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Serum estradiol and progesterone in the mid-luteal phase predict clinical pregnancy outcome in IVF/ICSI cycles

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Abstract

In this prospective study, we tested the hypothesis if E2 and P serum levels significantly differ during the luteal phase following in vitro-fertilization/intracytoplasmic sperm injection (IVF/ICSI) therapy in conception (CC) versus non-conception (NC) cycles, and their potential in the prediction of pregnancy at the earliest point in time. Serum was sampled from the day of embryo transfer (ET) and throughout the luteal phase until ET + 14 from patients consecutively enrolling for IVF/ICSI therapy. The luteal phase was supported by vaginal P suppositories only, clinical pregnancies were detected by ultrasound and followed up until the 20th week. Overall pregnancy rate was 30.9% constituting the two study groups of CC ($n = 22$) and NC cycles ($n = 49$). Significantly, higher E2 (3326 ± 804 versus 1072 ± 233 pmol/l, $p = 0.014$) and P (244 ± 68 versus 73 ± 10 nmol/l, $p = 0.023$) were present in CC versus NC from as early as ET + 7. In the CC group, patients with ongoing pregnancies (CC-OG) as compared with miscarriages (CC-MC) had significantly higher E2 and P from ET + 7, predicting ongoing pregnancy in receiver operator characteristics analysis.

Keywords

Estradiol, IVF/ICSI, luteal phase, pregnancy outcome, progesterone

History

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Introduction

Implantation is the key limiting step in assisted reproduction techniques (ARTs) and determined by embryo quality as well as endometrial receptivity [1]. Preparation of the endometrium depends on ovarian steroid secretion and in natural cycles leads to an optimal time window for implantation and successful pregnancy at 8–10 days following ovulation [2]. Early signalling from the implanting embryo via secretion of human chorionic gonadotrophin (hCG) is indispensable for the rescue and maintenance of the corpus luteum (CL) and its role in supporting early pregnancy [3].

In ART cycles, controlled ovarian stimulation (COS) leads to supraphysiological levels of steroid hormone secretion and the subsequent need for luteal phase support (LPS) [4]. Therefore, existing data on the steroid secretion profile in the luteal phase of spontaneous conception cycles and its predictive value for pregnancy outcome [5] need to be discussed against the background of preceding COS.

The purpose of this study was to (i) confirm our previous results from a retrospective study on the differences in luteal phase E2 secretion between conception (CC) and non-conception (NC) cycles following in vitro-fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatment in a prospective study design, (ii) analyse the predictive role of mid-luteal phase P for CC and (iii) validate the role of luteal phase E2 and P as predictors of (ongoing) pregnancy.

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Methods

Patients and study design

All patients undergoing IVF/ICSI therapy at the infertility clinic between March 2004 and September 2005 were consecutively invited at the time of embryo transfer (ET) to participate in this prospective study, which was approved by the local ethics committee of University Hospital Münster. Additional to regular cycle monitoring, serum samples were drawn for later measurement on the day of ET, as well as ET + 2/3, ET + 4/5, ET + 7 and ET + 14. Of all patients willing to consent ($n = 112$), those with frozen-thawed ET cycles ($n = 28$) and those not applying recombinant follicle stimulating hormone (FSH) under a long protocol ($n = 13$) were not included into the final evaluation.

Ovulation induction and IVF/ICSI procedure

Ovulation induction (OI) and IVF/ICSI procedure was performed according to the standard long protocol as described previously [6]. Briefly, in the mid-luteal phase of the preceding cycle, a gonadotropin releasing hormone (GnRH) agonist (Decapeptyl 0.1 mg s.c. daily, Ferring, Kiel, Germany or Synarela 0.4 mg intranasally, Pfizer/Pharmacia, Erlangen, Germany) was applied. Pituitary down-regulation was confirmed by vaginal bleeding and oestradiol serum concentration <110 pmol/l followed by ovarian stimulation within 14 days later. Ovarian stimulation was performed with recombinant FSH preparations (Gonal F®, MerckSerono, Darmstadt, Germany or Puregon®, MSD, Germany) at a standard dose of 150 IU that could be adjusted according to the expected ovarian response. Criteria for OI with either 10 000 IU urinary hCG (Choragon®, Ferring) or 250 µg recombinant hCG (Ovitrelle®, MerckSerono) were fulfilled in patients with at least three follicles ≥17 mm. Embryo quality

