Induction of multifollicular growth in patients non responding to clomiphene citrate and gonadotropins or gonadotropins only

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Summary: *Objective:* We aimed at inducing multifollicular recruitment in patients with chronic anovulation resistant to Clomiphene Citrate (CC) and/or exogenous gonadotropins. *Design:* At the II Department of Obstetrics and Gynecology we treated our patients with exogenous gonadotropins and concomitant endogenous gonadotropin suppression obtained by the use of a GnRH analog (Buserelin). *Patients:* We studied 14 patients with chronic anovulation due to either hypothalamic dysfunction or polycystic ovarian syndrome. *Measurements:* We monitored the follicular growth ultrasonographically from the eighth day of the menstrual cycle, and assessed E_2 and LH daily dosage from the tenth day. *Results:* With this protocol we obtained 2 or 3 mature follicles, and reached an ovulatory rate of 80.7% and a pregnancy rate of 71.4%. Premature luteinization never occurred, and progesterone increase took place only after human chorionic gonadotropins administration.

Key words: Chronic anovulation; GnRH agonist (Buserelin); Gonadotropins; Ovulation-induction.

INTRODUCTION

Chronic anovulation is quite a common clinical condition. Its most frequent causes can be subdivided into:

– causes due to dysfunction of the hypothalamohypophyseal tract (Hypothalamic Chronic Anovulation) (HCA), (Table 1);

– causes due to anomalies in the feedback system (AN FB), (Table 2).

One of the causes for HCA is psychogenic anovulation. It implies a timelag with a psychologically involving event, though psychic stress cannot be easily determined because of the variability in the receptivity of the individual's psychoemotional unit.

As for the forms linked to malnutrition (loss of weight), a continuum from food reduction (incongruous diet) for aesthetic reasons to anorexia nervosa can be outlined, throughout intermediate stages such as neurotic and anorexic reactions to food intake; psychological aspects play a major role, also in these cases.

The pathophysiology of anovulation from psychic causes and loss of weight is complex and still undefined.

Substantial evidence (Barchas *et al.*, 1978, Delitala *et al.*, 1984, Yen *et al.*,

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Table 1. – *Chronic anovulation due to hypothalamohypopheseal tract dysfunction.*

1) Dysfunctional (Hypothalamic chronic anovulation)
A) Psychogenic
B) Due to malnutrition - Anorexia nervosa - Simple loss of weight
C) Associated with physical activity
D) Hormonal congenital deficiencies - Gonadotropin isolated deficit - Laurence-Biedl syndrome.

Table 2. – *Chronic anovulation due to feed-back system anomalies.*

Polycystic ovarian syndrome
Cushing's syndrome
Addison's syndrome
Adrenal delayed congenital hyperplasia
Associated with androgenous tumors
Associated with thyrohyoid hypo and hyperfunction.

1988) shows a common denominator in a reduction of GnRH release, caused by an increase of opiodergic and dopaminergic levels.

In the forms linked to loss of weight, anovulation occurs when the loss is over 15% of the ideal corporeal weight. Ovulation, in fact, seems to be connected with a minimum of adipose tissue, representing 22% of the total corporeal weight.

Basal and dynamic studies on the gonadotropic activity show different situations, ranging from prepuberal hypothalamic regression (prevailing FSH secretion) (anorexia nervosa) to normogonadotropinemia and oestrogenemia (simple loss of weight).

Chronic anovulation from feed-back system anomalies is the consequence of an anomalous secretion and peripheral steroid metabolism.

Acyclic oestrogenic production and the alteration of androgenous metabolism with a frequent reduction of sexual binding steroid globulins (SHBG) interfere with the delicate counterregulation mechanisms governing the ovulatory cycle over the reproductive age.

The commonest cause of chronic anovulation from ANFB is the polycystic ovarian syndrome (PCOS).

The first choice treatment for these pathologic conditions is Clomiphene Citrate (CC), which induces a high ovulatory rate (70%), resulting in an acceptable pregnancy rate; the best conditions for its use are normogonadotropinemia and normoestrogenemia.

The matter becomes more difficult when no response is given to CC. With gonadotropins (hMG, FSH), the pregnancy rate does not rise over 25-30% (Zimmermann *et al.*, 1982; Thompson and Hansen, 1970), and this percentage is also impugned by a large number of abortions. This depends on exogenous gonadotropin interference with endogenous hypophyseal secretion. Exogenous gonadotropins often induce high quantities of plasma estradiol (E_2) (before full follicular maturation). By a positive feed-back, they cause a premature LH surge with precocious follicular luteinization.

