawal bleeding following the administration of the progestagen: the progestagen-test is negative (grade 3). Since the progestagen-negative group comprises patients differing in the pituitary response towards an i.v. bolus of Gn-RH, this test allows a further characterization of the pathophysiological continuum of hypothalamic amenorrhoea within grade 3 (Text-fig. 2). (1) For the 'adult response' (grade 3a), the increase of LH in serum is larger than that of FSH after injection of 100 μg Gn-RH. This response is also observed in normal women. (2) For the 'prepubertal response' (grade 3b), the increase of LH is impaired and quantitatively and qualitatively similar to that of FSH. This pattern of LH and FSH after Gn-RH is also observed in prepubertal children (Roth, Kelch, Kaplan & Grumbach, 1972). (3) In grade 3c there is no pituitary reaction towards stimulation with 100 μg Gn-RH. This response is observed in the most serious forms of hypothalamic amenorrhoea and in patients with hypothalamic or pituitary stalk lesions.

Parallel to the decreasing response of the pituitary to the Gn-RH bolus, there is a tendency to lower basal serum levels of LH and FSH with progressive hypothalamic impairment. Preliminary data suggest that there is a correlation between the different grades of hypothalamic amenorrhoea as determined by the dynamic test of pituitary response and the 24-h pulse rate of LH in serum as determined by blood sampling every 15 min over a period of 24 h (L. Wildt & G. Leyendecker, unpublished observations) (Text-fig. 3).



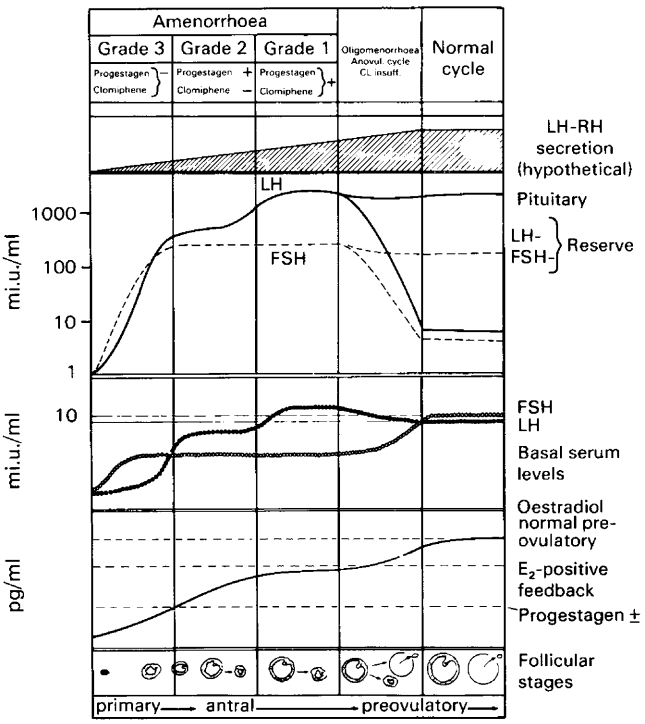
Text-fig. 2. The LH and FSH response to an i.v. bolus of 100 μg Gn-RH in women with hypothalamic amenorrhoea grades 3a, 3b and 3c (progestagen negative). The serum levels of prolactin are also depicted.



Text-fig. 3. The pulsatile pattern of LH in serum during a 24-h period as measured at 15-min intervals in patients with different grades of hypothalamic amenorrhoea (L. Wildt & G. Leyendecker, unpublished observations).

Ovarian biopsies in patients with amenorrhoea revealed that there is a considerable follicular growth (Nakano, Hashiba, Washio & Tojo, 1979). In progestagen-negative as well as in progestagen-positive patients with hypothalamic amenorrhoea antral follicles of large size were found, with an increased incidence of larger follicles in the latter group. Our own ultrasonographic studies demonstrated that, starting with hypothalamic amenorrhoea of grade 3a, antral follicles of more than 10 mm diameter can be detected. In patients of grades 3b and 3c no spontaneous growth of follicles to a size of up to 5–10 mm occurred (G. Leyendecker & L. Wildt, unpublished observations).

In summary, the data presented suggest that hypothalamic ovarian failure ranges from luteal-phase defects to severe hypothalamic amenorrhoea and is a pathophysiological continuum due to a gradually reduced hypothalamic activity (Text-fig. 4).



