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Pharmacodynamics and pharmacokinetics after repeated subcutaneous administration of three gonadotrophin preparations

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Recently, several new urinary gonadotrophin preparations have been developed, containing less luteinizing hormone (LH) activity than human menopausal gonadotrophin. Normegon is a gonadotrophin preparation with a follicle stimulating hormone (FSH)/LH ratio of 3:1; Follegon and Metrodin-HP are purified FSH preparations. The aim of the present randomized study was to compare pharmacodynamics, -kinetics and local tolerance of these preparations after repeated s.c. administration. Thirty-six healthy female subjects were treated with Lyndiol contraceptive pills for 5 weeks to suppress endogenous gonadotrophin concentrations. After 3 weeks of Lyndiol treatment, 150 IU of Normegon, Follegon or Metrodin HP were administered once daily, s.c. for 7 days. Blood samples were collected once daily during the fourth and fifth weeks of the study and assayed for FSH and oestradiol. After the last gonadotrophin injection, blood samples were collected more frequently to determine pharmacokinetic parameters of FSH. During the fourth and fifth study weeks, daily ultrasound measurements of follicular growth were performed. Endogenous FSH and LH values were extremely suppressed during Lyndiol treatment. Serum FSH values showed similar patterns in the three groups. The maximum FSH concentration was reached 9–11 h post-injection, the terminal half-life was 43–47 h. The preparations were bioequivalent with respect to FSH immunoreactivity. The number of follicles tended to be larger after Normegon than after Follegon and Metrodin HP treatment, though this was not statistically significant. Serum oestradiol concentrations were significantly higher after Normegon treatment. In general, s.c. injections were well tolerated. In conclusion, the three preparations were bioequivalent with respect to FSH immunoreactivity. Nevertheless, the biological activity of Normegon tended to be higher than that of Follegon and Metrodin HP in Lyndiol-suppressed women.

Key words: FSH/gonadotrophin/pharmacodynamics/pharmacokinetics/subcutaneous

Introduction

Human menopausal gonadotrophin (HMG) has been used on a large scale for more than 30 years. This urinary preparation contains equal amounts of follicle stimulating hormone (FSH) and luteinizing hormone (LH) activity. If the LH bioactivity is too low after extraction from urine, human chorionic gonadotrophin (HCG) is added to the preparation to increase the amount of LH bioactivity. Administration of exogenous LH does not appear to be necessary for adequate ovarian stimulation in normogonadotrophic women, even during pituitary suppression by a gonadotrophin-releasing hormone (GnRH) agonist (Devroey *et al.*, 1994). In gonadotrophin-deficient women, however, although multiple follicular growth is observed after treatment with pure (recombinant) FSH, serum 17 β -oestradiol concentrations remain very low (Schoot *et al.*, 1994; Balasch *et al.*, 1995). So a minor amount of LH is required for adequate steroid synthesis by the ovarian follicles. Lately several new urinary preparations have been developed, containing less LH bioactivity than HMG. Normegon is produced in the same way as HMG, but no HCG is added to this preparation. One ampoule contains 75 IU FSH and 25 IU LH in-vivo bioactivity. Follegon is a purified FSH preparation, containing 75 IU FSH and virtually no LH activity. Metrodin HP is a highly purified FSH preparation, from which non-FSH proteins are largely removed. The primary objective of the present study was to compare the effects of Normegon, Follegon and Metrodin HP on follicular growth and oestradiol production.

Gonadotrophin preparations are usually administered i.m. These preparations can also be administered s.c., in particular highly purified preparations such as Metrodin HP or recombinant human FSH (Howles *et al.*, 1994; Recombinant FSH Study Group, 1995). S.c. injections are preferable to i.m. injections, because they are more suitable for self-administration by the patient. Therefore, in the present study the three gonadotrophin preparations were administered s.c.. A second objective of the study was to investigate the 7 day multiple-dose pharmacokinetics of Normegon, Follegon and Metrodin HP and the local tolerance of the s.c. route. The study subjects used Lyndiol contraceptive pills to suppress endogenous gonadotrophin concentrations, enabling investigation of both pharmacodynamic and pharmacokinetic parameters of exogenous FSH preparations.

