

DOI: <http://dx.doi.org/10.18203/2320-1770.ijrcog20190303>

Original Research Article

## Elevated progesterone level and its consequences on IVF

Sankar Kumar Das<sup>1,2\*</sup>, Priyanka Roy<sup>1,3</sup>, Krishna Kalita<sup>1,4</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Swagta Hospital and Research Center, Bongaigaon Assam, India

<sup>2</sup>Department of Obstetrics and Gynecology, Gauhati Medical College and Hospital, Guwahati, Assam, India

<sup>3</sup>Department of Obstetrics, Assam Medical College and Hospital, Dibrugarh, Assam, India

<sup>4</sup>Department of Obstetrics and Gynecology, Assam Agricultural University, Jorhat, Assam, India

**Received:** 19 December 2018

**Accepted:** 11 January 2019

**\*Correspondence:**

Dr. Sankar Kumar Das,

E-mail: [drsankarkumardas@rediffmail.com](mailto:drsankarkumardas@rediffmail.com)

**Copyright:** © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

### ABSTRACT

**Background:** Understanding of the embryo-endometrium dialogue is still far from being understood. During conventional in vitro fertilization cycles, progesterone elevation on the day of human chorionic gonadotropin administration refers to rising progesterone levels in the absence of either premature or a luteinizing hormone surge. Most research have reported that elevated progesterone had an adverse impact on the endometrial environment of fresh cycles, leading to a decrease in pregnancy rates.

**Methods:** The current study was undertaken at Swagat hospital and research Centre, Bongaigaon, Assam (India). Fifty patients undergoing in vitro fertilization (IVF) were selected for the study. Baseline estimation of follicle stimulating hormone (FSH), luteinizing hormone (LH), anti-Mullerian hormone (AMH), (estrogen) E2 and (transvaginal sonography) TVS was done. The patients were grouped on the basis of their progesterone levels on the day of hCG trigger, with the cutoff for defining premature progesterone rise being (progesterone)  $P4 \geq 3\text{ng/ml}$ . Final oocyte maturation was induced with hCG. Oocyte retrieval was performed 34-36 h after hCG. Two to three embryos of day 3 cleavage stage were transferred under TVS guidance. The parameters obtained from each cycle were recorded. Statistical analysis was performed. Probability (P) value  $<0.05$  was considered statistically significant. A sonographic confirmation of pregnancy was performed 2 weeks after  $\beta$ -hCG positive.

**Results:** On dividing the patients into two groups, based on the cut off of P4 as 3ng/ml, it was observed that the no of cases that conceived was significantly higher in the subjects whose P4 level was less than or equal to 3ng/ml than the subjects whose P4 level was more than 3ng/ml.

**Conclusions:** Elevated P4 i.e. Progesterone level just before trigger is a reflection of endometrial maturity that can cause disparity between endometrial and embryonic ageing and therefore hamper implantation or cause failure in assisted reproductive technology (ART).

**Keywords:** Embryo, Endometrium, IVF, Progesterone

### INTRODUCTION

Understanding of the embryo-endometrium dialogue is still far from being understood, not to know completely the parameters affecting the IVF or intrauterine insemination (IUI) success rate. The pivotal role of four

hormones namely FSH, LH, P4 (progesterone), E2 in various physiological phenomenon, specially menstruation and pregnancy have been extensively studied. During conventional in vitro fertilization (IVF) cycles, progesterone elevation on the day of human chorionic gonadotropin (hCG) administration refers to

