Progesterone Levels on the Day of hCG and Fertilization Rates in In-vitro Fertilization

IN VITRO FERTİLİZASYON SİKLUSLARINDA hCG GÜNÜ PROGESTERON DÜZEYLERİ VE FERTİÜZASYON ORANLARI ARASINDAKİ İLİŞKİ

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SUMMARY

*In this study serum progesterone (P) levels on human chorionic gonadotropin (hCG) administration day in in vitro fertilization (IVF) cycles with gonadotropin-releasing hormone agonist (GnRH-a) suppression were investigated whether an elevated P level had an adverse affect on oocyte quality and fertilization of oocytes. Seventy-eight cycles treated with GnRH-a (Buserelin) begun in the luteal phase of the prior menstrual cycle and continued until the day of hCG were studied. In 30 cycles P was < 1.5 ng/ml and in 48 cycles P > 1.5 ng/ml. Cycles with high P levels had significantly high estradiol **(E2)** levels on the day of hCG (p<0.01). The mean number of mature oocytes per cycle, the fertilization and cleavage rate and the number of embryos was significantly low and the mean number of postmature oocytes was significantly high when the P level over 1.5 ng/ml on the hCG day (p<0.05). In conclusion, the IVF cycles suppressed with GnRH-a; the fertilization, cleavage rate and oocyte quality is better when the P level lower than 1.5 ng/ml on the hCG day.*

Key Words: In vitro fertilization, Progesterone, Fertilization rate, Oocyte maturity

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ÖZET

*Bu çalışmada gonadotopin-releasing hormon analog (Gn-RH a) kullanılan in vitro fertilizasyon (IVF) siklusla rında human koryonik gonadotropin (hCG) yapılma günündeki progesteron (P) düzeyleri incelenerek, yüksek P düzeyinin oosit kalitesi ve fertilizasyonu üzerine olumsuz etkisi olup olmadığı araştırılmıştır. Bir önceki siklusun luteal döneminde başlanarak hCG gününe dek GnRH-a kullanılan yetmişsekiz siklus çalışılmıştır. 30 siklusta P değeri < 1.5 ng/ml iken, 48 siklusta P değeri > 1.5 ng/ml idi. P düzeyi yüksek olan sikluslarda hCG günündeki estradı'ol **(E2)** düzeyi anlamlı olarak yüksek idi (p<0.01). P düzeyi hCG gününde 1.5 ng/ml üzerinde olduğu grupta siklus başına ortalama matür oosit sayısı, fertilizasyon ve klivaj oranları anlamlı olarak düşük, ortalama postmatür oosit sayısı anlam olarak yüksek bulundu (p<0.05). Sonuç olarak GnRH-a ile süprese edilen IVF sikluslarında, fertilizasyon ve klivaj oranları ve oosit kalitesi hCG gününde P düzeyi 1.5 ng/ml altında ise daha iyidir.*

Anahtar Kelimeler: İn vitro fertilizasyon, Progesteron, Fertilizasyon oranı, Oosit maturitesi

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It is commonly accepted that an adjuvant use of gonadotropin-releasing hormone agonists (GnRH-a) improve oocyte quality (1-3), allow a more synchronous cohort of follicles to be recruited and prevent premature luteinizing hormone (LH) surges thus providing an opportunity to avoid exposure of ovarian follicles to endogenous gonadotropins (3-6). Premature luteinization may lead to postmature oocytes, decreased fertilization and a lower pregnancy rate (7). Progesterone (P) concentrations prior to, during and following the administration of human chorionic gonadotropin (hCG) are a valuable marker for the detection of early luteinization and premature ovulation cycles (8-10). According to the accepted criteria when P levels surpass 1.5 ng/ml on the day of hCG most In vitro fertilization (IVF) centers cancel treatment cycles. However, conflicting reports exist regarding P levels to predict IVF outcome (11-12). The purpose of this retrospective study was to examine how P levels on the day of hCG correlate with oocyte quality, fertilization and cleavage rates In our IVF Unit.

