A randomized, assessor-blind, group-comparative efficacy study to compare the effects of Normegon® and Metrodin® in infertile female patients undergoing in-vitro fertilization


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Introduction

The currently available human gonadotrophin preparations are natural hormones derived from urine of menopausal women. The standard human menopausal gonadotrophin (HMG) preparations contain follicle stimulating hormone (FSH) and luteinizing hormone (LH) bioactivity, standardized with a small amount of human chorionic gonadotrophin (HCG) in order to obtain the required FSH/LH ratio of approximately 1:1 (Stokman et al., 1993). These preparations are intended to induce ovarian stimulation in assisted procreation. It has been suggested that too high concentrations of LH during the follicular phase may have deleterious effects on reproductive processes, resulting in premature luteinization and lower rates of fertilization, cleavage and implantation as well as a higher risk of abortion (Stanger and Yovich, 1985; Chappel and Howles, 1991). It has therefore been suggested that HMG preparations with a higher FSH:LH ratio or purified FSH might be advantageous in clinical conditions in which normal or high concentrations of LH occur. Moreover, it has been claimed that a higher FSH:LH bioactivity ratio may lead to improved success rates in patients undergoing in-vitro fertilization (IVF) (Bernardus et al., 1985; Muasher et al., 1985; Jones, 1987a,b). Because of the above-mentioned arguments, an FSH-dominant HMG preparation, i.e. Normegon®, with an FSH:LH bioactivity ratio of 3:1, has been developed. The current study was performed to compare the efficacy of Normegon® with that of Metrodin® in infertile women undergoing IVF and embryo transfer.

Materials and methods

Patients

From January 1990 until October 1992, 158 infertile female patients were recruited and treated at the Centre for Reproductive Medicine, University Hospital, Dutch-speaking Brussels Free University, Belgium. Each individual patient who was willing to participate was given a full counselling and signed a witnessed informed consent. The study protocol was approved by the institutional ethical committee.

The inclusion criteria were as follows: (i) age between 18 and 37 years old, (ii) menstrual cycle ranging from 27 ± 3 to 32 ± 3 days, (iii) the cause of infertility solvable by IVF.

The exclusion criteria were (i) infertility caused by endocrine abnormality, especially polycystic ovarian disease (diagnoses made on ultrasound; Franks, 1989), recurrent corpus luteum insufficiency and absence of ovarian function; (ii) male infertility, i.e. <20×10⁶ spermatozoa/ml and/or <40% normal morphology and/or <40% normal motility; (iii) contraindications for the use of HMG and HCG, especially ovarian and endogenous LH.
pituitary tumours; (iv) extensive adhesions; (v) any ovarian
doctornaire and abdominal abnormalities that would interfere with
adequate ultrasound investigation; (vi) hypertension (sitting
diastolic blood pressure >90 mmHg and/or systolic blood
pressure >150 mmHg); (vii) chronic cardiovascular, hepatic,
renal or pulmonary disease; (viii) history or current abuse of
alcohol or drugs, and (ix) use of investigational drugs within
3 months prior to entry to the study.

Study design
This was a randomized, assessor-blind, prospective study
comparing Normegon (N.V. Organon, Oss, The Netherlands)
and Metrodin (Serono, Geneva, Switzerland) and was part of
a multicentre study. Eligible patients were randomized in
blocks of five with a ratio between treatment with Normegon
and with Metrodin of 3:2. The number assigned from the
randomization list corresponded to the number assigned to
the patient at the start of the first treatment cycle. Each patient
was treated for a maximum of three cycles with either of the
two drugs. The drug assignment list was only available to
the study-coordinator, who prepared and administered the
medication, but who in no way took part in any decision
concerning the HMG/FSH dose during the first treatment cycle.
Second and third treatment cycles were not assessor-blind but
performed as in an open study.

Study medication
Normegon [HMG (FSH:LH = 3:1), Org 31338, batch numbers
CP 89044 and CP 90033; N.V. Organon] was supplied as a
lyophilized powder in ampoules each containing 75 IU FSH
and 25 IU LH expressed as units of in-vivo bioactivity, as
determined in the ovarian weight augmentation assay (Steelman
and Pohley, 1953) and seminal vesicle weight assay (Van
Hed el et al., 1964) respectively. Metrodin (urinary FSH, batch
numbers 89C16, 90G23, 91B14; Serono) was supplied in its
commercially available dosage form containing 75 IU FSH
(Steelman and Pohley, 1953) and minor amounts of in-vivo
bioactive LH according to the ovarian ascorbic acid depletion
test (Parlow, 1961; FSH:LH ratio >60). Both preparations
were reconstituted with at least 1 ml of solvent (0.9% NaCl)
and administered i.m. in the upper lateral quadrant of the
buttocks.

