CEOG Clinical and Experimental Obstetrics & Gynecology

Original Research

Luteal phase progesterone and estradiol concentrations and their relation to the outcome of IVF/ICSI cycles with controlled ovarian stimulation

P. Bakas[1], M. Simopoulou[1], P. Panagopoulos[2], N. Salakos[1], Ch. Siristatidis[2]

[1]2nd Department of Obstetrics and Gynecology, Aretaieion Hospital, University of Athens, Athens
[2]3rd Department of Obstetrics and Gynecology, Attiko Hospital, University of Athens, Athens (Greece)

Summary

Purpose of Investigation: To examine if the potential changes in progesterone (P) and estradiol (E2) levels during the luteal phase can predict the outcome in IVF/ICSI cycles. *Material and Methods:* A prospective study of 282 patients with infertility undergoing IVF/ICSI have been included. Serum E2 and P measurements at day of hCG triggering and days 6 and 10 after embryo transfer in conception and non-conception cycles. *Results:* The ROC curve in predicting clinical pregnancy was calculated at 0.864 (95% CI: 0.841-0.92). A maximum reduction of ≤ 32 % demonstrated sensitivity of 84.3% and specificity of 80.4%. The corresponding ROC curve for percentage change in P levels was 0.733 with 95% CI: 0.334-0.96. *Conclusion:* A maximum reduction of 32 % in E2 levels between days 6 and 10 after embryo transfer demonstrated a predictive ability for clinical pregnancy after IVF/ICSI of 86.4% with sensitivity 84.3% and specificity 80.4%.

Key words: Estradiol; Progesterone; Ganirelix; Controlled ovarian stimulation; COS; IVF; ICSI.

Introduction

The use of GnRH analogues to achieve pituitary down regulation and prevent a premature LH surge in order to improve the outcome of IVF/ICSI cycles has been used in assisted reproduction techniques (ART) daily practice [1]. The final goal is through the ovarian stimulation with gonadotropins to achieve an adequate number of mature oocytes providing an improved potential for better quality embryos to select from for embryo transfer and freezing of surplus embryos for future use [2].

During the luteal phase of these ART cycles, an abrupt decline of steroids levels between the ovulation triggering day and the midluteal phase has been reported, but it is not deleterious for implantation after in vitro fertilization and embryo transfer [3]. Also, the ability of the altered luteal phase hormonal profile in predicting pregnancy after IVF/ICSI cycles remains controversial. Several reports have resulted in conflicting conclusions, concerning the affected fertilization, implantation and pregnancy rates [4-8]. All studies examine the outcome of the IVF/ ICSI cycles in relation to the mean estradiol (E2), peak E2 levels, and mean progesterone (P) and FSH levels in the luteal phase, and although an association with mean E2 levels has been suggested, no cut- off values could be recognized that could distinguish achievement of pregnancy on an individual basis.

In the present study, the authors evaluated the E2 and P levels and their percentage and actual changes between days 6 and 10 after the embryo transfer and estimated their predictive ability with the achievement of clinical pregnancy in subfertile couples undergoing IVF/ICSI cycles.

Materials and Methods

This prospective study was conducted at the Assisted Reproductive Unit of the 2nd Department of Obstetrics and Gynecology, Aretaieio Hospital of Athens, Greece, during the period from March 2013 to March 2017. The study was approved by the Hospital's Ethics Committee and informed consent from the patients was obtained.

The inclusion criteria entry in the study were: age 25-42 years, BMI ≤ 35 and ≥ 19, normo-ovulatory patients, and basal FSH ≤11 mIU/mL. Definition of expected normal ovarian response was based primarily on antral follicle count (AFC ≥ 5 and ≤ 12) and secondary on the absence of polycystic morphology of the ovary. The exclusion criteria were: history of more than three previous unsuccessful IVF/ICSI cycles, FSH > 12 mIU/mL, BMI > 35 or < 19, poor ovarian response according to the 2011 Bologna criteria [9] history of autoimmune, endocrine or metabolic disorders, ovarian cystectomy or oophorectomy. A cohort of 282 participants that met the inclusion criteria was enrolled in the study.