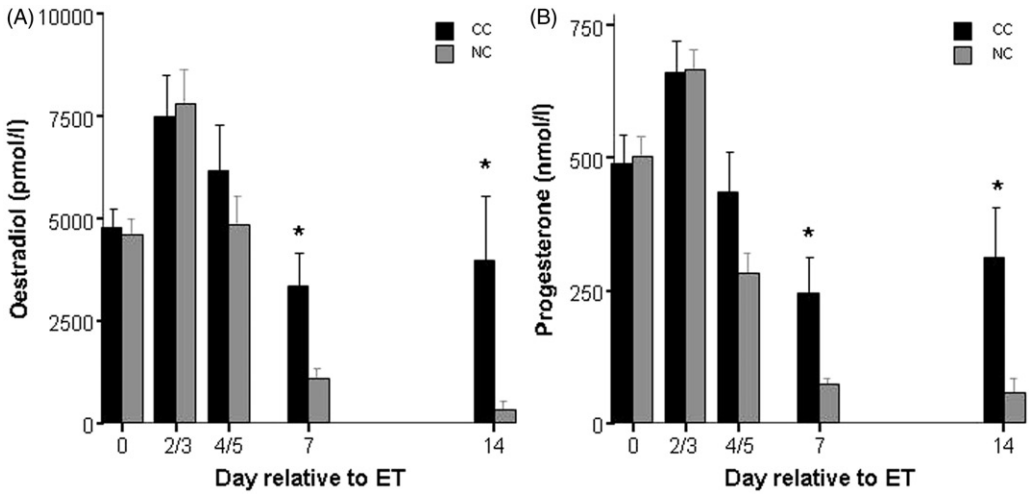

Figure 1. E2 (A) and P (B) serum levels between CC and NC. A significant difference (*) is detected from as early as day 7 following ET for E2 as well as P serum levels.

following oocyte retrieval and IVF/ICSI was assessed with a scoring system by Steer et al. [7]. Preceding ETs on day 2 or 3, LPS was commenced on the day after FP with vaginal P only with two different formulations that had been previously proven to be comparable in terms of clinical and ongoing pregnancy rates (3×200 mg Utrogest®, Kade, Berlin, Germany or 90 mg Crinone® 8%, MerckSerono [8]). Pregnancy was detected by hCG measurement on ET + 14, and transvaginal ultrasonography (TVUS) was performed 1 week later and onwards. Only clinical pregnancies with detection of an intrauterine foetal sac were counted and followed for at least 20 weeks. All clinical pregnancies lost until that time were considered as miscarriage (CC-MC).

Estradiol and progesterone measurement

Concentrations of E2 (pmol/l) and P (nmol/l) were measured using chemiluminescence immunoassay (ECLIA, Roche, Mannheim, Germany) on Modular E170 automated analyser in the Central Laboratory of the University Hospital Münster.

Statistics

Statistical analysis was performed with SPSS statistical software package version 11.5 (SPSS Inc., Chicago, IL). Clinical and hormonal data were analysed using the unpaired student's *t*-test or one-way ANOVA in case of more than two groups. Correlation between clinical parameters was analysed by Pearson correlation coefficient (two-sided, $p < 0.01$ considered significant) where appropriate. For categorical variables, chi-square test was performed. Receiver operator characteristics (ROCs) analysis was performed to assess E2 and P as a predictive test for CC and the course of pregnancy. In general, values are presented as mean ± SEM, and a p value of <0.05 was considered statistically significant.

