

Hormonal serum profiles and follicular development after intramuscular and pulsatile intravenous administration of human menopausal gonadotrophin

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A study was performed to compare, in a randomized way, the effect of pulsatile intravenous (iv) and intramuscular (im) human menopausal gonadotrophin (hMG) administration on hormonal serum profiles and follicular development in in vitro fertilization (IVF). Fourteen IVF patients participated in the study, aged between 20 and 40 years, with a normal endocrine profile, no hormonal medication used for at least 3 months previously, no endometriosis, both ovaries present and a normal male factor. Seven patients were treated with im hMG at a daily dose of 150 IU and seven patients with pulsatile iv hMG at a daily dose of 112.5 IU, in both cases in combination with buserelin. Ultrasonography was performed every other day during the stimulation phase and blood samples were collected once daily up to five times a day during the entire IVF cycle. Serum concentrations of follicle-stimulating hormone, luteinizing hormone, 17 β -oestradiol, progesterone and human chorionic gonadotrophin were determined. There were no differences in hormonal profiles between the two groups. The numbers of retrieved oocytes, fertilization rates and mean embryo quality were identical in this study, as was follicular growth. In conclusion, in the present randomized study no differences were observed in hormonal levels or follicular development after im and pulsatile iv hMG treatment.

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Human menopausal gonadotrophin (hMG) has been used for almost 30 years to stimulate ovarian follicular growth. Urine-derived hMG preparations comprise follicle-stimulating hormone (FSH), luteinizing hormone (LH) and human chorionic gonadotrophin (hCG), and are usually administered once daily by intramuscular (im) injection. The first reports on pulsatile intravenous (iv) administration of hMG were by Afnan et al. (1) and Ho Yuen et al. (2). By this route of administration, pulsatile gonadotrophin release by the pituitary gland is mimicked more closely than when the im route is used. Later, other authors compared im and pulsatile iv administration of hMG in ovulation induction cycles in women with refractory anovulation (3) and in in vitro fertilization (IVF) cycles, in low-responder patients and women with normal ovarian function (4, 5). Prak et al. (5) reported higher pregnancy rates after pulsatile iv than after im hMG treatment. However, as with all the investigations mentioned, their study did not have a randomized design, meaning that the higher pregnancy rates could

have been due to pre-existing differences between the study groups.

We decided to perform a randomized study to investigate whether the previously reported superior results obtained with pulsatile iv hMG treatment as against routine im treatment could be confirmed. The study was carried out in a well-defined group of women undergoing IVF treatment who had normal endocrine profiles. Administration of hMG was combined with gonadotrophin-releasing hormone agonist (GnRHa) treatment. We evaluated the effects of im and pulsatile iv hMG treatment on hormonal profiles in serum and on follicular development. Both hormonal profiles and follicular growth were monitored very closely to detect any differences between the two methods that might explain the possible increase in pregnancy rates. During the initial treatment days, multiple samples were taken to compare the increase of FSH and 17 β -oestradiol (E₂) concentrations. In addition, the numbers of retrieved oocytes, fertilization rates and mean embryo quality were assessed in both groups.

Subjects and methods

Patients

Fourteen women undergoing IVF treatment for tubal pathology or unexplained infertility participated in the present study. The inclusion criteria were: age between 20 and 40 years, normal endocrine serum profile in the early follicular phase of the cycle (FSH concentration < 8 IU/l, LH/FSH ratio < 3, testosterone concentration < 2.5 nmol/l, prolactin concentration < 700 mIU/l and thyroxine concentration between 58 and 148 nmol/l), no hormonal medication for at least 3 months prior to the study, no endometriosis observed on laparoscopy, both ovaries present and a normal male factor (a normal semen analysis, i.e. at least 50% motile sperm, motility grade at least fairly good (i.e. score 4 on a scale from 1 to 6), at least 60% morphologically normal sperm and absence of antisperm antibodies as confirmed by the direct immunobead test (6); or a fertilization rate of at least 50% if the patient had IVF before).

The study was approved by the hospital's Ethical Committee and all the women gave their informed consent. The patients were allocated randomly to one of two groups. Seven patients received hMG (Humegon[®], NV Organon, Oss, The Netherlands) via the im route (Group A), and the other seven patients received hMG via the pulsatile iv route (Group B).

The indication for IVF in Group A was tubal infertility in three patients and unexplained infertility in four patients. Two patients had had IVF treatment previously. In Group B the indication for IVF was also tubal infertility in three patients and unexplained infertility in four patients. Three patients had had IVF treatment previously. The groups were comparable with respect to mean age, weight and early follicular FSH concentrations (33 years, 66 kg and 5.6 IU/l in Group A; 30 years, 61 kg and 7.2 IU/l in Group B).

Induction protocol

Intranasal administration of the GnRHa buserelin (Suprefact[®], Hoechst AG, Frankfurt, Germany) was started on day 21 of the menstrual cycle, at a dose of 300 µg three times a day. On the tenth day of buserelin administration, transvaginal ultrasonography was performed to exclude the presence of ovarian cysts. Serum E₂ concentration was determined the same day by time-resolved fluoroimmunoassay (Delfia, Wallac Oy, Turku, Finland). If the serum E₂ concentration was below 500 pmol/l, administration of hMG was started the next day (day 3 of the IVF cycle) and the buserelin medication was continued. If the E₂ level remained higher than 500 pmol/l, the administration of buserelin was continued but hMG administration was postponed until the serum E₂ concentration had fallen below 500 pmol/l (determined every 2 or 3 days).