rising progesterone levels in the absence of either premature luteinization or a luteinizing hormone (LH) surge.<sup>1</sup> Although the premature luteinization is suppressed by gonadotropin-releasing hormone (GnRH) analogues, early rises in progesterone levels still occur in 5%-50% of all down-regulated IVF cycles.<sup>2-4</sup> The impact of premature progesterone elevation on ART-cycle outcomes has been a subject of some debate in the last two decades.<sup>5-7</sup> In recent years, several large trials and meta-analyses have suggested a negative impact of elevated progesterone on pregnancy rates in GnRH antagonist cycles.<sup>8-12</sup> Most research have reported that elevated progesterone had an adverse impact on the endometrial environment of fresh cycles, leading to a decrease in pregnancy rates. According to some studies, P4 rise more than 1.5ng/ml may have deleterious effects on endometrial receptivity. It causes acceleration of the endometrial maturation process that subsequently narrows the time frame for implantation and thus decreases the pregnancy rate.<sup>13,14</sup> High P4 levels on the day of hCG trigger induce advanced endometrial histological maturation and differential endometrial gene expression, which lead to implantation failure.<sup>15-17</sup> However, to the embryo-endometrial cross-dialog, the embryo quality is as important as endometrial receptivity. Thus, another possibility is that the elevated progesterone has negative effects on the quality of the oocyte or resulting embryo.<sup>18</sup>

## METHODS

The current study was undertaken at Swagat hospital and research Centre, Bongaigaon, Assam (India). After approval from the Institutional Ethics Committee and informed consent from the patients, fifty patients undergoing IVF were selected for the study. Fresh embryos were transferred in all the cases.

### Inclusion criteria

- Patients between 25 to 38 age group
- Basal E2 less than 60pg/ml
- Both ovaries present
- Normal uterine cavity on hysteroscopy
- Basal FSH less than 18 IU/ml.

### Exclusion criteria

- Presence of endometriosis of grade 3-4
- Endometrial tuberculosis- subjects who were EB-PCR positive
- Antral follicular count of more than 12 in baseline scan.

Most of the cases were subjected to antagonist protocol, only two cases were subjected to agonist protocol. Baseline estimation of (Day 2) FSH, LH, AMH, E2 and TVS was done. In GnRH agonist protocol lupride 20mg sc once daily was given from day 25<sup>th</sup> of the preceding cycle till downregulation i.e. LH less than 6 IU/L, E2 less

than 60pg/ml, ET less than 5mm and follicle size less than 10mm. In GnRH antagonist protocol, gonadotropin was started from day 2 of cycle, if basal LH less than 6 IU/L and E2 less than 60pg/ml. Sanrox 0.25 microgram started, once daily from day6 or when the largest follicle was more than or equal to 14mm and continued till the morning of the day of trigger. The dose of gonadotropin was individualized according to patient's response. The gonadotropin that was used was mostly HMG. Serial monitoring with TVS was done to determine follicular size with hormonal profiling to determine LH, E2, and progesterone levels on day 8 of stimulation and on the day of trigger. The patients were grouped on the basis of their progesterone levels on the day of hCG trigger, with the cut off for defining Premature Progesterone Rise being P4 $\geq$  3ng/ml. Final oocyte maturation was induced with hCG (10,000 IU i.m.) when at least three follicles of size 17-18 mm were observed in both ovaries. Oocyte retrieval was performed 34-36 hour after hCG. The oocytes retrieved were inseminated. Fertilization check was performed on day 1 of insemination, and embryos cultured in sequential medium. Embryos were graded according to Veck's criteria. Two to three embryos of day 3 cleavage stage of grade A/B were transferred under TVS guidance.

### Statistical analysis

The parameters obtained from each cycle were recorded. Statistical analysis was performed with the Statistical Package for the Social Sciences trial version 23.0 software for Windows. The categorical data were compared among groups using Chi-square test. Groups were compared for quantitative data, which were presented as mean and standard deviation and were compared using ANOVA test. Probability (P) value<0.05 was considered statistically significant. Serum  $\beta$ -hCG levels were recorded 15 days after embryo transfer. Those with positive  $\beta$ -hCG, that is  $\geq$ 50mIU/ml, were considered to calculate conception rate. A sonographic confirmation of pregnancy was performed 2 weeks after  $\beta$ -hCG positive.

