Ovarian stimulation for assisted reproduction with HMG and concomitant midcycle administration of the GnRH antagonist Cetrorelix according to the multiple dose protocol: a prospective uncontrolled phase III study

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A total of 346 women with normal ovulatory function were stimulated with human menopausal gonadotrophins (HMG) to attain ovarian stimulation for IVF or intracytoplasmic sperm injection (ICSI). Stimulation with HMG started on cycle day 2 or 3. After 6 days of stimulation, Cetrorelix in its minimum effective multiple dose of 0.25 mg/day, was administered daily until induction of ovulation. In total, 333 patients (96.2%) reached the day of HCG administration, and 324 (93.6%) underwent oocyte retrieval. A mean of 25.2 ampoules of HMG was applied for a mean of 10.4 days. Cetrorelix was administered for a mean time lapse of 5.7 days. The mean normal fertilization rate was 60% in the IVF group and 59% in the ICSI group. Seventy pregnancies were attained, reflecting an ongoing clinical pregnancy rate of 24% per transfer. The ongoing clinical implantation rate was 11.4%. Only three cases of raised luteinizing hormone (LH) (≥10 IU/l) with increased progesterone secretion (≥1 ng/ml) were observed after initiation of Cetrorelix administration, reflecting an incidence of premature luteinization of 0.9%. The abortion rate was 17%. The incidence of severe ovarian hyperstimulation syndrome (World Health Organization grade III) was as low as 0.6%.

Key words: Cetrorelix/gonadotrophin-releasing hormone antagonists/human menopausal gonadotrophins/ovarian stimulation

Introduction

One of the main causes for a relatively low efficacy of ovarian stimulation with human menopausal gonadotrophins (HMG) only was the onset of premature luteinization in about 15–20% of treatment cycles. The incidence of this event, with its negative impact on oocyte and embryo quality as well as on the pregnancy rates attained, could be lowered to below 2% by the introduction of gonadotrophin-releasing hormone (GnRH) agonists into stimulation protocols for assisted reproduction techniques (Porter et al., 1984; Stanger and Yovich, 1985; Wildt et al., 1986). The long agonistic protocol, which aims to achieve complete desensitization of GnRH receptors before the start of HMG administration, has become the ‘gold standard’ for ovarian stimulation in assisted reproduction techniques (Biljan and Tan, 1998). Recently, the GnRH antagonist Cetrorelix (ASTA-Medica AG, Frankfurt/Main, Germany) has been investigated for ovarian stimulation in assisted reproduction techniques. Cetrorelix has proved to be reliable in preventing the onset of premature luteinization hormone (LH) surges during HMG stimulation for assisted reproduction techniques, as well as by daily injections from 5 or 6 days of stimulation onwards as by a single injection on stimulation day 7 (Diedrich et al., 1994; Olivennes et al., 1994, 1995; Felberbaum et al., 1995, 1996; Albano et al., 1996). Pregnancy rates of about 30% per transfer have been described (Albano et al., 1997). However, reports regarding the possibility of lowering the amount of HMG ampoules to obtain satisfactory follicular maturation by using GnRH antagonists instead of a desensitizing protocol have been contradictory (Albano et al., 1996; Felberbaum et al., 1996). No data regarding the incidence of serious ovarian hyperstimulation syndrome (OHSS) under these treatment modalities have been available until now. Overall, there was clearly a need to obtain more data regarding efficacy and safety aspects of this new therapeutic approach to ovarian stimulation for assisted reproduction techniques. This report details a prospective uncontrolled study including 346 patients treated with Cetrorelix in its minimum effective dose of 0.25 mg/day, according to the multiple dose administration protocol.

