IVF Performance of Women Who Have Fluctuating Early Follicular FSH Levels¹

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Objectives: The aim was to evaluate whether women who have early follicular follicle-stimulating hormone (FSH) levels >12 mIU/ml have reduced response to follicular stimulation for in vitro fertilization (IVF) in a following month, in spite of normal FSH levels.

Material and methods: In a 3-year period from January 1996 to December 1998, 303 women aged 38 years and above and/or who had previously responded poorly to superovulation for IVF gave blood samples for FSH, luteinizing hormone (LH), and estradiol (E_2) on day 2 of menstruation before commencing treatment.

Results: In 117 (38.6%) of these women, FSH levels were >12 mIU/ml (range 12–114 mIU/ml). Sixty-six of these women gave a further 130 blood samples for FSH measurement in the following months. Seventy-eight (60.0%) of the tests showed raised FSH value >12 mIU/ml. Thirty women whose repeat FSH levels were <12 mIU/ml underwent 41 IVF cycles (group I). Sixty-three other women, older than 38 and/or who had a poor response to superovulation previously and whose FSH levels were <12 mIU/ml, served as the control group (group II). There were no differences in the responses to superovulation and delivery rates between the two groups (14.6% vs. 12.7%).

Conclusions: Women whose early follicular phase FSH levels were raised >12 mIU/ml had an increased risk ($>50\%$) that in subsequent cycles levels would remain raised, and it was not possible to predict which individuals would have favorable FSH levels. If the cycle day 2 FSH level returns to a "normal" level of <12 mIU/ml, women aged 40 and above had substantial cycle cancellation rates (43%), but patients who achieved the stage of embryo transfer had a good chance of conceiving, regardless of their age.

KEY WORDS: Follicle-stimulating hormone; ovulation induction; ovarian reserve; in vitro fertilization; treatment outcome.

INTRODUCTION

A major factor in successful in vitro fertilization (IVF) treatment is the ability of the ovary to respond to gonadotropin stimulation and to develop several follicles. That response reflects the ovarian function or ovarian reserve. The reduction of ovarian reserve is due to reduced numbers of ovarian primordial follicles from about a million at birth to around 250,000 at menarche to very few at the end of reproductive life. This loss accelerates around the age of 37 and precedes the menopause by 10–12 years (1,2). There is wide variation in the number and rate of depletion of follicles. Some women fail to respond to gonadotropin stimulation and the cycles are canceled. This is common particularly in older women (3,4). Age of the female partner is an important factor in fertility and as a woman gets older, her fertility diminishes (5–9). It would be clinically and economically very helpful if we could predict a poor response before treatment. Once the ovary is more or less exhausted, increased pituitary production of follicle-stimulating hormone (FSH) follows. This event takes place a few years before the actual menopause. Currently, the serum FSH level is the best marker to assess ovarian reserve and of predicting response to superovulation with a good correlation to pregnancy rates (10–12). However, lack of a clear cutoff point, huge variations between different laboratories, and monthly variations in FSH secretion means that FSH measurement is only of limited value in assessing the prognosis of IVF treatment (13,14). Based on evidence from our historic data, women whose FSH level is >12 mIU/ml (upper limit of normal 10 mIU/ml) on day 2 of

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menstruation rarely achieved pregnancy and our policy is to recommend they do not proceed directly with treatment. They are advised to repeat the FSH test in the following months with the intention of starting a treatment cycle if the FSH level falls below 12 mIU/ml. The response of these women, with evidence of reduced ovarian reserve is not clear. Martin *et al.* (15) found very low pregnancy rates in women whose cycle day 3 FSH was increased at least once, but their cutoff point for normal basal FSH level was considerably higher at 20 mIU/ml. The objectives of this study were: (a) to evaluate the variability of follicular phase FSH levels in different IVF treatment cycles, and (b) to test the hypothesis that if FSH levels have been elevated to >12 mIU/ml, the response to follicular stimulation for IVF is reduced, even if the FSH level is normal at the start of the treatment cycle.

