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Low-dose exogenous FSH initiated during the early, mid or late follicular phase can induce multiple dominant follicle development

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This prospective, randomized trial in normo-ovulatory women was designed to test whether administration of low-dose exogenous FSH initiated during the early, mid to late follicular phase can induce multiple dominant follicle development. Forty normal weight women (age 19–35 years, cycle length 25–32 days) participated. A fixed dose (75 IU/day) of recombinant FSH was started on either cycle day 3 (n = 13), 5 (n = 13) or 7 (n = 14) until the induction of ovulation with human chorionic gonadotrophin. Frequent transvaginal ultrasound scans and blood sampling were performed. Multifollicular growth occurred in all groups (overall in 60%), although day 7 starters showed less multifollicular growth. Age, cycle length and initial FSH and inhibin B concentrations were similar between subjects with single or multiple follicle development. However, for all women the lower the body mass index (BMI), the more follicles emerged (r = –0.44, P = 0.007). If multifollicular growth occurred, the length of the luteal phase was reduced (P = 0.002) and midluteal serum concentrations of LH (P = 0.03) and FSH (P = 0.004) were decreased and oestradiol (P = 0.002) and inhibin A (P = 0.01) were increased. In conclusion, interference with decremental serum FSH concentrations by administration of low dose FSH starting on cycle day 3, 5 or as late as day 7, is capable of disrupting single dominant follicle selection. The role of BMI in determining ovarian response suggests that differences in pharmacokinetics of exogenous FSH are involved. Multifollicular growth *per se* has a distinct effect on luteal phase characteristics. These observations may be relevant for the design of mild ovarian stimulation protocols.

Key words: follicle development/FSH/luteal phase/menstrual cycle/ovarian stimulation

Introduction

Due to demise of the corpus luteum and the subsequent decrease in oestradiol (Le Nestour *et al*., 1993) and inhibin biosynthesis (Roseff *et al*., 1989), FSH serum concentrations start to increase during the late luteal phase of the menstrual cycle (Hall *et al*., 1992). As soon as FSH concentrations surpass the 'threshold' for ovarian stimulation (Brown, 1978; Schoemaker *et al*., 1993) during the luteo-follicular transition, a cohort of small antral follicles is recruited and stimulated to ongoing growth (Fauser and van Heusden, 1997). These recruited follicles are of comparable potential to gain dominance and to continue their development. As a result of negative feedback from inhibin B (Groome *et al*., 1996) as well as oestradiol (Zeleznik *et al*., 1985) produced by this cohort of growing follicles, FSH concentrations decrease during the mid to late follicular phase effectively limiting the number of days on which FSH is above the threshold (referred to as the FSH window) (van Santbrink *et al*., 1995). Decremental follicular phase FSH appears to be essential for the selection of a single dominant follicle (with a diameter >9 mm) from the recruited cohort (Hodgen, 1982; Zeleznik *et al*., 1985; van Santbrink *et al*., 1995). Despite this decline in FSH concentrations, the most mature (dominant) follicle escapes from atresia and continues its growth by increased sensitivity for stimulation by FSH (presumably due to up-regulation by intraovarian factors (Erickson and Danforth, 1995; Fauser and Van Heusden, 1997) and the induction of LH receptors (Sullivan *et al*., 1999). Remaining follicles from the recruited cohort lack sufficient stimulation by FSH and enter atresia.

A brief but distinct elevation of FSH concentrations above the threshold in the early follicular phase does not affect dominant follicle development, although a transient increase in the number of small antral follicles could be observed (Schipper *et al*., 1998). On the contrary, a moderate but continued elevation of FSH concentrations during the mid to late follicular phase extending the FSH window does interfere with single dominant follicle selection and induces ongoing growth of multiple follicles (Schipper *et al*., 1998). This

confirms previous observations in the monkey showing that interference with decremental FSH can override the selection of a single dominant follicle (Zeleznik *et al*., 1985).

It is known from ovarian stimulation for IVF or intrauterine insemination (IUI), that a marked and continued elevation of FSH during the entire follicular phase will induce growth of large numbers of dominant follicles of different size (Hillier *et al*., 1985). These stimulation protocols overrule single dominant follicle selection by extending the FSH window by serum FSH concentrations far above the threshold. Recently, serious concerns have been expressed concerning the stimulation of growth of large numbers of follicles for assisted reproduction (Edwards *et al*., 1996; Fauser *et al*., 1999). Considering the risks, side-effects and the high costs of ovarian hyperstimulation and multiple gestation, current approaches for ovarian stimulation regimens should be re-evaluated (Hughes *et al*., 1998; de Jong *et al*., 2000a; Macklon and Fauser, 2000). Additional insight into the significance of the timing of initiation of exogenous FSH on follicle recruitment and selection may help to further develop and optimize milder ovarian stimulation protocols for assisted reproduction.

Materials and methods

Subjects and study design

This study was approved by the local Ethics Review Committee. A total of 40 healthy subjects was selected from responders to advertisements in local newspapers and interviews on the local radio and television. Written informed consent was obtained from each participant, and all subjects were paid for their participation.