The relation between E_2 increase and the diameter of the dominant follicle is, consequently, suddenly interrupted, and an altered quality of oocytes follows (Zimmerman *et al.*, 1982).

Precocious luteinization occurs when progesterone is 1.5 ng/ml 24 hours before the diameter of the dominant follicle reaches 20 mm (Fleming *et al.*, 1986).

This phenomenon can be observed in 33% (Vargyas *et al.*, 1984), 51% (Fleming *et al.*, 1986) of cycles treated with gonadotropins and it is higher than the rate found in patients treated with Clomiphene Citrate only.

On account of all this, there are reasons for using GnRH agonists in multiple ovulation patterns in patients with chronic anovulation HCA and AN FB.

In this study we have used Buserelin associated with gonadotropins (Gn) (FSH-hCG) for ovulation induction in patients non-responding to CC and Gn or Gn only treatment.

MATERIALS AND METHODS

Fourteen women with a mean sterility period of five years due to chronic anovulation were selected for this study. Ten of them had clinical features and hormonal pattern of PCOS, four of HCA.

Tables 3 and 4 show the main clinical and hormonal characteristics of patients with PCOS.

Table 3. – *Clinical characteristic of PCOS patients.*

Patients	Age	Sterility period years	Menstrual cycle	Previous cycles of treatment No.
1	25	3	ameno	6
2	28	4	ameno	6
3	24	2	ameno	6
4	32	8	oligo	8
5	36	6	oligo	10
6	27	4	ameno	8
7	34	6	oligo	6
8	32	4	oligo	11
9	31	3	ameno	9
10	26	4	ameno	10

They were aged 29.5 on the average, and had been previously treated with a daily 100-150 mg CC dose for five days and/or with individual patterns of gonadotropins for six cycles at least.

The sterility period ranged from 2 to 8 years. Six women were amenorrhoic, the ones left had oligomenorrhoic cycles. All the patients with amenorrhea responded to medroxyprogesterone acetate test (MAP test), showing a satisfactory endogenous estrogen level. Prolactin (PRL) was normal in all cases. Plasma androgen values had increased as well as LH/FSH ratio, which was always >2. Reaction to luteinizing hormone realeasing hormone test (LHRH test) was always considerable.

Table 5 shows the characteristics of HCA patients. They were 27.5 years old on the average; the infertility period ranged from 3 to 5 years; 3 of them were amenorrhoeas, and one was oligimenorrhoeal. Even these amenorrhoeal patients responded to MAP test showing a satisfactory endogenous estrogen level.

Our therapy protocol consisted of Buserelin (Suprefact Hoechst Spray) associated with human gonadotropins (Gn) (Metrodin and Profasi Serono).

The agonist was administered 4 times a day (200 g each time) by inhalation (IN) from the first day of a menstruation which could be either spontaneous or induced with MAP test.

The Gn posology pattern provided for a pure FSH administration (150 IU/die), on the first and second day of the cycle in order to have, with the agonist "flare-up" effect, the follicular recruitment. Successively patients were given 75 IU/die pure FSH for six more days. From the eighth day, an individual pattern in relation

Table 4. – *Hormonal characteristics of PCOS patients.*

Number of patients	PRL ng/ml	A ng/ml	FT ng/ml	FSH mIU/ml	LH mIU/ml	LH after GNRH test
1	10	6	3.8	4	18	64
2	21	4.4	2.5	5	12.6	50.4
3	15	3.5	4	3	9	52
4	9	2.8	4.1	6	16	80
5	14	7.1	3.6	5	20	120
6	18	3	3.4	2	9	51
7	12	6.2	5.6	6	30	105
8	14	7.2	4	4	26	85
9	16	2.9	3.6	5	21	80
10	12	6.5	4.6	3	14	60

Normal hormones values (RIA); PRL<24.8 NG/ML; A<3.1 NG/ML; FT<3.2 PG/ML.

Table 5. – *Clinical characteristic of HCA patients.*

Patients	Age	Sterility period years	Menstrual cycle	Previous cycles of treatment No.
1	28	4	oligo	5
2	25	3	ameno	8
3	31	3	ameno	6
4	26	5	ameno	7

to follicular maturation and plasmatic estradiol was followed.

From the same day follicular growth was followed by means of transabdominal ultrasonography with a 3.5 Mhz convex explorer, while plasmatic estradiol level was daily monitored from the tenth dav.

From the same day also plasmatic LH level was assayed daily every four hours. When the ultrasonographic test showed 2 or more follicles with a diameter ≥16 mm and plasmatic estradiol >300 pg/ml per follicle ≥16 mm, but with a total value ≤2200 pg/ml, according to the ovarian diameter 5,000-10,000 IU hCG were given (O day) and the agonist was interrupted.

