Elevated day 3 FSH/LH ratio: a marker to predict IVF outcome in young and older women

Berna Seckin · Figen Turkcapar · Gulnur Ozaksit

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Abstract

Purpose To evaluate the value of elevated day 3 FSH/LH ratio in predicting IVF results in young and older women.

Methods One hundred seventy-four women with normal day 3 FSH levels undergoing IVF treatment were studied. Patients were divided into two groups according to basal FSH/LH ratio: Group 1 (FSH/LH ≥ 3 , $n=43$) and Group 2 (FSH/LH <3 , $n=131$). The effects of FSH/LH ratio on IVF outcomes were compared. Also, the impact of elevated FSH/LH levels on younger (<35 years; $n=113$) and older (≥ 35 years; $n=61$) women was evaluated.

Results Group 1 had significantly lower mean day 3 LH levels ($p=0.001$), lower number of oocytes retrieved ($p=0.004$) and lower clinical pregnancy rate ($p=0.04$). Older women with elevated FSH/LH ratio ($n=23$) had significantly lower transferred good grade embryo counts ($p=0.04$) and lower pregnancy rate ($p=0.03$) versus older women with lower FSH/LH ratio. But in younger women treatment outcomes were similar in both subgroups.

Conclusion Elevated day 3 FSH/LH ratio is useful in predicting IVF outcome in older women, but does not seem to be an accurate predictor in younger women.

Keywords FSH/LH ratio · IVF outcome · Ovarian reserve · Pregnancy

Capsule An elevated basal FSH/LH ratio in the presence of a normal basal FSH level is associated with poor IVF outcome in older women but not in younger women.

B. Seckin (✉) · F. Turkcapar · G. Ozaksit
Assisted Reproduction Unit,
Zekai Tahir Burak Women's Health
Education and Research Hospital,
Güvenlik Avenue, Yazanlar Street 28/6, A.Ayrançi,
06540 Ankara, Turkey
e-mail: bernaseckin1@hotmail.com

Introduction

The aim of controlled ovarian hyperstimulation (COH) for in vitro fertilization (IVF) is to induce women to produce a larger number of mature oocytes available for retrieval and to provide a sufficient number of high-quality embryos for transfer. Adequate ovarian reserve is essential for the success of this treatment. Identifying potential low responders is of clinical importance for predicting the chance of pregnancy with treatment and required dose of medication.

Advancing female age is associated with diminished ovarian reserve [1]. On the other hand, chronologic age does not always show a linear correlation with the ovarian age and some young women respond poorly to COH. It has been suggested that there is a fixed time-interval between accelerated decline of fertility and the menopause and poor response to ovarian stimulation most likely represents this stage [2]. The variability of ovarian ageing among women is evident from the large variation in age at menopause [3]. It is estimated that up to 10% of women in general population may have experienced an accelerated decline of fertility before the expected time. They may continue to be otherwise asymptomatic for several more years and they have been classified as “early ovarian ageing” [2]. It is necessary to distinguish this separate group of patients from the physiologic ovarian ageing for proper interpretation of ovarian reserve screening tests and altering stimulation regimens [4].

The most commonly used marker for predicting ovarian reserve is the day 3 follicle stimulating hormone (FSH) level [5]. Despite the presence of a normal day 3 FSH, some patients respond poorly to controlled ovarian hyperstimulation. Serum FSH levels increase early in reproductive ageing and a rise in luteinizing hormone (LH) levels is observed at a later stage [6]. As a result, an exaggerated day 3 FSH/LH ratio even with normal basal FSH has been reported to be a sign of

diminished ovarian reserve and poor IVF outcome [7–12]. However, in the studies investigating the impact of elevated FSH/LH ratio as a predictor of prognosis in IVF success, analysis according to age was not performed.

In the present study we aimed to investigate the effectiveness of an elevated FSH/LH ratio as a marker to predict IVF outcome in young and older reproductive age women with normal day 3 FSH concentrations.

Materials and methods

This study included 174 women who were treated in the IVF unit of a tertiary referral hospital between January 2008 and February 2010. Inclusion criteria were age <42 years, body mass index of 18–29 kg/m², regular menstrual cycles every 25–35 days, total antral follicle count of 8–18 and basal day 3 FSH levels <12 mIU/mL at the unstimulated cycle. Patients with polycystic ovarian syndrome (PCOS), hypothalamic hypogonadism, hyperprolactinemia, history of ovarian surgery and endometriosis were excluded. Therefore we selected patients who were considered to have favourable ovarian reserve. Each patient was evaluated at single IVF treatment cycle. Institutional Review Board approval was obtained for this prospective cohort study and informed consent was obtained from all participants.