Inclusion criteria were: (i) age 19–35 years; (ii) history of regular menstrual cycles (cycle lengths 25–32 days) and no use of oral contraceptive pills or other medical or hormonal treatment for ⩾3 months prior to study initiation; (iii) body mass index (BMI) 19–27 kg/m^2; (iv) midluteal progesterone concentrations, assessed 7 days before expected menses, >25 nmol/l; (v) no prior treatment for infertility; (vi) willingness to use contraceptive measures (intrauterine device, condoms or prior tubal ligation) or to refrain from intercourse during the study period. Detailed oral and written information concerning the importance of contraception was given before and during the study period.

All subjects were studied during a single menstrual cycle. At the onset of menses, subjects were assigned to one of three interventions using a computer-generated randomization schedule, assigned via numbered sealed envelopes. Group cycle day (CD) 3 received a daily fixed dose of 1 ampoule (75 IU) recombinant FSH (rFSH, Gonal-F®; Serono Benelux BV, The Hague, The Netherlands), starting on CD 3 until the administration of human chorionic gonadotrophin (HCG). Group CD 5 and group CD 7 received a similar daily dose rFSH, but started on CD 5 or CD 7 respectively. rFSH was administered s.c. by self-injection at 22.00 h. Participants were instructed by qualified nurses. As soon as the largest follicle reached a diameter of ⩾18 mm, a single dose of 5000 IU HCG (Profasi®; Serono Benelux BV) was administered i.m. at 22.00 h to induce ovulation.

Monitoring consisted of transvaginal sonography (TVS) and blood sampling was performed between 8.00 and 10.00 h, every 2 days starting on CD 3. As soon as the largest dominant follicle reached a diameter of ⩾15 mm, TVS and blood sampling were performed on a daily basis until the day of HCG. Finally, TVS and blood sampling were repeated on day HCG+2 and HCG+8. The day of initiation of the following menstrual period was recorded. TVS was performed by a single observer (F.H.), using a 6.5 MHz transvaginal transducer (EUB-420; Hitachi Medical Corp., Tokyo, Japan). Follicle diameter was calculated as the mean diameter (measured in two dimensions when <9 mm, and in three dimensions if at least one diameter was ⩾9 mm) as published previously (Pache *et al*., 1990; van Santbrink *et al*., 1995).

Hormone assays

Blood samples were centrifuged within 2 h after withdrawal and stored at –20°C until assayed. Serum FSH, LH, and progesterone concentrations were measured by chemiluminescent immunoassay [Immulite, Diagnostic Products Corporation (DPC), Los Angeles, CA, USA] in single assays. Addition of various doses of rFSH to serum without FSH yielded a curve parallel to that of the standard. Recovery of rFSH was 55.5 ± 3.7% (SD, $n = 4$). Serum oestradiol concentrations were measured in duplicate using radioimmunoassay kits provided by DPC (Los Angeles, CA, USA), as described previously (Fauser *et al*., 1991). Dimeric inhibin A and inhibin B concentrations were also determined in duplicate using an immunoenzymometric assay (Serotec, Oxford, UK), as described previously (Groome *et al*., 1996). Intra- and inter-assay coefficients of variation were <5% and <7% for FSH, <5% and <6% for LH, <10% and <10% for progesterone, <5% and <7% for oestradiol, <8% and <15% for inhibin A and <8% and <14% for inhibin B respectively. All samples from one subject were run in the same assay.

Data analysis

Results are presented as the median and range. Comparisons of outcome measures between the three randomized groups were performed using the Kruskal-Wallis *H*-test for continuous data and using the χ^2-test for binary variables. Two group comparisons (between single and multiple dominant follicle selection) were performed using the Mann-Whitney *U*-test. Comparisons of means of values in time between two groups were performed using analysis of variance (ANOVA). Correlation coefficients given are Pearson's. *P* values are two-sided and 0.05 was considered the limit for statistical significance. A dominant follicle was defined as a follicle with a mean diameter of ⩾10 mm (Pache *et al*., 1990; van Santbrink *et al*., 1995). A pre-ovulatory follicle was defined as a follicle with a mean diameter of ⩾15 mm. This distinction seems clinically important, since not all follicles of ⩾10 mm necessarily develop into pre-ovulatory follicles. Arbitrarily, progesterone concentration (day of HCG) ⩾3.2 nmol/l (1.0 ng/ml) was considered as premature luteinization (Harada *et al*., 1995; Ganirelix dose-finding study group, 1998). The four subjects showing premature ovulation were not included in the analysis of hormone serum concentrations on the day of HCG. Data were analysed using the commercially available software package SPSS, Inc. (Chicago, IL, USA).

Results

Baseline characteristics and follicle development

Forty normo-ovulatory women entered the study protocol. There were no drop-outs during the study. All subjects were ovulatory in the intervention cycle, as assessed by TVS and elevated serum progesterone concentrations in the midluteal phase. With regard to the distribution of age, BMI, cycle length and baseline endocrine serum concentrations (FSH, oestradiol and inhibin B), no significant differences were found between the three groups (Table I). Parity was equally distributed over the three groups (nullipara versus multipara

Table I. Clinical, endocrine and menstrual cycle characteristics (median and range) in 40 normo-ovulatory volunteers receiving exogenous FSH (75 IU/day) starting on cycle day (CD) 3, 5 or 7