Materials and methods

After having obtained approval from the ethical boards of all medical centres involved in the study, 346 healthy female partners were treated in this open-label, multicentre, multinational phase III study with Cetrorelix in its minimum effective multiple dose of 0.25 mg/day and HMG according to the multiple dose protocol, which has been described extensively before (Diedrich et al., 1994). Fourteen centres in eight European countries—forming the European Cetrorelix Study Group—participated in this study.
Study Group—participated in this study, as outlined in Table I. The infertility could be treated by ovarian stimulation for IVF or intracytoplasmic sperm injection (ICSI), as shown in Table II. The women had to be between 18 and 39 years of age, with normal menstrual cycles of range 24–35 days, and an intra-individual variation of ±3 days. The mean age of the patients was 32 ± 4 years, and the mean body weight 62 ± 10 kg. No more than three IVF procedures were to have taken place in the past. A normal uterus and at least one functioning ovary were basic requirements.

All patients started treatment with two ampoules of HMG and remained on that dose for 5 days. Thereafter, the stimulation procedure with respect to the amount of HMG administered per day was handled individually. If sterilized, reversed sterilization was considered a minor protocol exclusion. In case, of premature LH rise (LH ≥10 IU/l and progesterone ≥1 ng/ml) before HMG day 6, Cetrorelix was administered immediately in order to interrupt a possible spontaneous ovulation and therefore to rescue the cycle. Each Cetrorelix injection was given after a blood sample had been taken for hormone assessments [follicle stimulating hormone (FSH), LH, oestradiol and progesterone] at intervals of 24 ± 2 h. In cases of cycle cancellation before HCG administration due to multifollicular development (≥12 follicles >15 mm in diameter) and/or oestradiol concentrations >4000 pg/ml, the Cetrorelix administration was continued for at least one week. This procedure was followed in order to prevent spontaneous ovulation accompanied by the release of multiple oocytes, the increased risk of development of OHSS, and multiple pregnancies.

Since the investigation was designed as an open study, all patients were treated in accordance with the same schedule.

To proceed with the administration of 10 000 IU of HCG (Predalon®, Serono, Geneva, Switzerland/Choragon®, Ferring GmbH), the following prerequisites had to be fulfilled: at least one follicle with a diameter of ≥20 mm or an oestradiol concentration ≥1200 pg/ml.

Luteal phase support was given according to the centre’s rules regarding start, duration, medication and dosage. The protocol allowed two medications: either injections of HCG according to the centre’s rules (though not in the case of oestradiol concentration ≥2000 pg/ml) or vaginal administration of progesterone (e.g. 600 mg per day for 14 days).

Concentrations of LH, FSH, oestradiol and progesterone were measured regularly on the day of screening, HMG day 1, Cetrorelix day 1, and during the Cetrorelix administration on each morning and on the evening of ovulation induction before HCG administration. Furthermore, hormone concentrations were measured at the day of oocyte retrieval, day of embryo transfer, and 6–8 days after embryo transfer. All analyses were performed locally at each centre’s laboratory. However, to enable an additional analysis of these hormones in a central laboratory, a second serum sample was taken at the same time as the morning blood collection. The second serum samples were immediately frozen and stored at −20°C until being analysed at the clinical chemistry laboratory of ASTA-Medica AG.

**Statistical methods**

All statistical evaluations and analyses were performed using SAS™ 6.09 (SAS Institute Inc., Cary, NC, USA). The primary aim of the present study was to estimate the response rate defined as successful ovarian stimulation, the number of patients reaching the day of HCG, the rate of patients with oocyte retrieval, embryo transfers and pregnancies, as well as to assess serious adverse events. As a response rate of at least 95% was to be expected in order to obtain lower one-sided 95% confidence limits (CL) of 92.5%, a sample size of 319 patients was required. Assuming a rate of about 6% for non-evaluable patients, the inclusion of 340 patients seemed to be appropriate. A sample size of 340 patients and a pregnancy rate of about 20% would lead to about 65 babies being delivered. For this reason at least one adverse experience would have been observed with 95% probability given an assumed incidence of ≥4.5%. One-sided 95% lower CL were calculated according to Clopper–Pearson for the efficacy parameters.