All participants were treated with a GnRH antagonist protocol: ovarian stimulation began on the second day of the cycle and the antagonist, Ganirelix was initiated as soon as the leading follicle reached a diameter of 14 mm. For the ovarian simulation, recombinant FSH in the form of either follitropin alpha or follitropin

Revised manuscript accepted for publication May 21, 2018

Clin. Exp. Obstet. Gynecol. - ISSN: 0390-6663
XLVI, n. 5, 2019
doi: 10.12891/ceog4741.2019

7847050 Canada Inc.
www.irog.net

Published: 10 October 2019

Table 1. — *Mean values of parameters under investigation with their standard deviation, 95% CI for the mean, and range.*

	Arithmetic mean	95% CI for the mean	Standard deviation	Range
Day of hCG triggering estradiol level pg/ml	1802.2	1633.1-1971.4	1139.9	234-5660
Day of hCG triggering progesterone level ng/ml	1.27	1.15-1.38	0.80	0.2-5.2
Day 6 estradiol level* pg/ml	404.3	3395 –469.2	549.4	19-3700
Day 10 estradiol level* pg/ml	268.9	206.5- 331.4	529.1	12-4250
Day 6 progesterone level* ng/ml	45.8	37.4-54.0	70.3	7.7-687
Day 10 progesterone level* ng/ml	33.4	27.8-38.9	46.6	3-469
% change of estradiol level	-73.0	(-77.3)- (-68.7)	28.8	(-110) - 123
% change of progesterone level	-12.4	(-20.2)-(-4.53)	66.5	(-98)-366
Difference estradiol level**	-128.7	(-184.2)-(-73.2)	470.3	(-3449)-1364
Difference progesterone level **	-12.2	(-19.0)-(-5.3)	58.0	(-616)-161

*CI: confidence interval, *Day 0 : the day of embryot ransfer, **Difference: the difference in levels between day 6 and day 10.*

beta was administered subcutaneously.

For cycle monitoring, transvaginal ultrasonography every 2~4 days (or as required) was performed. Starting doses were adjusted individually according to the age, FSH, AMH levels, and previous response to IVF cycles of each participant, while further adjustments and monitoring frequency were dependent upon participant's response to stimulation. When two or more follicles reached a diameter of 18 mm, hCG [(10,000 IU) was administered prior to transvaginal ultrasound–guided oocyte retrieval (OR) 36 hours later. Intracytoplasmic sperm injection (ICSI) was performed both in cases of male subfertility, and in assisted reproductive history of low fertilization rate. Embryo transfer was performed either two or three days after the OR. Luteal phase support was achieved by transvaginal administration of progesterone vaginal suppositories 200 mg tds. Clinical pregnancy was confirmed by a transvaginal ultrasound scan at seven weeks of gestation, suggested after a positive β-hCG 12- 14 days after ET.

The Manuscript Ethics Committee Number is Φ-6/28/03/2013.

Both E2 and P levels were measured on day of hCG ovulation triggering and on days 6 and 10 after embryo transfer along with β-hCG on days 12-14 after embryo transfer in all patients. Clinical pregnancy was defined as the presence of intrauterine sac with yolk sac and fetal heart detected at transvaginal ultrasound performed at about seven weeks gestation. The primary analysis was performed to evaluate possible changes in E2 and P levels on days 6 and 10 after embryo transfer and to associate them with the occurrence or absence of clinical pregnancy.

The following kits were used for both hormones: the measurement range for E2 kit was from 9 to 3000 pg/mL. The analytic detection limit with a probability of 95% was 9 pg/mL. The functional detection limit, defined as the smallest measurable concentration of E2 with a coefficient of variation of 20%, was 25 pg/mL. The within-run reproducibility was 4.6% and the between-run reproducibility was 6.4%.

The measurement range for the P kit was from 25-80 ng/mL. The functional detection limit with a probability of 95% was < 0.25 ng/ml. The within-run reproducibility was 4.0% and the between-run reproducibility was 3.8%.

D'Agostino-Pearson test was used to assess if variables were following normal distribution or not. Because not all of the examined parameters did not follow normal distribution comparison of parameters was performed with the use of either Wilcoxon or Student's *t*-test.

Logistic regression analysis was performed for the dichotomous variables and coefficients, standard error and statistical significance of variables under investigation were estimated.

