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Premature progesterone rise incidence & its effects on in vitro fertilization cycles

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Abstract

Background: Ovarian stimulation using gonadotropins is associated with premature progesterone rise (PPR) in late follicular phase compared to natural cycle. Two schools of thoughts exist concerning implications of PPR during late follicular phase with one insisting that outcome of in vitro fertilization (IVF) is adversely affected when PPR occurs while the second school of thought argues that PPR has no adverse effect on IVF outcome. The study is aimed at determining the incidence of PPR in the cohort data evaluated; pregnancy rates across the sides of adopted cut-off level of progesterone and association if any between PPR & pregnancy rates.

Methods: It was a descriptive retrospective cohort study of data of 114 patients and egg donors.. Analysis of continuous & categorical data was done using IBM SPSS Statistics 25.

Results: Mean serum progesterone on HCG day of IVF cycle among the cohort was 2.490±1.355 with a PPR incidence of 55%. The odds of having PPR was 6.7 times higher among subjects with follicular number more than 13 compared to subjects with follicular numbers ≤13 . Number of follicles retrieved & age of subjects were strongly associated with progesterone level. The odds of subject with PPR getting pregnant was found to be 1.5 times less compared to the subjects with pre-HCG P4 < 1.5ng/ml.

Conclusion: Pre-HCG progesterone level is positively associated with pregnancy outcome in IVF cycles

Keywords: ovarian stimulation, premature progesterone rise, IVF outcomes.

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Incidence de la montée prématurée de la progestérone et ses effets sur les cycles de fécondation in vitro

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Résumé

Contexte général de l'étude: La stimulation ovarienne à l'aide de gonadotrophines est associée à une augmentation prématurée de la progestérone (APP) en phase folliculaire tardive par rapport au cycle naturel. Deux écoles de pensée existent concernant les implications de l'APP au cours de la phase folliculaire tardive, l'une insistant sur le fait que le résultat de la fécondation en vitro (FEV) est affecté négativement lorsque l'APP se produit, tandis que la deuxième école de pensée soutient que l'APP n'a aucun effet négatif sur le résultat de la FIV.

But et objectifs de l'étude: Déterminer l'incidence de l'APP dans les données de cohorte évaluées ; taux de grossesse sur les côtés du niveau seuil de progestérone adopté et association, le cas échéant, entre l'APP et les taux de grossesse.

Méthode de l'étude: Il s'agissait d'une étude de cohorte rétrospective descriptive des données de 114 patients et donneurs d'ovules. L'analyse des données continues et catégorielles a été effectuée à l'aide d'IBM SPSS Statistics 25.

Résultats de l'étude : La progestérone sérique moyenne le jour HCG du cycle de FEV parmi la cohorte était de 2,490 ± 1,355 avec une incidence d'APP de 55 %. La probabilité d'avoir une APP était 6,7 fois plus élevée chez les sujets ayant un nombre folliculaire supérieur à 13 par rapport aux sujets ayant un nombre folliculaire $\leq$13 . Le nombre de follicules récupérés et l'âge des sujets étaient fortement associés au niveau de progestérone. La probabilité que les sujets atteints de PPR tombent enceintes était 1, 5 fois inférieure à celle des sujets avec P4 pré-HCG < 1, 5 ng/ml.

Conclusion: Le niveau de progestérone pré-HCG est positivement associé à l'issue de la grossesse dans les cycles de FEV

Mots-clés : Stimulation ovarienne, augmentation prématurée de la progestérone, résultats de la FEV.

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INTRODUCTION

Ovarian stimulation using gonadotropins is associated with premature progesterone rise (PPR) in late follicular phase compared to natural cycle (1). The incidence of PPR could vary so widely from 13% to 71% (1,2,3) depending on the cut off value used for progesterone level . The PPR incidence occurs in ovarian stimulation cycles whether the pituitary down regulation is achieved using agonist 5-35%[1,4] or antagonist 20-85%(5,6) drugs

Premature rise in progesterone level prior to or on the day of human chorionic gonadotropin (HCG) administration in invitro fertilization (IVF) cycles has been shown to be inversely related to pregnancy outcomes from adverse endometrial receptivity.(1,2) The cut offs at which pregnancy rate start to decline range from 0.8-2.0ng/ml with cut-off value of 1.5ng/ml being widely used(7,8,9).

Possible reasons adduced for PPR include luteinizing hormone (LH) rise in follicular phase; rise in human chorionic gonadotropin (HCG) indirectly from the LH component of human menopausal gonadotropin (HMG); enhanced LH receptor sensitivity of the granulosa cells and disproportionate sensitivity in favor of LH sensitivity compared to ovarian response giving higher progesterone/estradiol ratio of more than one.

