In Vitro Fertilization Outcome According to Age and Follicle-Stimulating Hormone Levels on Cycle Day 3

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Purpose: In a retrospective study, the prognostic factors of in vitro fertilization outcome were studied in women of 39 years of age or older, with an elevated cycle day 3 follicle-stimulating hormone (FSH) level.

Methods: Ovarian stimulation was achieved with a combination of gonadotropin-releasing hormone agonist/human menopausal gonadotropin in a short protocol. All patients underwent FSH dosage on cycle day 3 prior to stimulation. The pregnancy rate was studied according to age, FSH levels, and stimulation parameters.

Results: There was a negative correlation between day 3 FSH levels and the number of oocytes retrieved. Cycles canceled because of the absence of ovarian response had a significantly higher mean FSH value (18.2 mIU/ml) than cycles ending in oocyte retrieval (14.6 mIU/ml). Patients with three or more growing follicles during stimulation achieved a significantly higher pregnancy rate per egg retrieval (16%) compared to patients with fewer than three growing follicles (6%). Eighty percent of those pregnancies were obtained during the first two IVF cycles. Even with an elevated FSH level, some patients developed three or more follicles after stimulation. In such cases, the number of embryos available for transfer was the only significant limiting factor to achieving pregnancy.

Conclusions: As our results suggest, there is a discrepancy between biological and chronological ovarian age. In patients with an elevated cycle day 3 FSH level and over 40 years of age, alternatives to fertility treatments (ovum donation, adoption, or no treatment) should not be considered as first choices. Indeed, even with elevated FSH levels, a 16% pregnancy rate per egg retrieval may be obtained if three or more growing follicles can be seen during ovarian stimulation. However, in the presence of fewer than three growing follicles during ovarian stimulation, the patient

should be informed about the discouraging prognosis of the running cycle.

KEY WORDS: age; FSH; IVF; poor responders.

INTRODUCTION

Regardless of the type of stimulation protocol used, the collection of a suitable number of mature oocytes is a prerequisite for successful in vitro fertilization (IVF). During treatment cycles, about 15% of patients end with poor follicular recruitment and inappropriate serum estradiol production. These patients are defined as "poor responders." They show a response with few follicles and/or low estradiol levels when stimulated with standard or high doses of gonadotropins. A higher proportion of such responders is observed with advanced maternal age (1). There are many tests available which can identify poor responders (2,3). All are based on the follicle-stimulating hormone (FSH) profile during spontaneous cycles. Determination of basal FSH levels in the early follicular phase (days 2–3 of the cycle) seems to be the simplest method with reliable results for such screening (4). A poor outcome is associated with poor responders (5): the fertilization rate is noted to be significantly lower per oocyte retrieved (5), and the rate of embryo cleavage slower (6). Many empirical treatments have been proposed for the management of poor responders (6–8) but none have proved entirely successful.

In this study, we tried to determine the most accurate parameters that influence IVF outcome in such a group of women. In a retrospective study, we investigated the relationship between maternal age, basal FSH concentrations on day 3 of the cycle, and the results of ovarian stimulation and IVF outcome. We tried to define the prognosis of IVF treatment for these patients.

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MATERIALS AND METHODS

Sixty-four patients aged ≥ 39 years and with an elevated cycle day 3 FSH level were included in the study. A total of 127 started cycles were accomplished in these women. In all cycles, the cycle day 3 FSH level was over the upper limit, defined in our laboratory as 8 mIU/ml. All the cycles were pooled together for statistical analysis. Besides the evaluation of fertilization rates and IVF outcome, we studied the relationship between FSH levels and stimulation results according to the patients' age and ovarian response. All patients received the same protocol of ovarian stimulation. A short protocol of combined gonadotropin-releasing hormone agonist (GnRHa)/human menopausal gonadotropin (hMG) therapy was used. Patients began their GnRHa (Buserelin; Hoechst, Brussels, Belgium) on the first day of the cycle at a daily dose of 900 mg; hMG (Humegon; Oss, The Netherlands), 300 IU/day, was added to the GnRHa treatment, beginning on the third day of the cycle, for 3 days. On the sixth day, gonadotropin doses were adjusted according to ultrasound results and serum estradiol levels. Triggering of ovulation was performed with 10,000 IU of human chorionic gonadotropin (hCG) im in the presence of one or two follicles over 17 mm in diameter, with an estradiol level of 100 pg/ml per follicle. Ovocyte retrieval was carried out transvaginally under vaginal ultrasound guidance. Embryo transfer (ET) took place 48 hr after ovocyte retrieval as described previously (7). The patient was considered pregnant and included in the analysis in the presence of a clinical pregnancy confirmed by ultrasound 4 weeks after ET.