Treatment
Each subject received in each treatment cycle a starting dose
of two or three ampoules of Normegon or Metrodin during the
first 4 treatment days. After 4 days of treatment, the
daily dose was adjusted individually, based on hormonal and
ultrasound parameters. The treatment was started on the first,
second or third day of the menstrual cycle. The first day of
the menstrual cycle was defined as the first day of menstrual
bleeding.

Serum 17β-oestradiol, progesterone and luteinizing hormone
(LH) concentrations were assessed on the first day of FSH/
HMG treatment and every day or every other day from day 5
onwards, up to the day of human chorionic gonadotrophin
(HCG, Pregnyl®, N.V. Organon) administration. Ultrasound
examination was carried out on the fourth or fifth day and
thereafter whenever needed.

In principle, 10000 IU HCG was administered when at
least three follicles ≥17 mm were seen on ultrasound. However,
whenever rises of endogenous LH and/or progesterone were
noted, HCG was administered earlier. Rises of LH were defined
as at least two consecutive measurements indicating that serum
LH concentrations were >10.0 IU/l after 4 days of HMG/FSH
treatment. Rises of progesterone were defined as at least one
measurement indicating that serum progesterone was >1.0 µg/l
after 4 days of HMG/FSH treatment. In such cases, 10 000 IU
of HCG was injected to sustain the endogenous surges. All
oocytes were inseminated as previously described (Staessen
et al., 1988). Embryonic development was assessed 40 h
after insemination. The embryos were classified according to
morphological criteria (Staessen et al., 1988, 1992). Up to
three embryos were replaced and the remaining embryos were
cryopreserved for later use (Van Steirteghem et al., 1987). The
luteal phase was supplemented by 1500 IU HCG (i.m.) 2, 5
and 8 days after embryo transfer. When serum oestradiol
concentrations were ≥1500 ng/l, HCG administration was
postponed.

Clinical pregnancies were defined as gestations with
embryonic sacs, as identified by ultrasound. Ongoing pregnan-
cies were confirmed when positive fetal heart activity was
detected 12–16 weeks after embryo transfer. In this study
miscarriage rates represent fetal loss after establishment of a
clinical pregnancy but before 16 weeks of gestation.

All children born had an extensive paedriatic examination
at birth and at 2 months of age. Major malformations were
defined as those that caused functional impairment or required
surgical correction.

Data analysis
The data presented are part of a multicentre study. Summary
tables of hormones, duration of treatment and the number of
ampoules are presented as median values with ranges. Other
parameters are presented as mean values with ranges. Statistical
analysis was restricted to first treatment cycles, which were
investigator-blind. The primary outcome variables were the
number of well-dispersed cumulus—oocyte complexes, and
the ongoing pregnancy rate per started cycle and per transfer
as assessed by ultrasound scanning at least 12 weeks following
embryo transfer. Secondary variables were the number of
follicles ≥14 mm, serum oestradiol on the day of HCG
administration and the total number of ampoules. The clinical
and ongoing pregnancy rates were analysed using the
Mantel—Haenszel test. All other parameters were statistically
analysed by means of the Wilcoxon ranked sum test. A
treatment difference was significant whenever P < 0.05.

Results
Patien characteristics
A total of 158 patients started hormonal treatment, of whom
93 were allocated to the Normegon group and 65 to the
Metrodin group. The mean age of these patients and their
cause and duration of infertility were comparable for both
treatment groups (Table I). The incidence of primary infertility

333
was 59% in the Normegon group and 46% in the Metrodin group.

**Cycle cancellations**

Table II summarizes the number of started cycles, cycles with HCG injection (10,000 IU), oocyte retrievals, inseminations and transfers during three treatment cycles in the two medication regimens.

The total number of started treatment cycles was 248, i.e. 146 in the Normegon group and 102 in the Metrodin group. Embryo transfer was not performed in 20 and 28% of first treatment cycles and in 20 and 23% of all treatment cycles with Normegon and Metrodin respectively. Evaluation of all started cycles revealed that cancellation before oocyte retrieval (5 and 4%) was mainly due to poor-ovarian response. In 90 out of 248 cycles (36%), premature rises of serum LH and/or progesterone were noted but these were all continued with HCG administration and oocyte retrieval. The overall incidence of cycles with oocyte retrieval but without embryo transfer was relatively high (15 and 19% respectively for Normegon and Metrodin). This was mainly due to cycles with premature rises of LH and/or progesterone, i.e. in 21 out of these 90 cycles (23%) embryo transfer was not performed due to fertilization failure or insufficient embryo quality (see last paragraph of Results).