	CD 3	CD 5	CD 7
n	13	13	14
Age (years)	29 (21–35)	29 (22–34)	28 (20–35)
BMI (kg/m^2)	22 (19–27)	23 (19–27)	22 (19–27)
Cycle length (days)	28 (26–31)	28 (25–31)	28 (26–30)
Cycle day 3			
FSH (IU/l)	6.5 (2.8–13.5)	6.3 (3.3–9.8)	7.7 (5.4–13.1)
Oestradiol (pmol/l)	127 (64–220)	175 (91–404)	137 (109–264)
Inhibin B (ng/l)	87 (21–192)	130 (12–213)	114 (57–179)
Day of HCG			
FSH (IU/l)	5.8 (4.5–9.6)	6.1 (5.5–10.9)	6.9 (4.7–20.4)
LH (IU/l)	6.8 (1.5–19.1)	4.6 (1.0–21.1)	7.9 (0.3–57.2)
Oestradiol (pmol/l)	945 (660–2840)	1106 (470–2302)	985 (682–1610)
Progesterone (nmol/l)	4.3 (2.5–5.4)	3.6 (1.4–27.3)	4.1 (2.4–8.7)
Inhibin A (ng/l)	51 (28–224)	69 (27–143)	73 (38–195)
Follicle number (⩾10 mm)	2.5 (1–6)	2 (1–7)	1 (1–5)
Follicle number (⩾12 mm)	1.5 (1–6)	2 (1–5)	1 (1–4)
Follicle number (⩾15 mm)	1.5 (1–4)	1 (1–3)	1 (1–3)
No. of subjects with multiple DF[a]* (%)	9 (69)	10 (77)	5 (36)
No. of subjects with multiple PF[b] (%)	7 (54)	6 (46)	4 (29)
Total amount rFSH (IU)**	750 (600–1125)	525 (375–825)	450 (75–900)
Follicular phase length (days)	13 (12–19)	13 (11–17)	14 (9–20)
Luteal phase length (days)	12 (9–15)	12 (8–16)	13 (11–17)
Progesterone (nmol/l)[c]	53 (23–287)	44 (18–109)	61 (17–96)

[a]DF = dominant follicle (diameter ⩾10 mm).
[b]PF = pre-ovulatory follicle (diameter ⩾15 mm).
[c]Midluteal.
*P = 0.07 (χ^2-test).
**P = 0.001 (Kruskal–Wallis H-test).

in group CD 3, CD 5 and CD 7: 54 versus 46%, 54 versus 46% and 57 versus 43% respectively).

A daily dose of 75 IU of rFSH was able to interfere with single dominant follicle selection in 24 subjects (60%) in all three groups. There was no difference in multifollicular growth (defined as at least two follicles ⩾10 mm) or the development of multiple pre-ovulatory follicles (defined as a follicle ⩾15 mm) between the three groups, although a tendency was seen towards less multifollicular growth in group CD 7 (P = 0.07) (Table I). Group CD 7 versus all other subjects (groups CD 3 and CD 5) did show a statistical difference in the development of multiple dominant follicles (36 versus 73%; P = 0.02). Figure 1 shows the mean follicle number in both ovaries during the follicular phase in subjects presenting with multiple dominant follicle development, in each group.

Table II shows the comparison of women presenting with single (n = 16) or multiple (n = 24) dominant follicle development following the administration of rFSH. With regard to age, BMI, cycle length and initial (CD 3) hormone concentrations, no differences were found between the two groups. There was no difference in parity between the groups with mono- or multifollicular growth (nullipara versus multipara: 62 versus 38% and 50 versus 50% respectively). The lower the BMI, the more follicles developed (Figure 2: r = −0.44, P = 0.007). All subjects with a BMI ⩾24 kg/m^2 (n = 10) did not show multiple pre-ovulatory follicles. BMI was significantly higher in women presenting with single pre-ovulatory follicle development [median BMI 23 kg/m^2 (range 19–27) versus 22 kg/m^2 (range 19–24), P = 0.03]. There was no correlation between age and the number of follicles developing (r = 0.03, P = 0.88). The growth rate of the largest follicle in subjects presenting with single or multiple dominant follicle selection was similar (Figure 3).

Serum hormone concentrations

Figure 4 shows the hormone concentrations during the follicular phase (separate for women presenting with single or multiple dominant follicles), comparing groups CD 3, CD 5 and CD 7. In group CD 7, FSH concentrations of the subjects with multifollicular growth did not decrease during the early to midfollicular phase, whereas the subjects exhibiting monofollicular growth presented with decremental FSH concentrations (Figure 5, P = 0.02). In group CD 7, oestradiol concentrations were lower on cycle day 7 if multiple dominant follicles developed [Figure 5: median oestradiol 151 pmol/l (range 137–253) versus 210 pmol/l (range 183–983), P = 0.03]. In group CD 3 and CD 5 no differences were seen in FSH and oestradiol concentrations at the day of initiation of rFSH between the subjects who showed mono- and multifollicular growth [group CD 3: median FSH 6.4 IU/l (range 6.2–13.5) versus 7.9 IU/l (range 2.8–12.2) and median oestradiol 138 pmol/l (range 64–220) versus 126 pmol/l (range 93–156); group CD 5: median FSH 7.7 IU/l (range 5.7–9.5) versus 6.9 IU/l (range 6.4–8.5) and median oestradiol 176 pmol/l (range 106–1163) versus 176 pmol/l (range 144–196)]. In all groups (CD 3, CD 5 and CD 7) inhibin B concentrations at the day of initiation of rFSH were similar between subjects showing mono- or multifollicular growth [median inhibin B