RESULTS

The mean age of patients was 42.4 ± 2 years, with a mean FSH level, on day 3 of the cycle, of 15.5 ± 0.7 mIU/ml. The stimulation duration was 12.9 ± 3 days, with a mean number of 59.1 ± 25.8 ampoules used per started cycle. A total of 127 cycles were included in the study. Eighty-three cycles (65.4%) ended in ovocyte retrieval. A mean number of 3.9 ± 2.8 mature ovocytes was obtained and the mean number of embryos available for transfer was 2.7 ± 2 . The mean number of embryos replaced was 2.2 ± 0.9 per cycle.

The cancellation rate was thus 34.6%. The mean value of FSH levels on day 3 was significantly higher ($P = 0.003$) in canceled cycles (18.2 mIU/ml) than in noncanceled cycles (14.6 mIU/ml), despite the similar

mean age in both groups. Transfer of at least one embryo was possible in 61 cycles (73.5%/ovocyte pickup, 48%/treatment cycle).

Ten clinical pregnancies were obtained (16.4%/transfer, 12%/ovocyte pickup, and 7.8%/treatment cycle). Eight of the 10 pregnancies occurred during the first and second IVF attempts. Only one pregnancy was observed during the third attempt and one during the fourth. The results of each attempt are listed in Table I. The stimulation parameters, the FSH levels on day 3, and the mean age of patients were similar during all the attempts. Also, a similar number of embryos was replaced during each attempt. However, pregnancy rates of 20% and 16.6% per embryo transfer were observed during the first and second attempts respectively, compared to a 10% pregnancy rate during further attempts. This difference was statistically significant ($P = 0.03$).

A negative correlation was found between day 3 FSH levels and the number of retrieved ovocytes. On the contrary, a positive correlation was found between day 3 FSH levels and the total number of ampoules of hMG administered (Figs. 1 and 2). In Fig. 1, filled circles represent the basal FSH values and the number of ovocytes obtained from women who conceived. Open circles represent the basal FSH values and the number of ovocytes obtained from women who did not conceive. Eight of the 10 pregnancies occurred in women with three or more ovocytes available for insemination, and only 2 in women with fewer than three ovocytes for insemination. In Fig. 2, it is clearly shown that the consumption of gonadotropins increased with the basal FSH levels.

The results of cycles with pregnancy and those without pregnancy after embryo transfer are shown in Table II. Levels of FSH on day 3 and the length of stimulation were not different in cycles with and without pregnancy. In addition, the mean number (8.9 ± 2.9) of ovocytes in pregnant cycles was similar to the mean number (9.1 ± 3) of ovocytes retrieved in nonpregnant cycles. However, the mean total number (8 ± 2.9) of embryos was significantly different in cycles with conception compared to those without conception (mean number of embryos: 2.5 ± 2).

