Global Journal of Medical Research: E
Gynecology and Obstetrics
Volume 17 Issue 1 Version 1.0 Year 2017
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Circulating Progesterone Levels and Ongoing Pregnancy Rates in Controlled Ovarian Stimulation Cycles in Assisted Reproduction Techniques

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Abstract- How can we prevent premature leutinization in our IVF cycles? To help find the solution a study designed with specific exclusion criteria in an assisted reproductive centre of a tertiary care hospital was designed. In a period of 2 years 100 patients were followed up prospectively. GnRH antagonist protocol was implemented for all recruits because of shorter duration of treatment and as it prevented hyperstimulation. Specific criteria was chosen for GnRH anatagonist initiation. Serum Progesterone measurements were used to predict premature leutinization. Electro-chemiluminiscence Immunoassay using COBAS 6000 used for the measurements. Three different groups analysed in above cohort and we concluded that despite of progesterone rise pregnancy outcome was unaffected. Why is the question answered in discussion.

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GJMR-E Classification: *NLMC Code: WJ 190*

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Circulating Progesterone Levels and Ongoing Pregnancy Rates in Controlled Ovarian Stimulation Cycles in Assisted Reproduction Techniques

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Abstract- How can we prevent premature leutinization in our IVF cycles? To help find the solution a study designed with specific exclusion criteria in an assisted reproductive centre of a tertiary care hospital was designed. In a period of 2 years 100 patients were followed up prospectively. GnRH antagonist protocol was implemented for all recruits because of shorter duration of treatment and as it prevented hyperstimulation. Specific criteria was chosen for GnRH anatagonist initiation. Serum Progesterone measurements were used to predict premature leutinization. Electro-chemiluminiscence Immunoassay using COBAS 6000 used for the measurements. Three different groups analysed in above cohort and we concluded that despite of progesterone rise pregnancy outcome was unaffected. Why is the question answered in discussion.

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I. Introduction

One of the essential components of a successful *in vitro* fertilization/embryo transfer (IVF – ET) cycles is the procurement of mature oocytes that will develop into good embryos with good implantation potential. There are a variety of ovulation induction protocols being utilized to achieve this goal but the markers of optimal maturity remain controversial.

The progesterone increase at, or just before, the onset of LH surge, was thought to be the response of granulosa cells to increased pituitary LH pulses. On the other hand, progesterone can also augment the positive feedback of estradiol on the LH surge[1]. During controlled ovarian stimulation (COS) cycle, progesterone levels rapidly increase following Human Chorionic Gonadotropin (hCG) administration given to induce final oocyte maturation[2]. However, we have also noticed premature LH (Leutinizing hormone) surge caused by the modulatory action of estradiol (E2) levels induced by gonadotropins which have led to premature luteinization and further more cancellation of treatment cycles in patients who are undergoing *in vitro* fertilization (IVF). We can now suppress the release of endogenous gonadotropins from pituitary by introduction of Gonadotropin releasing hormone (GnRH) agonists and antagonists which can decrease incidence of premature LH surge[2]. Despite use of GnRH analogues, subtle increase in serum progesterone levels (beyond an arbitrary threshold value) have been observed at the end of follicular phase in stimulation cycles for IVF and ICSI – ET[3].

This pre-hCG progesterone increase is referred as 'premature luteinization'[4], the term though is misleading as the increasing levels of serum progesterone occurs with GnRH analogues, i.e. it is taking place under low serum LH concentration.

Rather than the excessive amount of progesterone being produced by granulosa cells as a part of early luteinization, it is more likely that excess number of follicles are responsible for elevated progesterone levels as each one may be producing a normal amount of progesterone, consistent with late follicular phase[5]. Whether the presence of increased levels of progesterone on the day of hCG will affect ongoing pregnancy rate is the subject of debate.

Hence, we have investigated association between serum progesterone levels on the day of human Chorionic Gonadotropin (hCG) administration and the probability of ongoing pregnancy in woman undergoing Controlled Ovarian Stimulation for IVF (*in vitro* fertilization)/ICSI-ET (Intracytoplasmic sperm injection with Embryo transfer) in our this study.

