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Research Article

Risk of premature luteinization in IVF cycles and its impact on clinical pregnancy rate

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ABSTRACT

Background: Premature luteinization (PL) refers to a rise in serum progesterone (P4) levels on the day of HCG administration (1-4). Most studies use an absolute P4 level on the day of HCG administration as an indicator of PL. This study is carried out with the aim to evaluate the incidence of premature luteinizaition (P4 >1.5ng/ml on the day of HCG) and factors influencing it in both agonist and antagonist cycles & its effect on clinical outcome.
Methods: 400 Patients treated by IVF/ICSI at Jaipur fertility centre (ART Unit of Mahatma Gandhi University of Medical Sciences and Technology) from January 2014 to June 2015 were included in this retrospective clinical study. 200 patients were taken in agonist group and 200 in antagonist group. Ovulation induction was given with r-FSH/HMG in both protocols. P4 along with E2, LH and number of oocytes on the day of HCG were taken for study.
Results: Premature luteinization or PL (P4 > 1.5 ng/ml) was noticed in 16% cases in the agonist group and 6% in the antagonist group (p-0.002). In our study the factors predisposing to PL were agonist protocol (16% Vs. 6%, p-0.002), total dose of gonadotrophins > 2000 IU (17.69 % Vs. 2.29, p-0.000), >10 follicles of > 14mm on the day of HCG with E2 Levels > 2500 pg/ml (33.33 % Vs. 0%, p-0.000) in agonist protocol and (17.39% Vs. 2.59%, p-0.000) in antagonist protocol.
The clinical pregnancy rate was significantly reduced in cases with PL (32.73% Vs 12.5%, p-0.037) in agonist group. Though the difference was not statistically significant in antagonist group (32.97% Vs 8.33 %, P-0.144),this could be due to large difference in the proportion of sample size.
Conclusions: Despite the use of GnRH analogues, risk of premature rise of progesterone is still there. The risk mainly depends on ovarian response. The high responders with high no of > 14mm follicles, high E2 Levels > 2500 pg/ml and high doses of gonadotrophins used are associated with high risk of PL. As premature rise in P4 level has significant Impact on clinical pregnancy rate (CPR), identification of high risk factors & their proper management can reduce the incidence of PL & cycle cancellation rate as well as can improve the clinical outcome.

Keywords: GnRH agonist, GnRH antagonist, Progesterone, Premature luteinization

INTRODUCTION

Premature luteinization (PL) refers to a rise in serum progesterone (P4) levels on the day of HCG administration.[1-4] Most studies used an absolute P4 level on the day of HCG administration as an indicator of PL, and the cut off level differed from 0.8 to 2ng/ml.[2,5-9] The cutoff of 1.5 ng/ml cannot be considered arbitrary, as it signifies the transition from the follicular phase to the luteal phase in the natural cycle (Hoff et al 1983).[10]

Serum P4 concentration increase gradually 12-24 hrs before the onset of the LH surge.[10] Despite the administration of GnRH analogues, moderate elevation in serum P4 concentration can be observed on the day of HCG, Which could influence endometrial receptivity.[11]

No association has been reported between P4 elevation and fertilization rates or oocyte /embryo quality.[5,6,12-14] Which exclude the effect of PL on oocyte quality, as previously suggested.[1,3,5] However a progesterone rise during the late follicular phase has been considered a negative predictive factor for clinical outcome in both GnRH agonist, Schoolcraft et al 1999, Silverberge et al 1991, Elnashar 2010, Papanikolaou et al 2011 and GnRH antagonist protocol, Bosch et al 2003, D Kyron et al 2012 papanikolaou et al 2011.[3,15-19] This could be due to advanced histological endometrial maturation, kyrou D et al 2009, Papanikolaou et al 2009, Saadat et al 2004 or may be differential endometrial gene expression Labrata et al 2011, Van Vaerenbergh et al 2011 bosch et al 2010 which may be related to implantation failure.[20-25]