**Outcome of first treatment cycles**

Baseline levels of serum LH, oestradiol and progesterone assessed just prior to the first HMG/FSH injection were comparable in both treatment groups (Table III). In both groups, the median duration of stimulation was 7 days (range 5–15 days for patients treated with Normegon and 4–14 days for those treated with Metrodin). No significant difference was found with respect to the number of ampoules used. The median number (ranges) of ampoules of Normegon required was 21 (6–40) and of Metrodin 21 (11–36).

Mean (± SE) concentrations of serum LH, oestradiol and progesterone assessed during each treatment cycle are presented in Figure 1. The hormone profiles of each treatment group appeared to be almost identical both during the follicular phase and during the luteal phase. Mean concentrations of serum LH first decreased and reached their lowest values of 2–3 IU/l after 4 days of HMG/FSH treatment. Thereafter, serum LH increased rapidly up to mean concentrations of 8–9 IU/l (median values 6.5 and 5.5 IU/l for Normegon and Metrodin respectively; see Table III) at the day of HCG administration.

The degree of ovarian stimulation as reflected by the number of follicles ≥14 mm and serum oestradiol concentrations, both measured on the day of HCG administration, was not significantly different between the two treatment regimens (Table III). The relatively low concentrations of serum oestradiol, i.e. 1041 (430–2686) pg/ml in the Normegon group and 985 (370–2671) pg/ml in the Metrodin group, were in good agreement with the number and size of antral follicles (Table III).

The total mean number and quality of retrieved oocytes and replaced embryos are presented in Table IV. The mean numbers of well-dispersed cumulus–oocyte complexes were not different in the two treatment groups (9.6 and 9.5 respectively). Also, the mean (range) fertilization rates [55% (0–100) and 58% (0–100)] and embryo cleavage rates [86% (0–100%) and 80% (0–100%)] were comparable for both treatment regimens. In both groups the same number and quality of embryos were replaced.

The clinical and ongoing pregnancy rates per attempt, per oocyte retrieval and per embryo transfer of first treatment cycles are shown in Table V. No statistically significant differences between the two treatment groups were found for these pregnancy rates. The implantation rates were 13.5% (25 implanted embryos/184 replaced embryos) and 10.4% (12 implanted embryos/115 replaced embryos) in Normegon- and Metrodin-treated patients respectively. In first treatment cycles the ongoing pregnancy rates per transfer were 15 and 17% respectively.

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### Table I. Patients' characteristics. Values represent means (ranges are given in parentheses where applicable)

<table>
<thead>
<tr>
<th></th>
<th>Normegon</th>
<th>Metrodin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>93</td>
<td>65</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.5 (21–37)</td>
<td>30.1 (20–37)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.1 (45–90)</td>
<td>59.5 (47–79)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166 (130–182)</td>
<td>167 (152–178)</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>3.6 (&lt;1–10)</td>
<td>3.6 (&lt;1–12)</td>
</tr>
<tr>
<td>Cause of infertility (%)</td>
<td>Tubal</td>
<td>48.4</td>
</tr>
<tr>
<td></td>
<td>Unexplained</td>
<td>41.9</td>
</tr>
<tr>
<td></td>
<td>Endometriosis</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Tubal/endometriosis</td>
<td>4.3</td>
</tr>
</tbody>
</table>

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### Table II. Number of patients that started treatment with hormone regimen in three subsequent treatment cycles

<table>
<thead>
<tr>
<th></th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normegon</td>
<td>Metrodin</td>
<td>Normegon</td>
</tr>
<tr>
<td>HMG/FSH started</td>
<td>93</td>
<td>65</td>
<td>43</td>
</tr>
<tr>
<td>HCG injected</td>
<td>89</td>
<td>62</td>
<td>40</td>
</tr>
<tr>
<td>Oocyte retrieval</td>
<td>88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62</td>
<td>41&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insemination</td>
<td>87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Transfer</td>
<td>74</td>
<td>47</td>
<td>33</td>
</tr>
</tbody>
</table>

<sup>a</sup>One cycle without retrieval because partner had left.
<sup>b</sup>In one cycle oocyte retrieval was carried out without HCG administration; this patient had an endogenous LH rise.
<sup>c</sup>No spermatozoa available in one cycle.

