Original Article

Progesterone/Estradiol Ratio as a Predictor in the ART Cycles with Premature Progesterone Elevation on the Day of hCG Trigger

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Abstract

Background: The purpose of the study was to evaluate the role of Progesterone/ Estradiol (P_4/E_2) ratio as a predictive tool for clinical pregnancy in ART cycles with a premature progesterone rise of >1.5 *ng/ml* on the day of human chorionic gonadotropin (hCG) trigger.

Methods: Retrospective analysis was done on 569 fresh embryo transfer cycles from January 2011 to December 2012 at the infertility unit of a tertiary care hospital. Age, BMI, number of cycles and number of clinical pregnancies have been considered.

Results: The overall clinical pregnancy rate per embryo transfer was 42.8% (244/569). The clinical pregnancy rate in the 36 cycles with progesterone (P_4) level >1.5 *ng/ml* was significantly lower than the 533 cycles with normal p_4 ≤1.5 *ng/ml* (22.2% *vs*. 44.2%; p=0.0092). The 36 cycles with progesterone level >1.5 *ng/ml* were divided into subgroups of P_4/E_2 >1 (n=20) and P_4/E_2 ≤1 (n=16). The 20 cycles with P_4/E_2 >1 and P_4 >1.5 *ng/ml* had a significantly lower pregnancy rate than the cycles with P_4 ≤1.5 *ng/ml* (15% *vs*. 42.8%; p=0.0103). The 15 cycles with P_4/E_2 ≤1 and P_4 >1.5 *ng/ml* had a similar pregnancy rate as the cycles with P_4 ≤1.5 *ng/ml*.

Conclusion: A premature progesterone elevation in ART cycles is possibly associated with lower clinical pregnancy rates; this adverse impact of elevated progesterone seems to be limited mainly to a subgroup with an elevated P_4/E_2 ratio >1.

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Introduction

Since the advent of assisted reproductive technology (ART), there has been continued effort to identify factors affecting embryo implantation which have an important bearing on the final treatment outcome. The inability to predict successful implantation even after transfer of "good quality" embryos in a normal uterus without any obvious intracavity pathology, can lead to disappointment for the couple and treating clinician.

One of the reasons for failure of implantation is due to an alteration in the implantation potential of the endometrium (1). A suggested cause is a premature progesterone elevation which causes endometrial advancement and impairs receptivity (2). Controlled ovarian stimulation with high dose exogenous gonadotropins does increase the oocyte yield but adversely affects the implantation potential of the endometrium (3).

It has been suggested that the decrease in the endometrial receptivity due to ovarian stimulation is due to an alteration in the protein profile expression (4). In addition, new metabolomics (5) and genomic (6) data have brought the possibility of predicting and thereby improving the implantation potential. Currently, such technologies are not widely used and also suffer from a lack of standardization (7). A rapid, reliable non-invasive test capable of predicting endometrial implantation could be an important tool which can help optimize the ART outcomes (8).

Premature elevated progesterone level during

controlled ovarian stimulation has been associated with decreased endometrial receptivity (2). A prematurely elevated progesterone level advances the endometrial window of receptivity leading to an asynchrony between the endometrium and the embryo (3) which in turn leads to a failure of implantation (9). However, the literature has been conflicting due to factors such as discrepancies in cut-offs, heterogeneous patient characteristics and the different stimulation protocols used (10).

Varying cut-offs have been used to define an elevated progesterone during ART cycles, ranging from 0.8 to 2.0 *ng/ml* (11-15). However, recent studies have defined an elevation of serum progesterone using a cut-off of 1.5 *ng/ml* since there has been evidence of a marked difference in endometrial gene expression profiles above and below serum progesterone levels of 1.5 *ng/ml* (16, 17).

A premature progesterone elevation does not uniformly mean a failed implantation, because there are still clinical pregnancies recorded in cycles with high progesterone levels. Hence, there is a need to identify the subgroup of patients who have a good chance of conception in spite of elevated progesterone levels. One earlier study has suggested that there is a subgroup of high responders in whom elevated progesterone doesn't negatively affect the outcome (18).

Progesterone/Estradiol (P_4/E_2) ratio has been suggested as a marker for predicting clinical pregnancy rates, but the evidence is conflicting (19-21).

In this study, an attempt was made to evaluate the usefulness of P_4/E_2 ratio to predict implantation and clinical pregnancy in cycles with prematurely elevated progesterone during ART.