Although several authors did not find any negative effect of PL on IVF outcome.[4,6,7,9] There is a marked variation in the incidence of PL, 13% to 71% due to discrepancies in definition, population characteristics and/or treatment protocols.[2,5-9]

The pathogenesis of PL in COH is still poorly understood. Increased LH receptor sensitivity of the granulosa cells to FSH especially in hyper responders is one of the important hypothesis of PL Elnashar et al.[17]

The use of GnRH antagonist is increasing day by day in IVF cycles. Nonetheless, there is still a considerable debate regarding their efficacy to achieve similar reproductive outcome, when compared with the most commonly used GnRH agonist.

So, the aim of this study is to explore whether progesterone control in the late follicular phase differs when GnRH antagonist is used as compared with GnRH agonist. Simultaneously to evaluate different factors predisposing to early progesterone rise in late follicular phase & impact of this P4 rise on clinical outcome.

METHODS

In this retrospective study, four hundred patients treated by IVF / ICSI at the jaipur fertility centre from January 2014 to June 2015 were included. 200 cases with agonist protocol (Group A) and 200 cases with antagonist protocol (Group B) were selected for comparison.The inclusion criteria were age <38 years, basal FSH <10 mIu/ml and E2 <60pg/ml, BMI <29 kg/m2. The etiological factors included,were tubal factor, male factor, Endometriosis stage 1 & 2 and anovulation (mild PCOS).

In Group A, the GnRH agonist (Ing lupride 1 mg s/cdaily, Sun Pharma) was started on day 21 of the preceding cycle. Gonadotrophins (Inj Gonal – F, Merck – Serono or Inj Menogon, Ferring) were started from day 3 of period after complete down regulation (LH <5 mIu/ml, E2 < 50 pg/ml).The dose was individualized according to age, BMI & ovarian reserve of the patient.

In Group B, gonadotrophins (rFSH/ HMG) were started from day 3 (if basal LH <5 mIu/ml and E2 <60 pg/ml). GnRH antagonist (Inj Cetrotide 0.25 mg s/c daily, Merck-Serono) was started from day 6 of stimulation (fixed protocol) and continued till morning of triggering day. For both study groups, final oocyte maturation was induced with HCG 10,000 IU (Inj Sifasi, Serum Institute) when at least three follicles of >18 mm were present. Oocyte retrieval was performed 36 hrs after HCG. 2-3 cleavage stage embryos (Day 3) of grade A & B were transferred under sono guidance. Pregnancy was confirmed by beta HCG 15 days after embryo transfer (ET). Clinical pregnancy rate was defined as the presence of heart beat on day 30 of ET.

All blood samples for hormonal measurement were analyzed in our laboratory with fully automated enzyme linked fluorescent assay (ELFA) method on the day of collection.

Statistical analysis

Standard error of proportion (Z-test) was applied for qualitative data & t-test was applied for quantitative data to calculate the p value. α - error was kept at 0.05 with 80% power to detect the difference.

RESULTS

Table 1 shows baseline characteristic and stimulation data of the two groups. Age, BMI, days of stimulation, 2 PN embryos and no. of embryos transferred were all comparable between GnRH agonist and antagonist group. Only the no of >14 mm follicles & E2 levels > 2500 pg/ml on the day of HCG and no of oocytes retrieved were significantly higher in agonist group in comparison to antagonist group. The incidence of premature progesterone elevation (P4>1.5ng/ml) was significantly high in agonist group as compared to antagonist (16%, Vs. 6%, p-0.002).

Table 2 shows the effect of PL (P4 >1.5ng/ml) on the day of HCG on clinical outcome. The clinical pregnancy rate was significantly high in patients with P4 levels<1.5ng/ml on the day of HCG in agonist group (32.73 % Vs. 12.5% p-0.037).While in antagonist group (32.97% Vs. 8.33%, p-0.144), the result was not statistically significant. This could be due to large difference in the proportion of sample size. This shows that high P4 levels > 1.5ng/ml on the day of HCG impairs the clinical outcome.