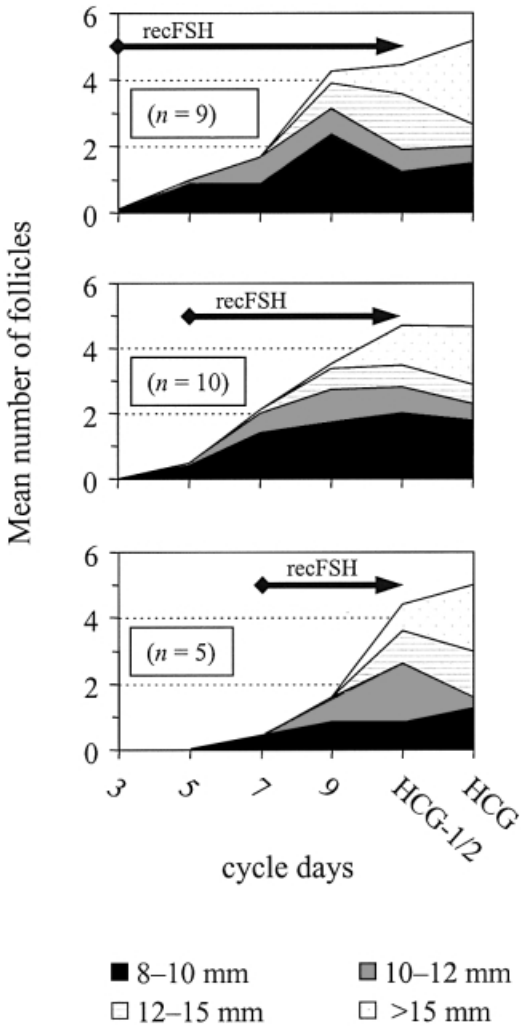

Figure 1. Number of follicles (⩾8 mm) during the follicular phase in 24 normo-ovulatory women receiving fixed low daily doses (75 IU) of exogenous FSH (starting on either cycle day 3, 5 or 7), who developed multiple dominant follicles (⩾10 mm) on the day of human chorionic gonadotrophin (HCG) administration. Areas represent the mean number of follicles in both ovaries on a given day in all subjects, with shaded areas representing different size classes. The time scale on the *x*-axis is divided into cycle days (day of onset of menses = cycle day 1) and days prior to administration of HCG.

in group CD 3, CD 5 and CD 7: 62 ng/l (range 26–91) versus 90 ng/l (range 21–192), 80 ng/l (range 76–151) versus 115 ng/l (range 51–412) and 113 ng/l (range 53–160) versus 114 (range 75–180) respectively].

Serum hormone concentrations on the day of HCG, comparing subjects presenting with single- and multiple dominant follicle selection, are presented in Table II. Differences in oestradiol and inhibin A concentrations on the day of HCG were even more pronounced in subjects presenting with single compared to multiple pre-ovulatory follicles [median oestradiol 759 pmol/l (range 470–1464) versus 1516 pmol/l (range 839–2840), $P < 0.001$; median inhibin A 49 ng/l (range 27–143) versus 119 ng/l (range 43–224), $P < 0.001$]. Premature luteinization (defined as a progesterone concentration on the day of HCG ⩾3.2 nmol/l) was observed in 80.6% of all subjects, distributed over all groups (CD 3, CD 5 and CD 7: 89, 70 and 70% respectively) and both in single or multiple dominant follicle development (75 versus 82%). Oestradiol concentrations on the day of HCG were comparable between the subjects presenting with or without premature luteinization [median oestradiol 1106 IU/l (range 470–2840] versus 985 IU/l (range 536–1541)].

Midluteal serum hormone concentrations between women exhibiting single and multiple dominant follicle selection are presented in Table II. The differences in midluteal serum concentrations were more pronounced in single versus multiple pre-ovulatory follicle development [median FSH 2.6 IU/l (range 0.2–7.3) versus 0.7 IU/l (range 0.1–1.7), $P < 0.001$; median LH 4.1 IU/l (range 0.1–8.5) versus 0.4 IU/l (range 0.1–13.8), $P = 0.006$; median oestradiol 497 pmol/l (range 158–969) versus 729 pmol/l (range 323–2486), $P = 0.001$; median inhibin A 61 ng/l (range 9–122) versus 91 ng/l (range 37–205), $P = 0.01$]. Midluteal serum hormone concentrations between the three randomization groups were similar (data not shown).

Cycle characteristics

Reported previous cycle length of the subjects correlated positively with the cycle length in the intervention cycle ($r = 0.44$, $P = 0.005$; data not shown). The intervention cycle was shorter compared to the normal cycle length [median length 26 days (range 17–33) versus 28 days (range 25–33), $P < 0.001$]. Four subjects showed premature ovulation (follicle collapse before the dominant follicle reached a diameter of 18 mm) and did not receive HCG. The difference in the length of the intervention cycle and the normal cycle remained when corrected for subjects showing premature ovulation [median length 26 days (range 21–33) versus 28 days (range 25–31), $P < 0.001$].