Humegon[®] was given either by im administration

between 17.00 and 18.00 h at a fixed dose of two ampoules (150 IU) daily, or by pulsatile iv administration at a fixed total daily dose of 1.5 ampoules (112.5 IU) with a pulse interval of 90 min. The lower daily dose for the iv route as compared with the im route was based on the work of Ho Yuen et al (2), who had described an approximately 40% reduction in the dose. We reduced the daily dose for the iv route to 1.5 ampoules of hMG per day. An iv catheter was inserted into a forearm or upper arm vein. Six ampoules of hMG were diluted in 2.6 ml of prednisolone-heparin solution and 40 µl was administered in each pulse via a computerized pump (MiniMed[®] Infusion Pump 404-SP, Minimed Technologies, Sylmar, CA, USA; Microdose Pump MRS 5, Disetronic AC, Burgdorf, Germany). The pump was refilled with fresh hMG solution every 4 days. Eleven patients were treated with Humegon[®] from the same batch, while the other three patients were treated with Humegon[®] from another batch with similar FSH and LH in vivo bioactivity (85 IU of FSH and 81 IU of LH, and 79 IU of FSH and 68 IU of LH, respectively).

The administration of gonadotrophins was discontinued as soon as at least three large follicles were present (diameter ≥ 15 mm), one of which had a diameter of ≥ 20 mm, and the E₂ concentration was higher than 1000 pmol/l per large follicle. On the day these criteria were satisfied no hMG was administered im and the infusion pump for iv hMG administration was stopped immediately before the im hCG injection of 10 000 IU of hCG (Pregnyl[®], NV Organon, Oss, The Netherlands) at 23.00 or 24.00 h. Buserelin was administered until 24.00 h that night.

Transvaginal ultrasound-guided oocyte retrieval was performed 35 h after the hCG injection. After oocyte retrieval, 5000 IU of hCG was injected im, followed by 1500 IU of hCG im between 10.00 and 12.00 h every other day until day 10 after follicle aspiration. Two batches of hCG (5000 IU and 1500 IU) were used for all patients. The retrieved oocytes were counted and their quality (immature, mature, postmature, luteinized) was scored. The number and quality of the embryos were scored also, on a scale from 1 (bad) to 5 (good). Embryo transfer was performed on day 3 after oocyte retrieval.

Blood sampling

Blood samples were collected between 08.00 and 10.00 h on days 20, 22, 24, 27 and 30 of the cycle prior to the IVF cycle (before and during buserelin treatment). If the hypo-oestrogenic state had not yet been reached, further samples were obtained every 2 or 3 days hereafter until the serum E₂ concentration was lower than 500 pmol/l. On the first day of hMG administration (day 3 of the IVF cycle) blood samples were taken immediately before the first im injection (at 17.00 h) or before the insertion of the iv catheter (between 14.00 and 17.00 h), and also at 20.00 and 23.00 h. The next day, blood samples were drawn at

08.00, 12.00, 17.00, 20.00 and 23.00 h. On day 5 blood was collected at 08.00 and 17.00 h. Thereafter, blood samples were taken once daily between 08.00 and 10.00 h for the rest of the IVF cycle until the day of the pregnancy test or the first day of the next period. The blood was allowed to clot at room temperature; it was then centrifuged at 1000 *g* for 5 min and the serum samples were stored at -20°C until assayed.

Ultrasound

Vaginal ultrasound examinations were performed on day 20 of the previous cycle, on day 10 of busserelin administration and subsequently every other day from day 5 of the IVF cycle until the day of hCG administration.

Hormonal assays

Concentrations of FSH, LH and E_2 were determined in all serum samples. Progesterone concentrations were determined during the luteal phase of the previous cycle, i.e. the cycle before the actual treatment, on day 7 of the stimulation phase and daily from day 12 of the stimulation phase onwards. Serum hCG concentrations were determined once daily during the treatment cycle. Concentrations of FSH and LH were determined by immunoradiometric assays that have been described previously (7). Concentrations of E_2 and progesterone were determined by radioimmunoassays (8). Human chorionic gonadotrophin concentrations were measured by an immunoenzymetric assay specific for intact hCG (Tandem-E hCG, Hybritech Europe SA, Liège, Belgium).

Statistics

Concentrations below the detection limit were assigned the minimum detectable concentration for the assay.

Differences between the groups were evaluated using Wilcoxon's rank sum test. In order to evaluate hormonal profiles, a limited number of characteristics were selected, namely the increment in serum FSH and E_2 concentrations in the stimulation phase, plateau FSH concentrations and peak E_2 concentrations in the stimulation phase.

Results

The serum FSH, LH and E_2 concentrations in the two groups are shown in Figs. 1, 2 and 3. One cycle in Group B was cancelled on cycle day 16 because of poor ovarian response. On ultrasonography no follicular growth was seen and serum E_2 concentrations did not exceed 500 pmol/l. In another cycle in Group B none of the retrieved oocytes were fertilized, despite good semen motility. The results for the luteal phase of the previous cycle and for the stimulation phase in Figs. 1, 2 and 3 include the values for the cancelled cycle and the cycle

without fertilization. The results for the luteal phase of the IVF cycle do not include the values of the said two cycles.