Table 3 shows the factors influencing P4 levels on the day of HCG. The incidence of PL was significantly high with agonist protocol (16% vs. 6%, p-0.002). It was also high with high doses of gonadotrophins used in either protocol (17.69 % Vs. 2.29 %, p-0.000) high no. of >14mm follicles & E2 levels > 2500 pg/ml on the day of HCG in both agonist (33.33%Vs. 0%, p-0.000) and antagonist protocol (17.39 % Vs. 2.59 %, p-0.000).

Table 1: Demographic and stimulation parameters.

S. No	Parameters	Agonist group (n = 200)	Antagonist group (n = 200)	p-Value
1.	Age (years)	30.01 ± 3.7	30.02 ± 3.6	0.099
2.	BMI (kg/m^2)	21.4 ± 2.7	21.8 ± 2.6	0.136
3.	Days of stimulation	9.8 ± 1.7	9.6 ± 1.6	0.226
4.	>10 follicles of > 14 mm on the day of HCG	96/200 (48%)	46/200 (23%)	0.000
5.	E_2 levels > 2500 pg/ml on the day of HCG	96/200 (48%)	46/200 (23%)	0.000
6.	No. of oocytes retrieved	14.2 ± 2.2	12.8 ± 1.9	0.000
7.	2 PN Embryos	10.2 ± 1.9	9.9 ± 1.7	0.104
8.	No. of Embryos transferred	2.5 ± 0.5	2.4 ±0.49	0.686

Table 2: Effect of p4 levels on clinical outcome.

S.No	Parameters	P_4 < 1.5(ng/ml)	P_4>1.5 (ng/ml)	C.I.	p-Value
A	Total study population (n=400)				
1	Clinical pregnancy rate (CPR)	117/356 (32.86%)	5/44 (11.36%)	0.070 – 0.359	0.006
2	Miscarriage rate	14/117 (11.96%)	1/5 (20%)	0.374 – 0.213	0.873
B	Agonist Group (n=200)				
1	Clinical pregnancy rate (CPR)	55/168 (32.73%)	4/32 (12.5%)	0.029 – 0.374	0.037
2	Miscarriage rate	8/55 (14.54%)	1/4 (25%)	0.469 – 0.260	0.874
C	Antagonist group (n=200)				
1	Clinical pregnancy rate (CPR)	62/188 (32.97%)	1/12 (8.33%)	0.024 – 0.517	0.144
2	Miscarriage rate	6/62 (9.60%)	0/1 (0%)	0.313 – 0.504	0.437

Table 3: Factors influencing P4 levels on the day of HCG.

S.No	Parameters	P_4 <1.5 (ng/ml)	P_4>1.5 (ng/ml)	C.I.	p-Value
1.	***Stimulation protocol (n=400)***				
a	Agonist (n=200)	168/200= 84%	32/200 = 16%	0.038 – 0.161	0.002
b	Antagonist (n=200)	188/200= 94%	12/200= 6%		
2.	***Type of Gn***				
a	r- FSH (n=88)	78/88= 88.63%	10/88= 11.36%		
b	HMG (n=176	154/176= 87.50%	22/176= 12.5%	0.094 – 0.072	0.916
c	r FSH + HMG (n=136	124/136= 91.17%	12/136= 8.82%	0.025 – 0.112	0.300
3.	***Total dose of Gn***				
a	<2000 IU (n=174)	170/174= 97.70%	4/174= 2.29%		
b	>2000 IU (n=226)	158/226= 69.91%	40/226= 17.69%	0.215 – 0.092	0.000
4.	***No. of follicles >14 mm on the day of HCG***				
a	Agonist				
	<10 follicles (n=104)	104/104= 100%	0/104= 0%		
	>10 follicles (n=96)	64/96= 66.66%	32/96= 33.33%	0.435 – 0.231	0.000
b	Antagonist				
	<10 follicles (n=154)	150/154= 97.40%	4/154= 2.59%	0.226 – 0.069	0.000
	>10 follicles (n=46)	38/46= 82.60%	8/46= 17.39%		
5.	***E_2 levels on the day of HCG***				
a	Agonist				
	<2500 pg/ml (n=104)	104/104= 100%			
	>2500pg/ml(n=96)	64/96= 66.66%	32/96= 33.33%	0.435 – 0.231	0.000
b	Antagonist				
	<2500 pg/ml (n=154)	150/154= 97.40%	4/154= 2.59%	0.226 – 0.069	0.000
	>2500 pg/ml(n=46)	38/46= 82.60%	8/46= 17.39%		