Methods

A retrospective analysis of all ART cycles was done at the infertility unit of a tertiary care hospital from January 2011 to December 2012. During the study period, a total of 569 fresh embryo transfers were carried out. These included 289 antagonist cycles, 188 long protocol, 49 ultralong protocol and 43 short protocol cycles.

Cycle regulation before the IVF cycle was done using oral contraceptive pills. The choice of protocol and dose of recombinant gonadotropins was chosen according to the patient's age, BMI, antral follicle count and the indication for IVF.

For antagonist protocol, recombinant follicle stimulating hormone (rFSH) (Recagon, Organon pharmaceuticals, Dublin, Ireland) was started from the first or second day of menstrual cycle. Follicular monitoring was initiated from the 5th day of controlled ovarian hyperstimulation (COH) and antagonist (Ganirelix, Organon pharmaceuticals, Dublin, Ireland) was added at a dose of 0.25 *mg* subcutaneously once the lead follicle reached a size of 14 *mm* or more (multiple dose, flexible regime). Daily doses of antagonist were given till the day of human chorionic gonadotropin (hCG) trigger.

In case of long protocol, down regulation was achieved using leuprolide acetate (Lupride, Sun pharmaceuticals, Gujarat, India) at a dose of 0.5 *mg* subcutaneously daily starting from day 21 of the previous menstrual cycle till the day of hCG administration. Recombinant FSH was started from the first or second day of menstrual cycle and follicle monitoring by ultrasound was initiated from the sixth day of COH.

For ultralong protocol, which was used in cases of endometriosis undergoing ART, long-term pituitary down regulation was achieved by administering leuprolide acetate in monthly depot doses (3.75 *mg* intramuscularly) starting three months before the ART cycle. The down regulation was continued by using leuprolide acetate 0.5 *mg* subcutaneously daily till the day of hCG administration.

For anticipated poor responders, short protocol was used. Leuprolide acetate was started from the first day of menstrual cycle at a dose of 1 *mg* subcutaneously for initial three days (for the flare effect), and then, daily doses of 0.5 *mg* subcutaneously were continued daily till the day of hCG administration. Recombinant FSH was started from the third day of starting leuprolide acetate and follicle monitoring by ultrasound was initiated from the sixth day of stimulation.

Cycle monitoring was done using serial ultrasound till the leading follicle cohort reached 18 *mm*, when triggering was done using Injection of hCG 5000 *IU* (Pregnyl, Organon pharmaceuticals, Dublin, Ireland).

Serum estradiol and progesterone levels were estimated on the day of hCG trigger using electrochemiluminescence assay by Roche e411 (22) with an assay range for estradiol from 0.005 *ng/ml* to 4.3 *ng/ml* and for progesterone from 0.030 to 60.00 *ng/ml*.

Transvaginal ultrasound guided oocyte retrieval was scheduled 35 *hr* after hCG injection. The retrieved oocytes were incubated for 3-4 *hr* in ferti-

lization medium, and then decision for IVF or ICSI was taken depending on the indication, the number of oocytes and previous fertilization rates. Short incubation insemination (2 *hr*) and group culture were followed for IVF. Oocyte denudation was done before ICSI. The oocytes were incubated overnight in mini incubator with triple gas mixture and observed 16-18 *hr* post insemination/ injection for fertilization. Embryo transfer was done at the cleavage or blastocyst stage using Sydney IVF (SIVF) catheters. Luteal support was given with vaginal micronized progesterone pessaries 400 *mg* twice daily post-oocyte retrieval and intramuscular progesterone 100 *mg* twice weekly.

Pregnancy was confirmed by taking a positive serum beta hCG 14 days after embryo transfer. Transvaginal ultrasound was done two weeks after a positive serum beta hCG and a clinical pregnancy was defined as the ultrasonographic visualization of one or more gestational sacs (23).

Statistical analysis was done using SPSS version 17.0 (24) using Fischer's exact test for 2x2 contingency table taking a p-value of <0.05 as significant.

Results

A total of 569 fresh embryo transfer cycles were carried out during the study period. Thirty six cycles had a premature progesterone (P_4) elevation with a level >1.5 *ng/ml* on the day of hCG. The baseline clinical characteristics of age and BMI were not significantly different between the elevated progesterone and the normal progesterone group (Table 1).