There were no significant differences between the groups treated by im and pulsatile iv hMG administration (Groups A and B, respectively). The rates of increase in serum FSH and E_2 concentrations during the initial days of hMG administration were similar (see insets in Figs. 1 and 3). The plateau FSH concentration, defined as the mean concentration from day 5 to day 10, was 8.9 IU/l in group A and 9.8 IU/l in Group B. The median values of the peak E_2 concentrations on the day before oocyte retrieval were 17 000 pmol/l in Group A and 15 500 pmol/l in Group B.

Serum progesterone concentrations in the luteal phase of the previous cycle reached maximum values on day 24. The median value in Group A was 78 nmol/l (tenth percentile (p_{10}) = 53 nmol/l and ninetieth percentile (p_{90}) = 110 nmol/l) and in Group B it was 58 nmol/l (p_{10} = 36 nmol/l and p_{90} = 110 nmol/l). During the stimulation phase of the IVF cycle, progesterone concentrations were undetectably low, increasing just before or just after the hCG injection. During the luteal phase of the IVF cycle high progesterone concentrations were observed, reaching maximum values 9 days after oocyte retrieval. The maximum progesterone concentration in the non-pregnant patients in Group A was 450 nmol/l (median value; p_{10} = 230 nmol/l, p_{90} = 710 nmol/l) and in Group B it was 580 nmol/l (p_{10} = 490 nmol/l, p_{90} = 1600 nmol/l). There were no significant differences between the groups.

The administration of hMG, which contained a certain amount of hCG, hardly influenced serum hCG levels. In the follicular phase of the IVF cycle serum hCG concentrations remained below the detection limit of the assay (2.5 IU/l), only increasing slightly on the last day before hCG administration (median value of 2.5 IU/l in Group A and 3 IU/l in Group B). One patient in Group B had raised hCG levels throughout the follicular phase (3.7–8.4 IU/l), even before the start of hMG administration. When other assays for hCG were applied for these serum samples (another sandwich assay and a radioimmunoassay), hCG concentrations were below the detection limit of the assay (2.0 IU/l and 1.0 ng/ml, respectively). The increased hCG values, therefore, will not be of clinical relevance. After the administration of 10 000 IU of hCG, serum hCG levels increased. The maximum serum hCG concentration was reached on the first day after oocyte retrieval (the median value in Group A was 320 IU/l (p_{10} = 150 IU/l, p_{90} = 460 IU/l) and in Group B it was 390 IU/l (p_{10} = 270 IU/l, p_{90} 670 IU/l)). Thereafter, a gradual fall in hCG concentrations was observed.

The median duration of the stimulation phase seemed shorter for Group B than for Group A (Table 1), but the difference was not statistically significant. The total number of ampoules used was lower for Group B than

