In Vitro Fertilization: Influence of Women's Age and Basal Follicle Stimulating Hormone Levels

IN VİTRO FERTİLİZASYON: KADININ YAŞI VE BASAL FOLLİKÜL STİMULE EDİCİ HORMON DÜZEYLERİNİN ETKİSİ

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SUMMARY

Prior studies have demonstrated that gonadotrophin stimulating quality is better in vitro fertilization (IVF) patients with low basal follicle stimulating hormone (FSH) levels. In this study 78 women (83 cycles) who had undergone IVF attempts in our clinic were studied to determine the potential impact of basal FSH and age. Analyses were performed to predict the factors that were contributing the fertilization and cleavage rates. No correlation was found between the age and the group with high basal FSH (>10 IU/L) and that with low basal FSH level (< 10 IU/L) with regard to fertilization, cleavage rate and oocyte quality.

Key Words: Basal FSH, Women's age, In vitro fertilization

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ÖZET

İn vitro fertilizasyon (IVF) hastalarında basal follikül stimule edici hormon (FSH) düzeyi düşük olduğu zaman daha başarılı gonadotropin stimülasyonu sağlandığı çalışmalar ile gösterilmiştir. Bu çalışmada basal FSH ve yaşın potansiyel rolünü incelemek amacıyla IVF uygulanan 78 kadının 83 siklusu incelenmiştir. Fertilizasyon ve klivaj oranlarına etki eden faktörler araştırılmıştır. Yüksek basal FSH (>10 IU>L) ve düşük basal FSH (<10 IU/L) ile yaş ve fertilizasyon ve klivaj oranları ve oosit kalitesi arasında bir korelasyon bulunamamıştır.

Anahtar Kelimeler: Basal FSH, Yas, in vitro fertilizasyon

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Exogenous gonadotrophin stimulation for IVF does not achieve superovulation in all women despite the use of increasing amounts of human menapousal gonadotrophin (hMG). The proposed factors for this responsivelessness are the age of patient (1,2), in some cases the causes of infertility eg. like previous pelvic surgery, chemotherapy and radiotherapy and the elevated basal level of gonadotrophins (3).

Increasing age is one of the processes that reduce the number of follicles (3). As the older patients tend to have a reduced response to gonadotrophins compared to the younger patients the question whether the ovarian status or the chronological age affects this situation arises.

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The serum level of FSH increases as ovarian functon declines. On the other hand in so, ,ie patients wide fluctuations in their basal FSH levels have been observed between cycles. This altered gonadotrophin secretion probably affects the activity of the developing cohort of follicles. Whatever the mechanism, this predictive ability of the basal serum FSH seems to predict IVF outcome (4).

Since some patients with elevated basal FSH value of with increased age do conceive spontaneously, In this study we attempted to define the effect of age and the relative power of basal FSH in predicting their value for the fertilization and cleavage rates.

MATERIALS AND METHODS

The records of 78 patients (83 cycles) in our IVF program were reviewed retrospectively. These cycles included all women taken into IVF program regardless of etiology of infertility. The infertility reasons in the study group were tubal, male factor, endometriosis and unexplained infertility. Unexplained infertility was defined when none of these infertility factors were present

and ovulation was detected by midluteal progesterone value in 6 subsequent cycles. According to our routine protocol all of our patients had undergone endocrinological (FSH, LH, estradiol **(E2),** progesterone (P), prolactin, total testosterone) and ultrasonographic evaluation prior to IVF program. At our IVF clinic, we do not treat patients with a basal FSH>20 IU/L. The cycles which were cancelled before oocyte retrieval because of inadequate response and the cycles in which no transfer occured despite oocyte recovery were excluded from the study. The basal FSH value in the cycle of stimulation was the basis for analysis in all cases. The FSH value > 10 IU/L was accepted as high and < 10 IU/L as low. In the patients in whom more than one IVF is attempted the basal FSH value from the prior cycle was analyzed. According to their age patients were divided into 3 groups; < 30 years of age; between 30 and 34; and the patients > 35 years of age.