**Results**

After having obtained fully informed consent, 352 patients were screened for enrolment into the study and 346 patients started HMG stimulation and concomitant Cetrorelix treatment. Six patients were screened, but received neither HMG nor
Cetrorelix. Four patients out of these six did not fulfil the criteria set for the start with HMG, namely because their FSH concentration was >10 mIU/ml or progesterone concentration >1 ng/ml. One patient showed a Papanicolaou (PAP) IV result at screening, which was defined as an exclusion criterion. One patient withdrew due to spontaneous pregnancy after screening. All of the remaining 346 patients were evaluated for efficacy and safety criteria, as well as with respect to compliance.

As expected, the HMG stimulation had an important impact on hormone concentrations. FSH rose continuously to a mean concentration of 13.8 mIU/ml (day of HCG administration) as long as the HMG treatment continued. Concentrations of oestradiol increased to a mean of 1544 pg/ml (morning value; central laboratory) on the day of HCG injection.

LH concentrations were clearly suppressed during the entire stimulation period (Figure 1). The median evening value on the day of HCG injection was 1.0 IU/l (evening value, central laboratory).

The median duration of Cetrorelix treatment was 5 days (mean 5.7 days; range 1–15 days). Among 346 cycles, none had to be cancelled because of allergic, anaphylactoid, local hypersensitivity or other adverse reactions of the patient.

A mean of 25.2 ampoules (median 23 ampoules) of HMG were given for a mean of 10.4 days. HMG was administered in all patients for a minimum of 6 and up to a maximum of 19 days.

**Primary parameters of efficacy**

Among 346 patients treated according to the described treatment modalities, 333 fulfilled the criteria for administration of HCG to induce final oocyte maturation. This reflected a 96.2% efficacy rate in respect of the primary parameter of success with a lower 95% CL of 94.1%.

Two of the patients without HCG administration showed a rise in LH, one on HMG day 7 and one on HMG day 13. In the case of the first patient, no central laboratory analysis was available. However, this patient was considered to be one of the major protocol violators, as the FSH concentration at HMG day 1 was >12 mIU/ml, and HMG started on day 4 of the cycle instead of day 2 or 3. Finally, the cycle was cancelled in this case as the woman refused to continue ovarian stimulation. In the case of the second patient, the response to ovarian stimulation was very poor, and although progesterone did not rise in LH, one on HMG day 7 and one on HMG day 13. In

**Premature rises of LH**

The incidence of premature LH rises above 10 IU/l during stimulation, and premature luteinization of the follicles as indicated by progesterone values above 1 ng/ml, were analysed once by the results from the local laboratory, and once by the results from the central laboratory (Tables III and IV).

Generally, the central laboratory provided slightly lower LH values. Including HMG day 6 for analysis led to an incidence of LH rises as defined by the study protocol (LH >10 IU/l and progesterone >1 ng/ml) of 3.5% with 95% CL of 5.6% according to local laboratory values, and 1.8% (95% CL = 3.5%) according to central laboratory data. This incidence
dropped after having started Cetrorelix administration on HMG day 6 to as low as 2.9% (95% CI = 4.9%) according to local laboratory values and 0.9% (95% CI = 2.3%) according to central laboratory data.

HCG was administered in 12 out of 13 cases with elevations of LH above 10 IU/l without rising concentrations of progesterone. Oocyte retrieval and subsequent fertilization were performed as planned in eight of these patients; cumulus–oocyte complexes were obtained in seven of these cases. The fertilization led to embryos of good to excellent quality in six of the eight cases, and to embryos of fair quality in two cases. The treatment cycle of one of these patients led, after replacement of two embryos of excellent quality, to a viable ongoing clinical pregnancy. On HMG day 6, the frequency of patients with follicles of a given size was distributed as follows: 80% of patients had follicles of 11–14 mm, 33% of 15–19 mm, and 3% large follicles (≥20 mm). This turned on the day of HCG injection when 72% of patients had follicles of 11–14 mm, 96% of 15–19 mm, and 89% had large follicles (≥20 mm). On the day of HCG administration, 10.1 follicles were seen on average, and the mean number of follicles with a diameter ≥20 mm was 2.4.