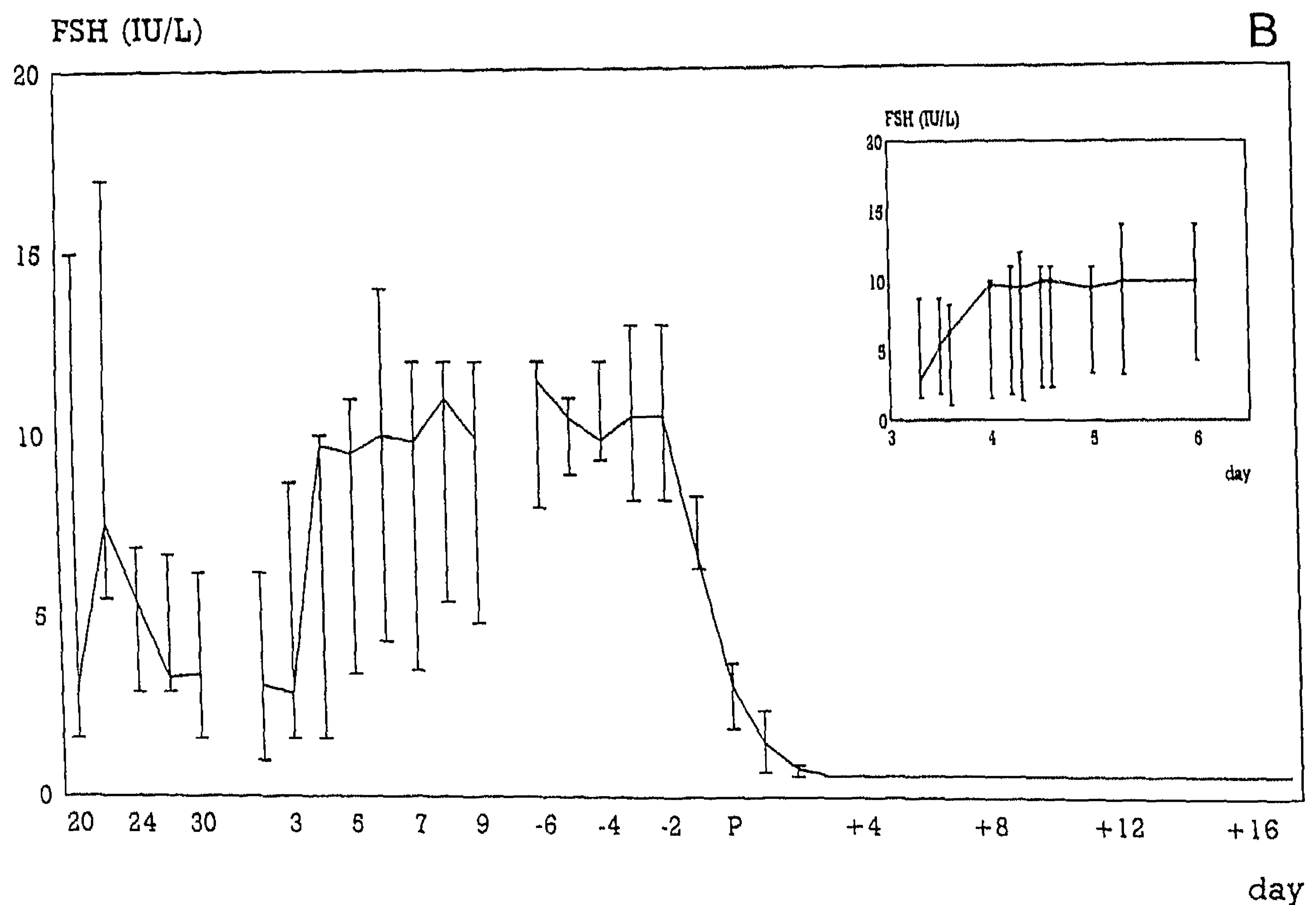
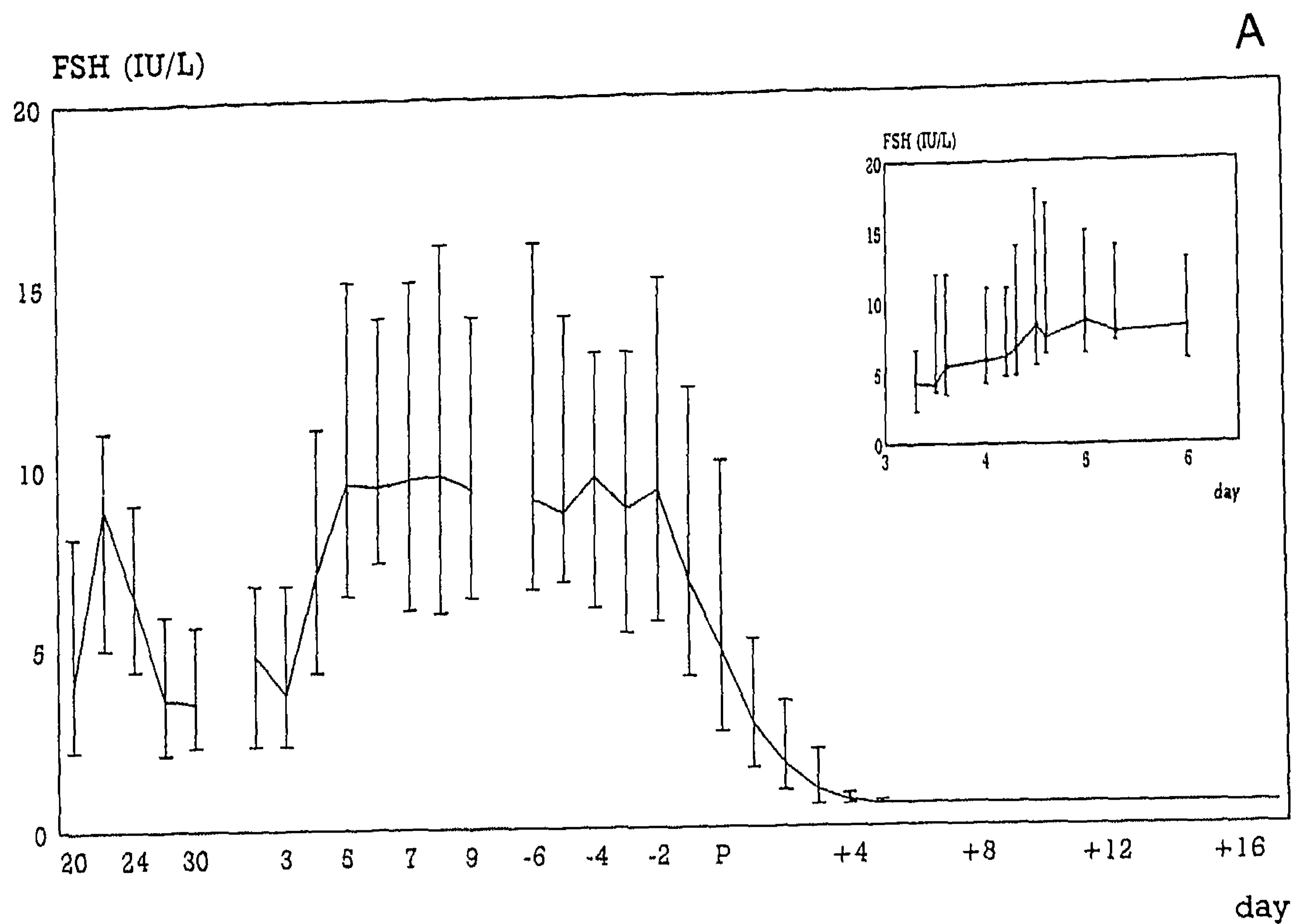


Fig. 1. Median serum follicle-stimulating hormone (FSH) concentrations (10th and 90th percentiles) during in vitro fertilization cycles in seven patients treated with human menopausal gonadotrophin (hMG) im (A) and seven patients treated with pulsatile iv hMG (B). From day 6 to the day of oocyte retrieval (P) Group B comprised six patients, and during the luteal phase it comprised five patients. FSH concentrations during the initial days of hMG treatment, when blood sampling was performed more frequently, are shown in the inset.