Materials and methods

Subjects

Thirty-six healthy female volunteers participated in the study. Inclusion criteria were: age between 18 and 39 years, use of oral

contraceptives for at least 3 months (not originally prescribed for menstrual irregularities), body weight between 50 and 75 kg and between 80 and 130% of the ideal body weight (according to the Metropolitan Life Insurance Company tables). The most important exclusion criteria were: endocrine abnormalities such as hyperprolactinaemia, polycystic ovary syndrome and absence of ovarian function, hypertension, chronic cardiovascular, hepatic, renal or pulmonary disease, abuse of alcohol or drugs, protein allergy. Prior to the start of the study, all subjects underwent a physical and gynaecological examination including a cervical smear and transvaginal ultrasonography. Blood samples were obtained to determine routine haematology and blood biochemistry.

All subjects gave their written informed consent. The study was approved by an independent ethical committee (STEG).

Study protocol

The subjects were divided in a randomized way into three study groups of 12 subjects each. After a pill-free period of 7 days, all subjects were treated with Lyndiol contraceptive pills (NV Organon, Oss, The Netherlands; 50 µg ethinyl oestradiol and 2.5 mg lynestrenol per tablet) during 5 weeks to suppress endogenous gonadotrophin activity. The first day of Lyndiol treatment was called study day 1. After 3 weeks of Lyndiol pretreatment, from day 22 onwards, 150 IU of either Normegon (NV Organon), Follegon (NV Organon) or Metrodin HP (Laboratoires Serono SA, Geneva, Switzerland) were injected once daily s.c. in the abdominal wall. Gonadotrophin treatment was given for 7 days. Lyndiol treatment was continued for 1 week after the last gonadotrophin injection.

Gonadotrophin injections were given at a fixed time point. The injection site of the previous day was inspected and local reactions (redness, itching, swelling, pain, bruising) were scored as either none, mild, moderate, or severe. Transvaginal ultrasonography was performed once daily from study day 20 until day 35 to measure all visible follicles. Blood sampling was performed once daily from day 20 until day 35 between 7.30 and 9.00 a.m., just prior to the gonadotrophin injection. On day 28, the seventh day of gonadotrophin administration, a blood sample was obtained prior to the last injection and subsequently 1, 2, 4, 6, 8, 10, 12, 16, 24, 30, 36 and 48 h after the injection. The subjects kept bed rest from 1 h before until 12 h after the last injection. Heavy exercise and alcohol consumption of more than one glass per day were not allowed during the week of gonadotrophin treatment until 3 days thereafter.

Gonadotrophin preparations

One batch of each preparation was used for the study. The FSH immunoreactivity in five ampoules from each batch was determined by a time-resolved fluoroimmunoassay (Delfia; Pharmacia, Woerden, The Netherlands). FSH isohormone profiles of the three batches were determined by chromatofocusing according to Matikainen *et al.* (1994).

Assays

Blood samples were allowed to clot for 30 min, then centrifuged for 15 min at 2000 g. Sera were stored at -20°C until assayed. Serum concentrations of FSH and oestradiol were determined in each sample by time-resolved fluoroimmunoassays (Delfia; Pharmacia). Serum LH concentrations were determined on days 21, 24, 28 (before injection) and 32, using a time-resolved fluoroimmunoassay (Delfia; Pharmacia). These two-site assays employ a β -directed capturing monoclonal antibody (MCA) and an α -directed europium labelled detection MCA. The assays were performed as described by the manufacturer using the Delfia instrumentation software and MultiCalc software (Pharmacia). FSH and LH immunoreactivity was expressed in terms of the Second

International Reference Preparation (IRP) of pituitary FSH (code no. 78/549) and the Second International Standard (IS) for pituitary LH (code no. 80/552). The intra- and interassay coefficients of variation (CV) were <7.5% (range 4.3–7.5) and <6.4% (range 5.1–6.4) for FSH and <5.4% (range 2.0–5.4) and <10.5% (range 8.6–10.5) for LH, respectively. The intra- and interassay coefficients of variation of the oestradiol assay were <7.7% (range 3.3–7.7) and <10.7% (range 4.4–10.7), respectively.

Data analysis

Several pharmacokinetic parameters were calculated: the concentration as measured just before each dosing and 24 h after the last dosing, the peak concentration (C_{max}) and the time of its occurrence (t_{max}). The elimination half-life ($t_{1/2}$) was estimated using log-linear regression on the terminal data points of the concentration-time curve, the area under the curve (AUC_{0-24}) over one dosing interval was calculated by means of the linear trapezoidal rule. The clearance was calculated by dividing the administered immunoreactive dose by the AUC_{0-24} , and was expressed in relation to the body weight (Cl/kg).