There were 244 clinical pregnancies from 569 fresh embryo transfer cycles, with a clinical pregnancy rate per embryo transfer of 42.8% among all cycles.

Among the 533 fresh embryo transfer cycles with progesterone level ≤1.5 *ng/ml*, there were 236 clinical pregnancies giving a clinical pregnancy rate of 44.2%. Among the 36 fresh embryo transfer cycles with progesterone level >1.5 *ng/ml*, there were eight clinical pregnancies giving a clinical pregnancy rate of 22.2% (Table 2).

Table 1. Baseline characteristics

	Progesterone >1.5 *ng.ml*	Progesterone ≤1.5 *ng/ml*	p-value
Age (years) *	31.5±4.9	31.7±4.4	0.804
BMI (*kg/m²*) *	23.3±4.9	25.2±4.1	0.06

* M±SD

Table 2. Clinical pregnancy rate in all ART cycles

	Number of cycles	Clinical pregnancies	Clinical pregnancy rate	p-value
All cycles	569	244	42.8%	
P ≤1.5 *ng/ml*	533	236	44.2%	0.0092
P >1.5 *ng/ml*	36	8	22.2%	

Subgroup analysis was done within the cycles with elevated progesterone (>1.5 *ng/ml*). Out of 36 cycles, 20 cycles had a progesterone/estradiol (P_4/E_2) ratio of >1 and 16 cycles had a P_4/E_2 ratio of ≤1. Of the 20 cycles, with P_4/E_2>1, there were only 3 clinical pregnancies with a clinical pregnancy rate per embryo transfer of 15%. And of the 16 cycles with P_4/E_2 ≤1, there were 5 clinical pregnancies with a clinical pregnancy rate of 31.3% (Table 3).

Subgroup analysis was done for antagonist and long protocol cycles because only these cycles had sufficient numbers for a statistical analysis. Out of 289 antagonist cycles, 136 had a clinical pregnancy with a clinical pregnancy rate of 47.1%. The group with elevated progesterone >1.5 *ng/ml* had a significantly lower clinical pregnancy rate of 17.6% (3/17) when compared to the group with progesterone ≤1.5 *ng/ml* where a clinical pregnancy rate of 48.9% (133/272) was obtained (Table 4).

There were a total of 188 long protocol cycles and a clinical pregnancy rate of 43.6% (82/188) was recorded. The group with elevated progesterone (>1.5 *ng/ml*) had a lower clinical pregnancy rate of 28.5% (2/7) compared to the group with progesterone ≤1.5 *ng/ml*, which had a clinical pregnancy rate of 44.1% (80/181), but the difference did not reach statistical significance (Table 5).

Table 3. Clinical pregnancy rate-subgroup analysis

	Number of cycles	Clinical pregnancies	Clinical pregnancy rate	p-value
P ≤1.5 *ng/ml* (Group 1)	533	236	44.2%	
P >1.5 *ng/ml* and P_4/E_2 >1 (Group 2)	20	3	15%	0.0103 (Group 1 *vs.* Group 2)
P >1.5 *ng/ml* and P_4/E_2 ≤1 (Group 3)	16	5	31.3%	0.4442 (Group 1 *vs.* Group 3)

Table 4. Subgroup analysis of antagonist cycles

	Number of cycles	Clinical pregnancies	Clinical pregnancy rate	p-value
All cycles	289	136	47.1%	--
P ≤1.5 *ng/ml*	272	133	48.9%	0.013
P > 1.5 *ng/ml*	17	3	17.6%	

Table 5. Subgroup analysis of long protocol cycles

	Number of cycles	Clinical pregnancies	Clinical pregnancy rate	p-value
All cycles	188	82	43.6%	--
P ≤1.5 *ng/ml*	181	80	44.1%	0.47
P > 1.5 *ng/ml*	7	2	28.5%	

Discussion

In our study, progesterone elevation on the day of hCG trigger was associated with a significant reduction in clinical pregnancy rate (44.2% *vs*. 22.2%; p=0.0092). In the subgroup analysis of the patients with progesterone elevation, the group with P_4/E_2 >1 showed a significant decrease in the pregnancy rate (44.2% *vs*. 15%; p=0.0103). Importantly, in the group with p>1.5 *ng/ml* but P_4/E_2 ≤1, the pregnancy rate was similar to the group with normal progesterone level (44.2% *vs*. 31.3%; p=0.44).