HMG = human menopausal gonadotrophin; FSH = follicle stimulating hormone; HCG = human choriionic gonadotrophin; LH = luteinating hormone.
Efficacy of Normegon in ovarian stimulation

## Table III. Median values (range) of hormones at baseline and on the day of HCG administration. The mean number (range) of follicles of various size classes is based on the last ultrasound examination before HCG injection

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Normegon</th>
<th>Metrodin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pg/ml)</td>
<td>34 (17-122)</td>
<td>34 (20-80)</td>
</tr>
<tr>
<td>Luteinizing hormone (IU/l)</td>
<td>6.5 (2-20)</td>
<td>5.5 (1-13)</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>0.3 (0.1-1.3)</td>
<td>0.3 (0.1-1.1)</td>
</tr>
</tbody>
</table>

### Overall treatment outcome

The outcome of first, second and third cycles with respect to the various above-mentioned response parameters was comparable between the two treatment groups, but within each treatment group the pregnancy rates of second and third cycles tended to be higher than those of the first cycles, especially in the Normegon group. The overall implantation rates were 15.7% (47 implanted embryos/300 replaced embryos) and 10.4% (20 implanted embryos/193 replaced embryos) in Normegon- and Metrodin-treated patients respectively. The overall pregnancy rates per attempt, per oocyte retrieval and per embryo transfer were the same for Normegon and Metrodin (Table V). The ongoing pregnancy rates per embryo transfer were 21 and 19% respectively.

During 248 started cycles, hospitalization was twice required because of ovarian hyperstimulation syndrome. One patient treated with Normegon experienced a mild ovarian hyperstimulation (grade II) and one patient treated with Metrodin experienced a severe ovarian hyperstimulation (grade III).

In total, 57 embryos were cryopreserved after Normegon treatment and 32 embryos after Metrodin treatment. So far, in the Normegon group 23 replacements of these embryos (total of 35 embryos) have been performed, resulting in four ongoing pregnancies. In the Metrodin group eight replacements (22 embryos) have been done and pregnancy has not occurred. The additional four ongoing pregnancies in the Normegon group increased the ongoing pregnancy rate from 18 to 21% per oocyte retrieval (29/139).

### Cycle outcome of patients with premature LH rises

During HMG/FSH treatment, rises of serum LH to >10.0 IU/l were observed in 77 out of the 248 started cycles (31%). Concomitant or subsequent rises of progesterone to >1.0 µg/l were noted in 33 of these 77 cycles (43%); thus, in 44 out of 77 cycles progesterone did not increase up to values >1.0 µg/l. In addition, rises of serum progesterone without previous or concomitant rises of LH >10 IU/l were noted in 13 of the 248 treatment cycles (5%). These patients showed clear rises of LH but maximal concentrations ranged between 4.2 and 9.6 IU/l. The incidence of LH and/or progesterone rises was comparable in both treatment groups.

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**Table IV.** The total mean number and quality of retrieved cumulus–oocyte complexes and replaced embryos

<table>
<thead>
<tr>
<th></th>
<th>Normegon</th>
<th>Metrodin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulus–oocyte complexes/retrieval</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9.7 (1-25)</td>
<td>9.5 (1-33)</td>
</tr>
<tr>
<td>Well-dispersed</td>
<td>9.6 (1-25)</td>
<td>9.5 (1-33)</td>
</tr>
<tr>
<td>Embryos/transfer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>0.2 (0-2)</td>
<td>0.1 (0-1)</td>
</tr>
<tr>
<td>Good</td>
<td>1.9 (0-3)</td>
<td>2.1 (1-3)</td>
</tr>
<tr>
<td>Fair</td>
<td>0.3 (0-2)</td>
<td>0.1 (0-2)</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Mean (± SE) serum luteinizing hormone (LH), oestradiol (E₂) and progesterone (P) concentrations of patients treated with Normegon® and Metrodin® for ovarian stimulation. Values represent the mean (range) concentrations [at each time point number of measurements varied: n = 48 (4-88) for Normegon® and n = 31 (3-61) for Metrodin®].
In all 90 cycles with premature LH and/or progesterone rises, treatment was continued with 10000 IU HCG and oocyte retrieval. The mean (range) number of retrieved oocytes was 8.5 (1–27) in those treated with Normegon and 8.5 (1–24) in those treated with Metrodin. However, embryo transfer was not performed in 14 cycles due to fertilization failure and in seven cycles because of inadequate embryo quality. As a consequence, the number of cycles including embryo transfer after oocyte retrieval was relatively lower than in cycles without premature LH and/or progesterone rises, i.e. 77 versus 94%. In cycles with premature rises of LH and/or progesterone, clinical pregnancy and ongoing pregnancy rates per transfer were 29 and 22% respectively.