## RESULTS

On dividing the patients into two groups, based on the cut off of P4 as 3ng/ml, it was observed that the no of cases that conceived was significantly higher in the subjects whose P4 level was less than or equal to 3ng/ml than the subjects whose P4 level was more than 3ng/ml.

**Table 1: Result of IVF based on P4 cut off 3ng/ml.**

	P4 $\leq$ 3ng/ml	P4 >3ng/ml	Total	P value
No. of cases	26	24	50	0.000014
Conceived	24	8	32	
Failure	2	16	18	

On dividing the subjects into three groups based on P4 level as less than 1.5ng/ml, 1.5ng/ml to 3ng/ml and more than 3ng/ml. It was observed that there was a significant decrease in the AMH level in the group with P4 more than 3ng/ml. There was a significant increase in the LH level in the groups along with increasing P4 level. There was a significant decrease in the number of oocytes retrieved and embryo transferred with rising P4 level. No significant difference among the groups in relation to age group, basal FSH, peak E2 level and endometrial thickness was observed.

**Table 2: Hormone parameters, age group, endometrial thickness, no of oocytes and embryo transferred in the different P4 groups.**

	P4 <1.5ng/ml	P4 1.5 - 3ng/ml	P4 >3ng/ml	P value
No. of cases	10	16	24	
	<b>Mean±SD</b>			
Age	29.7±2.4	28.5±3.5	30.5±4.2	0.255
Peak E2 pg/ml	740±183	862±257	761±246	0.326
AMH ng/ml	2.47 ±0.7	2.6±0.7	1.9 ±0.7	0.007
Basal LH IU/L	5.7±1.4	9.6 ±4.6	11.3±4.9	0.005
Basal FSH IU/L	13.8±5.1	14.5±4.8	16.3±6.3	0.418
Oocyte retrieved	6.4±2.0	7.2±2.2	4.88±2.4	0.006
Embryo transferred	2.4±0.7	2.47±0.6	1.83±0.8	0.016
Endometrial thickness (mm)	8.95±1.0	10.1±1.5	9.2±1.4	0.066
conceived	9	15	8	
Failure	1	1	16	

On dividing the patients into two groups, based on the cut off of basal LH as 6IU/L, it was observed that the number of cases that conceived was significantly higher in the subjects whose basal LH level was less than or equal to 6IU/L than the subjects whose basal LH level was more than 6ng/ml.

**Table 3: Result of IVF based on basal LH cut off 6 IU/L.**

	Basal LH ≤6IU/L	Basal LH >6IU/L	Total	P value
No. of cases	14	36	50	0.008025
Conceived	13	19	32	
Failure	1	17	18	

## DISCUSSION

Pre-hCG elevation of P4 is poor predictor of IVF outcome. In our retrospective study authors studied the relationship between P4 concentration just before trigger

and clinical pregnancy outcome in fresh embryo transfer cycles. The present study showed an adverse relationship between higher circulating serum concentration of P4 and clinical pregnancy rate. In the study by Fanchin R et al, to find out the effect of premature elevation of plasma progesterone on pregnancy rates of in vitro fertilization and embryo transfer, it was found that despite the similarity in the oocytes retrieved, no of embryos transferred in the subjects; there was a decrease in pregnancy rate and a trend for decrease in embryo implantation rate in those with P4 more than 2.9nmol/L. They concluded that pre hCG elevation in P4 does not lead to decreased oocyte quality but alter endometrial receptivity to embryo implantation.<sup>19</sup> In the study done by Bosch et al in 4032 patients undergoing IVF/ICSI using GnRH analogues for pituitary downregulation, ongoing pregnancy rates were inversely proportional to P4 level on the day of hCG trigger. Patients with P4 less than 1.5ng/ml had significantly higher ongoing pregnancy rates than those with P4 more than 1.5ng/ml.<sup>20</sup> Li M et al, in his study divided the subjects into two groups taking P4 cut off 2ng/ml and 2.5ng/ml. The clinical pregnancy rate was lower in the elevated P4 group than in the control group, but the difference was not statistically significant.<sup>8</sup> In the present study however, with the P4 cut off as 3ng/ml, the difference in the clinical pregnancy rate is significant. Venetis et al, in their meta-analysis, concluded that premature progesterone rise diminishes the probability of achieving pregnancy in women undergoing fresh IVF cycles, even at concentrations in the range of 0.8-1.1ng/ml, and conception rates are further reduced when the progesterone concentration reaches 1.2-1.4ng/ml or higher.<sup>21</sup> In the study by Mascarenhas et al, P4 elevation was associated with a significant reduction in clinical pregnancy rate.<sup>22</sup>