MATERIALS AND METHODS

Subjects

In a period of 3 years from January 1996 to December 1998, 303 women aged 38 years and above and/or who had previously responded poorly to superovulation for IVF gave blood samples for FSH, luteinizing hormone (LH), and estradiol (E_2) on day 2 of

their cycle before commencing treatment. Poor response to stimulation was defined by one of the following criteria: a previous abandoned cycle (less than three follicles >14 mm), retrieval of fewer than four oocytes, and a requirement to increase the recombinant FSH dose up to 450 IU daily. In 117 (38.6%) of these women, the FSH levels were >12 mIU/ml. They were advised to postpone their treatment and to repeat the FSH test the following month. Sixty-six of these women gave a further 130 blood samples for FSH measurements and 78 (60.0%) of these tests returned raised FSH levels of >12 mIU/ml. Thirty women whose repeat FSH levels were <12 mIU/ml underwent 41 IVF cycles (group I) (Fig. 1). Sixty-three other women, older than 38, and/or had poor response to superovulation previously and whose FSH levels were <12 mIU/ml served as controls (group II). The indications for IVF were similar in both groups (Table I).

Ovulation Induction Protocol. Ovarian stimulation was induced in both groups using a “short” gonadotropin-releasing hormone agonist (GnRH-a) protocol with 300–450 IU recombinant FSH daily ($n = 34$ in group I and $n = 36$ in group II) or the long luteal phase GnRH-a protocol with a similar daily dose of recombinant FSH ($n = 7$ and 27, respectively). These protocols are used routinely in our unit for patients who are known to be poor responders and the starting dose is never higher than 450 IU of recombinant

Fig. 1. Chart flow for patients' management according to day 2 FSH levels.

Table I. Indications for IVF Treatment in the Study and Control Groups

Diagnosis of infertility	Study group N = 30	Control group N = 64
Tubal factor	10 (33.3%)	22 (34.4%)
Unexplained infertility	11 (36.7%)	27 (42.2%)
Male factor	7 (23.3%)	7 (10.9%)
Endometriosis	2 (6.7%)	5 (7.8%)
Anovulation	—	3 (4.7%)

FSH daily (16). Age and basal hormonal levels were similar in patients treated by either protocol. Briefly, in the short GnRH-a protocol, following administration of either buserelin acetate (Suprecur, Shire Pharmaceuticals Ltd., Andover, UK) or naferaline (Synarel, Searle, High Wycombe, UK), from the second day of the period, the ovaries were stimulated with highly purified FSH (Metrodin HP, Serono Laboratories Ltd., Welwyn Garden City, UK) or recombinant human FSH (Gonal-F, Serono Laboratories Ltd., Welwyn Garden City, UK) from the following day. Transvaginal ultrasound assessment of follicular growth, endometrial thickness, and measurement of E₂ levels were begun on stimulation day 8. Human chorionic gonadotropin (hCG) (Profasi, Serono, Welwyn Garden City, UK) 10,000 IU was injected subcutaneously when there were at least two follicles of ≥ 18 mm in average diameter. In the long luteal phase protocol, GnRH-a was started on day 21 of the cycle for 10–14 days followed by FSH stimulation in a similar manner to the short protocol. Transvaginal ultrasound directed oocyte recovery, mainly under general anaesthesia, was performed 32–36 hr after the hCG injection (17). In vitro culture and insemination were carried out according to our normal laboratory procedures (18).

Pregnancy Monitoring. Plasma samples were taken from the women 14 days after embryo transfer for measurement of hCG and progesterone concentrations. If hCG levels were >10 IU/l, then monitoring was continued at 5-day intervals. A transvaginal scan was performed about 35 days after embryo transfer to confirm the pregnancy. Clinical pregnancy was defined as the presence in the uterus of a fetus within a gestation sac, with evidence of fetal cardiac activity. Transient rises in hCG (“biochemical pregnancy”) without evidence of a gestation sac, “blighted ova,” or ectopic pregnancies were recorded but are not included in this analysis of data.

FSH Measurement. Serum FSH was measured with a commercially available microparticle enzyme

immunoassay (MEIA) (Abbott Laboratories Diagnostics Division USA, diagnostic pack No. 2223-20). The sensitivity of the FSH assay is calculated to be better than or equal to 0.2 mIU/ml, with an average recovery of 100%. The cutoff level of 12 mIU/ml is calibrated to the World Health Organization (WHO) Second International Reference Preparation (IRP) for human FSH (standard 78/549). The intra-assay and interassay coefficients of variation were 3.8% and 7.0%, respectively.