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MATERIALS AND METHODS

72 patients (21 to 39 years of age) undergoing IVF with a standard regimen of GnRH-a/human menopausal gonadotropins (hMG) following pituitary suppression in 78 cycles are retrospectively reviewed. Cycles with abnormal semen analysis were excluded from the study to avoid the influence of male infertility on the results of IVF-ET. The overall distrubution of infertility etiology was as follows: tubal factor 62.6%, unexplained 32% and endometriosis 6.4%.

GnRH-a (Buserelin, Hoechst, Frankfurt, Germany) was administered subcutaneously (s.c.) once daily (0.5 mg/d) starting on day 20 of the pretreatment cycle and continued until the day of hCG. Ovarian suppression was indicated by estradiol (E2) < 30 pg/ml, endogenous gonadotropins < 4 IU/L and follicles or cysts > 1.2 cm viewed by vaginal ultrasound (U/S) (13). When the suppression criteria were satisfied, hMG (Humegon, Organon, Istanbul, Turkey) 3 ampules daily, intramuscularly (i.m.) was started. Five days later, ovarian response was assessed by transvaginal U/S and serum **E2** and P assays. hMG dosage was then adjusted by the serum **E2** and follicular growth. When the leading follicle was > 15mm, the patients were followed on daily basis again with **E2,** P and U/S. Cycles with a P value > 2.5 ng/ml on two alternate days were cancelled. hCG (Pregnyl, Organon, Istanbul, Turkey) 10 000 IU was administered i.m. when sonography revealed at least three follicles > 17mm in diameter and serum E2 concentration was between 200 pg/ml/follicle > 15mm. Patients did not get any hMG injection on hCG day but got the last 0.5mg dose of GnRH-a s.c.

34-36 hours after hCG administration, oocytes retrieved by ultrasound guided transvaginal aspiration. The maturational status of the oocytes was recorded according to the criteria of Abdalla et al (3). Sperm processing, insemination and embryo transfers (ET) were performed by standard techniques (14). Up to 4 embryos were transferred 48 hours after oocyte retrieval. Luteal phase was routinely supported with Progesterone in oil (50 mg/day i.m.) starting the day of embryo transfer. p-hCG levels were determined 20 days after ET. The pregnancy was confirmed by visualization of gestational sac 4 weeks after ET.

Retrospectively, the cycles were divided into two groups: cycles in which P < 1.5 ng/ml on the day of hCG (low P group) and cycles in which P > 1.5 ng/ml on the day of hCG (high P group).

Data were collected on a data base program written for IVF clinic and transferred to SPSS/PC package program. Student's t- test and chi-square tests were used for statistical analysis, where appropriate. Values are given as mean ± SEM. A p value < 0.05 was considered significant.

RESULTS

Table 1 presents characteristics of patients and cycle stimulation data of the two groups. The groups were similar in terms of patients age, duration of infertility, etiology of infertility, amount of gonadotropin administered and the day of hCG administration. Patients in the high P group had a significantly greater E2 level ($p < 0.01$) on the day of hCG. A slight but significant correlation existed between P and E2 (r-0.37, $p<0.05$).

The mean values of the serum P levels for the days -4, -3, -2, -1, 0 (day 0 as the day of hCG) is given in Figure 1. Serum P levels of the high P group are compared with the serum P levels of the low P group for each day. Statistical difference was observed only on day 0.

In Table 2 the oocyte and embryo data of the cycles in the two groups are presented. The mean number of oocytes retrieved per cycle was similar in both

Table 1. Personal and cycle proporties of patients with low P and high P on the day of hCG

	p<1.5 (ng/ml)	p>1.5 (ng/ml)
No. of patients	27	45
No. of cycles	30	48
Age	33.4 ± 1.1	31.2 ± 0.9
Duration of infertility	9.8 ± 0.9	8.7 ± 1.1
Infertility cause		
Tubal	17(56.6%)	32(71.1%)
Endometriosis	1(3.3%)	4(8.3%)
Unexplained	13(43.3%)	12(25%)
Ampules of hMG	31.1 ± 3.4	29.7 ± 2.3
Day of hCG	11.8 ± 1.1	12.9 ± 1.2
E2 on day of hCG (pg/ml)	1476 ± 130	1973 ± 83*
P level on day of hCG (ng/ml)	1 ± 0.2	1.9 ± 0.3**