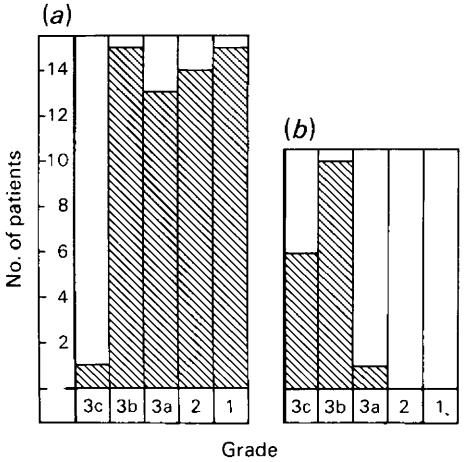
Text-fig. 4. A schematic representation of the pathophysiology of hypothalamic ovarian failure. The pituitary gonadotrophin reserve is depicted on the basis of a cumulative net increase of serum gonadotrophin levels during Gn-RH infusion. The separating lines for LH and FSH pituitary reserve indicate cyclically occurring changes of pituitary reserve due to gonadotrophin discharges. (Modified from Leyendecker, 1979.)

Diagnosis of hypothalamic amenorrhoea

Endogenous Gn-RH cannot be measured in peripheral blood and therefore direct evaluation of hypothalamic function is presently not possible and the diagnosis of hypothalamic amenorrhoea is essentially based on the exclusion of other causes of amenorrhoea, such as hyperprolactinaemia, hyperandrogenaemia, primary ovarian failure, genital tract defects, general systemic and neurological diseases. Primary pituitary failure is excluded by the ability to stimulate pituitary gonadotrophic function by pulsatile administration of Gn-RH. Once the diagnosis of hypothalamic

amenorrhoea has been established, the severity of hypothalamic impairment can be readily assessed by the above described tests (Table 1).

In 58 women with secondary hypothalamic amenorrhoea, these tests revealed a wide distribution of grades ranging from 1 to 3b. Grade 3c was only once found in secondary hypothalamic amenorrhoea. On the other hand, in 17 women with primary hypothalamic amenorrhoea mainly grades 3b and 3c were observed. Only one patient with primary hypothalamic amenorrhoea presented with grade 3a (Text-fig. 5).



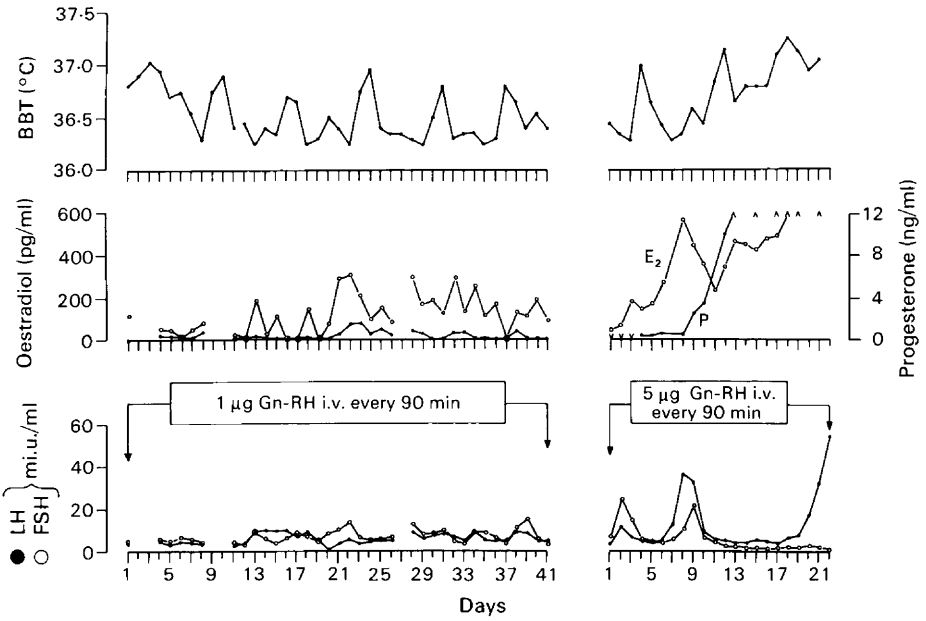
Text-fig. 5. The grades of severity in (a) 58 patients with secondary and (b) 17 patients with primary hypothalamic amenorrhoea.