The data were statistically analyzed by the paired and unpaired Student’s *t*-test and χ^2 test as indicated. The results are presented as mean $\pm$ standard deviation (SD)

RESULTS

Of the 303 women aged 38 years and above and/or who had previously responded poorly to superovulation for IVF, 117 (38.6%) had cycle day 2 FSH levels >12 mIU/ml (range 12–114 mIU/ml). Sixty-six women (56.4%) returned for repeat FSH measurement, while 51 failed to do so. There was no difference in the mean age of these two subgroups of patients (39.5 ± 4.7 vs. 39.8 ± 5.5). However, the mean FSH levels were significantly higher in patients who did not repeat their FSH tests (29.0 ± 27.0 mIU/ml vs. 17.0 ± 6.0 mIU/ml, $P = 0.004$). Moreover, 19 women (38%) who did not come back for a repeat test had initial FSH levels >20 mIU/ml, which is considered a postmenopausal level in our unit, compared to 10 women (14.9%, $P = 0.06$) in the group who returned for further tests. It might be therefore that some women decided or were advised not to check their FSH levels again in the belief that they were early menopausal and therefore had a negligible chance of success. Some of these women were counseled about the option of egg donation.

Our data show that there are intercycle variations in the FSH levels (Fig. 2). The average FSH level was 2.1 mIU/ml lower in the second test, but the changes ranged from 24.2 mIU/ml lower up to 57.8 mIU/ml higher. In 45 patients the second FSH levels were lower than the first test and in 20 others it was higher. Out of the 66 women who repeated their FSH test, 40 (59.7%) again had levels higher than 12 mIU/ml. There were no differences in mean age, day 2 E₂, or FSH and LH levels between women with increased and “normal” repeat FSH levels (Table II). It was impossible therefore to predict in which patient the

Fig. 2. Trends in day 2 FSH levels of 66 women who gave blood samples in two different cycles.

FSH level would return to normal and in whom it would remain abnormally high.

In total, 30 women whose repeat FSH levels were <12 mIU/ml (mean FSH 9.3 ± 1.8 mIU/ml) underwent 41 IVF cycles. Day 2 serum LH and E₂ levels, cancellation rates due to poor response to superovulation, and performance in IVF were similar in both groups. FSH level on day 2 was higher in the study group (9.3 ± 2.3 vs. 7.7 ± 2.1 , $P = 0.001$) (Table III). Six (14.6% per cycle) women from the study group and eight (12.7%) of the control group achieved successful clinical pregnancies and deliveries. Two other

women from group I and five patients from group II had transient raised hCG levels (biochemical pregnancy). There was one ectopic pregnancy in each group (12.5% vs. 9.1%, $P =$ nonsignificant) without any miscarriages in either groups.

In the study group, women aged 40 years and above had a significantly higher risk of cycle cancellation than younger women (42.9% vs. 13.3%, $P = 0.05$). There were no differences in mean age, basal FSH, E₂, and LH between patients with adequate response to superovulation compared to the nine whose cycles were abandoned. However, if women 40 and above

Table II. Characteristics of Women with Increased FSH Levels on Day 2 in Two Cycles Compared to Women Whose Repeat FSH Level Was Normal

	Elevated repeat FSH	Normal repeat FSH	<i>P</i> value
Number of patients	40	26	
Age (years)	39.0 ± 5.2	40.2 ± 4.1	NS
Day 2 FSH (mIU/mL) ^a	17.1 ± 6.6	17.0 ± 4.1	NS
Day 2 LH (IU/L) ^a	8.1 ± 6.8	15.6 ± 32.7	NS
Day 2 E ₂ level (pg/ml) ^a	29.9 ± 16.4	30.5 ± 21.3	NS

^a Hormonal levels are in day 2 of the first cycle.

Table III. Comparison of Day 2 Hormone Levels and Outcome of IVF Treatment of Patients with Initial Raised FSH Levels (Study Group) and Patients with Normal FSH Levels (Control Group)

	Study group	Control group	P value
Number of cycles	41	63	
Age (years)	39.2 ± 4.8	37.4 ± 5.6	NS
Day 2 FSH (mIU/mL)	9.3 ± 2.3	7.7 ± 2.1	0.001
Day 2 LH (IU/L)	5.5 ± 2.2	5.1 ± 3.2	NS
Day 2 E ₂ level (pg/ml)	58.3 ± 83.3	41.3 ± 23	NS
Abandoned cycles (%)	11 (26.8%)	8 (12.5%)	NS
Mean No. of oocytes (range)	6.4 (1–14)	8.6 (0–32)	NS
Fertilization rate (%)	56.3%	58.6%	NS
ET cycles	24 (58.5%) ^a	47 (74.6%) ^b	NS
Delivery rate per cycle	6 (14.6%)	8 (12.7%)	NS
Delivery rate per ET	25%	17%	NS

^a Four cycles: complete failure of fertilization, one cleavage arrest, and one embryos frozen because of uterine bleeding.