The predictive ability of days 6 and 10 E2 and P levels, their percentage change between days 6 and 10 post-embryo transfer and the absolute and percentage reduction of both levels between days 0 and 6 post-embryo transfer on clinical pregnancy were analyzed using the receiver operating characteristic (ROC) curve. Statistical analysis was performed using the Medcalc software (version 17.5.5-64 bit). A $p < 0.05$ was considered statistically significant.

Table 2. — *Logistic regression analysis. Coefficients, standard error, and statistical significance (p) of variables under investigation.*

Variable	Coefficient	Std. Error	Wald	*p*
%_progess_change	0.0067085	0.0055293	1.4720	0.2250
E2_%_change	0.021133	0.0042016	25.2995	<0.0001
E2_D6	-0.0090008	0.031680	0.08072	0.7763
E2_D10	0.0090991	0.031704	0.08237	0.7741
Pr_D6	-0.35319	0.29472	1.4362	0.2308
Pr_D10_OOR	0.35332	0.29462	1.4381	0.2304
E2_differ	-0.0094470	0.031670	0.08898	0.7655
Prog_differ	-0.33536	0.29437	1.2979	0.2546
Constant	-0.16534	0.24632	0.4506	0.5021

Results

The study consisted of 282 women, with a mean age (±SD) of 34.3 ± 4.8 years and a mean BMI (±SD) of 25.8 ± 4.06 (range 20, 7-31).

Table 1 presents the mean values of E2 and P level on the day of hCG ovulation triggering, day 6 E2 level, day 10 E2 level, day 6 P level, day 10 P level, percentage change of estradiol level, percentage change of P level, absolute difference of E2 level, absolute difference of P level, their corresponding range, standard deviation, and 95% CI for the mean.

A mean reduction of absolute E2 levels of 1407.5 ± 1118.4 ng/ml and of 73% ± 28.8, between days 0 (day of hCG triggering) and six post-embryo transfers were found, but none were associated with the outcome of IVF/ICSI (percentage E2 decline $p = 0.91$, and absolute E2 decline $p = 0.57$). Similarly, the mean levels and the absolute difference between E2 and P levels and the percentage change of P on days 6 and 10 post-embryo transfer had no correlation with the outcome of IVF/ICSI (Table 2).

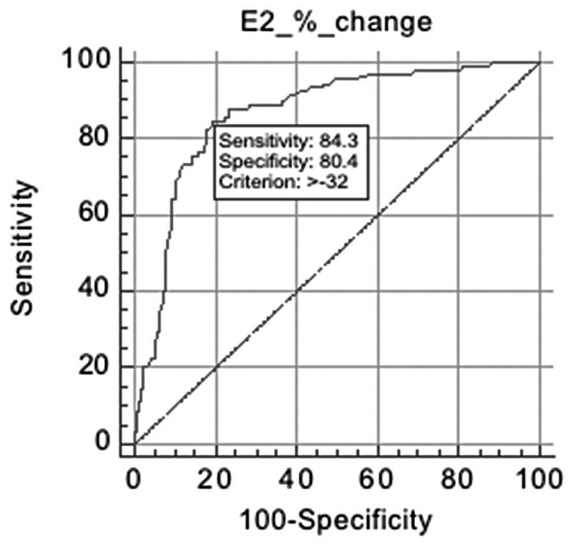

Figure 1. — ROC curve analysis of percentage change of E2 levels between days 6 and 10 of luteal phase to clinical outcome of IVF/ICSI.

In contrast, the percentage of E2 change between days 6 and 10 post-embryo transfer was found to be associated with the outcome of IVF/ICSI ($p < 0.0001$). The ROC curve in predicting clinical pregnancy was calculated at 0.864 (95% CI: 0.841-0.92). A cut-off value of reduction of > 32 % demonstrated a sensitivity of 84.3% and a specificity of 80.4% (Figure 1).

Discussion

In the present study, the authors evaluated the E2 and P levels and their percentage and actual changes between days of hCG triggering, on days 6 and 10 after embryo transfer and estimated their predictive ability with the achievement of clinical pregnancy in subfertile couples undergoing IVF/ICSI cycles, through GnRH antagonist protocols. The present authors observed that a reduction of at least 32 % in E2 levels between days 6 and 10 post-embryo transfer carries a predictive ability for clinical pregnancy after IVF/ICSI of 86.4%, with sensitivity of 84.3% and specificity of 80.4%.

