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Influence of gonadotrophin-releasing hormone agonist total dose in the ovarian stimulation in the long down-regulation protocol for in-vitro fertilization

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The goal of our study was to assess whether the total dose of gonadotrophin-releasing hormone agonist (GnRHa) administered affects the success of an in-vitro fertilization (IVF) programme. A retrospective analysis was performed on a total of 72 IVF cycles carried out on 70 patients with different causes of infertility included in our assisted reproduction programme. Cycles were divided into two groups according to the number of days of GnRHa administration (leuprolide acetate 1 mg/day) necessary until ovarian desensitization occurred: group I (GI) <13 days (n = 27) and group II (GII) ≥13 days (n = 45). The following parameters were assessed: number of gonadotrophin ampoules, number of stimulation days, endometrial thickness on the day of human chorionic gonadotrophin (HCG) administration, number of recovered oocytes, pregnancy rate. Pregnancy rate/cycle and pregnancy rate/transfer were positively correlated with the dose of GnRHa (GI: 44 and 60% respectively versus GII: 20% and 25% respectively). It is concluded that a long administration of GnRHa has no effect upon ovarian response, although the pregnancy rate is subsequently decreased.

Key words: agonists/long down-regulation/total dose

Introduction

Ovarian follicles increase in size under the influence of gonadotrophins, mainly due to an expansion of follicular fluid (FF) volume and an acceleration in granulosa cell mitosis. In a natural cycle, granulosa cells forming the cumulus and corona layers, and the oocyte that they surround, undergo synchronous maturational changes. This synchrony may be disturbed in a gonadotrophin-induced cycle in which interference from endogenous luteinizing hormone (LH) may also cause premature luteinization (Thanki and Schmidt, 1990).

In the treatment of infertility, the transient suppression of pituitary function can improve the efficacy of gonadotrophin therapy. The impact of gonadotrophin releasing hormone (GnRH) on the clinical management of infertility and reproductive endocrinology has been widely reported. Clinical application of GnRH and its analogues falls into two broad categories: those dependent upon inhibitory effects on gonadotrophin secretion and those dependent upon stimulatory effects of GnRH on gonadotrophin secretion (Gordon *et al.*, 1993).

Gonadotrophin-releasing hormone agonists (GnRHa) are now used in conjunction with exogenous gonadotrophins as an integral part of most ovulation induction protocols for various forms of assisted reproductive technologies (ART). The principal objective of their use is to reduce the incidence of premature LH surges (and hence reduce the cancellation rate) and, by producing a hypogonadotrophic state, to enable the timing of follicular development to be controlled more precisely, thereby facilitating scheduling of patients for oocyte collection (Gordon *et al.*, 1993).

Current clinical practice combines GnRHa treatment with gonadotrophin therapy. Two stimulation protocols have been used by different groups: the 'short protocol' combines endogenous follicle stimulating hormone (FSH)/LH from the initial flare effect with exogenous gonadotrophin. The GnRHa treatment usually begins on menstrual cycle day 1 or 2. The 'long protocol' is often begun in the antecedent mid-luteal phase (day 19–23) to minimize the flare effect, controlled mainly by progesterone, oestrogen, and possibly inhibin, from the functioning corpus luteum (Hodgen, 1990).

The majority of investigators agree over the benefits of using agonists for ART. However, few IVF groups have assessed whether the ovarian response to exogenous gonadotrophins, cycle performance and pregnancy rate are affected by different periods of agonist administration in long down-regulation protocols. In this paper we have attempted to answer this question.

Materials and methods

Between January and December 1995, a retrospective study was performed of 72 cycles in 70 patients who were undergoing *in vitro* fertilization (IVF) treatment for infertility of diverse aetiology. All patients were scheduled for IVF–embryo transfer using the long protocol.

To achieve multiple follicular development we used the following procedure. Ovarian desensitization was induced by GnRHa (leuprolide acetate 1 mg/day) in the luteal phase of the preceding cycle. Ovarian quiescence was verified during the first days of the cycle, by the absence of follicular growth observed by transvaginal ultrasound, and oestradiol serum concentration <50 pg/ml. GnRHa administration ended on the day of human chorionic gonadotrophin (HCG) injection and ovarian stimulation was started with daily doses of pure FSH (Neo-Fertinorm; Serono, Madrid, Spain) or FSH combined with human menopausal gonadotrophin (HMG; Pergonal; Serono). On day 6 of ovarian stimulation the dose was modified according to the ovarian response observed. The criterion for administering 5000 IU human chorionic gonadotrophin (HCG; 2500 IU, Profasi; Serono) was the presence of three or more follicles of ≥17 mm in diameter. Oocyte retrieval was performed 36 h after HCG administration by transvaginal puncture under ultrasound guidance. These oocytes