All patients had been tested for basal serum FSH, LH, estradiol (E₂) levels on the third day of a spontaneous cycle, within 3 months of the IVF cycle. E₂, FSH and LH levels were determined with an electrochemiluminescence immunoassay (Elecsys 170 analyzers, Roche Diagnostics). The intra- and inter-assay coefficients of variation were, respectively, 4.0% and 6.0% for E₂, 2.0% and 1.2% for FSH, 2.9% and 2.4% for LH. The lower sensitivity of the assay for E₂, FSH and LH was 10 pg/mL, 0.3 mIU/mL and 0.07 mIU/mL respectively.

For ovarian stimulation, the standard long protocol was used. Pituitary suppression was achieved by gonadotropin releasing hormone (GnRH) agonist leuporelin acetate (Lucrin Daily, Abbott, Johannesburg). Then stimulation with recombinant FSH (r-FSH) was performed with an initial 150–225 IU daily dose. Gonadotropin dosage was adjusted accordingly by the sonographic findings and serum E₂ levels. Human chorionic gonadotropin (hCG) (Pregnyl, Organon, the Netherlands, 10,000 IU, IM) was administered when at least three follicles reached a mean diameter of 18 mm. Cycle cancellation was undertaken, with poor ovarian response defined as a peak E₂ levels were <500 pg/mL or fewer than three mature follicles.

Follicular aspiration was performed 36 h after hCG injection. Metaphase II oocytes were analyzed. ICSI procedure was performed in all cases as our clinical policy. Fertilization was assessed 16–18 h after ICSI and embryo quality

was evaluated 2 and 3 days after ICSI by determining the number of blastomers and the relative proportion of anucleate cell fragments. A good quality embryo was defined as four cells on day 2, eight cells on day 3 and no fragmentation and multinucleation. A maximum of four embryos were transferred 2 or 3 days after oocyte retrieval. The present study was conducted before the new legislation concerning assisted reproduction treatments that restricts the number of embryos to be transferred. Therefore, multiple embryos were transferred in the study period.

All patients received luteal phase support with 90 mg/day vaginal progesterone gel (Crinone 8%, Merk Serono, Germany) starting on the day of oocyte retrieval. Clinical pregnancy was defined as the presence of gestational sac containing fetal hearts on ultrasound scan. Clinical pregnancy rates and cancellation rates were calculated per the number of IVF treatment cycles.

IVF cycle characteristics; serum E₂ concentrations and endometrial thickness on the day of hCG injection, duration of stimulation, total dose of gonadotropins used, number of retrieved and fertilized oocytes, number of transferred good quality embryos and cancellation rates, clinical pregnancy rates per cycle were determined. Patients were divided into two groups according to day 3 FSH/LH ratio: Group 1 (FSH/LH ≥3) and group 2 (FSH/LH <3). This cutoff level for the FSH/LH ratio was selected according to previous studies demonstrated highest specificity and sensitivity of poor response [9, 10]. Also, participants were placed into subgroups for the further analysis as younger (<35 years) or older (≥35 years) women. Stimulation cycle characteristics, cancellation and pregnancy rates were compared between the Group 1 and Group 2, also analysis of younger and older subgroups women were performed.

Statistical analysis of the data was performed using SPSS for Windows v. 11.5 statistical package programme (SPSS, Chicago, IL, USA). If the normality assumption for the comparison of means between two groups was satisfied, Student's *t*-test was used for the comparisons of means. Alternatively, if there was evidence of non-normality the Mann–Whitney *U* test was used. Comparisons between proportions were performed with a Pearson's chi-squared test or Fisher's exact test. All *P*-values reported are two tailed and at *P*<0.05, the difference was considered to be statistically significant.

Results

One hundred seventy-four patients met the inclusion criteria were included in the present study. Group 1 consisted of 43 patients with basal day 3 FSH/LH ≥3, group 2 included 131 patients with day 3 FSH/LH <3.

The indications for IVF treatment included male factor infertility (48.85%), unexplained infertility (30.45%) and tubal factor infertility (20.68%). There were no differences in the causes of infertility between the groups. The mean age of group 1 was significantly higher than group 2 (33.93 ± 5.24 vs. 30.98 ± 5.43 , $p=0.002$).