Chronic intermittent (pulsatile) administration of Gn-RH

Since the first introduction of pulsatile administration of Gn-RH to women with hypothalamic amenorrhoea, 108 treatment cycles have been performed. Following the demonstration that follicular maturation and ovulation could be achieved in these women by repetitive i.v. injections of Gn-RH at doses ranging from 10 to 20 μg at intervals of 90 min (Leyendecker, 1979; Leyendecker *et al.*, 1980a), an automatic device was introduced (Zyklomat: Ferring GmbH, Kiel, FRG) for the application of this mode of treatment in clinical practice (Leyendecker *et al.*, 1980b; Leyendecker & Wildt, 1981). The patients were selected for pulsatile treatment on the basis of the criteria described above. Only patients with hypothalamic amenorrhoea of grades 2–3c were considered suitable for Gn-RH substitution.

Dose of Gn-RH

Intravenous administration of Gn-RH with a dose of 10 μg per pulse did not result in full follicular maturation over a treatment period of 17 days in a patient with primary hypothalamic amenorrhoea, grade 3c (Leyendecker *et al.*, 1980a). The same patient did, however, ovulate and exhibit normal luteal phases repeatedly when i.v. doses of 15–20 μg/pulse were used. Doses of 2·5 and 5 μg/pulse again resulted only in anovulatory bleedings (Leyendecker *et al.*, 1981). In contrast, women with hypothalamic amenorrhoea of grades 3b, 3a and 2, ovulated with i.v. doses ranging from 2·5 to 20 μg/pulse, indicating that in less severe cases than grade 3c a smaller dose of Gn-RH might be sufficient to induce menstrual cycles (Table 2). However, in these patients a dose of 1–2·5



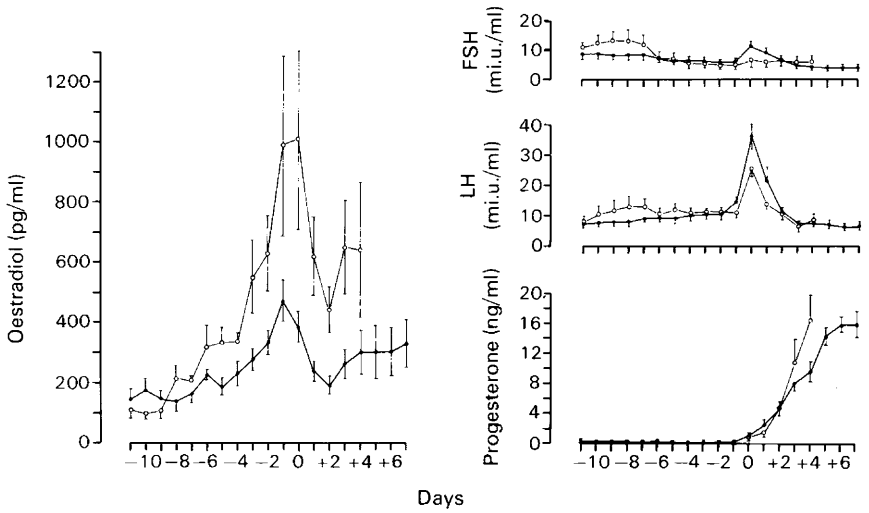
Text-fig. 6. Basal body temperature (BBT), pituitary and ovarian response during pulsatile administration of Gn-RH with a dose of 1 µg/pulse i.v. by means of the 'Zyklomat' over a period of 41 days in a patient with grade 2 secondary hypothalamic amenorrhoea. After the increase of the dose of Gn-RH to 5 µg/pulse ovulation and conception occurred. (Modified from Leyendecker & Wildt, 1981.)

µg/pulse might constitute a critical dose range for induction of ovulation. In a patient (Text-fig. 6) with secondary hypothalamic amenorrhoea, grade 2 ovulation could not be induced with a dose of 1 µg/pulse over a treatment period of 41 days. When the dose was increased to 5 µg/pulse ovulation was obtained and the patient conceived during the treatment course. As a consequence of these findings, patients with grade 3c hypothalamic amenorrhoea (usually patients with primary hypothalamic amenorrhoea grade 3c, or pituitary stalk and hypothalamic lesions exhibit this degree of severity) are now routinely treated i.v. with 15–20 µg/pulse and patients with grades 2–3b with 5 µg/pulse. With this dose regimen of Gn-RH all i.v. treatment cycles performed so far resulted in ovulation.

Once the critical threshold of the Gn-RH dose is surpassed there seems to be a dose–response relationship between the dose of Gn-RH administered per pulse and the ovarian response, as reflected by oestradiol and progesterone levels in serum (Text-fig. 7). The mean oestradiol and progesterone levels of the cycles induced with 15–20 µg/pulse were all above those obtained in cycles with 2·5–5 µg/pulse. Both were higher than those observed in normal menstrual cycles (Leyendecker, Hinckers, Nocke & Plotz, 1975). The results depicted in Text-fig. 7 were all obtained in patients suffering from grade 3b hypothalamic amenorrhoea.