The groups were compared with respect to age, height, weight and extent of suppression by a one-way analysis of variance (ANOVA) and the Kruskal-Wallis test. The Kruskal-Wallis test was used to compare the groups with respect to numbers of follicles and serum oestradiol concentrations. A repeated measures ANOVA was performed on the log-transformed values of the pre-dose values and the 24 h value after the last injection in order to check whether steady state had been reached. The predose concentration was corrected for differences in ampoule contents.

A one-way ANOVA was performed on the log-transformed values of the parameters C_{max} , $t_{1/2}$, AUC_{0-24} and Cl/kg. C_{max} and AUC_{0-24} were corrected for differences in ampoule contents. Differences in t_{max} were tested using the Kruskal-Wallis test. For the parameters C_{max} , $t_{1/2}$, AUC_{0-24} and Cl/kg, a 90% confidence interval (CI) for the true ratio 'test/reference' was derived from the ANOVA. The Metrodin HP treatment was designated as 'reference', Normegon and Follegon as 'test'. In the case of comparison between Normegon and Follegon, Normegon was designated as 'reference'. Two preparations were considered bioequivalent with respect to a certain parameter when the 90% CI of the ratio was fully contained within a range of 0.80–1.25.

Results

The three groups appeared to be comparable with respect to age (mean \pm SD: 23 \pm 4.7, 22 \pm 2.2, 24 \pm 4.1 years in the Normegon, Follegon and Metrodin HP group, respectively), height (1.69 \pm 2.9, 1.72 \pm 4.2, 1.71 \pm 6.8 m) and body weight (62 \pm 5.8, 65 \pm 3.7, 63 \pm 7.0 kg). The mean (\pm SD) FSH immunoreactivity was 36.6 \pm 0.7, 36.6 \pm 0.9 and 40.6 \pm 0.9 IU in the Normegon, Follegon and Metrodin HP ampoules, respectively. The isohormone profiles of the three batches appeared to be very similar (Figure 1).

In all groups serum LH concentrations were extremely low, <0.4 IU/l, during the entire study period. Serum FSH concentrations were also extremely suppressed after 3 weeks of Lyndiol treatment (Figure 2). Mean FSH concentrations before gonadotrophin treatment were <1.0 IU/l. During gonadotrophin treatment FSH concentrations increased until after 4 days a plateau phase was reached. FSH values were slightly higher in the Metrodin HP group than in the other two groups. However, when the serum FSH values were