*p<0.01 **p<0.05

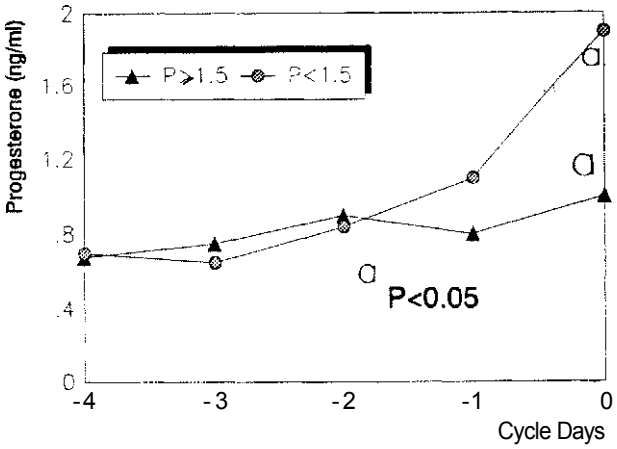

Figure 1. The mean values of serum P levels of the high P and low P groups for the days-4, -3, -2, -1, 0 (day 0 as the day of hCG.)

Table 2. Classification and outcome of oocytes retrieved in cycles with low P and high P on the day of hCG.

	p<1.5 (ng/ml) (n-30)	p> 1.5 (ng/mf) (n-48)
Total oocytes retrieved/cycle	7.4 + 1.3	6.3 ± 0.8
Mature	5.2 ± 0.7*	3.3 ± 0.6
Immature	1.7 ± 0,3	1.5 ± 0.4
Postmature	0.8 ± 0.3	1.5 ± 0.2*
Oocytes fertilized/cycle	3.9 ± 0.9	1.8 ± 0.7
Fertilization rate	53.1 ± 9.4*	29.6 ± 6.4
Embryos cleaved/cycle	3,7 ± 1*	1.5 ± 0.6
Cleavage rate	94.8 + 5.1*	81.2 + 4.1
Embryos transferred/cycle	1.3 ± 0.4	1.1 ± 0.5

*p<0.05

groups, however the mean number of mature oocytes was significantly higher in the low P group and the mean number of postmature oocytes was significantly higher in the high P group (p<0.05).

The fertilization rate (no. of oocytes fertilized/no. of oocytes collected) of the oocytes and the cleavage rate (no. of embryos cleaved/no. of oocytes fertilized) of the embryos was significantly low in the high P group (p<0.05) (Table 2).

DISCUSSION

Premature luteinization was defined clearly by Fleming and Courts (8) in patients who had functional pituitarios as serum P concentration > 1 5 ng/ml which had been associated with a rise in the serum LH concentration before developing follicles became mature. Several studies reported the absence of premature LH surge during gonadotropin therapy after desensitization of the pituitary gonadotropins by GnRH-a (15,16).

Despite suppression of endogenous gonadotropins with GnRH a, pre-hCG elevation in plasma P was reported in some cases of controlled ovarian hyperstimulation (COH) used for IVF (12.17,18). In the present study a serum P rise > 1.5 ng/ml was observed in 61.5 %(48/78) of the cycles using the protocol GnRH-a/hMG on the day of hCG. In the study of Edelstein et al (11), P levels on the day of hCG administration in cycles with GnRH-a suppression were measured in 101 patients and 72 patients had P < 0.9 ng/ml and 29 had P > 0.9 ng/ml. They found no correlation between LH and P on the day of hCG and they concluded that, premature P elevation in IVF cycles did not result from inappropriate pituitary suppression by GnRH-a. Kenigsberg et al (19) showed that in 2 of 13 monkeys pretrated with a GnRH antagonist and then stimulated with pure FSH, a definite elevation in P was defined in the absence of inci eased endogenous LH by either bioassay or RIA.