were washed with buffer medium (Flushing medium; Medi-Cult, Copenhagen, Denmark) and classified. All preovulatory oocytes were preincubated in culture medium (IVF medium; Medi-Cult) for 4–6 h prior to insemination in an atmosphere of 5% CO_2 in air at 37°C. Sperm concentration and motility were evaluated in a husband's semen sample. Simple preparative methods such as swim up and discontinuous Percoll gradients produced satisfactory sperm samples. Spermatozoa were inseminated in droplets at a concentration of 100 000/ml with normozoospermic samples, a higher concentration being used with samples from asthenozoospermic and teratozoospermic patients. Inseminated oocytes were cultured overnight (18–20 h) and examined for two pronuclei and two polar bodies. On the second day post-insemination, those embryos which had divided to two or more cells were classified (type 1, 2, 3, 4) and transferred (three or four embryos/patient) into the uterine cavity. Embryos that were not used in a fresh transfer were cryopreserved. The luteal phase was supported with 600 mg/day of progesterone (Utrogestan 100 mg; Laboratoires Besins-Iscovesco, Paris, France). Twelve or 13 days after embryo transfer, the serum β-HCG concentrations were determined, a value of >5 mIU/ml being considered to indicate a positive pregnancy. Pregnancy was confirmed by ultrasound 2 weeks later.

Data are presented as means ± SD and were normally distributed. Student's *t*-test was used to discriminate between the two groups. χ^2 test was used for frequency comparisons. Values of $P \leq 0.05$ were considered significant. All statistical evaluations were performed using the Statistical Package for Social Sciences (SPSS).

Results

Between January and December 1995, 72 cycles were studied in 70 patients who were undergoing IVF treatment. Cycles were divided into two groups according to the number of days of GnRHa administration necessary until ovarian desensitization. The median value of 13 days was chosen as the cut-off point: in group I (27 cycles), the down-regulation period before controlled ovarian hyperstimulation (COH) was <13 days, and in group II (45 cycles) the period was ⩾13 days.

The following parameters were determined: number of gonadotrophin ampoules, number of stimulation days, endometrial thickness on the day of HCG administration, the mean number of oocytes recovered and pregnancy rate.

Patients in the two groups were similarly distributed in age (32.9 ± 2.0 years versus 32.8 ± 2.8 years) and cause of infertility (Table I). Two patients (4.4 %) had ovarian cysts in group II. The number of cycles cancelled because of poor ovarian response was similar between the two groups (3.7 versus 4.4%) (Table I).

The amount of exogenous gonadotrophins required, length of the stimulation period, endometrial thickness on the day of HCG administration and the mean number of oocytes collected were very similar in the two groups (Table II). No statistically significant differences were found.

There was a relationship between the number of days of GnRHa administration and the clinical pregnancy rate. The pregnancy rate by transfer was significantly lower in the patients group suppressed for a longer period (group II) ($P < 0.05$; see Table II).

Table I. Distribution of patient characteristics and cancelled cycles

	GI	GII
Cycle number	27	45
Cancelled cycles (%)	1 (3.7)	2 (4.4)
Age (years; mean ± SD)	32.9 ± 2.0	32.8 ± 2.8
Causes of infertility		
Tubal (%)	33.3	33.3
Male (%)	22.2	24.4
Unexplained (%)	25.9	22.3
Endometriosis (%)	3.7	4.4
Mixed (%)	14.9	15.6

Discussion

The last few years have seen the increasing use of GnRHa during controlled ovarian hyperstimulation for ART. Today the majority of investigators agree over the benefits of using agonists, the main medical advantage consisting of the reduction of the rates of cancellation due to poor responses or to premature luteinization (Hughes *et al.*, 1992; Calhaz Jorge *et al.*, 1995; Senoz *et al.*, 1995; Devreker *et al.*, 1996; Ferrareti *et al.*, 1996). Other reports have indicated that GnRHa protocols might also establish a good uterine environment, although flare-up regimens have been associated with poor rates of fertilization and low embryo quality. Many authors advocate the use of long GnRHa protocols to obtain a complete down-regulation of pituitary gland, to establish basal conditions in the ovary, and to facilitate the programming of cycles (Edwards and Brody, 1995).