II. Methodology

This prospective single center study was done under the ethical guidelines of our institution. A total of 100 patients were included meeting the above criteria and received GnRH antagonist protocol for pituitary down regulation. Study period was of 2 years. Cases of endometriosis and women with above 35 years of age were excluded. The initial dose of gonadotropins was individualized for each patient according to age, BMI, basal FSH levels, antral follicle count and previous response to controlled ovarian stimulation. Dose adjustments performed according to ovarian response, monitored by TVS & serum E2 levels. As a part of routine

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clinical practice, determination of serum progesterone levels were performed on the day of hCG administration.

a) Progesterone measurement

Laboratory measurement of serum progesterone was done using electro-chemiluminiscence Immunoassay "ELISA" using COBAS 6000. Results are determined via a calibration curve which is instrument – specifically generated by 2-point calibration & a master curve provided via the reagent barcode. Quality control using Elecsys Preci Control Universal 1 and 2 are done at least once in 24 hours when test is in use, once per reagent kit, and after every calibration. Measuring range is 0.030 – 60.00 ng/ml. For purpose of statistical analysis serum progesterone was divided into 3 categories as follows: a) <1 ng/ml b)1-1.5 ng/ml c) >1.5 ng/ml.

b) Statistical analysis

The analysis was performed using SPSS version 16 software. The statistical analysis of the data was performed using Pearson Chi – square test or Fischer Exact test. *p* – value of < 0.05 was considered significant.

c) Results

Three groups were formed as described above. The results were compared with the levels of progesterone on the day of hCG administration in stimulation cycle with various outcomes as described below. Baseline characteristics of 100 cases were comparable in all the three groups. Outcomes were measured in terms of total number of mature eggs; quality of embryos; pregnancy outcomes.

Out of 93 women who had less than 1 ng/ml serum progesterone on day of menses, 64.5 % remained less than 1 ng/ml on the day of hCG, 21.5 % it was raised to 1-1.5 ng/ml and in 14 % to >1.5 ng/ml. Three women who had 1-1.5 ng/ml had equal proportion on the day of hCG whereas those who had more than 1.5 ng/ml of progesterone on day 2 of cycle (n = 4) had equal proportion in 1-1.5 ng/ml & more than 1.5 ng/ml. This shows that most of the women show a rise in levels of progesterone on the day of hCG (0.022).

Out of 61 women 59% had estradiol levels less than 3000 pg/ml, 32.8 % had estradiol levels in 3000-6000 pg/ml & 8.2 % had estradiol levels >6000 pg/ml, whereas in group with 1-1.5 ng/ml and more than 1.5 ng/ml progesterone only 4.3 % & 6.2 % women had estradiol more than 6000 pg/ml. Though there is a positive correlation between rising estradiol levels and progesterone levels, this was not seen in this study (Table 1). We saw good endometrial thickness in all three groups irrespective of serum progesterone levels.

Table 1: Comparing serum progesterone levels on the day of hCG with serum Estradiol level.

Serum progesterone on the day of hCG	Estradiol values (pg/ml) on day of hCG						Total (n=100)
	<3000		3000-6000		>6000		
	n	%	n	%	n	%	
<1 ng/ml (n = 61)	36	59	20	32.8	5	8.2	61
1-1.5 ng/ml (n = 23)	16	69.6	6	26.1	1	4.3	23
>1.5 ng/ml (n = 16)	11	68.8	4	25	1	6.2	16
Total (n = 100)	63	63	30	30	7	7	100

(P value is 0.92 by applying Fischer's Exact Test, hence not statistically significant)

In terms of retrieving good mature eggs also we see that out of 61 women with serum progesterone less than 1 ng/ml 78.7% had up to 8 mature eggs, 18% had 9-16 mature eggs whereas 3.3% had more than 16 mature eggs. In women with 1-1.5 ng/ml progesterone (n = 23) 56.5% had less than 8 mature eggs & rest 43.5% had 9-16 mature eggs, whereas out of 16 women with more than 1.5 ng/ml progesterone, 62.5% had up to 8 mature eggs whereas 31.5% had 9-16 mature eggs & 6.2% had more than 16 mature eggs.

Hence we see that good numbers of mature eggs were retrieved in equal proportion in all the three categories (Table 2).

Table 2: Comparing serum progesterone on the day of hCG and number of mature eggs retrieved

Serum progesterone on the day of hCG	Number of mature eggs retreived						Total (n=100)
	Up to 8		9 - 16		More than 16		
	n	%	n	%	n	%	
<1 ng/ml (n = 61)	48	78.7	11	18	2	3.3	61
1-1.5 ng/ml (n = 23)	13	56.5	10	43.5	0	0	23
>1.5 ng/ml (n = 16)	10	62.5	5	31.2	1	6.2	16
Total (n = 100)	71	71	26	26	3	3	100

(P value is 0.10 by applying Fischer's Exact Test, hence not statistically significant)

Sixty one women had progesterone levels less than 1 ng/ml, out of them 26.2% had no best grade embryos retrieved 39.3% had up to 2 & 34.4% had more than 3 best grade embryos. In comparison, group with 1-1.5 ng/ml progesterone 17.4% had no embryos, 39.1% had up to 2 & 43.5% had more than 3 best grade embryos.