The cycles in which three or more ovocytes were collected ($n = 50$) were compared to the cycles in which fewer than three ovocytes were collected ($n = 77$). Ninety percent of the former (44/50) ended in embryo transfer, with a clinical pregnancy rate of 18.2% per embryo transfer and 16% per cycle. However, among the 77 cycles with fewer than three ovocytes available, only 22% (17/77) ended in embryo

Table I. Results of Stimulation According to the Number of the Attempt

	Attempt 1 (n = 60)	Attempt 2 (n = 33)	Attempt 3 (n = 19)	Attempt 4 (n = 15)	t-test P
No. of canceled cycles (%)	24 (39%)	8 (24.2%)	9 (47%)	3 (20%)	ns
No. of cycles with embryo transfer (%)	25 (41%)	18 (54.4%)	9 (47%)	10 (66.6%)	ns
FSH levels (mean $\pm$ SD)	16.3 $\pm$ 8	14.9 $\pm$ 6.7	14.5 $\pm$ 7	14.4 $\pm$ 8.2	ns
No. of oocytes (mean $\pm$ SD)	4 $\pm$ 2.7	4 $\pm$ 2.7	4.2 $\pm$ 3.3	5.1 $\pm$ 2.7	ns
No. of pronuclei (mean $\pm$ SD)	2.4 $\pm$ 1.6	2.7 $\pm$ 1.7	3.4 $\pm$ 2	3.4 $\pm$ 3	ns
No. of embryos replaced (mean $\pm$ SD)	2.1 $\pm$ 0.9	2.5 $\pm$ 1	2.0 $\pm$ 1	2.3 $\pm$ 0.8	ns
No. of clinical pregnancies/embryo transfer (%)	5 (20%)	3 (16.6%)	1 (10%)*	1 (10%)*	

* Significantly different from the rate obtained after the first and second attempts.

transfer, with only two clinical pregnancies. Thus, the clinical pregnancy rate in these cycles was 11.7% per embryo transfer and 2.6% per cycle. In cycles where three or more oocytes were collected, when the number of oocytes obtained, the day 3 FSH levels, and the total dose of gonadotropins administered were correlated, none of these parameters significantly influenced IVF outcome. In fact, the correlation matrix showed a correlation only between the number of oocytes obtained and the levels of FSH on cycle day 3, and none of the other parameters had any significant impact on the issue of transfer (Table III).

DISCUSSION

It has been well demonstrated that a high FSH level on day 3 of the cycle or advanced maternal age are associated with a poor response during ovarian stimu-

lation (1,4,10). The increase in FSH levels as a consequence of follicle depletion can start very early during reproductive age and tends to accelerate over the age of 37 (1,12). In 1996, Buyalos *et al.* (13) demonstrated that a combination of basal (day 2 or 3) FSH levels and chronological age are useful predictors of fertility potential in women aged ≥ 35 years undergoing ovulation induction therapy. Women aged 39 years or more with elevated FSH levels are considered to have a poor prognosis for IVF treatment. However, it is often difficult to ascertain clinically when endogenous fertility potential is exhausted. The decision to abandon conventional fertility therapy and elect adoption, donor ova, or further therapy is an emotionally demanding one for infertile couples. On the other hand, practitioners should be fully aware of the medical possibilities and social cost when faced with patients with a poor prognosis for fertility treatment.

Our results show that increased FSH levels correlate negatively with the number of recovered oocytes and positively with the total dose of hMG administered.

Fig. 1. Correlation between FSH levels and the number of oocytes retrieved ($R^2 = 0.109$, $P < 0.002$).

Fig. 2. Correlation between FSH levels and the number of ampoules injected ($R^2 = 0.032$, $P < 0.03$).

Table II. Results of Cycles with and Without Pregnancy (Only Cycles with Embryo Replacement)

	Mean $\pm$ SD		<i>t</i> -test <i>P</i>
	Cycles with pregnancy (<i>n</i> = 10)	Cycles without pregnancy (<i>n</i> = 51)	
FSH on day 3 (mIU/ml)	14.5 $\pm$ 6.5	12.8 $\pm$ 5	0.3
Day of hCG administration	13.6 $\pm$ 2.5	13.2 $\pm$ 1.9	0.3
Total dose of hMG (ampoules)	46.5 $\pm$ 12	60.4 $\pm$ 20	0.06
No. of ovocytes	8.9 $\pm$ 2.9	9.1 $\pm$ 3	0.9
No. of embryos	4.8 $\pm$ 2.9	2.5 $\pm$ 2	0.0007
No. of replaced embryos	2.6 $\pm$ 0.8	2.1 $\pm$ 0.9	0.3