Results

A total of 71 cycles with ET following IVF/ICSI were included in the final data analysis. Overall, pregnancy rate was 30.9% constituting the two groups of CC ($n = 22$) and NC cycles ($n = 49$). Female age and mean cycle number, as well as measures of ovarian response to stimulation, FSH dosage and cumulative embryo score were comparable between groups (see supplemental file). Endometrial thickness was significantly higher in CC (11.5 ± 3.2 versus 10.2 ± 1.8 mm, $p = 0.032$), and only weakly correlated with E2 ($r = 0.219$, $p = 0.071$) in contrast to a highly significant correlation between E2 and follicle number ($r = 0.733$, $p = 0.000$) on the day of hCG administration. Of the resulting 22 clinical pregnancies, 8 were miscarriages (CC-MC, abortion rate: 36%). There were three twin pregnancies, all ongoing (multiple pregnancy rate: 13.6%) and no ectopic pregnancies.

E2 and P are not significantly different between groups on ET (Figure 1) and show a similar course during the luteal phase with an increase shortly after ET (ET + 2/3) followed by a decline on ET + 4/5 and ET + 7, and no further significant changes until ET + 14. E2 and P appear to be lower in the NC group as early as ET + 4/5, but not significantly (P, $p = 0.064$ and E2, $p = 0.277$). A significant difference is detected from ET + 7, with higher E2 (3326 ± 804 versus 1072 ± 233 pmol/l, $p = 0.014$) as well as P (244 ± 68 versus 73 ± 10 nmol/l, $p = 0.023$) in CC versus NC, as also on ET + 14 (E2: 3948 ± 1572 versus 319 ± 208 pmol/l, $p = 0.036$ and P: 310 ± 94 versus 58 ± 24 nmol/l, $p = 0.019$).

E2 and P analysed separately (Figure 2) for CC resulting in ongoing pregnancies (CC-OG) or miscarriages (CC-MC) demonstrate a statistically significant difference for both parameters from ET + 7 (E2 and P, $p = 0.000$) with higher values in *post hoc* analysis for CC-OG (E2: 4397 ± 1132 pmol/l, P: 318 ± 99 nmol/l) versus CC-MC (E2: 1491 ± 587 pmol/l, P: 117 ± 54 nmol/l) as well as NC. The E2-to-P-ratios were not significantly different between groups on ET or any given day during the luteal phase (data not shown).

Highly significant differences between CC and NC (Figure 3A) were seen in ROC analysis on ET + 7 for E2 (AUC = 0.729 and $p = 0.004$) and P (AUC = 0.708 and $p = 0.009$). When further evaluating differences between CC-OG and NC or CC-MC (Figure 3B), AUC values increased for E2 (AUC = 0.780 and $p = 0.003$) and P (AUC = 0.796 and $p = 0.001$). At a cut-off value of 2215 pmol/l (E2) and 126 nmol/l (P), the positive predictive value for ongoing pregnancy is 50 (E2) and 67% (P), and the negative predictive value is 81 (E2) and 83% (P).

Discussion

Differences between E2 and P between CC and NC measured as early as ET + 7 and despite LPS with exogenous P underline the paramount impact of early embryo signalling to the CL and resulting steroid secretion on the outcome of IVF/ICSI therapy. Furthermore, the results of this study highlight the value of P and E2 measurement during the luteal phase for the prediction not only of conception but also especially of an ongoing pregnancy.