Finally, out of 16 women with more than 1.5 ng/ml progesterone 25% had no best embryos, 43.8% had up to 2 and 31.2% had more than 3 best embryos. Hence, best grade embryos were retrieved in equal proportion in all groups (Table 3).

Table 3: Comparing serum progesterone on the day of hCG and best embryos retrieved

Serum progesterone on the day of hCG	Best embryos retreived						Total (n=100)
	Nil		Upto 2		More than 3		
	n	%	n	%	n	%	
<1 ng/ml (n = 61)	16	26.2	24	39.3	21	34.4	61
1-1.5 ng/ml (n = 23)	4	17.4	9	39.1	10	43.5	23
>1.5 ng/ml (n = 16)	4	25	7	43.8	5	31.2	16
Total (n = 100)	24	24	40	40	36	36	100

(P value is 0.89 by applying Pearson Chi-Square test, hence not statistically significant)

Ninety one cases came for follow up beta hCG testing to us out of them 41.8% were pregnant. We further followed these pregnant cases, 71% of these pregnant women had their first antenatal scan done with us showing cardiac activity present.

Three cases were chemical pregnancy whereas other 3 cases had ectopic pregnancies (Table 4).

Table 4: Comparing serum progesterone on the day of hCG and pregnancy

Serum progesterone on the day of hCG	Pregnant		Non pregnant		Total (n = 91)
	n	%	n	%	
<1 ng/ml (n = 56)	26	46.4	30	53.6	56
1-1.5 ng/ml (n = 20)	6	30	14	70	20
>1.5 ng/ml (n = 15)	6	40	9	60	15
Total (n = 91)	38	41.8	53	58.2	91

(P value is 0.47 by applying Pearson Chi-Square test, hence not statistically significant)

In the group with progesterone levels > 1.5 ng/ml 40% patient became pregnant & 60% patients were non pregnant, though statistically not significant we followed up these cases & compared their serum progesterone levels with their pregnancy outcome by the first trimester scan for presence of cardiac activity, excluding chemical pregnancy.

Progesterone estradiol ratio >1 as the definition of premature luteinization was proposed to be associated with low ovarian reserve as well as poor pregnancy outcomes in some studies[6].

Hence we found that though pregnancy outcome is higher in groups with ratio <1 but this difference was not statistically significant (Table 5).

Table 5: Comparing serum progesterone estrogen ratios on the day of hCG with pregnancy outcome

Progesterone estrogen ratio on the day of hCG	Pregnant		Non pregnant		Total (n = 91)
	n	%	n	%	
<1 (n = 84)	37	44	47	56	84
>1 (n = 7)	1	14.3	6	85.7	7
Total (n = 91)	38	41.8	53	58.2	91

(P value is 0.23 by applying Fischer's Exact test, hence not statistically significant)

III. Discussion

Several mechanisms have been proposed to explain subtle elevation of progesterone on the day of hCG administration. It has been suggested that excessive luteinization of the follicles occurs either due to late administration of hCG or exposure of granulosa cells to high concentration of LH forms, exogenous gonadotropin treatment[7, 8]. Both of them seem unlikely in our set up as GnRH antagonist protocol was used and

no premature LH surge or decline in estradiol was seen post hCG administration. Also, good fertilization rates are contradictory to excessive luteinization explanation. Another explanation is that follicle maturation may occur at a lower follicle diameter than expected, resulting in post-mature oocytes. However, hCG administration decision was not based solely on ultrasound findings; furthermore, no adverse effect was reported on the maturation phase of the oocytes, or fertilization or cleavage rates of embryos in the group with higher progesterone levels on the day of hCG administration[9].

However, treatment with GnRH antagonists has been reported to suppress both immuno-active and bioactive LH but not the subtle rise in progesterone on day of hCG administration[10.] Small elevation in progesterone levels on the day of hCG is not caused by elevation of LH because of higher pituitary output or external stimulation rather it may likely because of a local increase from the ovaries and an increase in receptivity of LH at the level of granulosa cells. There are also other intra-ovarian regulators which can be involved in the regulation of ovarian steroidogenesis[11], and also there is somatomedin-C which is found to augment significantly FSH induced progesterone biosynthesis in the rat *in vitro* culture[12].