The rationale of studying steroids after the embryo transfer lies on the fact that any kind of deviation of their physiological ovarian production might interfere with impaired implantation, due to a differentiated endometrial priming and receptivity and, possibly, with poorer embryo quality [3]. The conflicting results reported so far in the literature are associated with the lack of reliable tests for endometrial receptivity and probably the different protocols implemented and groups studied.

The present authors found reductions in both E2 and P levels in all their calculations between days of hCG triggering, on days 6 and 10 after the embryo transfer, all not being able to reach statistical significance, except for one comparison. Notably, the absolute midluteal E2 levels required for implantation have not yet been defined [3, 10-12] and furthermore, E2 support during this phase is not clear that it increases live birth rates [13-15].

The percentage reduction in E2 levels between days 6 and 10 post-embryo transfer was found to be associated with the occurrence of clinical pregnancy. The concept of this finding the present authors believe that, has to do with the theory of the regression or not of the corpus luteum. In the same context, such declines (concerning the difference between hCG triggering and midluteal phase and reaching up to 90%) have been reported either to be correlated with the outcome of IVF [8, 10, 16, 17] or not [3, 11], while this correlation was reported to be evident only in good or high responders [6, 7]. Interestingly, authors applying a multiple logistic regression analysis in a selected group of good and high responders undergoing a GnRH-agonist protocol, found that the hormonal profile of the midluteal phase, had no significant impact on implantation [3].

In the present study, the ROC curve for the percentage reduction in E2 in predicting clinical pregnancy was calculated at 0.864 (95% CI: 0.841-0.92). A cut-off value of reduction of > 32 % demonstrated a sensitivity of 84.3% and a specificity of 80.4%. Similarly, Ganesh *et al.* [17], comparing absolute levels found significant declines in E2 levels between pregnant and non-pregnant women at days 7 and 14 after embryo transfer, results that after a discriminant analysis, showed a classification accuracy of > 80%.

In contrast P levels were not associated with the occurrence of clinical pregnancy. This finding is in accordance with those reported so far in the literature [17], but not in frozen IVF cycles. The possible pitfalls with the calculations of P in the midluteal phase have to do with the presence of multiple corpora lutea, in addition to exogenous P supplementation.

The apparent limitations of this study are mainly attributed to its nature. The lack of power calculation, blinding, and randomization are linked with possible unknown confounders and selection bias, while partially explains the conflicting results so far. Moreover, residual confounding, such as antral follicle count and response to stimulation, cannot be excluded and this might potentially have an effect on the strength of the association between differences in hormonal levels and clinical pregnancy rates.

Conclusion

This prospective study on 282 subfertile women undergoing IVF, showed that a reduction of < 32 % in E2 levels between days 6 and 10 after embryo transfer demonstrated a predictive ability for clinical pregnancy after IVF/ICSI of 86.4% with sensitivity 84.3% and specificity 80.4%, and

can be used as a reliable marker for the prediction of success in terms of clinical pregnancy rates. Properly powered and conducted prospective studies are further needed to support these findings.

References

[1] Caspi E., Ron-El R., Golan A., Nachum H., Herman A., Soffer Y., Weinraub Z.: "Results of in vitro fertilization and embryo transfer by combined long-acting gonadotropin-releasing hormone analog D-Trp-6-luteinizing hormone-releasing hormone and gonadotropins". F*ertil. Steril.*, 1989, *51*, 95.

[2] Liu H.C., Lai Y.M., Davis O., Berkeley A.S., Graf M., Grifo J., *et al.*: "Improved pregnancy outcome with gonadotropin releasing hormone agonist (GnRH-a) stimulation is due to the improvement in oocyte quantity rather than quality". *J. Assist. Reprod. Genet.*, 1992, *9*, 338.

[3] Friedler S., Zimerman A., Schachter M., Raziel A., Strassburger D., Ron E.: "The midluteal decline in serum estradiol levels is drastic but not deleterious for implantation after in vitro fertilization and embryo transfer in patients with normal or high responses". *Fertil. Steril.*, 2005, *83*, 54.