The length of the follicular phase (from onset of menses until 2 days after HCG administration or until day of spontaneous ovulation) comparing the three groups, is presented in Table I. Within groups CD 3 and CD 5, no difference was seen in the length of the follicular phase comparing women with mono- or multifollicular growth [median follicular phase 13 (range 12–14) versus 14.5 days (range 12–19) in group CD 3 and 13 (range 11–16) versus 16 days (range 11–17) in group CD 5]. In group CD 7, the subjects exhibiting multifollicular growth had a significantly longer follicular phase [median follicular phase 15 days (range 14–20) versus 12 days (range 9–14), $P = 0.004$]. In this group, the mean diameter of the lead follicle on the day of initiation of rFSH (CD 7) was significantly larger in the group showing monofollicular growth compared with the group with multifollicular growth [median size lead follicle 10.6 mm (range 8.1–17.6) versus 7.8 mm (7.2–8.8)].

The luteal phase (from 2 days after HCG administration or from the day of spontaneous ovulation until the start of the next menstrual period) in the subjects with multiple dominant follicle selection was significantly shorter compared with the subjects with single dominant follicle development (Table II, $P = 0.002$). Two subjects were excluded for calculation of the luteal phase length, since these subjects conceived during the intervention cycle.

Table II. Clinical, endocrine and menstrual cycle characteristics (median and range) in 40 normo-ovulatory women presenting with either single (n = 16) or multiple (n = 24) dominant follicle (⩾10 mm) development following exogenous recombinant FSH starting on either cycle day 3, 5 or 7

	Dominant follicle		P[a]
	Single	Multiple	
n	16	24[b]	
Age (years)	29 (20–34)	29 (21–35)	–
Body mass index (kg/m^2)	23 (19–27)	22 (19–27)	–
Cycle length (days)	28 (26–31)	28 (25–31)	–
Cycle day 3			
FSH (IU/l)	7.0 (2.8–12.2)	7.2 (3.3–13.5)	–
Oestradiol (pmol/l)	137 (93–264)	153 (64–404)	–
Inhibin B (ng/l)	114 (26–179)	91 (12–213)	–
Day of HCG			
FSH (IU/l)	6.6 (4.5–20.4)	6.0 (4.5–15.0)	–
LH (IU/l)	7.4 (2.0–49.3)	4.6 (0.3–57.2)	–
Oestradiol (pmol/l)	793 (470–1353)	1176 (536–2840)	0.02
Progesterone (nmol/l)	4.1 (2.4–27.3)	4.3 (1.4–11.7)	–
Inhibin A (ng/l)	50 (28–143)	82 (27–224)	<0.05
Follicle number (⩾10 mm)	1	3 (2–7)	–[c]
Follicle number (⩾12 mm)	1	2 (1–6)	<0.001
Follicle number (⩾15 mm)	1	2 (1–4)	<0.001
Midluteal			
FSH (IU/l)	2.5 (0.2–6.0)	0.9 (0.1–7.3)	0.004
LH (IU/l)	4.2 (0.1–8.5)	0.8 (0.1–13.8)	0.03
Oestradiol (pmol/l)	482 (158–914)	694 (286–2486)	0.002
Progesterone (nmol/l)	50.2 (16.6–90.5)	54.9 (22.8–287)	0.08
Inhibin A (ng/l)	55 (9–122)	79 (37–205)	0.01
Menstrual cycle characteristics			
Follicular phase length (days)	14 (9–19)	13 (11–20)	–
Luteal phase length (days)	13 (8–17)	11 (9–14)	0.002

[a]Mann–Whitney *U*-test.
[b]Nine from group CD 3, 10 from group CD 5 and five from group CD 7.
[c]Based on this criterion both groups were separated.

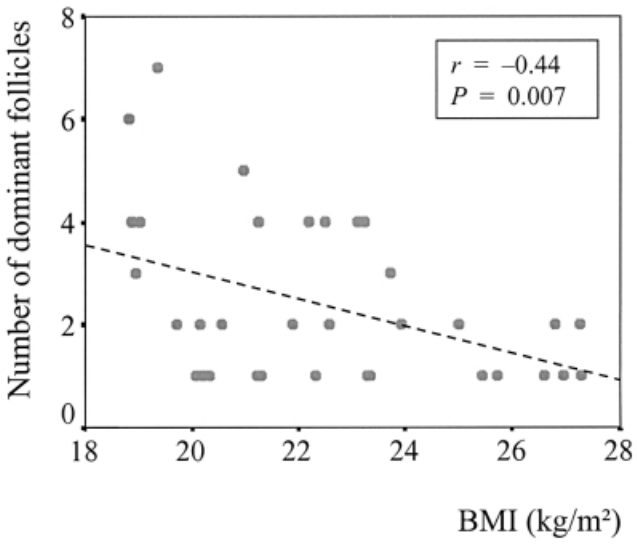

Figure 2. Distribution of the number of dominant follicles (⩾10 mm) observed in both ovaries on the day of HCG administration related to body mass index (BMI) in 40 normo-ovulatory women. Subjects received low daily doses of exogenous FSH during the follicular phase (starting on either cycle day 3, 5 or 7). Pearson's correlation: r = –0.44, P = 0.007.