The two mechanisms by which a premature progesterone surge can affect the ART outcome adversely is by having a deleterious effect either on the endometrium or the embryo. Elevated progesterone level has been implicated in accelerating the endometrial maturation leading to asynchrony between the endometrium and the developing embryo. Pregnancy is unlikely with asynchrony ≥3 days between expected and actual histological dating by Noyes' criteria (25). Endometrium with a discrepancy of 3 days express separate molecular cluster profiles and different gene expressions (16, 17, 26). Progesterone elevation does not have deleterious effect on embryo quality as evidenced by studies in donor-oocyte programs (13, 27, 28).

The effect of elevated progesterone on ART outcome has been controversial with some studies stating that there is no negative impact of an elevated progesterone on pregnancy rates (11-13, 15, 29-31). The recent studies (14, 32-35), however, show that a premature elevation of progesterone does decrease pregnancy rates. A meta-analysis in 2007 (which included five studies, n=700 patients) (36) stated that the negative effect of progesterone on pregnancy outcome did not reach statistical significance. However, two subsequent meta-analyses by the same group, the first of which included only antagonist cycles (five studies, n=585 patients) (37) and another which evaluated 63 studies (n=55199 patients) (38) came to the conclusion that progesterone elevation significantly reduced the clinical pregnancy rates in fresh embryo transfer cycles.

Among the antagonist cycles, there was a significant lowering of the clinical pregnancy rate with a P_4 level of >1.5 *ng/ml* (p=0.013) which concurs with most previous studies (32, 33, 39). With regard to long protocol cycles, the difference in clinical pregnancy rates between the normal and elevated progesterone groups did not reach statistical significance (p=0.47) which is in agreement with previous studies (11-13, 15, 29, 30, 40).

A meta-analysis by Griesinger et al. (18) manifested that a premature progesterone elevation does not affect clinical pregnancy rates in high responders.

Progesterone/Estradiol was introduced as a novel marker by Younis et al. in 1998 (41) and it was calculated as progesterone (*ng/ml*)×1,000/ estradiol (*pg/ml*). In this initial study (41) on superovulation and IUI cycles and in a later study (42) in ART cycles with the same group, the authors had put forth the hypothesis that a premature luteinisation defined as P_4/E_2 ratio >1 was associated with a significantly reduced pregnancy rate. They also concluded that premature luteinisation and elevated P_4/E_2 ratio are associated with dysmature follicles in poor ovarian reserve patients. The same cut-off was used for this study, but with a change of units as P_4/E_2 ratio equals progesterone (*ng/ml*) /estradiol (*ng/ml*). A subsequent study (19) concluded that P_4/E_2 ratio was a better predictor than serum progesterone alone for prediction of clinical pregnancy. As regarding the subgroup of normo-

responders undergoing ART, there is contradictory evidence (20, 21) as to whether elevated P_4/E_2 ratio has any impact on clinical pregnancy rate. A study in high responders (43) concluded that P_4/E_2 ratio is a more reasonable marker to predict pregnancy rate than serum progesterone alone, but P_4/E_2 ratio has low sensitivity and positive predictive value to predict pregnancy rates.

In our present study, among the cycles with a premature progesterone elevation (>1.5 *ng/ml*), the subgroup of cycles with an elevated P_4/E_2 ratio of >1 had a poorer outcome. In the other subgroup with a P_4/E_2 ratio of ≤1, the clinical pregnancy rates did not significantly differ from the cycles with a normal progesterone level of ≤1.5 *ng/ml*. Hence, the deleterious effect of progesterone elevation is possibly confined to only those cycles which have an elevated P_4/E_2 ratio as well. In clinical practice, a policy of cryopreservation of all embryos can be selectively considered for those ART cycles with P_4 >1.5 *ng/ml* and P_4/E_2 >1. The subgroup with progesterone elevation but P_4/E_2 ≤1 can be expected to have a similar pregnancy outcome as those with progesterone ≤1.5 *ng/ml*. However, given the limited sample size of our study, our results need to be validated in further studies with larger sample size. For the same reason, comparison between our study and previous studies is also limited by the sample size.

One possibility could be that the endometrial advancement is not dependent on progesterone levels alone but by the relative levels of both estrogen and progesterone in the circulation (44).