The mean basal day 3 FSH and E_2 levels were not significantly different between the groups. The mean basal day 3 LH concentration was significantly lower in group 1 (2.72 ± 1.28 vs. 5.02 ± 2.33 mIU/mL, $p=0.001$). Table 1 shows patient characteristics of the groups.

When the treatment cycle characteristics were compared between the groups, no differences were found with regard to total consumption dose of gonadotropin, serum E_2 level on the day of hCG injection, the duration of stimulation, number of oocytes fertilized, number of good quality embryos transferred and cycle cancellation rate. The mean endometrial thickness on the day of hCG injection was lower in group 1 (8.77 ± 1.98 vs. 9.85 ± 1.96 mm, $p=0.005$) and the mean number of oocytes retrieved was lower in group 1 (7.36 ± 4.33 vs. 9.95 ± 4.81 , $p=0.004$). The clinical pregnancy rate per cycle was significantly lower in group 1 (21.05% vs. 41.02%, $p=0.04$). Stimulation parameters of group 1 and group 2 are shown in Table 2.

In the subgroup analysis, older women with an elevated FSH/LH ratio ($n=23$) had significantly higher day 3 FSH levels (7.65 ± 1.76 vs. 6.46 ± 1.75 mIU/mL, $p=0.01$), lower day 3 LH levels (2.60 ± 0.81 vs. 4.71 ± 2.15 , $p=0.001$), lower transferred good grade embryo counts (2.47 ± 1.46 vs. 3.40 ± 1.56 , $p=0.04$) and lower pregnancy rate (5.26% vs. 31.25%, $p=0.03$) versus older women with lower FSH/LH ratio ($n=38$). In younger women with an elevated FSH/LH ratio ($n=20$), the mean day 3 LH levels were lower than younger women with lower FSH/LH ratio (2.85 ± 1.69 vs. 5.15 ± 2.41 mIU/mL, $p=0.001$), the mean endometrial thickness on the day of hCG injection was lower (9.03 ± 2.06 vs. 10.26 ± 1.93 mm, $p=0.02$), also the mean number of oocytes retrieved was lower, but the difference was of borderline significance (8.17 ± 3.87 vs. 10.20 ± 4.33 , $p=0.07$). There was no significant differences in the pregnancy and cancellation

rate between the two subgroups of young women. Patient and cycle characteristics of younger and older subgroup women are presented in Table 3.

Discussion

This study was mainly conducted to investigate the predictive value of an elevated FSH/LH ratio on IVF outcomes in different age groups. Our results showed that in all patients with elevated FSH/LH ratio, pregnancy rate was decreased. This decrease seems to result from the data that less number of oocytes were collected in this group. In detailed analysis according to age, we found that pregnancy rate was significantly lower also in the subgroup of older women with an elevated FSH/LH ratio. The only significantly different data in this group is the decreased number of good quality embryos transferred compared to the older women with lower FSH/LH ratio. In younger women we could not determine a predictive value of the elevated FSH/LH ratio on IVF outcomes. The only significantly different data between the two subgroups of younger women was endometrial thickness. Although it was statistically significant, it had no considerable effect on treatment outcomes.

The age related decline of the success in IVF is largely attributable to a progressive decline of oocyte quality and quantity [13]. It has been suggested that diminished ovarian reserve in younger women may be more reflective of quantity, rather than quality of oocytes [4, 14, 15]. Abdalla et al. [14] demonstrated that although younger cycling patients with high FSH had significantly lower number of oocytes collected and lower number of available and transferred embryos, their pregnancy rate was significantly higher than older women with normal FSH. Our study is consisted with these previous observations. In the present study, younger women with an elevated FSH/LH ratio tended to have lower number of oocytes retrieved than younger women with lower FSH/LH ratio, with borderline significance ($P=0.07$), but

Table 1 Patient characteristics of the groups

	Group1 (FSH/LH ≥ 3) ($n=43$)	Group 2 (FSH/LH <3) ($n=131$)	P value
Age(years)	33.93 ± 5.24	30.98 ± 5.43	0.002 ^a
Basal E_2 (pg/mL)	50.44 ± 29.62	42.73 ± 22.75	0.07
Basal FSH (mIU/mL)	7.39 ± 2.21	6.54 ± 1.82	0.13
Basal LH (mIU/mL)	2.72 ± 1.28	5.02 ± 2.33	0.001 ^a
Causes of infertility (n,%)			
Tubal factor	10 (23.2%)	26 (19.8%)	0.85
Male factor	21 (48.8%)	64 (48.8%)	
Unexplained	12 (27.9%)	41 (31.3%)	