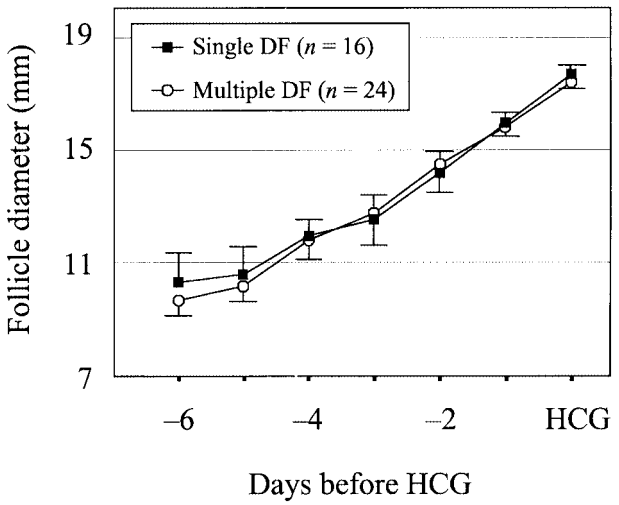

Figure 3. Diameter (mean ± SEM) of the largest dominant follicle in the follicular phase of intervention cycles in 40 normo-ovulatory women receiving low daily doses of exogenous FSH, starting on either cycle day 3, 5 or 7. The lines represent the subjects presenting with single- or multiple dominant follicle development (⩾10 mm). The scale on the *x*-axis is expressed as days prior to the late follicular phase administration of HCG. DF = dominant follicle(s).

Discussion

During the luteo-follicular transition, a cohort of small antral follicles at a given stage of development is recruited to gain gonadotrophin dependence and continued growth. Around the midfollicular phase, the most mature follicle gains dominance over other cohort follicles. This dominant follicle continues

its growth despite decremental FSH concentrations, whereas the remaining follicles from the recruited cohort enter atresia, due to insufficient stimulation by FSH (Fauser and van Heusden, 1997). Decreasing FSH concentrations and subsequent closure of the FSH window (Baird, 1987; Fauser *et al*., 1993) seems essential for single dominant follicle selection. This concept adds the element of time to the FSH threshold theory and emphasizes the importance of a transient increase of FSH above the threshold concentration for single dominant follicle selection. Interference with this decremental FSH—and hence extending the FSH window—can override the selection of a single dominant follicle as previously shown in primates (Zeleznik *et al*., 1985) and the human (Schipper *et al*., 1998). In case the dominant follicle is removed in the late follicular phase in monkeys, other cohort follicles can no longer be rescued and recruitment of a new cohort of follicles occurs (Goodman and Hodgen, 1979). The exact moment where single dominant follicle selection has become irreversible remains to be established.

The present study shows that the administration of low doses of exogenous FSH starting on CD 3, 5 or as late as CD 7, can overrule single dominant follicle selection in the majority of women. In subjects who did not respond, the amount of exogenous FSH might have been insufficient to elevate the FSH concentration long enough above the FSH threshold for the remaining non-dominant follicles from the recruited cohort. A negative correlation was found between BMI and the number of dominant follicles developed. Lower bodyweight women