The second reason could be that the progesterone elevation in P_4/E_2 ratio >1 sub-group is the result of a subtle LH elevation due to inadequate pituitary desensitization and the cause in the group with P_4/E_2 ≤1 is due to a combination of a high number of follicles and a supraphysiological FSH drive (45). This disparity in the clinical pregnancy rates with the two groups of P_4/E_2 ratio could also explain why previous studies have shown that clinical pregnancy rates in high responders are not affected by a premature progesterone rise (18). High responders would be expected to have high estradiol levels and hence the elevation in progesterone would possibly be compensated by a P_4/E_2 ratio of ≤1. The third possibility could be that the progesterone elevation in P_4/E_2 ≤1 is due to a "physiological" secretion by multiple follicles, and the progesterone elevation in P_4/E_2 >1 group is due to "premature luteinization" by dysmature follicles or in those with a low ovarian reserve (21, 41, 42).

Our current study had a retrospective design. The numbers of cycles with a progesterone level of ≥1.5 *ng/ml* are low, hence limiting the power of our statistical analyses. In addition, the stimulation strategies and doses were tailored to individual patients and hence were not uniform. This is a further limitation to our present study. Hence, our finding needs to be validated in a study with a prospective study design and with a larger sample size.

Conclusion

To conclude, our findings suggest that a premature progesterone elevation in ART cycles is possibly associated with lower clinical pregnancy rates. However, this adverse impact of elevated progesterone seems to be limited mainly to a subgroup with an elevated P_4/E_2 ratio. This hypothesis needs validation in larger trials. Elective cryopreservation of embryos is an option that needs to be discussed with this subgroup of patients.

Conflict of Interest

The authors report no declarations of interest. No funding has been taken from any funding agency.

References

1. Fatemi HM, Popovic-Todorovic B. Implantation in assisted reproduction: a look at endometrial receptivity. Reprod Biomed Online. 2013;27(5):530-8.
2. Al-Azemi M, Kyrou D, Kolibianakis EM, Humaidan P, Van Vaerenbergh I, Devroey P, et al. Elevated progesterone during ovarian stimulation for IVF. Reprod Biomed Online. 2012;24(4):381-8.
3. Fleming R, Jenkins J. The source and implications of progesterone rise during the follicular phase of assisted reproduction cycles. Reprod Biomed Online. 2010;21(4):446-9.
4. Li MQ, Jin LP. Ovarian stimulation for in vitro fertilization alters the protein profile expression in endometrial secretion. Int J Clin Exp Pathol. 2013;6 (10):1964-71.
5. Garrido-Gomez T, Dominguez F, Simon C. Proteomics of embryonic implantation. Handb Exp Pharmacol. 2010;(198):67-78.
6. Samborski A, Graf A, Krebs S, Kessler B, Reichenbach M, Reichenbach HD, et al. Transcriptome changes in the porcine endometrium during the preattachment phase. Biol Reprod. 2013;89(6):134.
7. Altmae S, Esteban FJ, Stavreus-Evers A, Simon C, Giudice L, Lessey BA, et al. Guidelines for the de-

sign, analysis and interpretation of 'omics' data: focus on human endometrium. Hum Reprod Update. 2014;20(1):12-28.