Our results have shown no difference in serum estradiol levels on the day of hCG, irrespective of higher or lower progesterone levels; none of our cases had significant premature LH surge; the number of oocytes recruited and their fertilization & cleavage rates did not differ between the three groups that we took in this study.

The possibility that small changes in the progesterone levels, on the day of hCG, may primarily effect the endometrium rather than on the ovary has already been proposed. An abnormal rise of progesterone prior to LH surge was reported to affect the uterine endometrium and it results in asynchronization between embryonic development and the endometrial environment[12].

The hormonal receptors present in the endometrium may be affected and may influence other factors responsible for implantation, like platelet activating factor (PAF), one of the factors reported to be involved in the process of implantation[13].

In a study of 115 cases, IVF/ET cycle outcomes were retrospectively correlated with progesterone levels on the day of hCG, as findings demonstrated that even modest increase in progesterone was associated with reduced pregnancy rates. So they recommended to cryopreserve all embryos rather than having a fresh embryo transfer[14]. Other changes in clinical practice may include administrating hCG at a smaller follicle size if progesterone levels start rising.

We require more studies to define the optimal markers of follicle maturation facilitating better timing of hCG administration[15].

In a study on one thousand twelve women undergoing 1,189 IVF-ET cycles, Fanchin et al demonstrated that when there is an adequately required response to controlled ovarian stimulation, an elevated P levels are not associated with lower pregnancy outcomes. This indicates that good embryo quality may compensate for the adverse endometrial effects of progesterone but when there is a weak response to controlled ovarian stimulation (poor responders), premature elevation can lead to lower pregnancy rates[16].

The cause of increase in serum progesterone levels is poorly understood. It may just reflect the mature granulosa cell response to high FSH exposure. It can be a possibility that the gonadotropin used in the stimulation regimen and the amount of LH suppression may play a role in the phenomenon, as both FSH & LH activity may differ in both quality & quantity between products and batches seperately.

Ubaldi et al showed some results indicating that there may be an association between the circulating FSH concentration an late follicular phase progesterone concentrations. This observation can lead to an alternate hypothesis for the increased progesterone secretion in the cycles which are treated with purified FSH[17]. It is also possible that reduced LH stimulation reduces the degree of catabolism of progesterone to androgens & estrogens, as reported above, which leads to excess progesterone secretion into the circulation.

Fleming in his study also raised the issue of methodology used for measuring progesterone in the studies, retained in meta – analysis, which were neither conceived nor validated for measuring low levels of progesterone in the follicular phase[18]. His own data showed that assay precision was varying depending on whether petrol – ether extraction step was used or not sufficiently supports this methodology concern. Also he provided with the evidence supporting the possibility that progesterone measurements at the end of controlled ovarian treatments could be flawed by patient specific matrix effects [18]. The method used in our study had a strict inclusion and exclusion criteria for case selection and the method used for progesterone measurement had a good precision hence unlikely that results are affected by above raised issue.

In Bosch et al study, the strength of association observed might be attenuated by the method used to group patients according to progesterone levels because their assay had sensitivity of 0.2 ng/ml, whereas assay used in this study has sensitivity of 0.030 ng/ml[19].

At present we have no consensus on whether the elevation of progesterone on day of hCG is associated with achieving of a pregnancy. Several studies have denied such association[4, 19, 20, 21], whereas other studies have concluded possibility of a negative association that is probability of pregnancy decreasing significantly when progesterone levels on the day of

hCG for final oocyte maturation, rises above a threshold [7, 16, 22]. In this study we noted that there is no association between elevation of progesterone on the day of hCG administration and pregnancy rate. It should be noted that if negative association between progesterone elevation on day of hCG and the chances of pregnancy do exists, it might be worth exploring the possibility of cryopreserving the resulting embryos and they can be transferred in a subsequent frozen – thawed cycle[23, 14] or another alternative is administrating hCG early in the follicular phase prior to progesterone elevation[18].

Different detrimental levels of serum progesterone on day of hCG in terms of pregnancy outcome has been proposed by above mentioned studies but in this study no such level could be indentified as in all cases we had good response to controlled ovarian stimulation protocol.

Limitation of this study was less sample size hence results were not statistically significant and larger studies size is required.

We found that though there is a rise in serum progesterone seen during controlled ovarian stimulation cycles on the day of hCG in GnRH antagonist cycle but this rise has no significant effect on endometrium thickness, number & quality of oocytes retrieved, number & quality of embryos retrieved and finally pregnancy outcome.

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