Uporabnost merjenja koncentracije estradiola in progesterona v folikularni tekočini pri napovedovanju izida IVF/ICSI postopka v naravnem ciklusu.

Usefulness of follicular fluid estradiol and progesterone concentration measurement in predicting outcome of IVF/ICSI natural cycles.

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Izvleček

Namen: *Namen raziskave je bil ugotoviti ali lahko na osnovi koncentracije estradiola (E2) in progesterona (P) ter njunega razmerja v folikularni tekočini predvidimo izhod IVF/ICSI postopkov v naravnih ciklusih.*

Metode: *V raziskavo smo vključili 91 žensk, pri katerih smo naredili 150 postopkov IVF/ICSI (78 IVF in 72 ICSI) v naravnem ciklusu. Folikularno tekočino smo zbrali pri aspiraciji folikov. S t-testom testom smo primerjali koncentracije E2 in P v folikularni tekočini (FT) in njuno razmerje (FT P/FT E2) med ciklusi z uspešno (z jajčno celico) in ciklusi z neuspešno (brez jajčne celice) aspiracijo folikov, med ciklusi z oploditvijo in ciklusi brez oploditve jajčne celice in med ciklusi z*

Abstract

Purpose: *The aim of the study was to establish whether follicular fluid (FF) estradiol (E2) and progesterone (P) measurement could be used to predict the outcome of unstimulated IVF/ICSI cycles.*

Methods: *91 women underwent 150 unstimulated IVF/ICSI cycles (78 IVF and 72 ICSI). Follicular fluid samples were collected at the time of oocyte recovery. Using the t-test, FF E2 and FF P levels and their ratios (FF P/FF E2) were compared between cycles with successful (with oocyte) and unsuccessful (without oocyte) oocyte recovery, between cycles with and without fertilization and between nonconception and conception cycles.*

zanositvijo in ciklusi brez zanositve.

Rezultati: *Jajčno celico smo dobili v 123 (86,7 %) ciklusih, do oploditve je prišlo v 84 (68,3 %) in zanositve v 21 ciklusih (delež zanosite na ciklus 14,0 %). Povprečna koncentracija E2 v FT je bila 3530 ± 1339 nmol/L, povprečna koncentracija P v FT 20649 ± 9489 nmol/L in povprečna vrednost razmerja FT P/FT E2 7,18 ± 6,42. Povprečne vrednost E2 , P in njunega razmerja v FT se niso statistično pomembno razlikovale med ciklusi z uspešno in neuspešno aspiracijo jajčnih foliklov, med ciklusi z in brez oploditve jajčne celice in med ciklusi z in brez zanositve.*

Zaključek: *Na osnovi vrednosti E2 in P v folikularni tekočini ne moremo predvideti uspešnost postopkov IVF/ICSI v naravnem ciklusu.*

Results: *The oocyte recovery rate was 86.7% (123/150), the fertilization rate 68.3% (84/123) and the pregnancy rate per oocyte recovery 14.0% (21/150). The average FF E2 level was 3530 ± 1339 nmol/L, average FF P 20649 ± 9489 nmol/L and average FF P/FF E2 ratio 7.18 ± 6.42. There were no statistically significant differences in FF E2, FF P levels and their ratio between cycles with unsuccessful and successful oocyte recovery, between cycles without and with fertilization, and between non-conception and conception cycles.*

Conclusion: *From the FF E2 and P levels and their ratio, it is not possible to make inferences to the likelihood of oocyte recovery, fertilization and conception in unstimulated IVF/ICSI cycles.*

INTRODUCTION

Follicular fluid is the fraction of extracellular fluid that accumulates in the antrum of ovarian follicles and provides a hormonal microenvironment for the oocyte (1). This environment is unique for each individual follicle (2). It has been demonstrated that follicular fluid (FF) estradiol (E2) and progesterone (P) levels increase during the follicular phase and that follicular maturity and health are reflected by their levels and ratios (2-4). So FF steroids have been suggested as potential markers for assessment of oocyte quality, its fertilization ability, embryo development and implantation in IVF cycles (1, 5, 6) along with some other hormones, growth factors, peptides and sugars (7).

The first studies of FF E2 and P concentration in relation to IVF outcome were done in completely natural cycles. They suggested that successful fertilization in vitro is associated with high levels of E2 and P in follicular fluid and that P concentration in FF can predict successful fertilization of an oocyte in more than 90% of cases (6, 7). Further studies were mostly done in stimulated cycles and gave conflicting results regarding relationship between oocyte maturity and FF E2 and/or progesterone concentration in stimulated cycles (7, 10), showing also the importance of the type of ovarian stimulation (11). Some studies suggest that oocytes that fertilized, cleaved in vitro and resulted in a pregnancy originated in those follicles with high E2 and/or high P concentrations (3, 5, 6, 12-14), others failed to show such a correlation (1, 4, 15-19).

The ratio of E2 to P has also been reported to predict pregnancy (3, 20).