All patients had undergone ovarian stimulating with gonadotropins [human menopausal gonadotrophs (hMG, Pergonal; Serono, Italy or Humegon; Organon, Turkey) or pFSH (Metrodin; Serono, Italy) or in combination] with pituitary desensitization using gonadotrophin releasing hormone analogues (GnRH-a, Buseralin, Suprefact; Hoechst, Germany) 0.5 ug subcutaneously. The GnRH-a are continued until the day of human chorionic gonadotrophin (hCG; Pregnyl; Organon, Turkey or Profasi; Serono, Italy) administration. 10 000 IU of hCG were administered intramusculary when two or more follicules were > 17mm and serum **E2** levels were > 200 pg/ml/per follicle > 15mm. Follicular development was monitored by **E2** and P measurements on alternate days starting from cycle day 5 with serial vaginal ultrasound examination. Oocyte recovery was performed by transvaginal ultrasound-guided needle aspiration 34-36 hours after the administration of hCG. Oocyte maturity was classified by the criteria of Abdalla et al (5). Preincubation of oocytes, sperm preperation, the concentration of sperm used for insemination, culturing conditions and techniques and general embryology laboratory procedures were employed as described by Dor J et al (6). The fertilization rate was calculated as the ratio of oocyets fertilized to number of oocytes collected, and the cleavage rate was described as the ratio of number of embryos cleaved to the number of oocytes fertilized.

All the hormonal determinations were performed in our laboratory using standard radioimmunoassay (RIA) techniques. The interassay and intra-assay coefficients of variation were 7.3% and 5.1% for FSH. Estradiol levels also were determined by a commercially available RIA (ICN Biomedicals, Inc. California) and had interassay and intra-assay coefficients of variation of 5.9% and 6.4%, respectively.

Data was collected on a database programme written for IVF clinic, and transfered to SPSS-PC package program. Unpaired t tests and chi-square analyses were performed as indicated.

Table 1. Patient characteristics according to the FSH

	FSH<10IU/L (n»59)	FSH>10IU/L (n-19)
Age	32.7 +/- 0.9	33.0 +/- 0.8
Age of husband	35.4+/-0.8	33.9+/-1.1
Age of marriage	20.5 +/- 0.6	22.9+/-0.9*
Cause of infertility		
Tubal	41 (69.5%)	11 (57.9%)
Endometriosis	6(10.2%)	3(15.8%)
Male	2 (3.4%)	2(10.5%)
Unexplained	10(3.4%)	3 (15.8%)

values are mean +/- SEM
*p<0.05

RESULTS

83 treatment cycles of 78 patients were evaluated between January and June 1992, 5 patients had two attempts. In Table 1 the patient characteristics according to their basal FSH were presented. The basal FSH values of the patients ranged between 2 to 18 IU/L on day 3 of their cycles. Fifty nine patients had FSH value below 10 IU/L. The mean value of the low FSH group was 7.2 +/- 1.2 IU/L Nineteen patients

Table 2. Patient characteristics according to the age

	<30y (n-24)	30-34y (n-42)	>35y (n-12)
Basal FSH (IU/L)	8.9 +/- 1.2	9.1 +/- 1.4	9.7 +/-1.5
Age of husband	32.8 +/- 1.1	34.1 +/- 0.9	36.4 +/- 0.8
Age of marriage	20.2+/-0.7	22.1 +/-0.4	21.2+/-0.6
Cause of infertility			
Tubal	18(75%)	27(64.3%)	7(58.3%)
Endometriosis	3(12.5%)	4(9.5%)	2(16.6%)
Male	1 (4.2%)	2 (4.8%)	1 (8.3%)
Unexplained	2(8.3%)	9(21.4%)	2(16.6%)

values are mean +/- SEM

Table **3.** Clinical characteristics of cycles

	FSH<10IU/L (n-63)	FSH>10IU/L (n-20)
Number of amps of hMG	20.6 +/- 0.6	23.1 +/-0.1*
Maximum hMG/per day	3.9 +/- 0.2	3.8+/-0.3
Duration of GnRH-a	22.1 +/-1.6	25.6+/-2.0
Day of hCG	11.5 +/-1.8	13.2+/-0.5
E2 on day of hCG	1885.1 +/-111.1	1822.6+/-93.8
P on day of hCG	1.9+/-0.2	2.1 +/-0.4
Endometrial thickness on day of hCG	11.2+/-0.4	10.6+/-0.5

values are mean +/- SEM
*p<0°5

Table 4. Classification and outcome of oocytes retreived in cycles with high or low FSH

	FSH<10IU/L (n-63)	FSH>10IU/L (n-20)
Number of total oocytes retreived per cycle	6.2+/-1.1	5.7 +/- 0.9
Oocyte muturity/per cycle		
Mature oocytes	4.5+/-0.7	3.8 +/- 0.8
Overmatuer oocytes	1.5+/-0.3	1.2+/-0.2
Immature oocytes	1.3+/-0.4	1.5+/-0.5
Total no of oocytes fertilized	2.8+/-0.7	2.2+/-0.6
Fertilization rate	46.6+/-8.1	39.2+/-6.8
Total no of embryos cleaved per cycle	2.6 +/- 0.7	1.9+/-0.4
Cleavage rate	93.1 +/-8.7	86.6 +/-6.3
Number of embryos transfered per cycle	1.2+/-0.3	0.9 +/- 0.4

values are mean +/- SEM

with high FSH group had a mean FSH value of 13.1 +/- 1.3 IU/L (p<0.01). The parameters that were evaluated in Table 1 were comparable except the age of marriage. In Table 2 the patient characteristics according to the age groups were presented and none of the parameters were statistically different.