^b One cycle, no oocytes; 3, complete failure of fertilization; and in four cycles all embryos were frozen: 1 due to risk of ovarian hyperstimulation, 1 uterine bleeding, and 2 because of personal reasons.

reached the stage of oocyte retrieval, there was no difference in their response to stimulation (number of ampoules of FSH, length of stimulation, peak E₂ levels, and number of oocytes retrieved) or pregnancy rates between them and the younger women (Table IV).

DISCUSSION

The ovaries of women in their late 30s and early 40s have depleted numbers of primordial follicles and therefore reduced ovarian reserve (19,20). It is important to develop independent markers to predict the ovarian response to superovulation before embarking on invasive, expensive, and demanding treat-

ment such an IVF. The most commonly used marker is the basal serum FSH level, which has been studied and validated extensively (12,14). There is wide consensus that women who have high basal serum FSH respond poorly to superovulation and achieve very low pregnancy rates, regardless of their age (10,11,14,15,21–28). However, there is no general agreement on what is the “normal” level of FSH. Studies in the late 1980s used a cutoff point of 25 mIU/ml FSH, but the introduction of a new FSH standard (WHO 2nd IRP) based on pituitary FSH values rather than menopausal urinary FSH values required a new threshold of probably 15 mIU/ml [(1 mIU/ml of 2nd IRP = 1 mIU/ml × 0.6 of 1st IRP (29)]. Our experience (unpublished data) showed that responses to superovulation and pregnancy rates

Table IV. Comparison of Day 2 Hormone Levels and Outcome of IVF Treatment of Patients Whose Repeat FSH Levels Were <12 mIU/ml Grouped by Age

	Women < 40 (n = 13) ^{a,b}	Women ≥ 40 (n = 18)	P value
Number of cycles	20	21	
Mean age	34.5 ± 3.6	42.7 ± 2.2	<0.001
Day 2 FSH (mIU/ml)	9.2 ± 2.1	9.6 ± 2.0	NS
Day 2 LH (IU/l)	6.3 ± 2.3	4.8 ± 1.9	0.04
Day 2 E ₂ (pg/ml)	62.8 ± 98	37.8 ± 30	NS
Abandoned cycles (%)	2 (10%)	9 (42.9%)	0.018
ET cycles	14 (70%)	10 (47.6%)	NS
Pregnancies (% per cycle)	4 (20%)	3 (14.3%)	NS
Pregnancy rate per ET	28.6%	30%	NS

^a Values are mean ± SD.

^b One patient had IVF cycle before age 40 and after 40.

in IVF were very poor when day 2 FSH levels were above 12 mIU/ml. A unit policy therefore was developed not to proceed with IVF treatment if the basal FSH levels were above this threshold. All tests were done "in house" because of the possibility of interinstitutional variability (30). There are two main reasons for this variability: first, FSH is a glycoprotein hormone composed of a protein dimer backbone with variable degrees of glycosylation; second, differences in the antibodies used to measure gonadotropin levels. Most of the commercial assay systems use polyclonal antibody systems that bind differently to separate haptens on the glycoprotein hormone (12). It is therefore meaningless to transfer results from one laboratory to another without verification that identical methods are being used.

When adopting a screening policy it is essential to determine whether there is intercycle and intracycle variation. Since the introduction of FSH screening (21) cycle day 3 has emerged as the standard in most studies. We chose cycle day 2 because our stimulation, the short (or flare) stimulation protocol starts from this day. Hansen *et al.* (29) have shown similar FSH results on day 2–5 of the same cycle, so the precise day on which to perform the test within this range is not crucially important and can give flexibility to patients and medical teams with the treatment regimens.