In the present study there was a concomitant significant rise in serum LH with rising P4 level; also, there was a significant decrease in the pregnancy rate in the cases with basal LH more than 6 IU/L; this might be due to premature luteinization and endometrial ageing due to elevated LH consequently leading to asynchrony between endometrium and embryo leading to the pregnancy failure with rising P4 level. Also, most of the subjects in the present study were subjected to antagonist protocol. Several studies have supported the incidence of premature progesterone rise with agonist protocol; a greater number of days of stimulation due to the suppression of the hypothalamo-pituitary-ovarian axis, a higher dose of gonadotropins, more number of intermediate follicles, and higher estrogen levels observed on the day of trigger may be plausible explanations favoring the same.<sup>2,14</sup> Also in GnRH agonist protocol, there's a high chance of premature endometrial maturity due to increase in basal LH level, owing to the initial flare up by the GnRH agonists, thus leading to endometrial-embryo asynchrony. In the present study the gonadotropin that was used was HMG. Anderson et al in his study concluded that the incidence of premature

progesterone rise was higher in RFSH treated patients than in HMG treated patients.<sup>23</sup>

In the present study it was also seen that there was a significant decrease in the oocyte retrieved and embryo transferred in the group with P4 more than 3ng/ml, suggesting a probability of detrimental effect of elevated P4 on oocytes and embryo. Bo huang et al, in their study demonstrated a negative effect of elevated progesterone levels on the day of HCG trigger on top quality embryo rate regardless of the basal FSH, the total gonadotropin, age of the woman or the time of ovarian stimulation.<sup>18</sup>

## CONCLUSION

Elevated P4 i.e. Progesterone level just before trigger is a reflection of endometrial maturity that can cause disparity between endometrial and embryonic ageing and therefore hamper implantation or cause failure in ART. The cut off level of P4 in the present study was taken to be 3ng/ml above which there was significant decrease in number of cases that conceived. Elevated baseline LH level of more than 6IU/L also can harm implantation and lead to failure of ART by causing premature luteinization and prior ageing of the endometrium that becomes unfavourable for the embryo. Either downregulation by the use of antagonists or freezing of the oocytes that are retrieved, instead of embryo transfer, in the face of an elevated P4 level just before trigger can prevent failure of such cases.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