Values are given as mean $\pm$ SD or number (percentage)

^a $P<0.05$ is considered as significant by Student's *t*-test

Table 2 Stimulation parameters of the groups

	Group1 (FSH/LH ≥ 3) ($n=43$)	Group 2 (FSH/LH <3) ($n=131$)	<i>P</i> value
Total dose of gonadotropins (IU)	2925 $\pm$ 1277	2583 $\pm$ 1328	0.16
Serum E ₂ on the day of hCG injection (pg/mL)	1692.48 $\pm$ 784.94	1989.13 $\pm$ 959.28	0.97
Endometrial thickness on the day of hCG injection (mm)	8.77 $\pm$ 1.98	9.85 $\pm$ 1.96	0.005 ^a
Duration of stimulation (days)	10.52 $\pm$ 2.15	10.56 $\pm$ 1.74	0.90
Number of oocytes retrieved	7.36 $\pm$ 4.33	9.95 $\pm$ 4.81	0.004 ^a
Number of oocytes fertilized	3.38 $\pm$ 2.15	3.83 $\pm$ 1.88	0.23
Number of good quality embryos transferred	3.13 $\pm$ 1.39	3.45 $\pm$ 1.22	0.19
Pregnancy rate (n,%)	9 (21.05%)	53 (41.02%)	0.04 ^b
Cancellation rate (n,%)	8 (18.6%)	14 (10.68%)	0.17

Values are given as mean $\pm$ SD or number (percentage)

^a $P < 0.05$ is considered as significant by Student's *t*-test

^b $P < 0.05$ is considered as significant by Pearson's chi-squared test

there was no significant difference in pregnancy rate in these subgroups of young women.

The two-cell theory suggests that both FSH and LH are needed for normal follicular growth [16]. It has been shown that a sustained secretion of LH is necessary for follicular development and ovulation could not be induced by FSH in the absence of LH receptor expression [17, 18]. The intra-ovarian regulators have regulatory function over follicle growth and development, acting by autocrine, paracrine or hormonal routes. The secretion of these proteins occurs under gonadotrophic stimulation [16]. It was speculated that

when day 3 LH concentrations are <3 mIU/mL there may be reduced activity of these ovarian regulators which can reduce number of follicles that become pre-ovulatory [18].

Likewise, several previous studies reported that a decreased day 3 LH concentration with normal basal FSH levels which could be reflected as an elevated FSH/LH ratio is predictive of a reduced ovarian response [9–11]. It was suggested that the exogenous addition of LH may be of benefit in this subgroup of women in order to improve IVF outcomes [10]. The use of LH for IVF stimulation is a debated issue in the literature and it has not been shown a

Table 3 Patient and cycle characteristics of younger and older women

	Older women (≥ 35 years) ($n=61$)			Younger women (<35 years) ($n=113$)		
	FSH/LH ≥ 3 ($n=23$)	FSH/LH <3 ($n=38$)	<i>P</i> value	FSH/LH ≥ 3 ($n=20$)	FSH/LH <3 ($n=93$)	<i>P</i> value
Age (years)	38.00 $\pm$ 2.44	37.76 $\pm$ 2.17	0.69	29.25 $\pm$ 3.29	28.21 $\pm$ 3.61	0.24
Basal E ₂ (pg/mL)	50.72 $\pm$ 32.77	43.71 $\pm$ 22.15	0.32	50.12 $\pm$ 26.37	42.33 $\pm$ 23.10	0.18
Basal FSH (mIU/mL)	7.65 $\pm$ 1.76	6.46 $\pm$ 1.75	0.01 ^a	7.09 $\pm$ 2.65	6.57 $\pm$ 1.86	0.29
Basal LH (mIU/mL)	2.60 $\pm$ 0.81	4.71 $\pm$ 2.15	0.001 ^a	2.85 $\pm$ 1.69	5.15 $\pm$ 2.41	0.001 ^a
Total dose of gonadotropins (IU)	3539 $\pm$ 1207	3381 $\pm$ 1577	0.70	2245 $\pm$ 1050	2273 $\pm$ 1067	0.92
Serum E ₂ on the day of hCG injection (pg/mL)	1764.5 $\pm$ 921.4	1938.0 $\pm$ 928.4	0.52	1606.9 $\pm$ 602.7	2008.4 $\pm$ 975.3	0.11
Endometrial thickness on the day of hCG injection (mm)	8.55 $\pm$ 1.95	8.82 $\pm$ 1.68	0.60	9.03 $\pm$ 2.06	10.26 $\pm$ 1.93	0.02 ^a
Duration of stimulation (days)	10.84 $\pm$ 1.74	10.75 $\pm$ 1.83	0.87	10.17 $\pm$ 2.54	10.49 $\pm$ 1.70	0.51
Number of oocytes retrieved	6.63 $\pm$ 4.68	9.31 $\pm$ 5.94	0.10	8.17 $\pm$ 3.87	10.20 $\pm$ 4.33	0.07
Number of oocytes fertilized	2.94 $\pm$ 2.81	3.84 $\pm$ 2.67	0.26	3.88 $\pm$ 0.85	3.83 $\pm$ 1.50	0.90
Number of good quality embryos transferred	2.47 $\pm$ 1.46	3.40 $\pm$ 1.56	0.04 ^a	3.88 $\pm$ 0.85	3.47 $\pm$ 1.08	0.14
Pregnancy rate (n,%)	1 (5.26%)	12 (31.25%)	0.03 ^b	8 (41.17%)	41 (44.7%)	0.78
Cancellation rate (n,%)	5 (21.73%)	6 (15.78%)	0.55	3 (15.0%)	8 (8.6%)	0.40