This is in accordance with the findings of Scott *et al.* (4) and Toner *et al.* (14). In their report, the latter concluded that basal FSH levels are a prognostic parameter for the follicular reserve regardless of the patient's age. Our findings corroborate these results, as only the mean FSH level was significantly increased (18.2 mIU/ml) in canceled cycles compared to other cycles, despite a similar mean age in cycles with and without ovocyte pickup. The cancellation rate was 34.6%, which is within the expected limits for such a population (15). However, embryo transfer was possible in only 48% of cases. This result corroborates the low fertilization rate observed (50%). It could be attributed to the decline in ovocyte quality observed in women over 39 years of age and in those with high FSH levels (15,16). Such a phenomenon may explain the low pregnancy (16.4%/ET) and implantation (8.9%) rates observed.

The pregnancy rate observed in our study is also within the expected range for patients aged 39 years or more with a poor response (14). However, the comparison of IVF cycles shows a significantly higher number of pregnancies obtained during the first two attempts (18.3%/ET) compared to further attempts (10%/ET). The mean FSH level on day 3, the mean age, the mean number of ovocytes retrieved, and the mean number of embryos transferred were similar in all the attempts. This may be a consequence of a decrease in ovocyte quality. In fact, aging of the ovary is associated with a reduction in the follicular population, affecting growing as well as reserve follicles, and with an acceleration of the atresic process (17). Thus, during the initial stimulation, gonadotropins may act on follicles which are healthier than those available during further attempts. It could also be the conse-

Table III. Correlation Matrix of Cycles with Four or More Ovocytes Available After Ovocyte Pickup

	Correlation	<i>P</i> value	95% inf.	95% sup.
FSH level/No. ovo.	-0.371	0.01	-0.6	-0.08
FSH level/No. PN2	-0.292	0.05	-0.5	-0.005
Age/No. ovo.	0.228	0.1	-0.07	0.491
Age/No. PN2	0.03	0.8	-0.3	0.321
FSH/IVF outcome hMG	0.182	0.2	-0.12	0.451
Total dose/No. ovo.	-0.05	0.7	-0.34	0.25
Total dose/No. PN2	-0.22	0.1	-0.48	0.08
Total dose/FSH level	-0.09	0.55	-0.38	0.21

quence of chronological aging because several (± 8) months separate the first and the third or fourth attempts. The decline in pregnancy rates after the first two IVF attempts was not observed in women aged 39 years or more undergoing ovulation induction therapy (13). In their model, Magarelli *et al.* (13), even with a starting pregnancy rate of 3.8% per cycle, reported an increase in the probability of pregnancy as the treatment cycles increased. In their patients, they used a combination of clomiphene citrate plus hMG or FSH for ovarian stimulation, while our protocol used GnRHa in combination with hMG or FSH. The role of GnRHa was studied in "poor responders" undergoing ovarian stimulation for IVF. It seems that protocols using a low dose of GnRHa might be best for such patients (18,19).

In contrast to observations made by other authors (4,13,14), in our study, once the ovarian response was initiated, ending in ovocyte retrieval, the elevated FSH level did not influence the IVF outcome. In fact, the mean FSH levels (14.3 $\pm$ 6.5 mIU/ml) in pregnant cycles, while higher than the normal range, were not significantly different from the mean FSH levels (12.8 $\pm$ 5 mIU/ml) observed in nonpregnant cycles (Table II). Although the number of cycles resulting in pregnancy was small (*n* = 10), these cycles, with a FSH level similar to that in cycles without pregnancy, ended with a similar mean number of ovocytes available for insemination but with a significantly higher mean number of embryos available for transfer. This highlights the role and limits of the FSH level as the only predictor of follicular recruitment and growth. In fact, once the ovocytes have been retrieved, other factors seem to influence the IVF outcome.