8. Edgell TA, Rombauts LJ, Salamonsen LA. Assessing receptivity in the endometrium: the need for a rapid, non-invasive test. Reprod Biomed Online. 2013;27(5):486-96.
9. Kolibianakis EM, Bourgain C, Papanikolaou EG, Camus M, Tournaye H, Van Steirteghem AC, et al. Prolongation of follicular phase by delaying hCG administration results in a higher incidence of endometrial advancement on the day of oocyte retrieval in GnRH antagonist cycles. Hum Reprod. 2005; 20(9):2453-6.
10. Elnashar AM. Progesterone rise on the day of HCG administration (premature luteinization) in IVF: an overdue update. J Assist Reprod Genet. 2010;27 (4):149-55.
11. Edelstein MC, Seltman HJ, Cox BJ, Robinson SM, Shaw RA, Muasher SJ. Progesterone levels on the day of human chorionic gonadotropin administration in cycles with gonadotropin-releasing hormone agonist suppression are not predictive of pregnancy outcome. Fertil Steril. 1990;54(5):853-7.
12. Givens CR, Schriock ED, Dandekar PV, Martin MC. Elevated serum progesterone levels on the day of human chorionic gonadotropin administration do not predict outcome in assisted reproduction cycles. Fertil Steril. 1994;62(5):1011-7.
13. Hofmann GE, Bentzien F, Bergh PA, Garrisi GJ, Williams MC, Guzman I, et al. Premature luteinization in controlled ovarian hyperstimulation has no adverse effect on oocyte and embryo quality. Fertil Steril. 1993;60(4):675-9.
14. Silverberg KM, Burns WN, Olive DL, Riehl RM, Schenken RS. Serum progesterone levels predict success of in vitro fertilization/embryo transfer in patients stimulated with leuprolide acetate and human menopausal gonadotropins. J Clin Endocrinol Metab. 1991;73(4):797-803.
15. Ubaldi F, Smitz J, Wisanto A, Joris H, Schiettecatte J, Derde MP, et al. Oocyte and embryo quality as well as pregnancy rate in intracytoplasmic sperm injection are not affected by high follicular phase serum progesterone. Hum Reprod. 1995;10 (12):3091-6.
16. Van Vaerenbergh I, Van Lommel L, Ghislain V, In't Veld P, Schuit F, Fatemi HM, et al. In GnRH antagonist/rec-FSH stimulated cycles, advanced endometrial maturation on the day of oocyte retrieval correlates with altered gene expression. Hum Reprod. 2009;24(5):1085-91.
17. Labarta E, Martinez-Conejero JA, Alama P, Horcajadas JA, Pellicer A, Simon C, et al. Endometrial receptivity is affected in women with high circulating progesterone levels at the end of the follicular phase: a functional genomics analysis. Hum Reprod. 2011;26(7):1813-25.
18. Griesinger G, Mannaerts B, Andersen CY, Witjes H, Kolibianakis EM, Gordon K. Progesterone elevation does not compromise pregnancy rates in high responders: a pooled analysis of in vitro fertilization patients treated with recombinant follicle-stimulating hormone/gonadotropin-releasing hormone antagonist in six trials. Fertil Steril. 2013;100 (6):1622-8.e1-3.
19. Keltz MD, Stein DE, Berin I, Skorupski J. Elevated progesterone-to-estradiol ratio versus serum progesterone alone for predicting poor cycle outcome within vitro fertilization. J Reprod Med. 2012;57 (1-2):9-12.
20. Cetinkaya ES, Berker B, Aytac R, Atabekoglu C, Sonmezer M, Ozmen B. The value of the progesterone-to-estradiol ratio on the day of hCG administration in predicting ongoing pregnancy and live birth rates in normoresponders undergoing GnRH antagonist cycles. Eur J Obstet Gynecol Reprod Biol. 2013;170(2):452-7.
21. Lai TH, Lee FK, Lin TK, Horng SG, Chen SC, Chen YH, et al. An increased serum progesterone-to-estradiol ratio on the day of human chorionic gonadotropin administration does not have a negative impact on clinical pregnancy rate in women with normal ovarian reserve treated with along gonadotropin releasing hormone agonist protocol. Fertil Steril. 2009;92(2):508-14.
22. COBAS® 4000 platform [Internet]. Dehli: Hoffmann-La Roche Ltd; 1996 [updated 2015; cited 2013 Dec 30]. Available from: http://www.roche-diagnosics.co.in/Products/Pages/COBAS4000platform.aspx
23. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. Hum Reprod. 2009;24 (11):2683-7.
24. SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.
25. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update. 2006;12(6):731-46.
26. Van Vaerenbergh I, McIntire R, Van Lommel L, Devroey P, Giudice L, Bourgain C. Gene expression during successful implantation in a natural cycle. Fertil Steril. 2010;93(1):268.e15-8.
27. Legro RS, Ary BA, Paulson RJ, Stanczyk FZ, Sau-

er MV. Premature luteinization as detected by elevated serum progesterone is associated with a higher pregnancy rate in donor oocyte in-vitro fertilization. Hum Reprod. 1993;8(9):1506-11.