In order to sustain the luteal phase, 2,000 IU hCG were given four times every three days, or 100 mg intramuscular (IM)/die of natural progesterone for 12 days. Even in this case our choice was made following the stage of ovarian stimulation ultrasonographically determined on the O day.

RESULTS

Tables 6 and 7 summarize the characteristics of the cycles of patients with PCOS and HCA treated with Buserelin+Gn.

Twenty-six treatment cycles altogether were carried out: 13 for the PCOS and 8 for the HCA patients respectively.

Table 6. – *Cycles' characteristics in PCOS patients treated with Buserlin+GN.*

Number of patients	Cycle	Ampoules of Gn	Days of Gn treatment	Number of follicles 16 mm	E_2 HCG	Pregnancy
1	1° cycle	15	12	2	280	–
	2° cycle	13	14	2	650	Successful pregnancy
2	1° cycle	20	15	2	900	–
	2° cycle	16	13	3	950	–
3	1° cycle	22	15	4	1900	Successful pregnancy
4	1° cycle	14	12	1	280	–
	2° cycle	18	14	2	635	Successful pregnancy
5	1° cycle	22	18	–	220	–
6	1° cycle	24	16	3	1450	Bigeminal pregnancy Abortion at 16 weeks
7	1° cycle	20	14	5	1980	–
	2° cycle	18	14	3	1350	Successful pregnancy
8	1° cycle	20	16	2	910	–
	2° cycle	22	16	4	1210	Successful pregnancy
9	1° cycle	16	13	–	200	–
	2° cycle	22	15	1	210	–
	3° cycle	30	18	–	180	–
10	1° cycle	22	11	2	760	–
	2° cycle	20	13	3	220	Abortion at 8 weeks

Table 7. – *Cycles' characteristics of HCA patients treated with Buserelin+GN.*

Number of patients	Cycle	Ampoules of Gn	Days of Gn treatment	Number of follicles 16 mm	E_2 HCG	Pregnancy
1	1° cycle	15	13	2	1000	–
	2° cycle	20	15	3	1400	Successful pregnancy
2	1° cycle	21	13	2	720	Abortion at 6 weeks
	2° cycle	28	18	2	840	–
3	1° cycle	12	11	2	1650	–
	2° cycle	20	13	4	2000	Bigeminal pregnancy
4	1° cycle	20	15	–	210	–
	2° cycle	28	19	–	89	–

The mean number of Gn ampoules used for each cycle was 20. There was a wide range in the number of Gn ampoules used for the ovulation induction (12-30), even for different cycles of the same patient. In five cases no ovulation was inducted. The ovulatory rate was 80.7% while the pregnancy rate was 71.4 with an outcome of 10 pregnancies.

No statistically significant difference was observed in the parameters taken for PCOS and HCA patients.

We had 8 cases of mild ovarian hyperstimulation and one of moderate hyperstimulation among the PCOS patients. We never observed premature luteinization of the follicle.

DISCUSSION

If no response is given to CC, GnRH pulsatile pump and Gn are also disappointing.

This might depend on the interference either between exogenous pulsatile GnRH and endogenous GnRH pulses or between endogenous and exogenous gonadotropins.

Furthermore, the use of the drugs mentioned above (CC, GnRH pulsatile, Gn) is likely to induce a high (51%) premature LH surge rate, with following precocious luteinization and low quality of the oocytes.

In administering 800 μg/die of Buserelin, we did not observe any premature LH surge. On the other hand, we had previously noticed that 600 μg/die still alowed some premature LH surges.

GnRH agonists, after a starting flare-up, suppress endogenous LH, FSH estradiol, and ovarian androgen secretions and cause hypogonadotropic hypogonadism, favourable to exogenous gonadotrapins which do not interfere with endogenous gonadotropins (Fleming *et al.*, 1987; Fleming *et al.*, 1988).

These characteristics improve the ovulatory and pregnancy rates in patients with chronic anovulation and conferm our satisfactory results (ovulatory rate 80.7%; pregnancy rate 71.4%).

Our choice for short stimulation cycles depends on the fact that they are better tolerated by patients. They require less effort compared with common stimulation patterns, where deposit from agonists are provided for.

It must be also added that in our cases, not intended for fertilization in vitro embryo transfer (Fivet) massive multiple ovulation was not as necessary as high quality oocytes.

This also explains the relative low quantities of Gn used and the relative low rate of ovarian hyperstimulation.

GnRH agonists can thus be considered a good alternative in ovulation induction in patients who have given no response to traditional theraphies; they can also provide the first choice treatment for some selected patients.

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