These findings in stimulated cycles are of little value for unstimulated cycles triggered with hCG, since studies have reported that FF E2 and P levels are significantly different in those cycles (1, 21, 22). Comparing completely natural cycles with unstimulated cycles triggered with hCG is also of limited value, since it has been shown that hCG has a profound effect on the steroid environment of the FF, also due to the longer half-life of hCG compared with LH (9, 21).

Some studies performed in unstimulated cycles showed no relationship between FF steroid levels and oocyte maturity and fertilizability (1, 15). However, the number of studied cycles was small and the results inconclusive.

The aim of our study was to establish whether FF E2 and P measurement could be used to predict oocyte recovery, fertilization and conception in unstimulated IVF/ICSI cycles.

MATERIALS AND METHODS

All patients with ovulatory menstrual cycles who intended to undertake IVF/ICSI procedure in stimulated cycle at the Department for Reproductive Medicine and Gynecologic Endocrinology of the University Clinical Centre Maribor, were offered participation in this research. We performed 150 unstimulated IVF/ICSI cycles in 91 patients who agreed to participate.

The age range of the women was 24-44 years (mean=32.9, SD= ± 5.2). The indications for IVF/ICSI procedure were only female infertility (n=40), only male infertility (n=27), female and male infertility (n=13) and unexplained infertility (n=11). In 72 cycles the ICSI procedure for male infertility and in 78 cycles the IVF procedure for female infertility was performed.

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 E2 was measured. The cycle was cancelled if baseline E2 was >0.3 nmol/L. Cycles with more than one growing follicle >12 mm were not included in the study. Transvaginal ultrasonography was performed and serum E2 concentration was measured every two days from day 7 onwards, and every day when the follicular diameter was >12 mm and serum E2 concentration >0.3 nmol/L. hCG (5000 IE) was administered when the average follicular diameter was >15 mm and E2 >0.5 nmol/L. Follicular puncture was carried out 35-36 hours after hCG injection in patients with negative LH before hCG administration. Only the dominant follicle was aspirated.

FF samples were collected at the time of oocyte recovery. Only blood free, clear FF aspirates were included in this study. After identification of oocytes, FF was immediately centrifuged and the supernatant was stored at –20° C until required for analysis. FF E2 and P levels were measured by using the chemiluminescent microparticle immunoassay technology (ARCHITECT; Abbott, Illinois, USA). High steroid concentrations in the FF necessitated the dilution of the follicular samples with control calibration provided with the kit.

Serum E2 concentrations were determined with the enzyme-immune technique (AXSYM; Abbott, Germany). Premature LH was assessed with the urine test (RAPITEST; Morwell Diagnostic; Swiss). The oocytes were cultivated, inseminated, the embryos transferred and the luteal phase was supported as described previously (23, 24). In ICSI cycles, the cumulus oophorus and corona radiata cells surrounding the oocyte were removed within 1 h after the recovery of oocyte-cumulus complexes and the oocytes were inspected for signs of meiotic maturation. Only mature oocytes were processed by ICSI. Fertilization was defined as the presence of two pronuclei at 24 hours. 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.

Using the t-test, FF E2 and FF P levels and their ratio (FF P/FF E2) were compared between cycles with successful (with oocyte) and unsuccessful (without oocyte) oocyte recovery, between cycles with and without fertilization and between nonconception and conception cycles. The data were processed with the Statistica program (StatSoft, Tulsa, Oklahoma, USA). Statistical significance was set at $p < 0.05$.

The study was approved by the Ethics Committee of the Ministry of Health of the Republic of Slovenia and sponsored by the Ministry of Science and Technology of the Republic of Slovenia, Grant No. J3-8764.

RESULTS

On the average, criteria for hCG administration were fulfilled on the 10.8 ± 2.3 day of the menstrual cycle at an average endometrial thickness of 8.2 ± 1.6 mm. Follicular diameter and serum E2 and P concentra-

Table 1: *Cycle characteristics on the day of hCG administration and follicle aspiration*

	Day of hCG	Day of aspiration
Mean follicular diameter	15.71 ± 1.46 mm	17.35 ± 1.73 mm
Serum E2 concentration	0.77 ± 0.24 nmolL	0.58 ± 0.17 nmol/L
Serum P concentration	0.83 ± 0.41 nmol/L	2.31± 1.24 nmol/L

Table 2: *Follicular fluid (FF) E2 and follicular fluid (FF) P levels and their ratios compared between cycles with different cycle outcome.*

	FF E2 (nmol/L)	FF P (nmol/L)	FF P/FF E2 ratio
Unsuccessful oocyte recovery (N=27)	3875 ± 1567	22763 ± 7867	7.42 ± 6.10
Successful oocyte recovery (N=123)	3437 ± 1280	19799 ± 7867	7.14 ± 6.56
Oocyte NOT fertilized (N=39)	3422 ± 1147	19562 ± 9692	6.28 ± 3.44
Oocyte fertilized (N=84)	3443 ± 1345	19933 ± 9678	7.53 ± 7.54
Nonconception cycles (N=129)	3528 ± 1412	20712 ± 9888	7.34 ± 6.79
Conception cycles (N=21)	3538 ± 788	20340 ± 6988	6.33 ± 3.59

tions are shown in the Table 1. No differences were established between cycles with successful and unsuccessful oocyte recovery, between cycles with and without fertilization and between conception and nonconception cycles regarding patient age, IVF/ICSI ratio, average and maximum follicular diameter, endometrial thickness and serum E2 concentration on the day of hCG administration.