It is not clear from the data available in the literature whether there are intercycle variations in FSH levels and what the clinical significance of these fluctuations is. Our data show that there are considerable intercycle variations in the FSH levels and that the changes are bidirectional, i.e., they are higher or lower than the initial tests, and more significantly, it is impossible to predict in which direction it will move. However, the second FSH levels were still above the threshold of 12 mIU/ml in two thirds of patients. Hansen *et al.* (29) found minimal changes between cycles in 19 subjects who were not at increased risk of diminished ovarian reserve, in contrast to our group of patients. Scott *et al.* (31) reported on a larger series of 81 women that patients with normal FSH levels (<15 mIU/ml) had very low intercycle variations with a mean value of 2.6 ± 0.2 mIU/ml, while patients with elevated basal FSH level had a much higher degree of variability with a mean value of 7.4 ± 0.9 mIU/ml. When they performed paired analyses of the high and low FSH cycles in these patients, there were no differences in stimulation quality, number of oocytes, or fertilization rate, and patients responded poorly in both cycles. They speculated that by the

time patients develop higher variability in their basal FSH concentrations, they already have a significant diminution in their ovarian reserve. Scott and Hofmann (14) suggested that serial screening of FSH levels to select the optimal cycle for stimulation would be of limited clinical value. Recently, Buyalos *et al.* (32) reported in 38 infertile women and/or older than 38 years of age who had normal FSH levels on day 3 of the cycle (FSH <13 mIU/ml) that 29% of them had raised levels in subsequent cycles. Martin *et al.* (15) conducted a large retrospective study to determine the effect of transitory raised basal FSH levels. They used a higher cutoff point than ourselves for normal levels (FSH = 20 mIU/ml). No pregnancies occurred in 53 cycles with day 3 FSH always >20 mIU/ml. In 54 cycles in which FSH was >20 mIU/ml, but <20 mIU/ml in the treatment cycle, the pregnancy rate was 5.6%, compared with 16.5% in cycles with always "normal" FSH levels. The serum FSH level is an important predictor for cancellation rates, but neither these data nor the variability of FSH levels between cycles are not available in Martin's study. However, we agree with their conclusion that the treatment cycle should be deferred in patients with high basal FSH levels until a normal level is achieved. Our results confirm Scott and Hofmann's (14) conclusions regarding the variability of FSH levels in women with some degree of reduced ovarian reserve. However, patients whose repeat FSH levels returned to normal and who continued with ovarian stimulation faced an increased risk of abandoning their cycles due to poor response to stimulation of up to 26%. Moreover, patients who reached the oocyte retrieval stage performed as well as the control group with the same treatment outcome (Table III).

The authors are aware of the limitations of retrospective study in which significantly higher proportions of patients from the study group were treated by the short protocol (83% vs. 57%, $P = 0.0124$). This difference is probably a result of the clinician's belief that women who are prone to poor response might succeed better in this protocol. It is possible therefore that the protocol itself has an effect on the outcome, but it does not change the findings that women with fluctuating basal FSH levels can still expect a reasonable successful outcome.

Most women younger than 40 years old with normal repeat basal FSH levels will reach the oocyte retrieval stage with favorable outcome, while women in the final years of their reproductive life face cancellation rate of more than 40% in spite of normal repeat FSH levels. However, patients who successfully reach

embryo transfer demonstrate a high pregnancy rate of 30%. In a previous study of more than 1000 started cycles in women above 40 (4) we showed that age alone is not an accurate predictor for IVF outcome, and the biological response is more important than the chronological age. While in the former study the cycle cancellation rate for women above 40 was 22.5%, in the current study the cancellation rate in this age group was higher and reflected the diminished ovarian reserve. It is possible that for women younger than 40, an incidental increase in FSH levels with a subsequent return to the normal does not necessarily indicate an imminent deterioration in the ovarian reserve. While for older women, even a transient rise may signal the beginning of the premenopausal stage, in which the FSH rise precedes the menopause by 5 to 6 years (33).

In summary, we have found in this study wide variations in basal serum FSH levels in women suspected of having reduced ovarian reserve, either because of their age (>38 years) or their previous poor response to superovulation. Although more than half of patients will have repeat FSH levels higher than 12 mIU/ml, it is worth trying ovarian stimulation for patients with normal levels. In spite of a higher cycle cancellation rate, especially for women 40 years old and above, patients who achieve the embryo transfer stage have a reasonable chance to conceive and deliver. It seems therefore that a transient high FSH level is not detrimental to women younger than 40 years of age, but is more relevant to the older women. Basal serum FSH screening should be offered to all patients with suspected reduced ovarian reserve and if the result is higher than the normal upper limit (each clinic should define its own normal range), then treatment should be deferred until a normal level is achieved. In this scenario, patients can be advised about the high cancellation rate but positively encouraged regarding their chances to conceive if they achieve the stage of embryo transfer.

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