The Preovulatory Serum Estradiol Pattern in Natural IVF/ICSI Cycles

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Purpose: The aim of the study was to find whether inferences to the possible success of natural IVF/ICSI cycles could be drawn from the estradiol (E_2) pattern.

Methods: Sixty-eight women who underwent oocyte recovery in 98 natural cycles were recruited for the study. Daily serum E_2 was measured in the preovulatory phase (-3 to $+2$ day). The E_2 pattern was compared among four groups: Group A, unsuccessful egg retrieval; Group B, no fertilization; Group C, no implantation; and Group D, implantation.

Results: There was no difference in mean E_2 levels between groups. Only the ratio of E_2 on day $+1/E_2$ on day 0 was significantly lower in conception cycles in comparison with nonconception cycles. In cycles with a decreased E_2 level on day $+1$, only the implantation rate was significantly higher in comparison with cycles with an increasing E_2 level.

Conclusions: From the E_2 pattern it is possible to make inferences about the likelihood of implantation but not the fertilization or oocyte recovery success.

KEY WORDS: estradiol pattern; implantation; natural cycles; in vitro fertilization.

INTRODUCTION

The first successful pregnancy resulting from in vitro fertilization (IVF) occurred during an unstimulated normal menstrual cycle (1,2). Shortly afterward, however, most IVF programs began using controlled ovarian hyperstimulation (COH) to achieve the development of several preovulatory follicles in an effort to increase the number of available embryos and consequently improve pregnancy success rates (3). Since then, improvements

occurred in oocyte culture technique, sperm preparation, and oocyte retrieval. Those improvements created a renewed interest in using spontaneous cycles in IVF (4).

In most recent reports of IVF in spontaneous cycles, human chorionic gonadotropin (hCG) has been administered to replace (and precede) the natural luteinizing hormone (LH) surge to facilitate the timing of oocyte recovery and planning of work schedules (5). The designation of this type of cycle has been natural or unstimulated IVF (6). Those cycles are successful and have several advantages compared with stimulated cycles. Despite this, natural cycles are not widely used in IVF programs (7). The main reason is the high failure rate at each step in the process and the unacceptably low delivery rate per egg recovery attempt, compared with stimulated cycles (8). To improve the pregnancy rate with natural cycles, it is important to select the patient according to age, infertility factors, basal LH level, etc. (6,9–11). Besides, we also know that the fecundability of all cycles is not equal in the same woman. So it is also important to identify the factors that predict the outcome of a certain cycle.

The aim of our study was to establish whether it was possible to make inferences about the success of natural IVF intracytoplasmic sperm injection (ICSI) cycles from the preovulatory serum estradiol (E_2) pattern.

MATERIALS AND METHODS

Sixty-eight women who underwent oocyte recovery in 98 natural cycles were recruited for this study. Their age range was 25–41 years (mean = 33 years, SD = ± 4.4 years). They were chosen from among those couples in which a stimulated IVF/ICSI procedure was anticipated. The indications for IVF/ICSI procedure were only female infertility in 45 (45.9%), only male infertility in 17 (17.3%), female and male infertility in

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11 (11.2%), and unexplained infertility in 25 (25.5%) cycles. In female infertility tubal infertility prevailed (54, or 55.1%); in male infertility, oligozoospermia (12, or 12.2%). In 24 (24.5%) women with tubal infertility, hydrosalpinx was established previously. A pelvic ultrasound examination was performed on day 2 of the menstrual cycle to evaluate the ovaries for the presence of cysts and on the same day serum follicle stimulating hormone (FSH) and E_2 were measured to confirm that the cycle has just begun. The cycle was canceled if the baseline E_2 was >0.3 nM. Transvaginal ultrasonography was performed and the serum E_2 concentration was measured every 2 days from day 7 onward, and every day when the follicular diameter was >12 mm and the serum E_2 concentration >0.3 nM. Cycles with more than one growing follicle >12 mm were not included in the study.

hCG (5000 IU) was administered at 2100 hr when the average follicular diameter was >15 mm and the serum E_2 >0.5 nM. Blood was drawn for E_2 estimation 17–18 hr after hCG application and just before oocyte recovery. At 36–37 hr after hCG injection, follicular puncture was carried out in patients with negative LH before hCG application. The oocytes were cultivated and inseminated, and the embryos transferred as described previously (12). The luteal phase was supported with hCG (1000 IU) on the day of embryo transfer and 4 and 9 days later.

Serological documentation of pregnancy was scheduled 16 days after embryo transfer. A pregnancy was confirmed only if there was ultrasonic evidence of a gestation sac.