The oocyte recovery rate was 86.7% (123/150). One hour after oocyte recovery, only 4 of all cleaned oocytes were not mature. The fertilization rate was 68.3% (84/123) and the pregnancy rate per oocyte recovery 14.0% (21/150).

The average FF E2 level was 3530 ± 1339 nmol/L, average FF P 20649 ± 9489 nmol/L and average FF P/FF E2 ratio was 7.18 ± 6.42.

There was no statistically significant difference in FF E2, FF P levels and their ratio between cycles with unsuccessful and successful oocyte recovery, between cycles without and with fertilization and between nonconception and conception cycles (Table 2.).

DISCUSSION

In normal ovulatory cycles prior to the LH surge, follicle growth is associated with a progressive and rapid increase in follicular fluid estradiol concentration, whereas progesterone levels remain relatively low and increase in small increments (13). Following the LH surge, a change in FF steroid content was measured, with the FF E2 level declining as ovulation approached, whereas the FF P level increased dramatically. With follicular maturation the number of LH/hCG receptors on granulosa cells increases. The accumulation of those receptors predetermines the extent of luteinization and the shift from E2 to P production (18). The follicle must be at the appropriate stage of maturity in order to respond to the ovulating stimulus. In normal cycle, LH release and final maturation of the follicle coincide (25). In stimulated and unstimulated cycles with hCG administration it is difficult to

foresee the right time for hCG administration, since E2 levels and follicular size at which the follicle reaches suitable maturity differ. Wide variations in FF content were found and also that maturity of the oocyte-corona-cumulus complex (OCCC) is reflected by the characteristics of the FF hormonal profile (3, 13, 21, 26). Unfortunately, it seems that OCCC morphology bears little relationship to oocyte maturity (27).

Seibel et al. showed that in spontaneous cycles the mean P/E2 ratio was significantly higher in FF containing metaphase I and metaphase II oocytes compared with FF containing oocytes with germinal vesicles or degenerating oocytes (4). Data on FF steroid concentrations in spontaneous cycles mostly used follicular steroid concentrations either before the onset of the spontaneous LH surge or after a variable period of LH exposure, which has a profound influence on FF steroid levels and oocyte maturation (1, 21). The precise assessment of the time at which the onset of the LH surge occurs is difficult and unreliable. Triggering the ovulation with hCG in unstimulated and stimulated cycles provided the opportunity to ensure a predictable duration of LH-like stimulation to the pre-ovulatory follicle. In a study including only stimulated ICSI cycles, in which oocyte maturity was assessed immediately after recovery, no differences were observed in FF P and E2 between follicles yielding mature, immature and degenerative oocytes. In unstimulated cycles, Enien et al. also found that FF P and E did not differ significantly in different oocyte maturity grades (1). Previous reports of FF steroid concentrations in relation to oocyte fertilization, cleavage and IVF outcome also gave conflicting results (15, 22).

In our study we included only unstimulated cycles, thus excluding the possible influence of ovarian stimulation on the intrafollicular hormonal milieu and ensuring that only one optimal follicle was aspirated. This is to our knowledge the largest study on FF steroid concentration in unstimulated cycles for IVF. We triggered ovulation with hCG to ensure a predictable duration of LH-like stimulation to the pre-ovulatory follicle, but we have to be aware that triggering ovulation with hCG as compared with endogenous LH alters the steroid metabolism. We were not surprised that no differences were observed in FF E2, FF P levels, and their ratio between cycles with successful and unsuccessful oocyte recovery, since we believe that the success of follicle puncture depends more on the technique than on follicle and oocyte characteristics (28). In our study ICSI procedures were also performed, so in these cycles oocyte maturity was directly estimated immediately after oocyte recovery. Unfortunately, the number of immature oocytes was too small for statistical comparing of FF compositions between follicles containing mature and immature oocytes. Like some other authors, we observed that FF steroids do not differ significantly as regards fertilization (1, 15). There is only a small possibility that our results were influenced by the choice of indications, since our study also included couples treated for male infertility, including ICSI cycles. We showed in a previous study that ICSI for severe male infertility in unstimulated cycles can generate results similar to those obtained in unstimulated IVF cycles for tubal infertility (29). Fertilization success and failure are influenced by oocyte maturity at the time of oocyte recovery and in our study almost all oocytes prepared for ICSI were in metaphase II. We also found no differences in FF E2, FF P and their ratio between conception and nonconception cycles. It seems that FF steroid concentrations are more relevant to granulosa cell proliferation and maturation than to oocyte quality, which is reflected by its fertilization ability and embryo implantation. We also have to bear in mind that the possibility of fertilization and conception depends on several factors, which may haze the value of FF E2, FF P, and their ratio in predicting fertilization and conception in unstimulated IVF/ICSI cycles.

However, our results suggest that FF E2 and P measurement cannot be used to predict the outcome of unstimulated IVF/ICSI cycles.

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