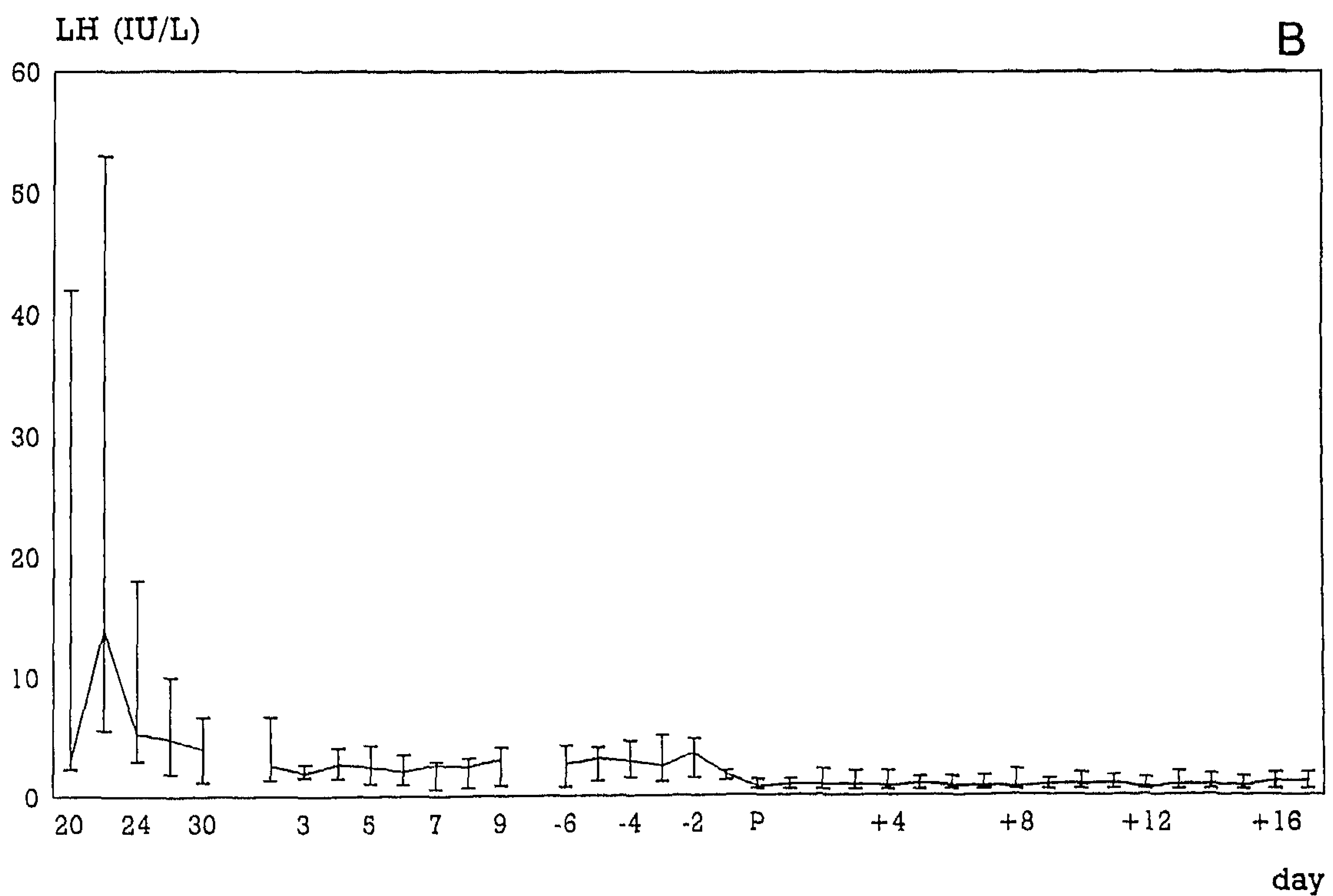
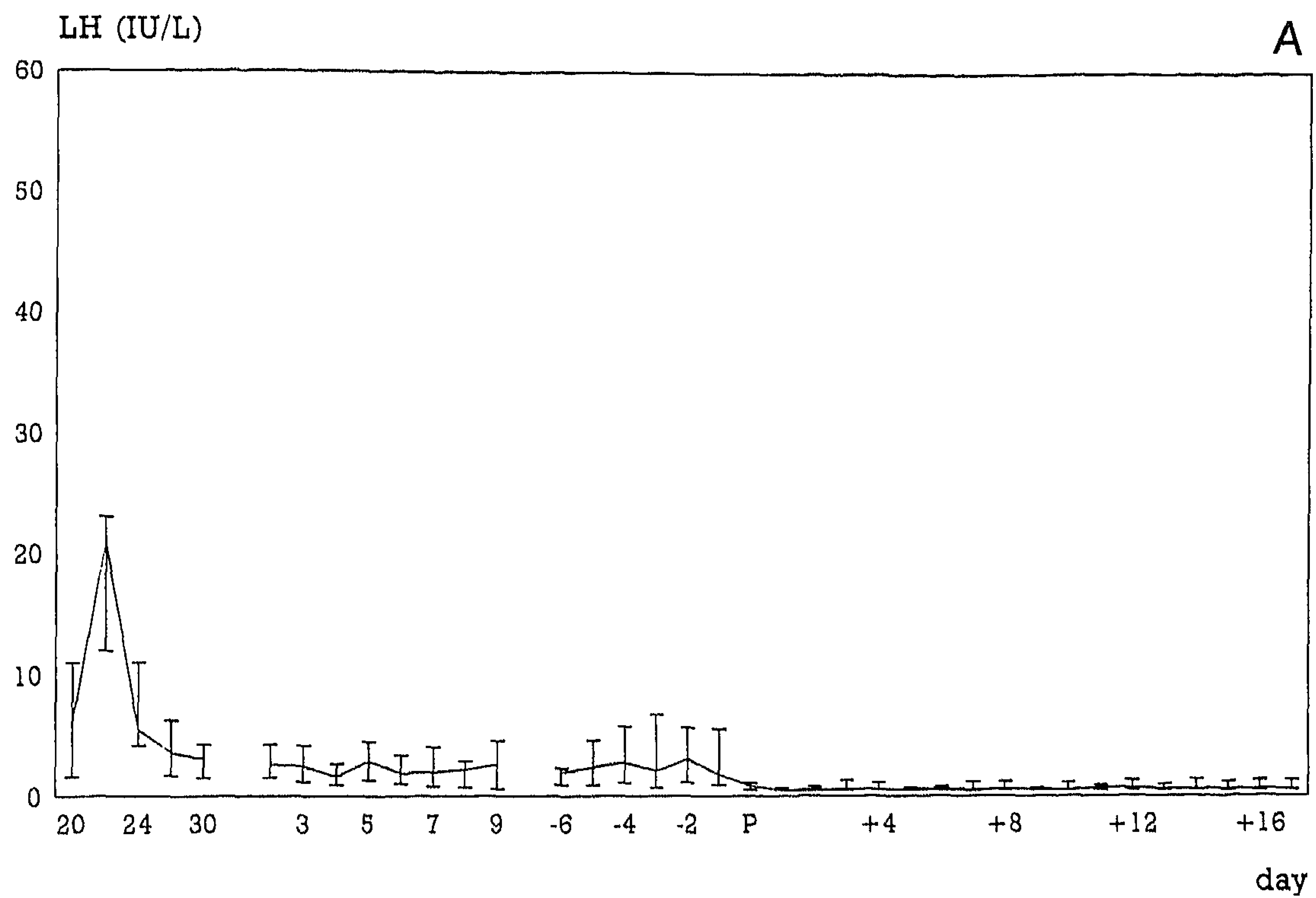