**Fertilization rate, quality of embryos and pregnancy rate**
Among a total of 324 patients with oocyte retrieval, 2971 cumulus–oocyte complexes were obtained. No oocyte was retrieved from two patients. The mean percentage of mature oocytes, as assessed according to microscopic criteria was 63.7% for patients submitted to IVF, while the mean percentage of metaphase II oocytes for ICSI patients was 75.1% of the oocytes retrieved (Staessen et al., 1989; Van Steirteghem et al., 1993). The fertilization rate, defined as the number of fertilized (two pronuclear, 2PN) oocytes per inseminated (IVF) or injected (ICSI) metaphase II oocyte, was nearly the same for both methods of fertilization, which means 60% for IVF and 59% for ICSI per cumulus–oocyte complex. In total, 149 patients were submitted to the IVF procedure, while metaphase II oocytes of 162 patients were the subject of the microinjection method, and 11 patients were submitted to both procedures (Table V).

A total of 2971 cumulus–oocyte complexes was retrieved, and these led to 1253 embryos; 34% of these embryos were of excellent quality according to morphological criteria, 46% were of good quality, and 19% were of fair quality (Staessen et al., 1989). The replacement of a mean of 2.7 embryos per transfer in 299 transfers induced 70 clinical pregnancies, as assessed by transvaginal sonography documenting fetal heart activity. Thus, the clinical pregnancy rate was 24% per transfer. The ongoing clinical implantation rate was 11.4 ± 22.6% when calculated by the number of embryos seen by transvaginal sonography on days 21–24 after embryo transfer divided by the total number of embryos replaced. This ongoing clinical implantation rate may reflect incomplete data, as the number of gestational sacs in cases of early pregnancy loss or ectopic pregnancies remains unknown. Twelve abortions were observed (abortion rate 17%).

**Adverse drug reaction**
Injection site reactions were seen in only three patients (0.9%), while one case of hot flushes was reported and attributed to the use of the GnRH antagonist Cetrorelix. None of these events led to premature discontinuation of the study medication.
**Severe OHSS (grade III)**

Only two cases of severe OHSS World Health Organization (WHO) grade III were reported, reflecting an incidence of 0.6% (2/346).