Comparison of the cycles with high and low FSH revealed no difference in any of the parameters except the total number of ampules used. In the high FSH group the mean total number of hMG ampules used was significantly higher than the mean total number of hMG ampules used in the low FSH group (p<0.05; Table 3).

The classification and the outcome of the oocytes retrived in cycles with high and low FSH groups presented in Table 4. Number of the total oocytes retrieved per cycle, oocyte quality per cycle and the total number of oocytes fertilized were comparable. Fertilization and cleavage rate seems better in the low FSH group but this difference was not statistically significant. Similarly no impact of age has been demonstrated on changes of fertilization, cleavage and oocyte quality (Table 5).

DISCUSSION

The clinical use of gonadotrophins to induce follicular maturation is associated with a wide variability as far as ovarian response is concerned. This variability induced clinicians to review the parameters (such as age, basal FSH, **E2)** (1,2,3). On the other hand in some cases, like polycyctic ovary syndrome, dissociated cyctic follicular growth could be detected.

Bas'! FSH concentrations reflect the balance between ovarian steroids and nonsteroidal factors during the follicular recruitment but before selection of the dominant follicle. These developing cohort follicles are

Table 5. Clasification and outcome of oocytes retrived in cycles according to age

	<30y (n-24)	30 to 34 (n-42)	>35y (n-12)
Number of total oocytes retrieved per cyclee	6.9+/- 1.2	5.9 +/- 0.9	5.1 +/-0.7
Oocyte maturity/per cycle			
Mature oocytes	4.5+/-1.2	3.5+/-1.3	2.7 +/- 0.9
Overmature oocytes	1.1 +/-0.3	1.2+/-0.4	1.1 +/-0.2
Immature oocytes	1.2+/-0.4	1.3+/-0.5	1.2+/-0.6
Total no of oocytes fertilized	3.6 +/- 0.7	2.4 +/-0.5	1.7+/-0.5
Fertilization rate	52.3+/-9.1	41.3+/-7.6	33.8 +/-5.6
Total no of ebryos cleaved per cycle	3.4 +/- 0.8	2.2 +/- 0.4	1.5+/-0.3
Cleavage rate	94.6 +/- 8.1	91.2+/-6.7	88.6+/-7.1
Number of embryos transfered per cycle	1.4+/-0.4	1.1 +/-0.6	0.7+/-0.3

values are mean +/- SEM

able to supress FSH levels by producing enough gonadal steroids during early follicular phase of the cycle. If the basal FSH levels are low for stimulation of development of the cohonot follicles a better response can be obtained by using hMG.

The maternal age is also considered as an important determinant in the outcome of IVF. A number of age associated decrements in IVF perfomance have reported regarding to oocyte quality. Sharma et al (2) and Piette et al (7) documented that number of oocytes retrieved and number of oocytes transfered were adversely affected by increasing age. There is a controversy about the relation between endometrial receptivity and age. The deleterious effect of recipient's age in the pregnancy rate on oocyte donation cycles was shown by Abdalla et al (8), while Serhal and Craft (9) reported that the pregnancy rate was not significantly different between those patients below or above the age of 40 years.

While most IVF centers employ a standard stimulation procedure for all patients -at least in their first cycles- we tried to evaluate the anticipated pattern of the ovarian response according to pretreatment FSH value and age. The basal E_2 levels were not taken into account in this study because we believe that the nonsteroidal factors originating from developing follicles also play a significant role in regulating gonadotrophin secretion during the early follicular phase.

In contrast to some other authors, our results indicate that both basal FSH and age are not strong influences on IVF performance regarding to parameters investigated in this study. We have only 4 patients with high basal FSH and > 35 years of age, though we haven't made any evaluation in this small group of patients. In our study the only parameter affecting the outcome was the total number of ampules of hMG.

Based on this data, patients with elevated basal FSH or increased age are not excluded from our IVF programme. Furthermore, it is possible that increasing the total dose of hMG may abolish the deleterious effect of these parameters.

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