In the present study protocol, GnRH-a was administered in all women until pituitary desensltization was evidenced by a serum E_2 of < 30 pg/ml and gonadotropins < 4 IU/L. We achieved adequate suppression in terms of immunoreactive (IR) LH, while in our laboratory we were unable to measure bioactive (BA) LH. Meldrum et al (20) reported the stimulation of IR LH with an associated reduction of BA LH and reduced BA/IR LH ratio in the human female following GnRH-a administration. Therefore if the suppression of IR LH is achieved it is not necessary to show the reduction of BA LH. Also the decreased levels of bioactive gonadotropins result in suppression of **E2** production (18). If we achieved the suppression of LH what is the mechanism responsible for the elevation of P levels.

One possible reason for the P rise on the day of hCG may be the effect of exogenous LH activity that is evenly present in the hMG. However, in the present study, there was no significant difference in the total administered dose of hMG between low and high P cycles.

Another possible reason for elevated P levels might be the excessive sensivity of granulosa cells (GCs) to LH. In this study it was demonstrated that the occurence of the P rise was significantly associated with increased concentrations of serum **E2.** This high concentration of serum **E2** induced by exogenous FSH resulted in the development of LH receptors on GCs and consequently an excessive sensitivity of the GCs to LH might Induce untimely P production even in low concentrations of serum LH (18,21).

A third possible reason for elevated P levels might be the P rise associated with non-LH dependent intraovarian mechanisms. During spontanous cycles, there is a marked increase in the rate of P rise, beginning 12 hours before the onset of LH surge (22). This P elevation occurs in the absence of detectable changes in the endogenous LH pulses and amplitude and may be secondary to some independent intra-ovarian mechanism that changes ovarian steroidogenesis toward P (23). In vitro studies have shown that human GCs obtained from late follicular phase follicles have an enhanced capacity to produce **E2** and P compared with GCs collected during the early follicular phase (24). Enhanced steroidogenesis reflects development and maturation of the enzyme complexes in the steroid cascade from cholesterol to **E2.** Furthermore, as final follicle maturation approaches the GCs use the delta 4 substrates more efficiently (24). In addition, the parallel increase in 3 6 hydroxysteroid dehydrogenase (3 p-HSD) activity progressively enhances the production of P from pregnenolone. Before the GCs are expressed to LH or LH-active substances (e.g. hCG), the 17-hydroxylase. C 17-20 desmolase, 3 p-HSD, 17 b-oxireductase, and aromatase support the increased E2 biosynthesis. Therefore, the preovulatory state is characterized by high serum levels of E2 and increasing

concentaritons of P (25). Increases in serum P levels up to 4-fold of previous levels may indicate with great probability a LH surge: however, in certain circumstances, it may represent a simple change in de novo biosynthesis in response to enzymatic competence or may represent an abnormality of granulosa cell function (10).

The results of this study showed that high serum P levels might be a marker for poor quality of oocytes. Silverberg et al (17) reported that although the number of collected oocytes was significantly correlated with increased serum P concentrations, the percentage of mature oocyte did not decrease with this increase. Moreover they also pointed out that highest fertilization rate was obtained in patients with increased concentration of the serum P (>0.9 ng/ml). However in our study, the number of mature oocytes collected was significantly low and the number of postmature oocytes collected was significantly high in the high P group. Therefore it is likely that the P rise may affect the maturation of oocytes. Furthermore, the differences in the rates of fertilization and cleavage and the number of embryos were also significantly different in two groups.

In the experimentally isolated mouse follicle, high P levels induced oocyte atresia and increased the number of inactive oocytes (26,27). Moreover reduced fertilization rates of ovine oocytes and abnormal embryo development in vitro was shown in the presence of elevated P levels (28).

The results of this study were highly similar to those reported by several authors (9,17,18). These results suggest that the P rise on the day of hCG might be a marker for poor quality of oocytes and poorer fertilization like premature luteinization does (8).

In conclusion, we suggest that the measurement of serum P concentration on the day of hCG may allow prediction of oocyte quality and fertilization rate.

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