**Discussion**

Although the clinical development of early generation GnRH antagonists was hampered by their ability to initiate systemic allergic as well as severe local hypersensitivity reactions due to mast cell degranulation and histamine release, these problems have been completely resolved by the introduction of GnRH antagonists such as Cetrorelix (Hahn et al., 1985; Reissmann et al., 1995; Schally and Comaru-Schally, 1997). On the basis of this extremely important observation, it has been possible to introduce Cetrorelix successfully into protocols for ovarian stimulation (Diedrich et al., 1994; Olivennes et al., 1995; Felberbaum et al., 1996; Albano et al., 1997). Daily midcycle administrations with concomitant HMG stimulation and single dose administration at day 7 of HMG stimulation were each able to reliably prevent the onset of premature LH surges. Subsequent phase II dose-finding studies defined 0.25 mg of Cetrorelix per day as the minimum effective dose for the prevention of premature ovulation induced by premature LH surges, according to the multiple dose protocol (Albano et al., 1996, 1997; Felberbaum et al., 1996). Using only 0.1 mg of Cetrorelix per day according to this administration scheme did not prevent the onset of premature rises of LH (Albano et al., 1997). However, to date the number of treated patients using 0.25 mg of Cetrorelix has been very few, and consequently experience with this new compound is limited. For this reason, a large phase III multicentre trial was initiated. This study evaluated the safety and efficacy of Cetrorelix at its minimum effective dose of 0.25 mg/day according to the multiple-dose protocol, and was the first to do so for a GnRH antagonist in ovarian stimulation. In particular, no such database on daily hormonal measurements during stimulation therapy has been reported to date. Patient compliance was excellent, and no patient had to be excluded due to allergic or hypersensitivity reactions, thus demonstrating that clinically relevant histamine release by this compound was avoided. In total, 333 patients showed satisfactory follicular development leading to administration of HCG for final oocyte maturation. In 324 patients, oocyte retrieval was undertaken. After having started Cetrorelix treatment, the incidence of premature LH rises followed by premature luteinization of the growing follicles was 0.9%. This was within the range to be expected after GnRH agonist administration for assisted reproduction techniques, according to the so-called ‘long’ protocol (Smits et al., 1992). By using Cetrorelix, follicular distribution at the day of HCG administration reflected a fairly homogeneous follicular development with only few small and intermediate follicles. It has been documented that the presence of several intermediate and small follicles at the day of HCG administration may increase the risk of OHSS (Ron El et al., 1991). A mean of 10.2 follicles on the day of HCG treatment seems to be almost favourable, notably in view of the need for softer ovarian stimulation procedure protocols (Edwards et al., 1996). A median of 23 ampoules of HMG used per stimulation cycle may indicate the possibility of reducing the amount of gonadotrophins in comparison to the long protocol (MacLachlan et al., 1989). Other authors have reported reduced amounts of gonadotrophins of about 28 ampoules per cycle using recombinant FSH (Out et al., 1996, 1997). The results of a large series is awaited to show whether a combination of GnRH antagonists with recombinant FSH will lead to a further reduction in gonadotrophins to be used for ovarian stimulation.

The number and quality of recovered oocytes, the percentage of metaphase II oocytes to be used for ICSI, and fertilization rates after ICSI or IVF must be considered absolutely normal as expected after normal oocyte maturation for assisted reproduction techniques (Küpper et al., 1995; Ubaldi et al., 1995). A clinical pregnancy rate of 24% per transfer is an acceptable result, and within the estimated limits of an open non-comparative study. These results are even higher than that reported in available European national IVF registers (French IVF Registry, 1995; Deech, 1996; Deutsches IVF-Register, 1997). However, this clinical pregnancy rate of 24% may be influenced by the fact that the population of patients recruited for this study excluded difficult cases such as patients suffering from polycystic ovarian disease, known low responders, or women aged more than 39 years.

It is reassuring to observe that, when using Cetrorelix, the percentage of babies born per embryo replaced was 10%.

An incidence of 0.6% of severe OHSS (WHO grade III) leading to hospitalization of the patient is low in comparison with the incidence (6.6%) reported after long-protocol agonist treatment (Ron-El et al., 1991). Taking into account all the complications and risks that severe OHSS implies for the patient (such as thrombosis, embolism, putative myocardial infarction and even occasional death), this suggests a major improvement in safety aspects. It is possible that modifications of the stimulation procedures, incorporating softer agents such as clomiphene citrate, may allow this severe, purely iatrogenic disease within assisted reproduction treatment to be abolished (Diedrich and Felberbaum, 1998).

As yet, it may be too early to predict an end of the agonist era for ovarian stimulation, and these agents will likely retain their importance in special indications such as endometriosis patients in whom assisted reproduction treatment is envisaged. However, if confirmation is obtained in large comparative studies, there may be clear advantages of antagonist co-treatment with gonadotrophins in ovarian stimulation with regard to the patient’s comfort, and reductions in treatment duration, medication and costs, while achieving the same range of therapeutic outcome.

In summary, this study has demonstrated the efficacy and safety of Cetrorelix in ovarian stimulation with HMG for IVF, with a pregnancy rate of 24% per embryo transfer being satisfactory. The incidence of severe OHSS grade III is as low as 0.6% indicated that Cetrorelix makes an important contribution to the safety of the treatment.

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