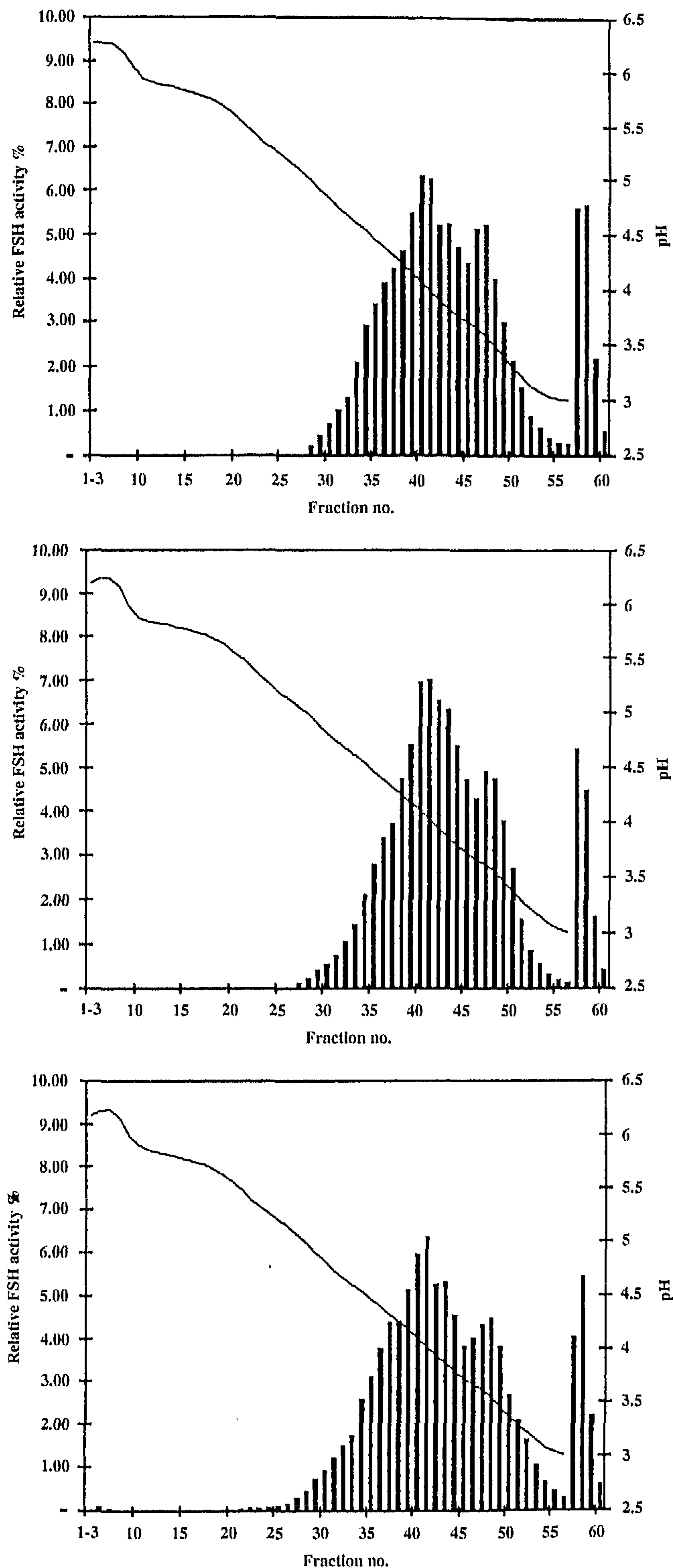


Figure 1. Follicle stimulating hormone (FSH) isohormone profiles of the batches of Normegon (**lower panel**), Follegon (**middle panel**) and Metrodin HP (**upper panel**) used for the study.

corrected for the differences in ampoule contents, as determined by immunoassay, the FSH curves of the three groups were almost identical. Pharmacokinetic parameters did not show significant differences between the groups (Table I). The preparations appeared to be bioequivalent with respect to FSH immunoreactivity.

Table II depicts median values of the total number of

follicles with a diameter >8 mm on each study day. Median values of the number of follicles with a diameter of 8 or 9 mm, 10 or 11 mm and 12 or 13 mm are shown in Figure 3. The largest number of follicles was seen on the second post-treatment day, study day 30. On day 30, the number of follicles with a diameter of 8 or 9 mm was 4.5 (1–15), 4 (0–10) and 1.5 (0–16), in the Normegon, Follegon and Metrodin HP group, respectively (median and range). The number of follicles with a diameter of 10 or 11 mm was 2.5 (0–7), 1 (0–7) and 0.5 (0–19); the number of follicles with a diameter of 12 or 13 mm was 0.5 (0–5), 0 (0–1) and 0 (0–19), in the Normegon, Follegon and Metrodin HP group. The number of follicles tended to be higher in the Normegon than in the Follegon and Metrodin HP group, and follicles tended to be larger in the Normegon group, but the differences were not statistically significant probably because of the large interindividual variation. Serum oestradiol concentrations were low during the entire study period. Only in the Normegon group was a small increase in serum oestradiol concentrations seen after several days of gonadotrophin treatment, and a median peak value of 127 pmol/l (range 56–420) was seen on the first post-treatment day (day 29). The median oestradiol concentration on day 29 was 67 pmol/l (range 50–122) in the Follegon group, 54 pmol/l (range 50–194) in the Metrodin HP group. Serum oestradiol concentrations were significantly higher in the Normegon group than in the other two groups ($0.01 < P < 0.05$ on days 25 and 26; $P < 0.01$ on days 27, 28 and 29).

Generally, the s.c. injections were well tolerated. The item 'redness' was scored as 'severe' by two subjects in the Normegon group on four and two treatment days, respectively, the item 'pain' was scored as 'severe' by these two subjects on three treatment days. The item 'bruising' was scored once by one of these subjects as 'severe'. The items 'itching' and 'swelling' were never scored as 'severe'. In the Follegon group none of the subjects scored any of the items as 'severe', on a few occasions 'moderate' was scored for redness, swelling or pain. In the Metrodin HP group none of the items was scored as 'moderate' or 'severe'.