Fig. 2. Median serum luteinizing hormone (LH) concentrations (10th and 90th percentiles) during in vitro fertilization cycles in seven patients treated with human menopausal gonadotrophin (hMG) im (A) and seven patients treated with pulsatile iv hMG (B). From day 6 to the day of oocyte retrieval (P) Group B comprised six patients, and during the luteal phase it comprised five patients.

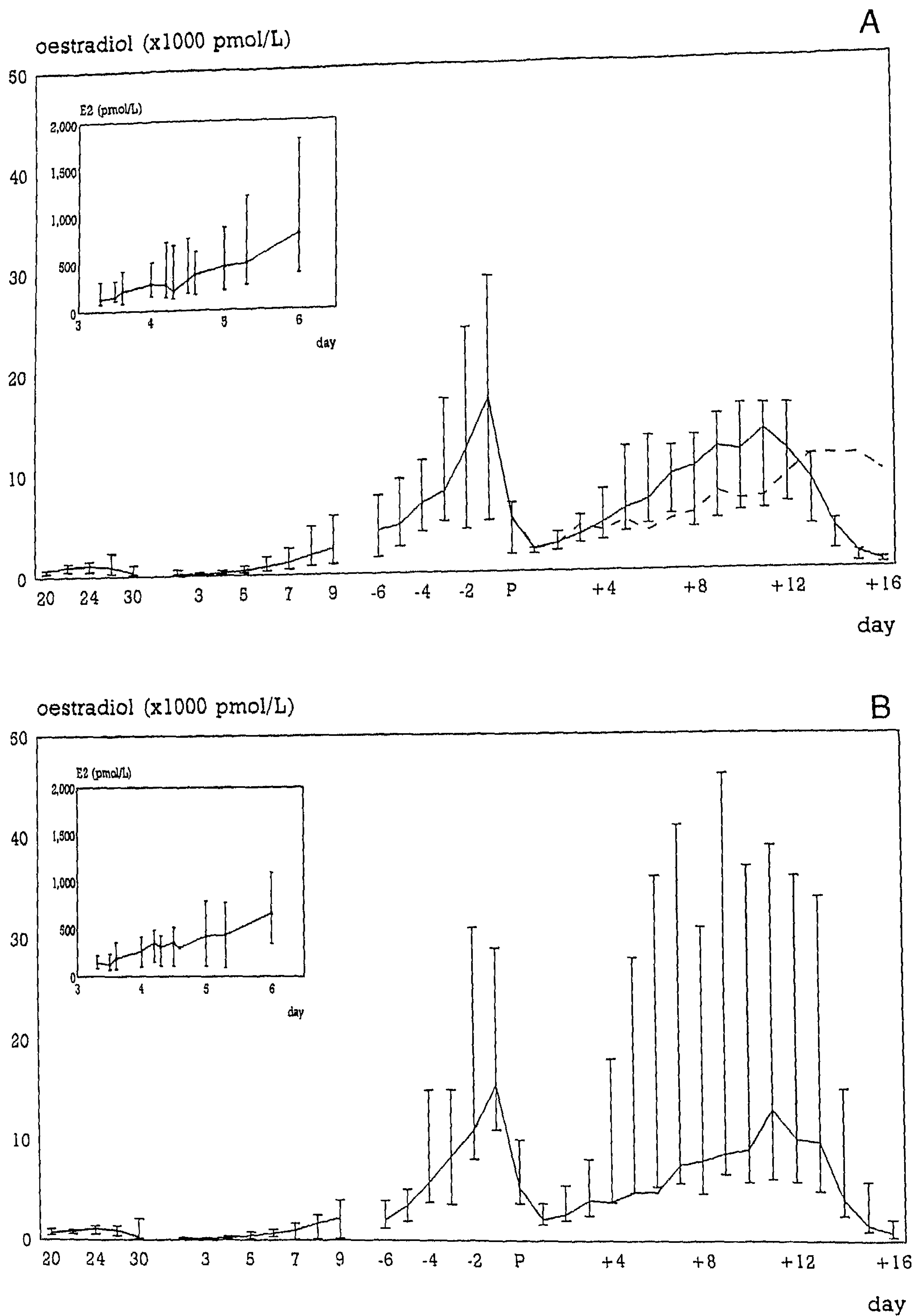


Fig. 3. Median serum 17β-oestradiol (E₂) concentrations (10th and 90th percentiles) during in vitro fertilization cycles in seven patients treated with hMG im (A) and seven patients treated with pulsatile iv hMG (B). From day 6 to the day of oocyte retrieval (P) Group B comprised six patients, and during the luteal phase it comprised five patients. The E₂ concentrations during the initial days of hMG treatment, when blood sampling was performed more frequently, are shown in the inset. In the luteal phase the results for four non-pregnant (solid line) and three pregnant (broken line) patients are shown.

Table 1. Cycle characteristics of seven patients treated with im human menopausal gonadotrophin (hMG) and seven patients treated with pulsatile iv hMG—median values with 10th and 90th percentiles in parentheses

	hMG im	hMG iv
Days of stimulation	12 (7–18)	9 (9–17)
Number of large follicles ^a	12 (5–24)	12 (0–22)
Number of retrieved oocytes	11 (2–17)	12 (7–17)
Fertilization rate (%) ^b	76 (50–100)	76 (0–94)
Mean embryo quality ^c	3.9 (3.0–5.0)	3.5 (2.4–4.4)

^a Largest diameter \geq 15 mm, as measured at the last ultrasound examination.

^b Six patients in the group treated with hMG iv.

^c Scale 1 (bad) to 5 (good); five patients in the group treated with hMG iv.

for Group A (105 ampoules versus 158 ampoules) owing to the lower daily dose. Table 1 shows median values of the numbers of follicles with a diameter greater than 15 mm at the last ultrasonography before oocyte retrieval, the numbers of retrieved oocytes, the fertilization rates and the mean embryo quality in the two groups. There were no significant differences between the groups.

One patient in Group B experienced leakage from the infusion pump system on day 13 of the stimulation phase of the cycle, leading to a drop in serum FSH concentrations from 12 to 4.9 IU/l. This problem was corrected within 24 h and serum FSH concentrations then increased again. Serum E₂ concentrations did not decrease during this leakage problem. None of the patients showed signs of phlebitis. Three patients in Group A had a positive pregnancy test. One of the three had bleeding 2 days later and a second pregnancy test was negative. There were no pregnancies in Group B.

Discussion

Hormonal profiles in the serum of women undergoing IVF treatment were monitored meticulously in the present study. A large interindividual variation in hormonal concentrations was observed, but median values in the two groups did not show significant differences. The increase in FSH concentrations after the start of hMG treatment was comparable in the two groups, as was the plateau FSH level between days 5 and 10 of the stimulation phase. In addition, the increase in E₂ concentrations during hMG administration and the peak E₂ concentrations were similar in the two groups. There were no differences between the groups as regards follicular growth, numbers of oocytes, fertilization rates or embryo quality. This indicates that pulsatile iv hMG administration does not lead to better results than the routine im hMG treatment.

It could be argued that the absence of better results

after pulsatile iv treatment was due to the lower daily dose in this group. This lower dose was based on the results of a previous study (2). However, the plateau FSH levels during hMG treatment were similar in the two groups, which means that the bioavailability of two ampoules of hMG administered im is comparable to that of 1.5 ampoules administered iv.

Previous studies on pulsatile iv hMG treatment showed that the pulsatile iv administration route was possibly more beneficial. Moreover, in one of these studies a lower daily hMG dose was used for pulsatile iv treatment as compared with im treatment. The first reports of the successful induction of ovulation by pulsatile iv hMG administration were by Afnan et al. (1) and Ho Yuen et al. (2). Ho Yuen and co-workers (3) reported the results of 107 pulsatile iv hMG treatment cycles in 30 women with refractory anovulation. Twelve women with polycystic ovary (PCO) syndrome had had previous im treatment cycles. Retrospective comparison of the two treatment methods reveals that less hMG was needed in the iv treatment cycles than in the im treatment cycles.