**Pregnancy outcome and follow-up of children**

In total, 44 patients had an ongoing pregnancy (40 treatment cycles and four frozen embryo cycles) which included 33 singleton pregnancies, 11 twin pregnancies and one triplet pregnancy. All ongoing pregnancies were uneventful and 40 children were born after Normegon stimulation and 18 children after Metrodin stimulation. Their birth weights were (mean ± SD) 2754 ± 749 and 2793 ± 947 g at a gestational age of 37 ± 2 and 37 ± 4 weeks respectively. An extensive paediatric examination revealed major malformations for three children. In the Normegon group, one baby died due to trisomy 18 and a set of twins was diagnosed with trisomy 21. In addition, in the Metrodin group one baby died due to entanglement by the umbilical cord.

**Discussion**

The current study demonstrates that Normegon is an efficacious preparation for the induction of ovarian stimulation in infertile women undergoing IVF. No differences were found between Normegon, containing 75 IU FSH and 25 IU LH bioactivity, and Metrodin, containing 75 IU FSH and ≤1.25 IU LH bioactivity. Moreover, the safety of Normegon treatment for patients and their offspring was supported by the pregnancy and children follow-up data included in this study.

Nowadays, the use of GnRH-agonists in ovulation induction regimens has become widespread and the majority of programmes are using them (reviewed by Loumaye, 1990). The main reason for the use of agonists is the avoidance of a premature endogenous LH rise. The drawback of a long desensitization protocol with these analogues is that an increased number of HMG/FSH ampoules is needed to obtain adequate folliculogenesis. Depending on the hormonal regimen of choice, such protocols may require 30–40 ampoules of HMG/FSH, and the average duration of treatment is 11–14 days (Polson et al., 1991; Smitz et al., 1992; Devroey et al., 1994). In the current study, performed without a GnRH analogue, the mean number of ampoules used was only 21 and the mean treatment period 7 days. On the day of HCG administration, follicular sizes were relatively small and, accordingly, oestradiol concentrations were relatively low. Nevertheless, the number and maturity of cumulus–oocyte complexes as well as the pregnancy rate per transfer were very comparable to those retrieved in conjunction with GnRH-agonists (Smitz et al., 1992).

HMG/FSH treatment without GnRH-agonists is frequently cancelled because of premature LH and/or progesterone rise, whereas in association with GnRH-agonists this incidence is largely reduced. Previous studies have demonstrated that premature luteinization, especially elevated progesterone, does not affect oocyte quality, fertilization rate or embryo quality (Hofmann et al., 1993). Whether premature luteinization reduces the chance of implantation was up to now unknown: for cycles with GnRH-agonist suppression contradictory data are available (Edelstein, 1990; Schoolcraft et al., 1991; Silverberg et al., 1993). Nevertheless, in cycles without pituitary suppression, pregnancy rates per transfer were not decreased due to premature luteinization.

In the current study, patients with rises of LH and/or progesterone continued HMG/FSH treatment. Premature rises or surges of LH have been frequently mentioned in the literature but appropriate definitions are lacking. In this study,
most patients had rises of endogenous LH during treatment, but significant rises of LH were defined as values >10.0 IU/l and of progesterone as values >1.0 ng/l on the assumption that such amounts might influence the clinical outcome of the study. According to the selected criteria, some patients showed rises of LH only and some of progesterone only, which is explained by the fact that some rises of LH >10.0 IU/l did not induce progesterone rises above 1.0 ng/l, whereas not all rises of progesterone >1.0 ng/l were accompanied by serum LH concentrations >10.0 IU/l. In addition, due to the time-intervals between measurements, it cannot be excluded that significant rises of LH and/or progesterone did occur but were not noted. Interestingly, the number of retrieved oocytes in cycles with endogenous rises of LH and/or progesterone tended to be lower but still within the normal range. On the other hand, fertilization and cleavage rates of inseminated oocytes from such cycles were clearly decreased, suggesting that the quality and/or maturity of these oocytes was affected. In addition, the clinical outcome of remaining cycles including transfer was similar to that of the overall-group, suggesting that in this study premature LH and/or progesterone rises did not affect endometrium quality or receptivity negatively. All together, these data demonstrate that continuation of treatment after observation of premature hormonal rises is justified and worthwhile.

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