Discussion

Endogenous serum FSH and LH concentrations after 3 weeks of Lyndiol treatment appeared to be suppressed more severely than after GnRH agonist treatment (Devroey *et al.*, 1994), and to resemble the gonadotrophin concentrations of gonadotrophin-deficient women (Schoot *et al.*, 1994). Serum LH values remained at very low concentrations during the entire study, so endogenous gonadotrophins were adequately suppressed during the gonadotrophin treatment. Administration of exogenous LH in the Normegon group did not result in an increase of serum LH values, probably because of the short half-life of LH. Serum FSH concentrations were slightly higher after Metrodin HP than after Normegon and Follegon administration. However, the amount of immunoreactive FSH in the Metrodin HP ampoules was somewhat higher than in the other ampoules. After correction of immunoreactive serum FSH values for the differences in content of the ampoules, no differences could be detected any more, and the three

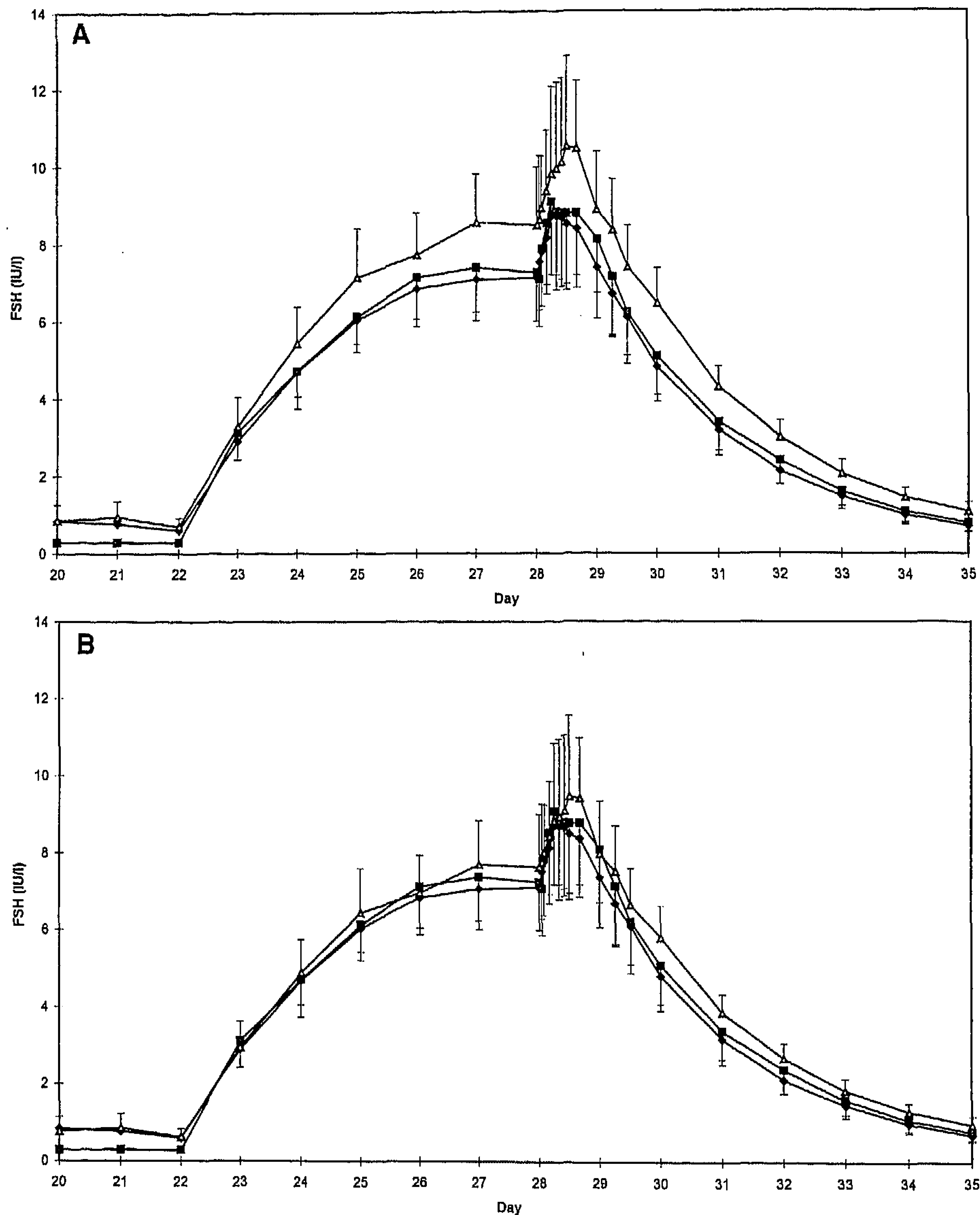


Figure 2. Mean (SD) serum FSH concentrations before (days 20–21), during (days 22–28) and after (days 29–35) 7 day s.c. administration of 150 IU Normegon (solid diamonds), Follegon (hatched squares) or Metrodin HP (stippled triangles); without (A) and with (B) correction for differences in ampoule contents.

Table I. Pharmacokinetic parameters of follicle stimulating hormone (FSH)

	Normegon	Follegon	Metrodin HP
t_{max} (h)	8.83 ± 2.89	9.83 ± 3.95	11.17 ± 4.13
C_{max} (IU/l)	8.98 ± 1.57	9.53 ± 1.85	10.92 ± 2.10
n- C_{max} (IU/l)	0.245 ± 0.043	0.260 ± 0.051	0.269 ± 0.052
AUC ₀₋₂₄ (IU.h/l)	197 ± 34.9	204 ± 37.6	234 ± 41.4
n-AUC ₀₋₂₄ (IU.h/l)	5.39 ± 0.95	5.59 ± 1.03	5.77 ± 1.02
Cl/kg (l/h)	0.0031 ± 0.0006	0.0028 ± 0.0005	0.0028 ± 0.0004
$t_{1/2}$ (h)	43.48 ± 3.65	44.01 ± 2.98	46.63 ± 4.71

Values are mean ± SD. n- C_{max} and n-AUC₀₋₂₄ are C_{max} and AUC₀₋₂₄ normalized for FSH ampoule content (C_{max} and AUC₀₋₂₄ divided by 36.6 or 40.6).