28. Melo MA, Meseguer M, Garrido N, Bosch E, Pellicer A, Remohi J. The significance of premature luteinization in an oocyte-donation programme. Hum Reprod. 2006;21(6):1503-7.
29. Bustillo M, Stern JJ, Coulam CB. Serum progesterone at the time of human chorionic gonadotrophin does not predict pregnancy in in-vitro fertilization and embryo transfer. Hum Reprod. 1995; 10(11):2862-7.
30. Urman B, Alatas C, Aksoy S, Mercan R, Isiklar A, Balaban B. Elevated serum progesterone level on the day of human chorionic gonadotropin administration does not adversely affect implantation rates after intracytoplasmic sperm injection and embryo transfer. Fertil Steril. 1999;72(6):975-9.
31. Levi Setti PE, De Cesare R, Sacchi L, Vaiarelli A, Drovanti A, Patrizio P. Elevated progesterone levels on the day of hCG administration have no influence on pregnancy and implantation rates: retrospective analysis of 5,258 cycles. Fertil Steril. 2013;100(3):S465–S465.
32. Bosch E, Valencia I, Escudero E, Crespo J, Simon C, Remohi J, et al. Premature luteinization during gonadotropin-releasing hormone antagonist cycles and its relationship with in vitro fertilization outcome. Fertil Steril. 2003;80(6):1444-9.
33. Bosch E, Labarta E, Crespo J, Simon C, Remohi J, Jenkins J, et al. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for in vitro fertilization: analysis of over 4000 cycles. Hum Reprod. 2010;25(8): 2092-100.
34. Fanchin R, de Ziegler D, Taieb J, Hazout A, Frydman R. Premature elevation of plasma progesterone alters pregnancy rates of in vitro fertilization and embryo transfer. Fertil Steril. 1993;59(5): 1090-4.
35. Hamori M, Stuckensen JA, Rumpf D, Kniewald T, Kniewald A, Kurz CS. Premature luteinization of follicles during ovarian stimulation for in-vitro fertilization. Hum Reprod. 1987;2(8):639-43.
36. Venetis CA, Kolibianakis EM, Papanikolaou E, Bontis J, Devroey P, Tarlatzis BC. Is progesterone elevation on the day of human chorionic gonadotrophin administration associated with the probability of pregnancy in in vitro fertilization? A systematic review and meta-analysis. Hum Reprod Update. 2007;13(4):343-55.
37. Kolibianakis EM, Venetis CA, Bontis J, Tarlatzis BC. Significantly lower pregnancy rates in the presence of progesterone elevation in patients treated with GnRH antagonists and gonadotrophins: a systematic review and meta-analysis. Curr Pharm Biotechnol. 2012;13(3):464-70.
38. Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: a systematic review and meta-analysis of over 60 000 cycles. Hum Reprod Update. 2013;19(5):433-57.
39. Papanikolaou EG, Pados G, Grimbizis G, Bili E, Kyriazi L, Polyzos NP, et al. GnRH-agonist versus GnRH-antagonist IVF cycles: is the reproductive outcome affected by the incidence of progesterone elevation on the day of HCG triggering? A randomized prospective study. Hum Reprod. 2012;27 (6):1822-8.
40. Hofmann GE, Khoury J, Johnson CA, Thie J, Scott RT Jr. Premature luteinization during controlled ovarian hyperstimulation for in vitro fertilization-embryo transfer has no impact on pregnancy outcome. Fertil Steril. 1996;66(6):980-6.
41. Younis JS, Haddad S, Matilsky M, Ben-Ami M. Premature luteinization: could it be an early manifestation of low ovarian reserve? Fertil Steril. 1998;69(3):461-5.
42. Younis JS, Matilsky M, Radin O, Ben-Ami M. Increased progesterone/estradiol ratio in the late follicular phase could be related to low ovarian reserve in in vitro fertilization-embryo transfer cycles with a long gonadotropin-releasing hormone agonist. Fertil Steril. 2001;76(2):294-9.
43. Lee FK, Lai TH, Lin TK, Horng SG, Chen SC. Relationship of progesterone/estradiol ratio on day of hCG administration and pregnancy outcomes in high responders undergoing in vitro fertilization. Fertil Steril. 2009;92(4):1284-9.
44. Abuelghar WM, Elsaeed MM, Tamara TF, Ellaithy MI, Ali MS. Measurement of serum estradiol/progesterone ratio on the day of embryo transfer to predict clinical pregnancies in intracytoplasmic sperm injection (ICSI) cycles. Is this of real clinical value? Middle East Fertil Soc J. 2013;18(1):31–7.
45. Xu B, Li Z, Zhang H, Jin L, Li Y, Ai J, et al. Serum progesterone level effects on the outcome of in vitro fertilization in patients with different ovarian response: an analysis of more than 10,000 cycles. Fertil Steril. 2012;97(6):1321-7.e1-4.