Values are given as mean $\pm$ SD or number (percentage)

^a $P < 0.05$ is considered as significant by Student's *t*-test

^b $P < 0.05$ is considered as significant by Fisher's exact test

clear beneficial effect of LH addition on IVF outcomes [19, 20]. Moreover, the role of the supplementation of LH in the women with high basal FSH/LH ratio and lower LH concentration has not been investigated.

Several previous studies had evaluated the predictive role of day 3 FSH/LH ratio on IVF outcomes [8–12]. In these studies, decreased ovarian response and lower pregnancy rates with an elevated FSH/LH ratio in the presence of a normal basal FSH were reported. Mukherjee et al. [8] and Liu et al. [11] showed that patients with a high FSH/LH ratio had significantly cycle cancellation rate.

As similarly, Ho et al. [21] assessed the relationship of serum FSH/LH ratio at the start of gonadotropin stimulation after pituitary down-regulation. They found that with an elevated FSH/LH ratio and lower endogenous LH levels, the number of recruited follicles was decreased and the chance of cancelling the cycle was increased. Orvieto et al. [12] examined the predictive role of day 3 FSH/LH ratio or LH levels in patients undergoing COH with GnRH agonist or antagonist cycles. They observed that elevated FSH/LH ratio, but not basal LH level, was associated with an inferior IVF outcome in both agonist and antagonist cycles.

However, subgroup analysis according to age was not performed in these studies. To the best of our knowledge, the predictive value of FSH/LH ratio on IVF results in younger and older reproductive age women has not been examined.

In this study, our data support the results of these earlier studies [8–12]. The elevated FSH/LH ratios were associated with the lower concentrations of day 3 LH levels both in all patients and subgroups.

Measurement of day 3 FSH and LH level is a rapid, easy screening test with low cost. Although previous studies have documented the intercycle variation of basal FSH levels in the same patient, it has been also demonstrated that women with a normal basal FSH level had a small range in the intercycle variation, in contrast to women with an elevated basal FSH showing a much greater variation [22]. In our study, patients with a previously documented abnormal day 3 FSH level were excluded so we considered that the possible effect of intercycle variation of day 3 FSH levels could be minimized.

Serum anti-mullerian hormone (AMH) level is a reliable test for predicting the ovarian response to ovulation induction. But, it is not in widespread use due to the cost of testing in our country and it is not routinely measured in our clinic. The main limitation of this study is that we could not correlate our results with AMH.

In conclusion, an elevated basal FSH/LH ratio in the presence of a normal day 3 FSH level is associated with a lower pregnancy rate in older women but not in younger women. According to the results of the present study, FSH/LH ratio does not seem to be an accurate predictor of IVF

success in younger women, so this measurement should be interpreted based on patient's age. The addition of exogenous LH can be considered to improve IVF outcomes in older women with an elevated FSH/LH ratio. Further studies are needed to clarify the accuracy of basal FSH/LH ratio in correlating with AMH for the prediction of IVF success in different age groups for choosing appropriate treatment regimens.

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Conflict of interest We declare that we have no conflict of interest.

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