AUC: area under the curve; C_{max} , t_{max} : peak concentration and its time; Cl: clearance.

preparations appeared to be bioequivalent with respect to serum FSH immunoreactivity. In addition, Out *et al.* (1996) have previously demonstrated bioequivalence between Folle-

gon and Metrodin. The elimination half-life of FSH of ~45 h was rather long in comparison with that found by Le Cottonnec *et al.* (1994a,b). They calculated an elimination half-life of 37 h after a single s.c. administration of recombinant human FSH (Gonal-F), and a half-life of 24 h after multiple s.c. administration. The difference may be caused by variation in FSH isohormone profiles of the applied preparations. If more acidic isohormones are present, the half-life will be longer (Wide and Hobson, 1986). Another explanation may be that the subjects in the study by Le Cottonnec *et al.* were treated with a GnRH agonist to suppress endogenous gonadotrophins. As was mentioned earlier, the endogenous gonadotrophins were less profoundly suppressed during GnRH agonist than during Lyndiol treatment. Exogenous FSH can be measured for a longer time period if endogenous FSH is more adequately suppressed. Since the half-life was estimated from the terminal part of the disappearance curve, the estimated value will be influenced by the degree of pituitary suppression. In a previous

Table II. Numbers of follicles >8 mm before (days 20–21), during (days 22–28) and after (days 29–35) 7 day s.c. administration of 150 IU Normegon, Follegon or Metrodin HP

Study day	Normegon	Follegon	Metrodin HP
20	0 (0–0)	0 (0–0)	0 (0–0)
21	0 (0–0)	0 (0–0)	0 (0–0)
22	0 (0–0)	0 (0–0)	0 (0–0)
23	0 (0–0)	0 (0–0)	0 (0–0)
24	0 (0–0)	0 (0–0)	0 (0–0)
25	0 (0–6)	0 (0–0)	0 (0–1)
26	0 (0–6)	0 (0–4)	0.5 (0–10)
27	2.5 (0–7)	0.5 (0–4)	1 (0–25)
28	5.5 (0–12)	2 (0–14)	2 (0–33)
29	6.5 (3–17)	3.5 (0–16)	3.5 (0–55)
30	7.5 (1–17)	5 (0–16)	2 (0–62)
31	5 (0–18)	3 (0–20)	1.5 (0–37)
32	6 (0–15)	2.5 (0–18)	2.5 (0–45)
33	6 (0–15)	3 (0–13)	1.5 (0–45)
34	3 (0–13)	0 (0–11)	0 (0–42)
35	2 (0–11)	0 (0–5)	0.5 (0–34)

Values are median (range).