There is also the notion that PPR is directly related to ovarian response *i.e* the more the follicles, the more the risk of raised progesterone level (1,10).

Although many studies found that premature progesterone rise before HCG trigger has no significant adverse effect on outcome of IVF (11-14), few epidemiological studies found that PPR is inversely proportional to outcome of IVF (15-19). Whether premature progesterone rise influences IVF outcome remains unclear.

What then exactly is the significance of premature progesterone rise in IVF outcome? Should determination of PPR continue routinely in IVF cycles or should it be stopped?

This study, therefore, aimed to accomplish the following: determine the incidence of PPR in antagonist versus agonist cycle; compared the incidence among antagonist cycles with different days of commencement of GNRH antagonist; determine if the number of follicles retrieved can be used as surrogate in place of progesterone assay in IVF cycles and determine pregnancy rates across divides of progesterone cut off levels.

MATERIALS AND METHODS

A descriptive cross-sectional study was employed. Subjects were seen during IVF cycles at Ayomide Women's Health Specialist Hospital & IVF Center, Osogbo, Nigeria. A total of one hundred and eleven (111) IVF cycles were carried out between January 1 and June 30, 2021 in which superovulation was done, with down regulation effected either by gonadotropin releasing hormone agonist or antagonist, and pre-HCG trigger progesterone assay was reviewed. All the IVF cycles took place within the period under review with 62 of the cycles being egg donor cycles. Patients were divided into two groups of progesterone level on pre-HCG trigger day $\leq$1.5 and >1.5 and further subdivided into Buserelin group (84 patients), day 5 Cetrotide group (9 patients) and day 6 Cetrotide group (18 patients)

Women undergoing superovulation in IVF cycles had routine infection screening (HIV, Hepatitis B, Syphilis), blood group, genotype, packed cell volume, and hormonal assay evaluation (Anti mullerian hormone, FSH,LH, PROLACTIN, TSH,T3,T4).

Ovarian hyper stimulation was done using human menopausal gonadotropin (HMG) for ten days using 225IU dose in the first six days & 150 I.U on day 7 to day 10 of stimulation. Down regulation was carried out with either buserelin injection 0.5mls subcutaneously administered from seven days before commencement of HMG administration (agonist cycle) or cetrotide injection 0.25mg administered subcutaneously from day 5 or day 6 of ovarian stimulation with HMG (antagonist cycle) Transvaginal ultrasound scans were done on day 1, day 6, day 10 of stimulation for follicular response monitoring while pre-HCG progesterone assay was done day 10 of stimulation. HCG 10,000IU was administered on day 10 if at least one oocyte was seen with diameter $\geq$ 16mm. In cycles where pre-HCG follicular count exceeded 18, HCG dose was reduced to 5000I.U.

In this study, premature progesterone rise was defined as progesterone assay level >1.5ng/ml on pre-HCG trigger day. Patient characteristics retrieved from electronic medical record data storage were age, body mass index (BMI), gravidity, parity, basal FSH, LH, AMH, serum progesterone levels on pre-trigger HCG day, number of follicles retrieved, pregnancy occurrence (quantitative B-HCG level of $\geq$ 15I.U/L on day 14 from embryo transfer using fine care machine/kit) and delivery rate.

Inclusion criteria in this study were: Down regulation with either buserelin or cetrotide injection; Superovulation with human menopausal gonadotropin; Progesterone assay done on pre-HCG trigger day 10. Exclusion criteria on the other hand included IVF cycles where no record of progesterone assay was found.

Progesterone measurement was done by collecting blood samples at HCG trigger. The samples underwent the test with 'The Finecare Progesterone Rapid Quantitative Test' - a fluorescence immunoassay used along with fine care FIA system (model no: Fs -112) for quantitative determination of progesterone in human serum, plasma or whole blood. The detection limit (analytical sensitivity) is 1.4ng/mL intra- and inter-assay precision, expressed as coefficients of variation (CV) is ≤ 15%, with correlation coefficient (R) is ≥0.9900.

Statistical Analysis

Chi-square test and an independent sample t-test were used for categorical and continuous variables respectively. Comparison of continuous variables among greater than 2 groups was carried out using analysis of variance (ANOVA). The relationship between biochemical parameters and progesterone levels was tested using Pearson's correlation analysis. Furthermore, we used a multivariate linear regression analysis to estimate the predictors of abnormal progesterone levels adjusting for other covariates. All statistical analyses were performed using SPSS (version 25) at a two-sided $P<0.05$.