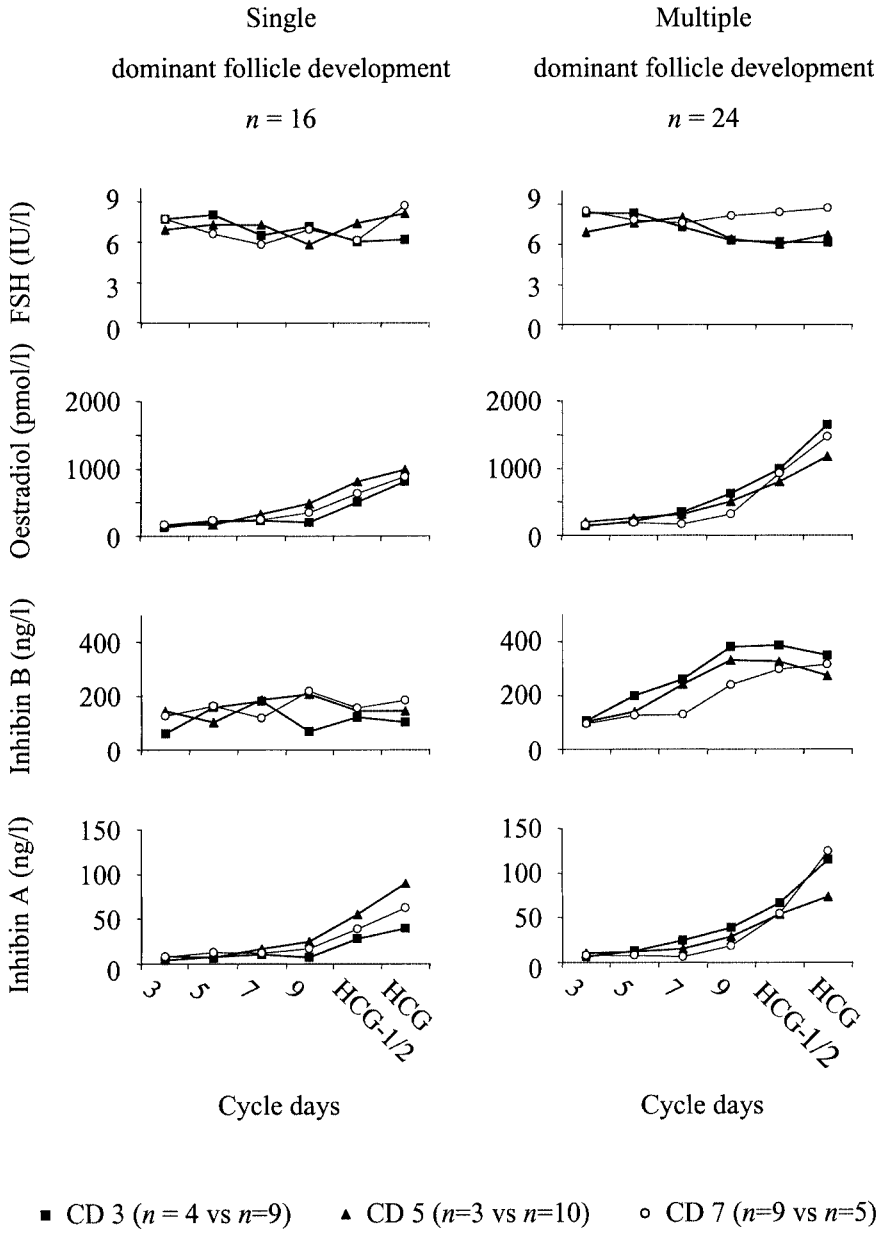

Figure 4. Follicular phase FSH, oestradiol and inhibin B and A concentrations in 40 normo-ovulatory women receiving low daily doses of exogenous FSH, starting on either cycle day 3, 5 or 7. The panels represent the subjects who developed single (left panels) or multiple (right panels) dominant follicles (⩾10 mm) on the day of HCG administration. The time scale on the *x*-axis is divided into cycle days (day of onset of menses = cycle day 1) and days prior to administration of HCG. Data are shown as the mean values.

received a higher FSH dose per kg. However, no correlation was found between BMI and FSH concentrations in the mid or late follicular phase (data not shown). Individual differences in metabolic clearance rate (Diczfalusy and Harlin, 1988) and distribution volume of FSH (Chong *et al*., 1986; Mannaerts *et al*., 1993), related to body weight may be involved. Moreover, the influence of weight on induction of ovulation for IVF has previously been stressed (Chong *et al*., 1986; Lashen *et al*., 1999). Women presenting with multiple dominant follicles exhibit higher mid to late follicular phase inhibin B concentrations and higher late follicular phase oestradiol and inhibin A concentrations. Serum FSH concentrations were not distinctly different, suggesting differential ovarian responsiveness to FSH being the predominant factor determining mono- or multifollicular response. On the other hand, an immunoassay was used to assess FSH concentrations (combining endogenous and exogenous FSH) and therefore differences in in-vivo bioactivity may not be disclosed (Mannaerts *et al*., 1991; Rose and Gaines-Das, 1998).

Surprisingly we did not find a significant difference in the number of women with irreversible single dominant follicle selection between the three groups. However, there was a tendency toward a lower percentage of women presenting with multiple dominant follicle development when FSH was initiated on CD 7 (36 versus 69 and 77%). A larger number of subjects is required to establish whether this tendency represents a true difference. There was a significant difference when CD 7 initiation was compared with the two other groups (CD 3 and CD 5) together ($P = 0.02$). The subjects who showed multiple dominant follicle selection after intervention in the mid to late follicular phase had a longer follicular phase and no signs of selection of the dominant follicle at the day of initiation of exogenous FSH. These results confirm the FSH window hypothesis: administration of low doses of exogenous FSH will induce multifollicular development, unless selection of the dominant follicle has occurred (defined as the appearance of a follicle ⩾10 mm), coinciding with a rise in oestradiol and a decrease in FSH concentrations.

We observed a substantial number of subjects presenting with premature luteinization (a rise in serum progesterone concentration on or before the day of HCG administration, which was based on ultrasound criteria only) in all intervention cycles. The definition used for premature luteinization and the threshold concentrations used for progesterone rise differ from study to study. Threshold concentrations for a subtle rise in progesterone on the day of HCG differ between 0.5 ng/ml (1.59 nmol/l) (Schoolcraft *et al*., 1991) and 1.5 ng/ml (4.77 nmol/l) (Sengoku *et al*., 1994). The occurrence of premature luteinization in our study was not dependent on the day of initiation of exogenous FSH, the occurrence of single or multiple dominant follicle selection or late follicular phase