It is well known that desensitization protocols require administration of an agonist for several days to achieve consistent suppression of ovarian activity. In our study, we demonstrated that higher doses of GnRHa (the degree of pituitary suppression) do not affect the response to stimulation.

Table II. Ovarian stimulation results and pregnancy rate

	Ampoules of gonadotrophin	Days stimulation	Endometrial thickness	Oocyte recovery (%)	Pregnancy rate/cycle (%)	Pregnancy rate/transfer
GI $n = 27$	36.0 ± 9.6	9.0 ± 1.9	11.2 ± 1.9	13.5 ± 7.1	44*	60*
GII $n = 45$	38.0±7.8	10.0 ±1.6	11.4 ± 2.7	11.8 ± 5.0	20*	25*

Results are expressed as mean ± SEM.
n = cycle number.
*Statistically significant differences were found among the groups ($P < 0.05$).

When the number of days of agonist administration was increased, despite inhibition of endogenous pituitary gonadotrophin secretion by GnRHa, the number of stimulation days and the number of gonadotrophin ampoules did not differ from those of patients with fewer administration days (Neven *et al.*, 1987). Similar amounts of exogenous gonadotrophins and similar duration of treatment yielded the same number of oocytes recovered from both groups, in agreement with results that have been described previously in more detail (Calhaz Jorge *et al.*, 1995; Senoz *et al.*, 1995; Ferrareti *et al.*, 1996). Olivennes *et al.* (1996) observed a better ovarian response and higher quality embryos in patients with a high basal FSH concentration on day 3 when they were treated with a low dose of GnRHa. In our study, it is possible that patients who needed a lower dose of GnRHa to produce suppression of ovarian activity had a high basal FSH concentration. However, this cannot be confirmed because we lack these data. On the other hand, in our IVF programme, basal FSH concentrations were obtained in patients with cycles previously cancelled due to poor response. In this group of patients, ovarian stimulation was achieved using the 'short protocol'.

Other reports have shown decreased number and quality of human oocytes and embryos obtained after exaggerated follicular stimulation using high doses of GnRHa (Testart *et al.*, 1986; Balasch *et al.*, 1993). In addition, expression of GnRH receptor messenger RNA in human ovaries has been demonstrated, providing evidence that the ovary may be a target for extrapituitary GnRH action (Minaretzis *et al.*, 1995). Moreover, it has been proved (Devreker *et al.*, 1996) that the analogue is able to diffuse into FF. With long-acting GnRHa, larger numbers of molecules persist in the blood circulation far beyond the injection of HCG and oocyte retrieval. The presence of GnRHa in the FF during oocyte maturation may interfere with embryo metabolism, impairing the potential of the future embryo to implant and giving rise to abnormalities that do not manifest themselves morphologically (Devreker *et al.*, 1996).

The comparison between IVF–embryo transfer cycles is complicated by differences between stimulation treatments. Different protocols have been designed for the administration of GnRH together with gonadotrophins for ovarian stimulation (Loumaye, 1990). One parameter subject to variation is the duration of GnRHa administration before starting the ovarian stimulation with gonadotrophins. Ultrashort, short and long protocols have been tested and overall results in the literature indicate that the long GnRHa protocol is associated with a higher pregnancy rate (Loumaye, 1990; Tan *et al.*, 1992). A second parameter subject to variation is the time of initiation of GnRHa therapy. The timing of GnRHa administration during the menstrual cycle may influence the time course of ovarian suppression and the IVF outcome (Ferrareti *et al.*, 1996). Moreover, different patterns of follicular growth were observed in humans according to the use of different GnRH molecules (buserelin, triptorelin or leuprorelin) (Testart *et al.*, 1993). These variations may explain discrepancies between our findings and those reported previously concerning the success of IVF. Clinical studies do not permit the identification of a direct effect of GnRHa either on the oocyte or on the uterus, since such effects are cumulative in the results of IVF–embryo transfer. Only research studies could demonstrate specific effects of GnRHa molecules on ovaries, oocytes and embryo implantation.

On the other hand, the endometrial thickness on the day of HCG administration was very similar in both groups, despite the remarkably higher pregnancy rate in group I.

Although we observed a similar number of cancelled cycles in both groups, our sample size is too small to demonstrate a significant difference between the two groups.

The present results show that a longer period of GnRHa administration to achieve ovarian desensitization has no effect on the ovarian response to gonadotrophin stimulation, although it leads to a decrease the clinical pregnancy rate (Neyro *et al.*, 1994). This may provide further information on the effect of long-acting GnRHa on uterine receptivity.

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