In transferred cycles, the number of ovocytes fertilized and the total number of embryos available were found to be significantly different in cycles with and without pregnancy. Having more embryos available

is a consequence of the higher number of ovocytes fertilized. This may allow embryo selection for transfer from a larger number. Thus, through the selection process, the best embryos may be transferred, increasing the chances of implantation and pregnancy. This parameter was found to be highly significant with regard to pregnancy outcome in previous studies, and seems to be independent of age (20,21). Also, a better-quality embryo may be a consequence of better ovocyte quality relating to the superior ovarian response obtained in these cycles.

Eighty percent of pregnancies were observed in cycles with three or more ovocytes available for insemination. In these cycles, the transfer rate was 90% and the pregnancy rate observed was 16% per egg retrieval. This percentage is higher than expected in patients aged 40 years or over (19,22). Moreover, in this group, no correlation was found between the stimulation parameters and the FSH levels. In these patients, the biological ovarian age may be different from the chronological ovarian age. This difference may be related to the intercycle variability of basal FSH (23), thus indicating that the basal FSH levels observed prior to treatment initiation may be different from those encountered during treatment cycles.

CONCLUSIONS

This study allows certain conclusions to be drawn about multiple IVF parameters in patients over 39 years of age with elevated basal FSH levels on day 3 of the cycle. It must be emphasized that although this study was not prospective and randomized, it nevertheless demonstrates the importance and influence of basal FSH levels, the patient's age, and the number of treatment cycles on the prognosis of IVF outcome. It strongly suggests that testing should be performed on all patients over 38 years of age undergoing IVF treatment, to provide couples with more information about the woman's chances of responding to ovarian stimulating agents. The combination of FSH concentration results and the woman's age may allow practitioners to formulate an adapted prognosis for success. However, as the results suggest, there is a discrepancy between biological and chronological ovarian age. Indeed, it is of utmost importance that alternatives to fertility therapy (ovum donation, adoption, no treatment) should not be automatic first choices, as even with elevated FSH levels in older patients, a 16% pregnancy rate per egg retrieval can hopefully be achieved in patients with three or more ovocytes. These results should help avoid the postponement of treat-

ment for infertility of short duration in patients aged 38 years or more. Our results clearly show that there is little to gain from repeated IVF attempts in such a population of women. They may also help patients and clinicians who are reluctant to accept results of purely biochemical markers and, at the same time, lead to an adapted decision if no conception occurs after two cycles of treatment. However, in the presence of two or fewer ovocytes during ovarian hyperstimulation, the patient should be informed of the discouraging prognosis of the running cycle and ovocyte pickup should be carried out with the oral consent of the couple.

REFERENCES

1. Spandorfer S, Avrech O, Colombero L, Palermo G, Rosenwaks Z: Effect of parental age on fertilization and pregnancy: Characteristics in couples treated by intracytoplasmic sperm injection. *Hum Reprod* 1998;13:334-338
2. Navot D, Rosenwaks Z, Margalioth E: Prognostic assessment of female fecundity. *Lancet* 1987;2:645-647
3. Loumaye E, Billion J, Mine J, Psalti I, Pensis M, Thomas K: Prediction of individual response to controlled ovarian hyperstimulation by means of Clomiphene citrate challenge test. *Fertil Steril* 1990;53:295-301
4. Scott R, Toner J, Muasher S, Oehninger S, Robinson S, Rosenwaks Z: FSH levels on cycle day 3 are predictive of IVF outcome. *Fertil Steril* 1989;51:651-654
5. Rysselberge M, Puissant F, Barlow P, Lejeune B, Delvigne A, Leroy F: Fertility prognosis in IVF treatment of patients with cancelled cycles. *Hum Reprod* 1989;4:663-666
6. Jenkins J, Davies D, Devonport H, Anthony F, Gadd S, Watson R, Masson G: Comparison of "poor" responders with "good" responders using a standard buserelin/human menopausal gonadotrophin regime for in vitro fertilization. *Hum Reprod* 1991;6:918-921
7. Ferlberg D, Farhi J, Ashkenazi J, Dieker D, Shalev J, Ben-Rafael Z: Minidose gonadotrophin-releasing hormone agonist is the treatment of choice of poor responders with high follicular stimulating hormone levels. *Fertil Steril* 1994;62:343-346
8. Van Hoof M, Alberda A, Huisman G, Zeilmaker G, Leerentveld R: Doubling the human menopausal gonadotrophin dose in the course of an in-vitro fertilization treatment cycle in low responders: A randomized study. *Hum Reprod* 1993;8:369-373
9. Bassil M, Magritte JP, Roth J, Nisolle M, Donnez J, Gordts S: Uterine vascularity during stimulation and its correlation with implantation in in vitro fertilization. *Hum Reprod* 1995;10:1497-1501
10. Buyalos R, Daneshmand S, Brzechffa P: Basal estradiol and follicle-stimulating hormone predict fecundity in women of advanced reproductive age undergoing ovulation induction therapy. *Fertil Steril* 1997;68:272-277
11. McNaughton J, Banah M, McCloud M, Hee J, Burger H: Age-related changes in FSH, LH, estradiol and immunoreactive inhibin in women of reproductive age. *Clin Endocrinol* 1992;36:339-345
12. Faddy M, Gosden R, Gougeon A, Richardson S, Nelson J: Accelerated disappearance of ovarian follicles in mid life,