The E_2 pattern was compared among four groups of cycles: Group A, unsuccessful egg retrieval; Group B, no fertilization; Group C, no implantation; and Group D, implantation. E_2 levels on a certain day and also the ratio of E_2 levels between 2 consequent days were compared using the one-way ANOVA. If a statistically significant association was found between the E_2 ratio and the cycle outcome, the cycles were divided into two groups according to the ratio value and the difference between two groups was evaluated with Fischer's exact and Mann–Whitney U tests. The data were processed with the statistical program by Statistica. Statistical significance was set at $P < 0.05$. The study was approved by the Ethics Committee at the Ministry of Health of the Republic of Slovenia.

RESULTS

The mean FSH level observed on day 2 of the cycle was 7.32 ± 3.12 IE. The mean follicular diameter on

the day of hCG injection was 16.4 ± 1.01 mm. In a total of 98 follicular punctures, 66 oocytes were recovered (recovery rate = 67.3). In 11 (16.7%) cycles ICSI was performed; and in 55 (83.3%), IVF. The overall fertilization rate was 66.7% (43/66). Ten clinical pregnancies resulted, representing an implantation rate of 23.3% (10/43) and a pregnancy rate of 10.2% (10/98) per attempted egg recovery. According to these results Group A consisted of 32, Group B of 23, Group C of 33, and Group D of 10 cycles. No differences were established between the groups as regards indications for the IVF/ICSI procedure, the percentage of ICSI procedures, the woman's age, the percentage of women with hydrosalpinx, the FSH level on day 2 of the menstrual cycle, and the mean follicular diameter on the day of hCG injection.

The pattern of serum E_2 in the periovulatory phase in all four groups is shown in Fig. 1. The mean E_2 level rose daily from day -3 up to hCG administration (day 0) and is similar in all four groups. In nonconception cycles (Groups A, B, and C), an increase in mean E_2 levels was observed from day 0 onward, peaking on day $+1$ and then decreasing toward day $+2$. In conception cycles (Group D), a continual decrease was observed from day 0 to day $+2$. However, there was no statistically significant difference in mean E_2 levels between groups on a certain day. Only the E_2 on day $+1/E_2$ on day 0 ratio was statistically significantly lower in conception cycles in comparison with nonconception cycles (Table I). If we divided the cycles into two groups according to different values of this ratio, we would find that only the implantation rate was different. There was no implantation if the E_2 level on day $+1$ increased more than 10% from the previous day. In cycles with the same or a decreased E_2 level on day $+1$, the implantation rate was statistically significantly higher in comparison with the implantation rate in cycles with an E_2 level increasing up to 10% on day $+1$ (Table II). No differences were established between the groups as regards indications for the IVF/ICSI procedure, the percentage of ICSI procedures, the woman's age, the percentage of women with hydrosalpinx, the FSH level on day 2 of the menstrual cycle, and the mean follicular diameter on the day of hCG injection and E_2 levels before and on day 0.

DISCUSSION

Serum E_2 measurement is important in monitoring natural cycles and, according to some opinions, also for predicting cycle outcome. In reviewing the characteristics of successful and unsuccessful cycles in natu-

Fig. 1. Preovulatory serum estradiol pattern in natural IVF/ICSI cycles.

Table I. E₂ Levels on Days 0 and +1 and E₂ on Day 0/E₂ on Day +1 Ratio in Four Groups, Analyzed by One-Way ANOVA

Group	E ₂ (mean $\pm$ SD nM)		E ₂ on day 0/E ₂ on day +1 (mean $\pm$ SD)
	Day 0	Day +1	
No oocyte retrieved (A)	0.709 $\pm$ 0.202	0.901 $\pm$ 0.254	1.315 $\pm$ 0.354
No fertilization (B)	0.760 $\pm$ 0.238	0.979 $\pm$ 0.418	1.291 $\pm$ 0.409
No implantation (C)	0.797 $\pm$ 0.313	0.980 $\pm$ 0.320	1.286 $\pm$ 0.364
Implantation (D)	0.798 $\pm$ 0.177	0.740 $\pm$ 0.221	0.914 $\pm$ 0.127*
All groups	0.760 $\pm$ 0.250	0.930 $\pm$ 0.323	1.259 $\pm$ 0.370

* Statistically significant ($F = 3.51$, $P = 0.0182$).

ral IVF programs, Paulson noted that cycles resulting in pregnancies tended to have a higher E₂ level on the day of hCG application than cycles which failed (6). However, this is of questionable practical value, because waiting for the attainment of a higher E₂ level may result in the occurrence of an endogenous LH surge and a potential loss of the cycle (6). On the other side, there is not always a correlation among oocyte maturity, cycle fecundibility, and E₂ level on the day of hCG application. So the E₂ level on a certain day

in the cycle is not convenient for predicting the cycle outcome. Our own results confirm this, as there was no statistically significant difference between groups with different cycle outcomes in mean E₂ levels on a certain day in the periovulatory phase.