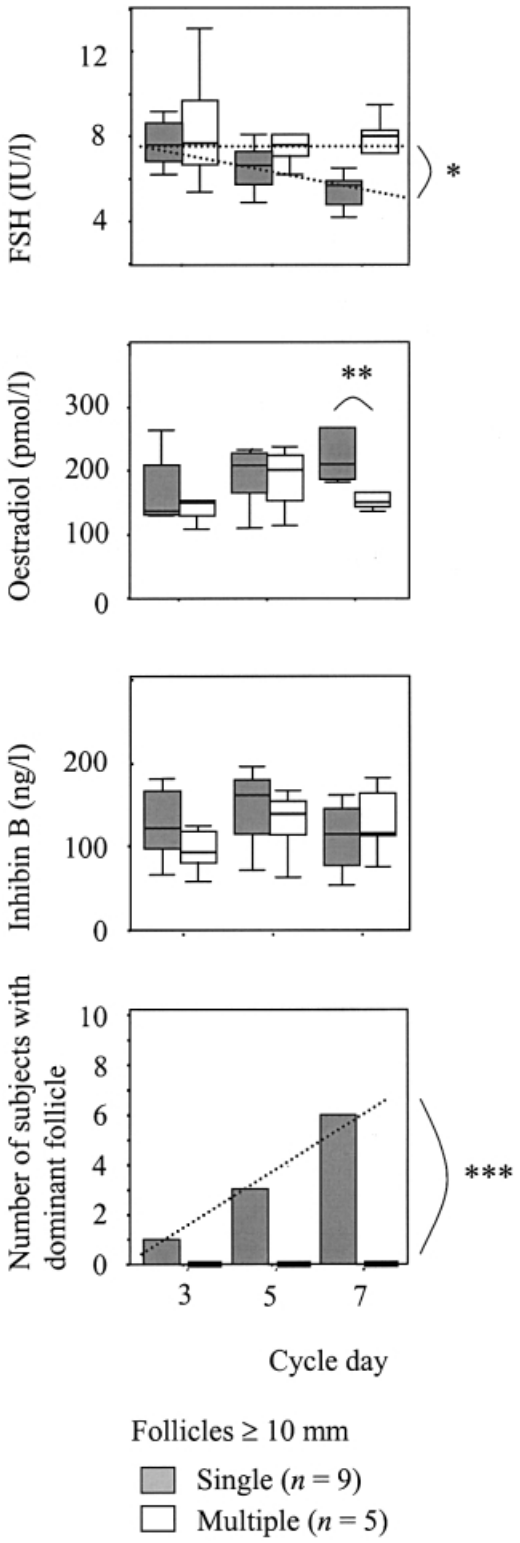

Figure 5. FSH, oestradiol and inhibin B concentrations and number of women presenting with a dominant follicle at various days during the early to midfollicular phase of 14 normo-ovulatory women receiving low daily doses of exogenous FSH starting on cycle day 7. Each panel shows the difference between the subjects presenting with single- or multiple dominant follicle development (⩾10 mm). Boxes indicate 25th and 75th percentiles, with the horizontal line representing the median value. Whiskers span the range between the 5th and 95th percentiles of the data. *Decremental FSH concentrations in women presenting with monofollicular growth and plateau FSH in multifollicular growth ($P = 0.02$, ANOVA). **Oestradiol concentration on cycle day 7 significantly different ($P = 0.03$, Mann-Whitney *U*-test); ***($P = 0.006$, ANOVA).

oestradiol concentrations (data not shown). The mechanism underlying a subtle progesterone rise during the late follicular phase after ovarian stimulation, the incidence and the implications for outcome of IUI or IVF are not yet clear. Some studies associate premature luteinization in IVF with poor oocyte quality, decreased fertilization rates, poor embryo quality and impaired implantation (Schoolcraft *et al*., 1991; Silverberg *et al*., 1991; Harada *et al*., 1995), whereas other studies suggest no difference in pregnancy outcome (Edelstein *et al*., 1990; Hofmann *et al*., 1996; Ubaldi *et al*., 1996). Studies regarding the effects of premature luteinization on clinical outcome in IUI are also contradictory (Sengoku *et al*., 1994; Manzi *et al*., 1995).

Although we found a correlation between the reported normal cycle length and the length in the intervention cycle, the intervention cycle was shorter. This phenomenon remained if we corrected for subjects showing a premature ovulation. There is no reason to believe that the administration of exogenous FSH will accelerate the growth of the lead follicle (Pache *et al*., 1990). However, the administration of HCG might shorten the follicular phase in some women since ovulation was triggered as soon as the lead follicle reached a diameter of 18 mm. In a spontaneous cycle, the median preovulatory follicle size is 21 mm, with a range of 18–30 mm (van Santbrink *et al*., 1995). A decreased luteal phase length in the subjects presenting with multiple dominant follicle development may represent an additional explanation for the shorter intervention cycle.

The length of the luteal phase was significantly reduced in all the cycles with multiple dominant follicle development. A short luteal phase in cycles stimulated with gonadotrophins for IVF has previously been documented (Laatikainen *et al*., 1988; de Jong *et al*., 2000b). However, it remains unclear if the reduction in luteal phase length is a consequence of gonadotrophin therapy, co-treatment with gonadotrophin-releasing hormone analogues, HCG or the follicle puncture procedure (Smitz *et al*., 1990). In the current study, the short luteal phase was independent from the administration of HCG or the amount of exogenous FSH administered. Midluteal phase endocrine concentrations in intervention cycles presenting with single dominant follicle development were comparable with non-intervention cycles in normo-ovulatory women (Macklon and Fauser, 2000). Although midluteal oestradiol and inhibin A concentrations were significantly higher in cycles with multiple follicle development, midluteal progesterone was similar. As midluteal gonadotrophin serum concentrations were significantly lower in cycles with multiple dominant follicle development, high oestradiol and inhibin A might trigger luteolysis through negative feedback mechanisms. *In vitro*, oestradiol was found to inhibit gonadotrophin-stimulated progesterone synthesis by luteal cells (Hahlin *et al*., 1986). Other studies suggest a luteolytic action of oestrogens mediated via prostaglandins (Auletta *et al*., 1976) or arachidonic acid (Fisch *et al*., 1994). In cycles with multiple dominant follicle development, high initial progesterone production may fall rapidly during the luteal phase, which in turn reduces the length of the luteal phase.

In conclusion, our findings are supportive of the FSH window concept. Subtle interference with decremental FSH by low-dose exogenous FSH can induce multiple dominant follicle development. Provided that no dominant follicle selection has occurred, initiation of FSH administration as late as cycle day 7 is sufficient to interfere with single dominant follicle selection. Multiple follicle development *per se* induces changes in the length and endocrine profile of the luteal phase. This information seems relevant for the design of mild ovarian stimulation protocols for IUI or IVF.

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