[4] Sonntag B., Loebbecke K.C., Nofer J.R., Kieasel L., Greb R.R.: "Serum estradiol and progesterone in the mid luteal phase predict clinical pregnancy outcome in IVF/ICSI cycles". *Gynecol. Endocrinol.*, 2013, *29*, 700.

[5] Chattopadhyay R., Chakraborty C., Chaudhury K., Chakravarty B.N.: "Luteal phase estradiol level: a potential predictive marker for successful pregnancy in in vitro fertilization/intracytoplasmic sperm injection". *Fertil. Steril.*, 2009, *91*, 1018.

[6] Moraroglu O., Tongue E.A., Ozel M., Ozaksit G., Var T., Surikaya E.: "The effects of peak and mid-luteal estradiol levels on in vitro fertilization outcome". *Arch. Gynecol. Obstet.*, 2012, *285*, 857.

[7] Aktan E., Bozkart K., Ozer D., Yucebilgin S., Karadadas N., Bilgin O.: "The effect of midluteal estradiol level on the outcome of ICSI-ET cycles". Arch. Gynecol. Obstet., 2004, *269*, 134.

[8] Vicdan K., Isik A.Z.: "Luteal phase hormonal profile in prediction of pregnancy outcome after assisted reprocuction". *Eur. J. Obstet. Gynecol.*, 2001, *96*, 98.

[9] Ferraretti A.P., La Marca A., Fauser B.C., Tarlatzis B., Nargund G., Gianaroli L.: "ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria". *Hum. Reprod.*, 2011, *26*, 1616.

[10] Sharara F.I., McClamrock H.D.: "Ratio of estradiol concentration on the day of human chorionic gonadotropin administration to midlutealoestradiol concentration is predictive of in-vitro fertilization outcome". *Hum. Reprod.*, 1999, *14*, 2777.

[11] Ng E.H.Y., Yeung W.S.B., Lau E.Y.L., So W.W.K., Ho P.C.: "High serum estradiol concentration in fresh IVF cycles do not impair implantationand pregnancy rates in subsequent frozen thawed embryo transfer cycles". Hum. Reprod., 2000, *15*, 250.

[12] Akman M.A., Erden H.F., Bener F., Liu J.E., Bahceci M.: "Can luteal phase estradiol levels predict the pregnancy outcome in in vitro fertilization cycles of good responders whose excess embryos yield blastocysts?" *Fertil. Steril.*, 2002, *77*, 638.

[13] Smitz J., Bourgain C., Van Waesberghe L., Camus M., Devroey P., Van Steirteghem A.C.: "A prospective randomized study on oestradiol valerate supplementation in addition to intravaginal micronized progesterone in buserelin and HMG induced superovulation". *Hum. Reprod.*, 1993, *8*, 40.

[14] Lewin A., Benshusan A., Mezker E., Yanai N., Schenker J.G., Goshen R.: "The role of estrogen support during the luteal phase in in vitro fertilization-embryo transplant cycles: a comparative study between progesterone alone and estrogen and progesterone support". *Fertil. Steril.*, 1994, *62*, 12.

[15] Zhang X.M., Lv F., Wang P., Huang X.M., Liu K.F., Pan Y., *et al.*: "Estrogen supplementation to progesterone as luteal phase support in patients undergoing in vitro fertilization: systematic review and meta-analysis". M*edicine (Baltimore)*, 2015, *94*, 459.

[16] Liu H.C., Davis O., Berkley A., Graft M., Rosenwaks Z.: "Late luteal estradiol patterns are better prognosticator of pregnancy outcome than serial beta human chorionic gonadotropin concentrations". *Fertil. Steril.*, 1991, *56*, 421.

[17] Ganesh A., Goswami S., Chattopadhyay R., Chakraborty C., Chaudhury K., Chakravarty B.N.: "Luteal phase estradiol level: a potential predictive marker for successful pregnancy in in vitro fertilization/intracytoplasmic sperm injection". Fertil. Steril., 2009, *91*, 1018.

Corresponding Author:
P. BAKAS, M.D.
Vas. Sofias Avenue 124A
Athens 11526 (Greece)
e-mail: pbakas74@gmail.com