Edelstein et al. (4) performed a study involving six IVF patients with a low response to high-dose gonadotrophin administration (450 IU of purified FSH daily). They were treated with 450 IU of FSH per day by pulsatile iv administration. Although the plateau FSH levels were significantly higher after iv than after im treatment, the numbers of oocytes and fertilization rates did not improve after iv treatment. The authors concluded that pulsatile iv gonadotrophin treatment was not of major benefit for the stimulation of low-responder patients.

Prak et al. (5) gave hMG by pulsatile iv administration over 82 IVF cycles in women with tubal or unexplained infertility. They compared the results with those for 231 cycles in which hMG was administered im. The hMG therapy was combined with GnRHa treatment. The dose of hMG was 40% lower than that used in a previous cycle of im treatment. The numbers of follicles and oocytes were similar in the two groups but the pregnancy rate was significantly higher in the group treated iv than in the im treatment group. The authors ascribed this higher pregnancy rate to improved oocyte quality and endometrial receptivity as a result of the more physiological method of ovarian stimulation. However, none of the previously published studies was well-controlled and randomized.

As stated above, the fact that the beneficial effect of pulsatile iv hMG administration could not be confirmed in a randomized trial indicates that differences between study groups in previous investigations may explain the results. However, further randomized studies are needed in patients suffering from PCO syndrome and patients whose response was poor in previous cycles.

The half-life of FSH, as present in hMG, is approximately 11 h after iv administration (9). Because of the

long half-life, pulsatile hMG administration will result in a gradual accumulation of FSH in the circulation, instead of a pulsatile FSH pattern. In contrast to the pulsatile iv GnRH treatment, where because of the very short half-life of GnRH a clear pulsatile pattern is present (10), the individual hMG pulses will contribute very little to the high plateau FSH level. This is supported by our results during the initial treatment days, showing a gradual increase in FSH concentrations leading to a steady-state level, with comparable patterns in the two groups. To investigate whether very small fluctuations in the plateau FSH level occur during pulsatile iv treatment, very frequent blood sampling is required. The long half-life of FSH and the similar patterns of serum FSH concentrations during the entire stimulation period explain why pulsatile iv hMG treatment did not lead to better results.

In conclusion, the results of the present study indicate that the effect of pulsatile iv hMG treatment is not superior to that of im treatment in women with normal endocrine profiles. Moreover, the pulsatile iv treatment has practical disadvantages as compared with im treatment, namely the vulnerability of the administration system, as illustrated by the leakage from the infusion system experienced by one of the patients. Inserting and refilling the infusion system are time-consuming procedures. Furthermore, there is the serious risk of flebitis and subsequent sepsis, complications that were seen during pulsatile iv GnRH treatment. The routine im hMG treatment remains, therefore, preferable to pulsatile iv treatment.

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References

1. Afnan AMM, Hillier SG, Margara RA, Franks S, Winston RML. Pulsatile gonadotropin administration in in-vitro fertilization. *Lancet* 1984;i:1239
2. Ho Yuen B, Pride SM, Sime MO. Successful induction of ovulation and conception with pulsatile intravenous administration of human menopausal gonadotropins in anovulatory infertile women resistant to clomiphene and pulsatile gonadotropin-releasing hormone therapy. *Am J Obstet Gynecol* 1984;148:508-12
3. Ho Yuen B, Pride SM, Burch Calligari P, Leroux A-M, Moon YS. Clinical and endocrine response to pulsatile intravenous gonadotropins in refractory anovulation. *Obstet Gynecol* 1989;74:763-8
4. Edelstein MC, Brzyski RG, Jones GS, Muasher SJ. Ovarian stimulation for in vitro fertilization in low-responder patients using pulsatile intravenous follicle stimulating hormone. *J In Vitro Fertil Embryo Transfer* 1990;7:275-9
5. Prak FM, Bots RSGM, Evers JH. Intravenous pulsatile administration of gonadotrophins in an in-vitro fertilization programme. *Hum Reprod* 1992;7:176-9
6. WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge: Cambridge University Press, 1992:66-8
7. Kremer JAM, Borm G, Schellekens LA, Thomas CMG, Rolland R. Pulsatile secretion of luteinizing hormone and prolactin in lactating and nonlactating women and the response to naltrexone. *J Clin Endocrinol Metab* 1991;72:294-300
8. Thomas CMG, Corbey RS, Rolland R. Assessment of unconjugated oestradiol and progesterone serum levels throughout pregnancy in normal women and in women with hyperprolactinaemia who conceived after bromocriptine treatment. *Acta Endocrinol (Copenh)* 1977;86:405-14
9. Diczfalussy E, Harlin J. Clinical-pharmacological studies on human menopausal gonadotrophin. *Hum Reprod* 1988;3:21-7
10. Handelsman DJ, Jansen RPS, Boylan LM, Spalliviero JA, Turtle JR. Pharmacokinetics of gonadotropin-releasing hormone: comparison of subcutaneous and intravenous routes. *J Clin Endocrinol Metab* 1984;59:739-46

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