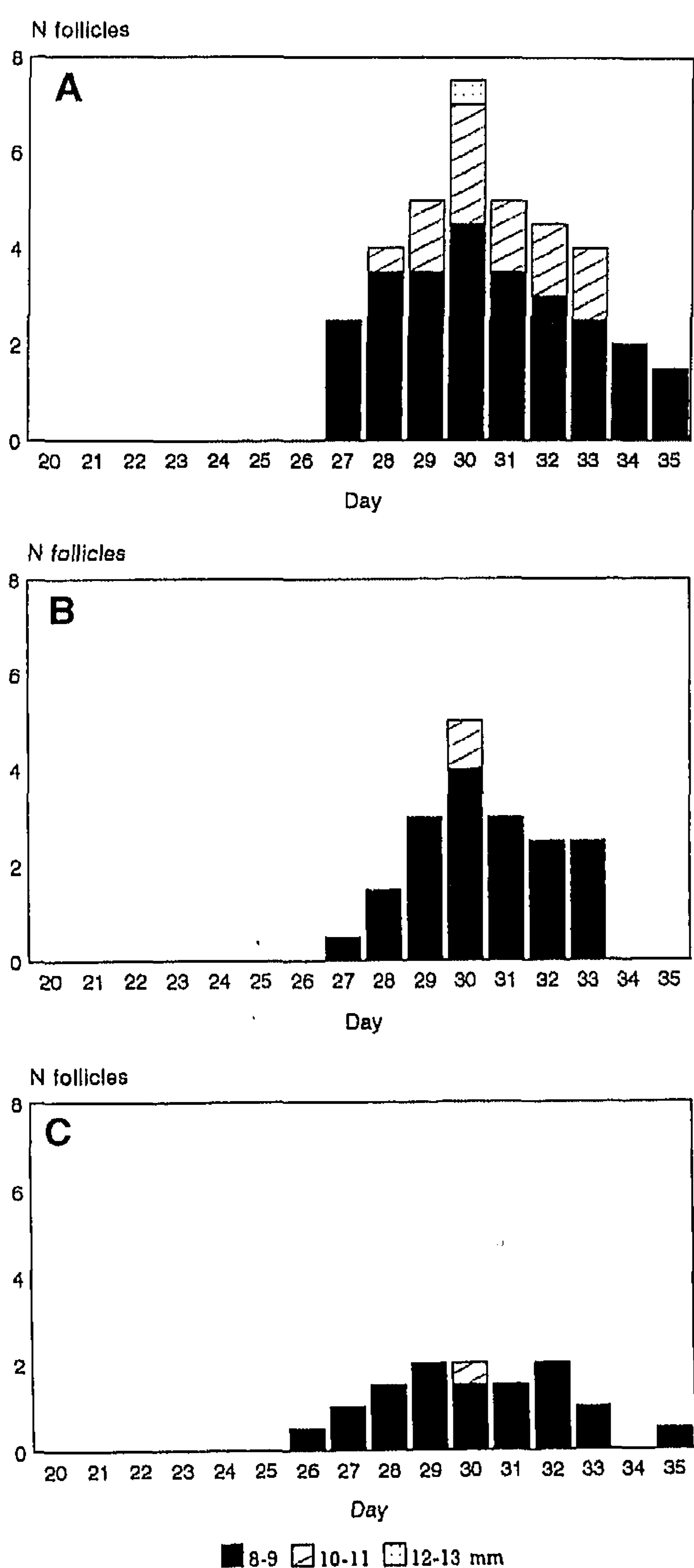


Figure 3. Median number of follicles with a diameter of 8 or 9 mm, 10 or 11 mm and 12 or 13 mm before (days 20–21), during (days 22–28) and after (days 29–35) 7 day s.c. administration of 150 IU Normegon (A), Follegon (B) or Metrodin HP (C).

study, pharmacokinetic parameters of Follegon and Metrodin were determined after a single i.m. injection in women treated with Lyndiol (Out *et al.*, 1996). The elimination half-lives calculated in this study (Follegon 38 h, Metrodin 44 h) were comparable to our results after s.c. administration.