But we found that it was possible to predict the outcome of IVF/ICSI cycles on the basis of the E₂ pattern. It is remarkable that we established a difference in E₂ pattern only in dependence on implantation, and not on fertilization or oocyte recovery success. Although other authors also do not find a connection between the E₂ pattern and fertilization (13), we admit the possibility that our results were influenced by the choice of indications for an IVF/ICSI cycle. Our study also included couples treated for male infertility, including ICSI cycles. Although we would expect the possibility of successful oocyte recovery to depend on the E₂ pattern and E₂ levels on the day of hCG application, we did not make this observation. Daya *et al.* reached similar conclusions. They believe that the success of follicle puncture depends more on the

Table II. Outcome of Natural IVF/ICSI Cycles According to Different E₂ on Day 0/E₂ on Day +1 Ratios

	E ₂ on day 0/E ₂ on day +1						Total
	≤ 0.9	> 0.9	≤ 1	> 1	≤ 1.1	> 1.1	
No. of cycles	14	84	26	72	39	59	98
Oocyte recovery rate	78.5	65.5	73.4	65.3	71.8	64.5	67.3
Fertilization rate	72.7	65.5	78.9	61.7	71.4	63.2	66.7
Implantation rate	71.4*	13.9	50.0*	10.3	52.6*	0.0	23.3

* Statistically significant ($P < 0.01$).

choice of the needle and the technique than the E_2 levels (9). As regards the possibility of predicting implantation, our findings are quite different. We established a difference in E_2 pattern between conception and nonconception cycles immediately after hCG application. In all conceptional cycles the E_2 level did not increase for more than 10% on the day following hCG application; in the majority it remained the same or dropped. The E_2 pattern in conception cycles in our study resembles the dynamics of E_2 in a normal menstrual cycle, in which, at about the time of the preovulatory LH surge, E_2 levels do not rise and decline within 24 hr after the onset of the LH surge (14). However, different E_2 patterns were observed by Jones *et al.* (15) and Laufer *et al.* (16) in conception COH cycles. They found the highest pregnancy rate among patients whose E_2 levels continued to rise on the day after hCG administration in COH cycles (15,16). Dor *et al.* also found that almost all (96%) women in their study with conceptional cycles had rising or plateauing E_2 levels on the day after hCG, whereas 37.5% of the women had decreasing E_2 levels (13).

Some observations in COH cycles and normal menstrual cycles as well as our own observations in natural cycles show that E_2 dynamics after hCG application may depend on follicular maturity. Dor *et al.* supposed that a decrease in E_2 levels in nonconceptional stimulated cycles was associated with follicular atresia and unruptured hemorrhagic follicles (13). The difference in E_2 patterns in stimulated and natural conception cycles can also be explained by the influence of follicular maturity on E_2 secretion. Numerous follicles grow in stimulated cycles, and since this growth is not necessarily synchronic, follicular maturity and E_2 pattern are different. In a normal menstrual cycle it is assumed that the endogenous LH release is synchronized with optimal follicular and oocyte maturity, so serum E_2 levels decrease after the initial rise in luteinizing hormone level due to a decrease in androgen production by theca cells and a similar fall in the aromatase activity of granulosa cells (17). These findings indicate that in our study, in those natural cycles in which there was no estradiol increase on day +1, the hCG application was performed in time, follicular maturity was optimal, and the possibility of conception was greater. In cycles with an E_2 increase it was too early, thus decreasing the possibility of conception. According to our observations, the E_2 levels on the day of hCG application do not influence further E_2 dynamics or reflect follicular maturity since no differences were found between average E_2 levels with regard to different E_2 patterns. This is understandable since, according to experience

in the normal menstrual cycle, accurate determination of follicle maturity is very difficult to recognize on the E_2 level if we know that the observed ranges for serum E_2 were 0.451–3.071 pM at the onset of the spontaneous LH surge (18).

On the basis of the importance of timely application, we might conclude that the pregnancy rate would be higher if the endogenous LH surge is used to determine the time of oocyte recovery, which is not the case. The reason lies in the advantage of hCG administration allowing much accurate timing of oocyte retrieval, which is probably of importance since the duration of oocyte maturation in vivo before aspiration appears to influence the chance of pregnancy (18). If it is possible to deduce the favorable time of hCG application retrogradely, the pregnancy rate per oocyte recovery can be improved by choosing cycles with a greater possibility of implantation, on the basis of E_2 levels on the day of hCG application and the day after, and performing the IVF/ICSI procedure in these only. We should realize that in view of the small number of cycles, the conclusions regarding predictability are preliminary. There is also the possibility that we may not have included all the factors bearing an influence on the E_2 pattern as well as on the implantation of embryos. Therefore, before clinical application, these results should be confirmed in a larger number of cycles, taking the remaining factors affecting the outcome of natural IVF/ICSI cycles into consideration.

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