## REFERENCES

- Hill MJ, Royster GD, Healy MW, Richter KS, Levy G, DeCherney AH, et al. Are good patient and embryo characteristics protective against the negative effect of elevated progesterone level on the day of oocyte maturation? *Fertil Steril.* 2015;103(6):1477-84 e1-5.
- 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.
- Huang B, Li Z, Zhu L, Hu D, Liu Q, Zhu G, Zhang H. Progesterone elevation on the day of HCG administration may affect rescue ICSI. *Reprod Biomed Online.* 2014;29(1):88-93.
- 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.
- Papanikolaou EG, Kolibianakis EM, Pozzobon C, Tank P, Tournaye H, Bourgain C, et al. Progesterone rise on the day of human chorionic gonadotropin administration impairs pregnancy outcome in day 3 single-embryo transfer, while has no effect on day 5 single blastocyst transfer. *Fertil Steril.* 2009;91(3):949-52.
- Venetis CA, Kolibianakis EM, Papanikolaou E, Bontis J, Devroey P, Tarlatzis BC. Is progesterone elevation on the day of human chorionic gonadotropin administration associated with the probability of pregnancy in in vitro fertilization? A systematic review and meta-analysis. *Human Reprod Update.* 2007;13(4):343-55.
- 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.
- 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. *Human Reprod.* 2010;25(8):2092-100.
- Ochsenkun R, Arzberger A, von Schonfeldt V, Gallwas J, Rogenhofer N, Crispin A, et al. Subtle progesterone rise on the day of human chorionic gonadotropin administration is associated with lower live birth rates in women undergoing assisted reproductive technology: a retrospective study with 2,555 fresh embryo transfers. *Fertil Steril.* 2012;98(2):347-54.
- Lahoud R, Kwik M, Ryan J, Al-Jefout M, Foley J, Illingworth P. Elevated progesterone in GnRH agonist down regulated in vitro fertilisation (IVFICSI) cycles reduces live birth rates but not embryo quality. *Archives Gynecol Obstet.* 2012;285(2):535-40.
- Yding Andersen C, Bungum L, Nyboe Andersen A, Humaidan P. Preovulatory progesterone concentration associates significantly to follicle number and LH concentration but not to pregnancy rate. *Reprod Biomed Online.* 2011;23(2):187-95.
- 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 editorial comment. *Obstet Gynecol Surv.* 2011;66(12):763-4.
- Kasum M, Radakovic B, Simunic V, Oreškovic S. Preovulatory progesterone rise during ovarian stimulation for IVF. *Gynecol Endocrinol.* 2013;29(8):744-8.
- Haouzi D, Bissonnette L, Gala A, Assou S, Entezami F, Perrochia H, et al. Endometrial receptivity profile in patients with premature progesterone elevation on the day of HCG administration. *Biomed Res Int.* 2014;2014.
- Saadat P, Boostanfar R, Slater CC, Tourgeman DE, Stanczyk FZ, Paulson RJ. Accelerated endometrial

- maturation in the luteal phase of cycles utilizing controlled ovarian hyperstimulation: Impact of gonadotropin-releasing hormone agonists versus antagonists. *Fertil Steril.* 2004;82:167-1.
16. Van Vaerenbergh I, Fatemi HM, Blockeel C, Van Lommel L, Schuit F, et al. Progesterone rise on HCG day in GnRH antagonist/rFSH stimulated cycles affects endometrial gene expression. *Reprod Biomed Online.* 2011;22:263-71.
  17. Labarta E, Martínez-Conejero JA, Alamá P, Horcajadas JA, Pellicer A, Simón 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;26:1813-25.
  18. Bo Huang, Xinling Ren, Li Wu, Lixia Zhu, Bei Xu, Yufeng Li, Jihui Ai, et al. Elevated Progesterone Levels on the Day of Oocyte Maturation May Affect Top Quality Embryo IVF Cycles. *PLoS One.* 2016;11(1):e0145895.
  19. Fanchin R, de Zeigler, Taieb J, Hazout A, Frydman R. Premature elevation of plasma progesterone alters pregnancy rates of in vitro fertilization and embryo transfer. *Fertil Steril.* 1993;59:1090-4.
  20. Li M, Xie Y, Park H, Kumar A, Hubert G, Buyalos R. The effects of elevated serum progesterone level at the day of hCG injection on clinical outcome in IVF-ET patients. *Fertil Steril.* 2013;100(3):S7.
  21. 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;19:433-57.
  22. Mascarenhas M, Kamath MS, Chandy A, Kunjummen AT. Progesterone/estradiol ratio as a predictor in the ART cycles with premature progesterone elevation on the day of hCG trigger. *J Reprod Infertil.* 2015;16:155.
  23. Andersen AN, Devroey P, Arce JC. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor blind controlled trial. *Hum Reprod.* 2006;21:3217-27.

**Cite this article as:** Das SK, Roy P, Kalita K. Elevated progesterone level and its consequences on IVF. *Int J Reprod Contracept Obstet Gynecol* 2019;8:672-6.