Serum oestradiol concentrations were significantly higher in the Normegon group than in the other groups. The higher oestradiol concentrations may be correlated with the larger number of follicles in the Normegon group. Another explanation for the increased oestradiol concentrations may be the higher amount of LH in Normegon. Previous studies in in-vitro fertilization (IVF) cycles showed that even pure (recombinant) FSH preparations could stimulate oestrogen production (Devroey *et al.*, 1994). However, in these cycles endogenous gonadotrophins were suppressed by GnRH agonists. In the present study, endogenous LH values were much lower, and resembled those of the gonadotrophin-deficient women in the studies by Couzinet *et al.* (1988), Schoot *et al.* (1994) and Balasch *et al.* (1995). In these women, serum oestradiol concentrations hardly increased during purified urinary FSH and recombinant FSH treatment, respectively. Possibly, in our study group, the minor amount of LH in Follegon and Metrodin HP was insufficient to stimulate oestrogen production.

Although the three preparations were bioequivalent with respect to FSH immunoreactivity, the number of follicles and the follicular diameter tended to be larger in the Normegon group. Apparently, although the immunological FSH activity was similar in the three groups, the biological effect was different. Two possible explanations can be given for the observed differences. A first explanation may be the presence of different amounts of urinary components other than FSH. The production process of the preparations differs in some way. Normegon is purified from female urine. To obtain a purified FSH preparation (Follegon), a polyclonal antibody to LH/HCG is used, which removes most of the LH activity. Metrodin HP is produced from the HMG bulk material using a monoclonal antibody to FSH and a number of high performance liquid chromatography steps, thus removing most of the contaminating urinary proteins. If these urinary proteins are capable of stimulating follicular growth, differences in biological activity between the preparations might occur. However, Normegon and Follegon are substantially the same products, apart from the removed LH activity, which makes this explanation less likely. A second explanation may be that the higher amount of exogenous LH in Normegon exerts a stimulatory effect on follicular growth. It is generally accepted that FSH, not LH, stimulates follicular growth. However, LH stimulates androgen production, and these androgens are converted into oestrogens. Oestrogens exert autocrine and paracrine effects within the ovary. For instance, oestrogens stimulate the proliferative activity of granulosa and theca cells and enhance the responsiveness of granulosa cells to FSH (Rao *et al.*, 1978; Richards, 1980). Thus, oestradiol may enhance the stimulation of follicular growth by FSH. Furthermore, LH might stimulate the production of growth factors within the ovary, which exert paracrine effects and may also play a role in the process of follicular growth.

Loumaye *et al.* (1995) described preliminary results of a study in gonadotrophin-deficient women, treated with recombinant FSH (Gonal-F, 150 IU daily) and different amounts of recombinant LH (0, 25, 75 and 225 IU daily). Interestingly, they observed that co-administration of LH led to a dose-related enhancement of the number of follicles and serum oestradiol concentrations. They concluded from their results that 75 IU LH per day was an effective dose for the majority of their patients. The results of this study would support our theory that, at least in hypogonadotrophic women, more exogenous LH might stimulate follicular growth. The results of a study by Fleming *et al.* (1996) also support this theory. They demonstrated that infertile women with low follicular phase serum LH concentrations (<1 IU/l), who were treated with Metrodin HP during 7 days, had significantly smaller follicles and lower serum oestradiol concentrations than patients with serum LH values ≥ 1 IU/l who were treated with HMG. Results from patients who had serum LH values >1 IU/l and were treated with Metrodin HP were intermediate.

In general, the s.c. gonadotrophin injections were well tolerated. In the Normegon group, more local skin reactions were observed than in the other groups, although hardly any severe reactions were seen. In clinical practice, especially Follegon and Metrodin HP are very suitable for s.c. administration.

In conclusion, treating female volunteers with Lyndiol contraceptive pills resulted in extremely suppressed endogenous gonadotrophin concentrations. Normegon, Follegon and Metrodin HP were bioequivalent with respect to FSH immunoreactivity. Despite this bioequivalence, the biological effect of Normegon tended to be greater in our study group of Lyndiol-treated subjects, possibly due to the higher amount of LH in this preparation. This observation will not have consequences for routine IVF practice, since endogenous LH concentrations of IVF patients are higher than in the present study group, even during GnRH agonist treatment, and the amount of exogenous LH appears to be not very relevant (Bentick *et al.*, 1988; Daya *et al.*, 1995). In women with extremely low endogenous LH concentrations, however, the amount of exogenous LH will probably influence the effect of gonadotrophin treatment.

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