DISCUSSION

The aim of our study was to assess the incidence of PL (P4 > 1.5 ng./ml) in two main protocols of IVF, its Impact on clinical outcome and to identify the factors influencing premature rise of P4 in late follicular phase.

The incidence was quite high in agonist group as compared to antagonist (16% Vs. 6%, p-0.002). The high no. of recruiting follicles in agonist protocol with large no of follicles >14 mm & high E2 levels > 2500pg/ml may be the predisposing factors for PL, although the incidence of PL varies in different study groups. In a large retrospective analysis of over 4000 cycles, the incidence of P4>1.5 ng/ml on the day of HCG was 8.4% in agonist and antagonist cycles.[25] Incidence up to 35% in agonist cycle & 38% in antagonist cycles have been reported.[2,3,6,7] The incidence of PL (with a cut off >1.5ng/ml) was same in both agonist & antagonist group (24.1 % Vs. 23%) in a study by Papanikolaou et al.[18]

Even modest rise in progesterone in late follicular phase may negatively affect implantation rates of a good quality cleavage stage embryos irrespective of the protocol used.[3,22] The high estradiol levels (associated with PL) cause up regulation of progesterone endometrial receptors and hence the higher the impact of even modest increase of P4 levels on premature endometrial advancement.[26] In our study all 32 cases in agonist group and 8/12 cases in antagonist group were having E2 levels > 2500 pg/ml along with P4>1.5 ng/ml (i.e. high responders.)

This endometrial advancement significantly impairs clinical pregnancy rate as seen in our study as well.[20-22] The clinical pregnancy rate was significantly low with P4 > 1.5 ng/ml in agonist group (32.73% Vs 12.5%, p-0.037). Though the result was not statistically significant in antagonist group (32.97% Vs. 8.33%, p-0.144). This could be due to large difference in the proportion of sample size.

Our study also demonstrated that serum P4 levels on triggering day were closely associated with the dose of gonadotrophin given irrespective of type of gonadotrophin. High responders with >10 follicles of >14 mm & E2 levels > 2500 pg/ml on the day of HCG have significantly high rise of P4 in both groups. This finding is in agreement with Kyrou 2012.[19] Gonadotrophin dose has been reported as a rise factor by Venetis et al & Filicori et al.[27,28] According to MERiT study, a higher incidence of serum P4 elevation was found in the FSH treatment group compared to the HMG treatment group (24.1 % Vs 11.8%).[29] In our study there was no significant difference in type of gonadotrophin used & P4 elevation.

CONCLUSION

Premature luteinization (P4 >1.5 ng/ml) on the day of HCG adversely affects clinical pregnancy rate probably due to embryo-endometrial asynchrony. The risk is high in high responders especially with agonist protocol.

The preventive measures include selection of antagonist protocol with milder ovarian stimulation (low gonadotropin doses) in anticipated high responders. Early HCG administration at follicular size of >17 mm in high responders can decrease the risk to some extent especially in agonist protocol. The decision of cycle cancellation & embryo freezing should be individualized according to quality and no of embryos, freezing facility, no of attempts or very early P4 rise (started few day before HCG trigger) . Still further studies required to decide the detrimental level of P4 on HCG day as an absolute indicator of cycle cancellation & embryo freezing.

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