Duration of the follicular phase

The duration of the follicular phase after pulsatile administration of Gn-RH is a reflection of the ovarian functional status at the beginning of the Gn-RH substitution. As indicated by ovarian biopsies (Nakano *et al.*, 1979) and ultrasonography of the ovaries, there is an increased chance of developed or even dominant follicles being present in grades 3a and 2 of hypothalamic amenorrhoea. Ultrasonographic follow-up demonstrates that there is a 'cyclic' growth of these



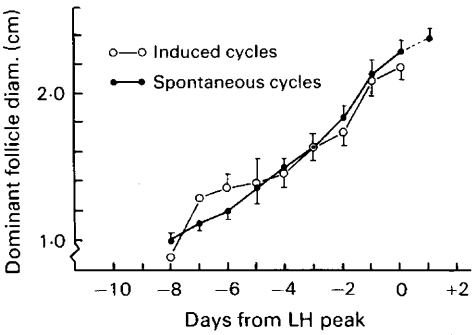
Text-fig. 7. The serum concentrations of FSH, LH, oestradiol and progesterone in patients with hypothalamic amenorrhoea grade 3b treated with Gn-RH doses of 15–20 μg/pulse (●, N = 6) or 2·5–5 μg/pulse (○, N = 12).

follicles ('occult anovulatory cycles'), which can reach a size of 14–18 mm in diameter before they become atretic (G. Leyendecker & L. Wildt, unpublished observations). This also resembles the prepubertal state, in which the ovaries contain a population of 'cyclically' growing large antral follicles which become atretic (Peters, 1979). Proliferative phases of short duration, ovulations and conceptions show that these follicles can be further stimulated and bear a competent egg. The first child born after induction of ovulation with Gn-RH resulted from such a large antral follicle which was present before initiation of treatment (Leyendecker *et al.*, 1980b). The presence of large antral follicles explains the observation that there is an immediate dramatic rise in serum oestradiol levels in some patients with the onset of Gn-RH substitution (Leyendecker *et al.*, 1980a).

The average length of the follicular phase was 17 days (range 15–19 days) in grade 3c, 14 days (range 12–18 days) in grade 3b, 10 days (range 6–15 days) in grade 3a and 9 days (range 2–14 days) in grade 2 of hypothalamic amenorrhoea. When a second treatment cycle was started immediately after the first in grades 3c, 3a and 2, the duration of the follicular phases equalled those of grades 3b and of the normal menstrual cycle. It is concluded that in grade 3b, before any treatment, there is a follicular status comparable with that of the beginning of the normal menstrual cycle. The duration of the follicular phases was independent of the dose of Gn-RH used. Regardless of the dose the growth rate of the dominant follicle was, with an average of 2 mm per day, comparable to that of the normal menstrual cycle (Text-fig. 8) (Hackeloer, Fleming, Robinson, Adam & Coutts, 1979).

Substitution during the luteal phase

The normal luteotrophic hormone in the human is pituitary LH (van de Wiele *et al.*, 1970). In severe hypothalamic amenorrhoea luteal function immediately ceases following termination of pulsatile Gn-RH substitution a few days after ovulation (Leyendecker & Wildt, 1981). Continuation of pulsatile administration of Gn-RH during the whole luteal phase resulted in normal luteal function as indicated by the length of the luteal phase, the progesterone levels in serum and conceptions (Table 2). Previously, it was suggested that luteal function should be supported by 1–3 injections of 2500 i.u. hCG once ovulation had been obtained by Gn-RH (Leyendecker *et al.*, 1980b). There is, however, no indication on the basis of our data (Table 2) that



Text-fig. 8. Growth of the dominant follicle in spontaneous cycles and those induced by chronic intermittent administration of Gn-RH. Values are mean ± s.e.m. for 12 spontaneous and 6 induced cycles.