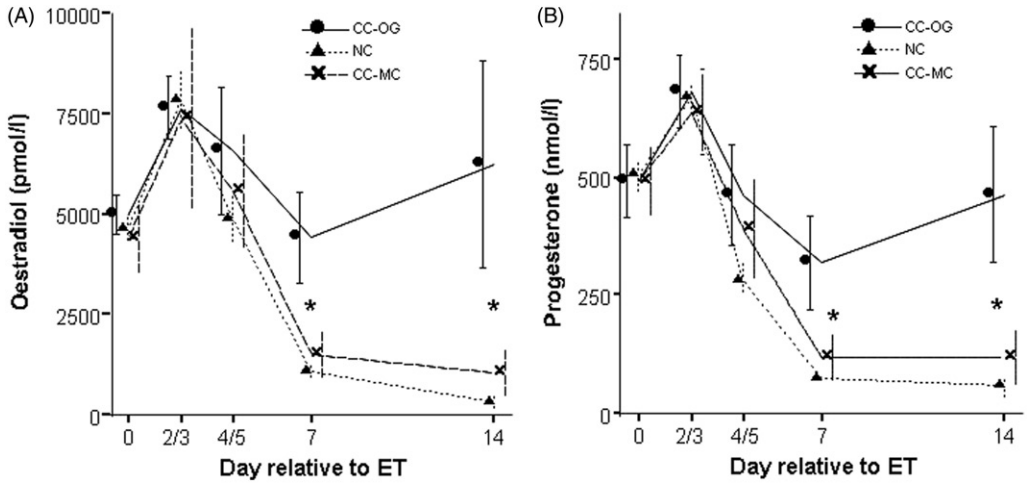

Figure 2. E2 (A) and P (B) serum levels between CC-OG, CC-MC and NC. A significant difference (*) is present from ET + 7 on between CC-OG and CC-MC or NC, but not between CC-MC and NC for E2 as well as P serum levels.

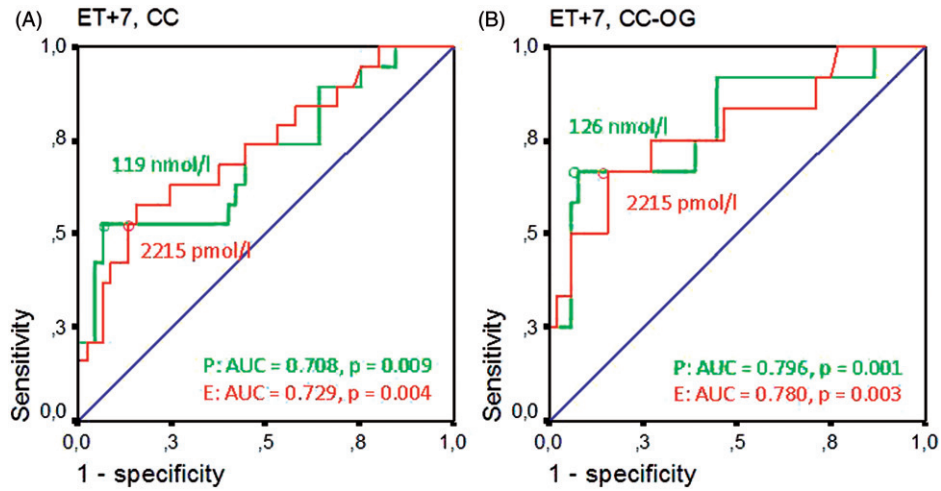

Figure 3. ROC analysis for E2 and P serum levels as a predictor of CC (A) and CC-OG (B). Significant differences were seen between CC and NC (A) for E2 (AUC = 0.729 and $p = 0.004$) and P (AUC = 0.708 and $p = 0.009$), as well as between CC-OG and CC-MC or NC (B): E2 (AUC = 0.780 and $p = 0.003$) and P (AUC = 0.796 and $p = 0.001$). On ET + 7, at an E2 threshold of 2215 pmol/l, the sensitivity was 52.5% for the CC and 66.7% for the CC-OG group with comparable specificity of 86.7 and 84.6%, respectively. At a P threshold of 119 and 126 nmol/l, respectively, the sensitivity was 52.6% for the CC and 66.7% for the CC-OG group with a specificity of 93.3 and 92.3%, respectively.