- implications for forecasting menopause. *Hum Reprod* 1992;7:1342–1346
13. Magarelli P, Pearlstone A, Buyalos R: Discrimination between chronological and ovarian age in infertile women aged 35 years and older: Predicting pregnancy using basal follicle stimulating hormone, age and number of ovulation induction/intra-uterine insemination cycles. *Hum Reprod* 1996;11:1214–1219
 14. Toner J, Philiput C, Jones G, Muasher S: Basal follicle stimulating hormone level is a better predictor of in vitro fertilization performance than age. *Fertil Steril* 1991;55:784–791
 15. Wilcox A, Weiberg C, O'Connor J, Baird D, Schlatterer J, Canfield R, Armstrong E, Nosula B: Incidence of early loss of pregnancy. *N Engl J Med* 1988;319:189–194
 16. Medrom D: Female reproductive ageing-ovarian and uterine factors. *Fertil Steril* 1993;59:1–5
 17. Gougeon A, Chainy GB: Morphometric studies of small follicles in ovaries of women at different age. *J Reprod Fertil* 1987;81:433–442
 18. Feldberg D, Farhi J, Ashkenazi J, Dicker D, Shalev J, Ben-Rafael Z: Minidose gonadotrophin-releasing hormone agonist is the treatment of choice in poor responders with high follicle-stimulating hormone levels. *Fertil Steril* 1994;62:343–346
 19. Olivennes F, Righini C, Fanchin R, Torrisi C, Hazout A, Glissant M, Fernandez H, Frydman R: A protocol using a low dose of gonadotrophin-releasing hormone agonist might be the best protocol for patients with high follicle stimulating hormone concentrations on day 3. *Hum Reprod* 1996;11:1169–1172
 20. Staessen C, Nagy ZP, Liu J, Janssenwillen C, Camus M, Devroey P, Van Steirteghem A: One year's experience with elective transfer of two good quality embryos in the human in-vitro fertilization and intracytoplasmic sperm injection programmes. *Hum Reprod* 1995;10:3305–3312
 21. Scott R, Hofmann G, Oehninger S, Muasher S: Intercycle variability of day 3 follicle-stimulating hormone levels and its effect on stimulation quality in in vitro fertilization. *Fertil Steril* 1990;54:297–302
 22. Hedon B, Galtier-Dereure F, Dechaud H: Women's age and assisted medical procreation. *In* Proceedings of the 15th World Congress on Fertility and Sterility, B Hedon, J Bringer, P Mares (eds). London, Parthenon, 1995, pp 379–385
 23. Lavy G, Pellicer A, Diamond MP, De Cherney A: Ovarian stimulation for in vitro fertilization and embryo transfer, human menopausal gonadotrophin versus pure human follicle stimulating hormone: A randomized prospective study. *Fertil Steril* 1988;50:74–78