Table 2. Results of pulsatile administration of Gn-RH in the treatment of infertility in favourable couples with primary (1°) or secondary (2°) hypothalamic amenorrhoea (HA)

Patient	Age (years)	Type of HA 1°/2°	Type of HA Grade	No. of cycles	Route	Dose per pulse (μg)	Ovul.	Luteal substit.	Pregnancy	Comment
S.J.	27	1°	3b	1	i.v.	5	+	hCG	0	
				2	i.v.	20	+	Gn-RH	0	
				3	i.v.	5	+	Gn-RH	+	Undelivered
P.S.	27	1°	3b	1	i.v.	20	+	hCG	+	Missed abortion
				2	s.c.	20	+	hCG	+	Missed abortion
				3	s.c.	16	+	hCG	0	
				4	i.v.	5	+	hCG	+	Term delivery
E.K.	29	2°	3a	1	i.v.	20	+	hCG	+	Term delivery
				1	i.v.	5	+	Gn-RH	0	
				2	s.c.	5	+	Gn-RH	0	
				3	s.c.	16	+	Gn-RH	0	
				4	i.v.	5	+	Gn-RH	0	
				5	i.v.	5	+	hCG	+	Undelivered
C.S.	35	1°	3b	1	i.v.	16	+	hCG	+	Term delivery
B.B.	26	2°	3a	1	i.v.	15	+	hCG	0	
				2	i.v.	15	+	hCG	+	Term delivery
W.Z.	26	2°	3b	1	i.v.	2·5	+	hCG	+	Term delivery
C.H.	31	2°	3b	1	i.v.	2·5	+	hCG	0	
				2	i.v.	2·5	+	hCG	+	Term delivery (twins)
M.H.	23	2°	2	1	i.v.	5	+	Gn-RH	+	Term delivery
H.R.	28	2°	3b	1	s.c.	5	+	Gn-RH	+	Term delivery
M.C.	36	2°	3b	1	i.v.	5	+	Gn-RH	0	
				2	s.c.	5	+	Gn-RH	+	Term delivery
H.K.	26	1°	3b	1	s.c.	20	+	hCG	0	
				2	s.c.	20	+	hCG	+	Term delivery
S.R.	26	2°	2	1	i.v.	5	+	Gn-RH	+	Abortion
				2	i.v.	5	+	Gn-RH	+	Undelivered
A.B.	26	2°	3b	1	i.v.	5	+	hCG	0	
				2	i.v.	5	+	hCG	0	
				3	i.v.	5	+	hCG	0	
				4	i.v.	16	+	Gn-RH	+	Undelivered (triplets)
S.H.	25	2°	2	1	i.v.	5	+	hCG	0	
				2	i.v.	5	+	hCG	0	
				3	i.v.	5	+	Gn-RH	+	Undelivered
U.K.	27	2°	3b	1	i.v.	5	+	hCG	+	Undelivered
R.R.	30	2°	2	1	i.v.	5	+	Gn-RH	+	Undelivered
E.St.	25	2°	3b	1	i.v.	5	+	hCG	0	
				2	i.v.	20	+	hCG	0	
				3	i.v.	20	+	hCG	0	
A.W.	27	2°	2	1	i.v.	5	+	Gn-RH	+	Undelivered

one method is superior to the other in terms of pregnancy rate obtained. Therefore, either mode of luteal substitution may be applied according to the convenience and practicability in each situation.

Intravenous versus subcutaneous application of Gn-RH

The same catheter used for the i.v. application of Gn-RH was also used for the subcutaneous route, but without the addition of heparin to the hormone-containing solution. The catheter was placed into the fat tissue of the lower abdominal wall. Ovulations could be induced with doses of 5–20 μg/pulse in patients with hypothalamic amenorrhoea of grades 2–3b and with 20 μg/pulse in a patient with grade 3c following removal of a craniopharyngeoma (Leyendecker & Wildt, 1981). Four pregnancies were obtained with the s.c. route. However, in contrast to the i.v. application with a 100% ovulation rate, in spite of the adequate dose per pulse provided, there were only 13 ovulatory cycles in 21 s.c. applications of Gn-RH. In some of the ovulatory cycles the follicular phase was considerably longer than had been expected for the particular grade. Additionally, in all the patients in whom the s.c. application of Gn-RH failed to induce an ovulatory cycle, ovulation and normal luteal function was obtained by applying Gn-RH intravenously at the same dose level. Delayed resorption of Gn-RH from the subcutaneous fat tissue might result in insufficient serum levels of Gn-RH for adequate stimulation of the pituitary gonadotrophs. The occurrence of anovulatory cycles and prolonged follicular phases is another indication of the dose–response relationship between Gn-RH stimulation of the pituitary and the ovarian response and may be considered as experimental support of the view of hypothalamic ovarian failure as a result of insufficient hypothalamic stimulation.