Luteal phase E2 and P measurement for the prediction of pregnancy in ART cycles has been discussed in several papers with conflicting results so far. Most of the study designs are retrospective and the results possibly influenced by differences regarding response to stimulation and peak E2 levels [9], or additional application of hCG during the luteal phase [10]. We have previously demonstrated by retrospective analysis, that luteal phase E2 secretion following IVF/ICSI is significantly different in CC versus NC cycles from as early as ET + 4 [6]. This result could have been hampered by the fact that a large proportion of patients received additional applications of hCG during the luteal phase. Oppositely, a study in good and high responder patients focussing on the mid-luteal E2 decline concluded that there is no significant difference between CC and NC, but reports a stronger decline of E2 in patients with early spontaneous abortions [11]. A detrimental effect on endometrial receptivity of high peak E2 levels as well as E2 decline during the luteal phase has been hypothesized. In a prospective study, Ganesh et al. have confirmed a potential predictive value of mid-luteal E2 for pregnancy in IVF/ICSI [12]. In that study, P levels were different only on ET + 7, but not ET + 14. As this is contradictory to previous data on the predictive value of a single P measurement for pregnancy outcome [13], the authors themselves have called for cross-validation of their results in different study populations.

The question had been debated for long if the preceding COS or the hCG stimulus deriving from an implanting embryo is responsible for differences in the luteal phase steroid secretion profile between pregnant and non-pregnant patients [6]. Preceding COS may negatively impact on endometrial receptivity, especially in high responder patients [14], and an approach to more individualized COS is discussed focussing on the best embryo quality with respect to limitations by patient's age and other individual factors [15,16]. Given that there were no differences between cycle parameters of ovarian response, our prospectively retrieved study data indicate that the interaction between a good quality embryo and responsive CL resulting in an optimally timed window of implantation may positively influence treatment outcome. Significantly higher endometrial thickness in CC combined with only weak correlation with peak E2 levels in our study may indicate a more receptive endometrium as an independent predictor for pregnancy as had previously been suggested in an oocyte donor model [17].

The debate on early cessation of LPS following IVF/ICSI in CC [18] has further stimulated out interest in the predictive value of mid-luteal E2 and P; ongoing P supplementation in the

case of pregnancy is still common practice in the majority of IVF centres around the world [19], despite potential hazards and increasing knowledge about a missing negative effect when it is stopped at the time of a positive pregnancy test [20,21]. The results of a recent prospective study have additionally supported the practice of early cessation of LPS in pregnant patients following IVF/ICSI [22]. Our data further indicate a discriminatory potential of E2 and P in the mid-luteal phase for CC-OG as compared with CC-MC; the best discriminatory capacity is given for P on ET + 7 with 66.7% of patients correctly determined as ongoing pregnant (CC-OG) and 92.3% as not (NC) or not ongoing pregnant (CC-MC) at a threshold of 126 nmol/l. This increased sensitivity for CC-OG as compared with CC in general reflects the early decline of P serum values during the mid-luteal phase in patients becoming pregnant but destined for miscarriage (see Figure 2B). At least in the prediction of ongoing pregnancy, P measurement alone or in combination with other parameters may turn out to be more helpful than single or repetitive hCG measurement at the time of pregnancy test or earlier [23,24]. It is postulated that the course of pregnancy is engrained in the developmental capacity of the implanting embryo actively promoting endometrial receptivity by regulating steroid secretion through hCG signalling. This is in line with a more integrated view on the continuum of follicular growth, oocyte and embryo developmental capacity and adequacy of the luteal phase independent of COS [25]. Following spontaneous conception, a threshold P of 5 ng/ml has been debated as an indicator of intact pregnancy and discriminator of ectopic pregnancy [26]. Interestingly, very recent *in vitro* data may help to explain the better discriminatory value of P versus hCG in the prediction of clinically intact and not intact pregnancies; hyperglycosylated hCG has been investigated in cultured human luteal granulosa cells, demonstrating differences in the biological activity and steroid secretion profile [27]. Based on the data presented here, the question may be addressed in future prospective studies, if cessation of LPS at an even earlier point in time is possible without detrimental effects on ongoing pregnancies depending on mid-luteal E2 and P measurement.

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Declaration of interest

The authors report no declarations of interest.

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