Ovulation and pregnancy rate

With a correct dose of Gn-RH (15–20 μg/pulse i.v. in grade 3c and 2·5–5 μg/pulse i.v. in grades 3b–2), ovulation and normal luteal function can be expected in every treatment cycle. Individual and uncontrollable factors influencing the resorption of the hormone from the fat tissue, however, reduce the ovulation rate when the s.c. route is chosen. Definite treatment failure (no ovulation) was only observed when the diagnosis of hypothalamic amenorrhoea was not correct. In our initial studies, mild hyperandrogenaemia was not carefully taken into account. In these patients pulsatile administration of Gn-RH could not induce ovulatory cycles. Patients with polycystic ovarian disease and related pathological entities are not considered to be suitable for pulsatile Gn-RH administration.

The pregnancy rate in these studies is remarkably high. Of 24 patients 21 became pregnant. Eleven children were born, amongst them one set of heterozygous twins. Three patients aborted, of whom one patient had two sequential abortions probably due to active cytomegaly. All these patients conceived again thereafter and had uneventful pregnancies so far. In total, 26 conceptions were obtained in 24 patients.

The pregnancy rate, however, is critically dependent upon whether or not additional factors causing infertility of the couple are present (i.e. tubal or andrological factors). In 'favourable couples', in whom the hypothalamic amenorrhoea constitutes the only cause of infertility of the couple, the pregnancy rate is comparable to that of the normal population: 17 of 18 favourable couples achieved a pregnancy (Table 2) and 10 conceptions occurred in the first, 4 in the second, 2 in the third and 1 in the fourth treatment cycle. Patient E.K., who had conceived in 1979 in her first treatment cycle and had delivered a healthy girl in 1980, underwent another treatment and has conceived in the fifth cycle. These data indicate a 60% chance for favourable couples to achieve a pregnancy in the first treatment cycle. In total, 40 treatment cycles were applied to 18 favourable couples and 21 pregnancies were obtained (1·9 cycles/pregnancy). The rate was 1·7 for Gn-RH cycles and 2·1 for cycles in which luteal function was maintained by hCG, indicating that the mode of luteal substitution is not of importance in terms of pregnancy rate.

In 6 unfavourable couples only 5 pregnancies were achieved in 28 treatment cycles. This poor result was mainly due to a patient with primary hypothalamic amenorrhoea who conceived in the 9th cycle after previous unsuccessful treatment with hMG/hCG.

Ovarian overstimulation and multiple pregnancies

The feedback mechanisms of ovarian steroids on the pituitary secretion of the gonadotrophic hormones are operative during pulsatile administration of Gn-RH. Clinical signs of ovarian overstimulation have, therefore, not been observed during 108 experimental and treatment cycles. As shown in Text-fig. 7, however, there is a dose-response relationship between the dose of Gn-RH and the ovarian response, which is mediated by a dose-related pituitary secretion of gonadotrophins. If it is considered that the selection of the dominant follicle and the suppression of the other accompanying follicle is dependent to a certain degree upon gonadotrophic stimulation, it has to be expected that a gonadotrophic stimulation of the ovaries resulting in discrete chemical overstimulation must cause an increased incidence of multiple pregnancies as compared to the normal population. In our study, 3 multiple pregnancies were obtained out of 26 conceptions. One of these multiple pregnancies (Patient A.B., Table 2) was obtained by too high a dose of Gn-RH for the particular grade of hypothalamic amenorrhoea. This patient did not exhibit clinical signs of ovarian overstimulation; she did not even have 'Mittelschmerz'.

Conclusions

Chronic intermittent (pulsatile) administration of Gn-RH by means of a portable pump has proved to be an efficient and practical method for the induction of ovulation as a treatment of infertility in hypothalamic amenorrhoea. The results obtained with this method of treatment are critically dependent upon the correct selection of patients as far as the diagnosis of hypothalamic amenorrhoea is concerned. Patients with hypothalamic amenorrhoea previously treated with human gonadotrophins are suitable for this mode of treatment. In total, 26 conceptions were obtained in 24 patients.

These favourable results are obtained due to a physiological stimulation of the ovaries during chronic intermittent administration of Gn-RH. On the basis of operating negative and positive feedback mechanisms of the ovarian steroids on the pituitary secretion of the gonadotrophins during treatment, the follicle itself regulates the required amount of gonadotrophic stimulation. However, since there is a dose relationship between the Gn-RH dose per pulse applied and the reaction of the pituitary–ovarian axis, the lowest dose of Gn-RH that reliably induces ovulatory cycles should be chosen. The method is well accepted by the highly motivated patient.

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