Outcome definitions were percentage cycle with progesterone level above 1.5ng/ml on HCG trigger day and pregnancy rate among women with progesterone (P4) level ≤1.5ng/ml and among women with P4 > 1.5ng/ml.

Other measures included age, number of oocytes retrieved & down-regulation groups were recoded into different variables to allow for bivariate analysis in exploring the data more extensively as follows: Age groups (years): ≤25; 26-35; 36-39; 40-49; ≥50; Age groups (years): ≤ 34; ≥35; Oocyte number groups: 1-4; 5-9; 10-18; > 18; Down-regulation groups: Buserelin; Cetrotide day 5 & Cetrotide day 6.

Ethical approval: Ethical approval was received from the educational committee of Ayomide Women's Health Specialist Hospital. All subjects undergoing ovarian hyper-stimulation gave written informed consent as matter of policy.

RESULTS

Table 1 shows the mean age across the different types of down regulation and progesterone level cut-off. A total of one hundred and eleven (87.4%) subjects met the inclusion criteria out of the one hundred and twenty seven women who had ovarian hyper-stimulation in the review period. The mean age of women included in this study was 28.59± 6.52 years with mean age of egg donors being 23.48± 2.47 years and was significantly lower than autologous (own) egg subjects mean age of 35.04± 3.69 years (p=0.000). Mean age of subjects who had down regulation with buserelin injection was significantly lower compared to those who had cetrotide injection and also the mean age of those who had PPP was significantly lower than those without PPP. The mean BMI of the patients was 26.88±4.46kg/m^2 with no difference in mean BMI across either down-regulation groups nor pre-HCG progesterone level cut off. Over four-fifth (82.1%) of the subjects were nulliparous while about a third (30.8%) of the subjects had had at least one pregnancy before.

The incidence of premature progesterone rise (PPR) in this study was 55% (Figure 1) being 48.8% among subjects down-regulated with buserelin and 74.1% among subjects down regulated with cetrotide (Table 2). Number of follicles was observed to be positively correlated with progesterone level (rho = 0.44; p<0.001) while age (rho = -0.39; p<0.001) and BMI (rho= -0.38; p<0.001) were inversely correlated with progesterone level. (Table 3).

The incidence of PPR was found to be directly proportional to increasing number of eggs being highest among the group with egg number above eighteen with incidence of 83.9%, also significantly higher among subjects donating eggs compared to autologous (own) egg subjects and among subjects down regulated with cetrotide injection compared to buserelin injection (Table 2). The odds of having PPR was 6.7 times higher among subjects with follicular number more than 13 compared to subjects with follicular numbers ≤13 (Table 4).

The chance of having PPR increased with reducing age with 57.4% of subjects in age group < than 26years and only 8.2% of subjects aged 36-39years having PPR (X2= 16.950, p= 0.001). Subjects aged 35 & above are 84% less likely to have PPR compared to those aged less than 35 years.(Table 4). Other association of egg ownership and mode of down regulation in relation to pre-HCG progesterone levels is as shown in Table 4.

In the multivariate regression analysis (Table 5), the number of follicles (β = 0.05; p=0.01), and age (β = -0.04; p=0.03) were the variables found to predict raised PPR.

Figure 2 shows that there is a direct dose response relationship between the number of follicles and progesterone levels with the association becoming significant when number of follicles retrieved ≥14.

Pregnancy rate among subjects whose pre-HCG progesterone was ≤ 1.5ng/ml was 31.0% compared to 22.2% among subjects with PPR (X^2= 0.439. p= 0.507) (figure 3), the odds of subject with PPR getting pregnant was found to be 1.5 times less compared to the subjects with pre-HCG P4 < 1.5ng/ml. Delivery rate among subjects whose pre-HCG progesterone was ≤ 1.5ng/ml was 20.7% compared to 16..7% among subjects with PPP (X^2= 0.118, p= 0.731), the odds of subject with PPR delivering with one fresh embryo transfer was found to be 1.3 times less compared to the subjects with pre-HCG P4 >1.5.

Pregnancy rates with different cut off values of progesterone in this study is as shown in Table 6. Most marked difference was found when cut off progesterone value of 2.0ng/ml was used 30.6% versus 18.2%. No pregnancy was recorded in this study when progesterone level on HCG trigger day was ≥5.0ng/ml

DISCUSSION

Baseline Characteristics: The mean age of own egg subjects in this Nigerian review (35.04± 3.69) is comparable to that of Martinez et al Barcelona, Spain (34.5± 3.2) (21) and that of Huang et al Taipei, Taiwan (34.9± 0.10) (23) but our subjects appear older than the review subjects of Sangisapu et al Mumbai, India whose mean age was (28.94± 3.65) (22). This may speak to ease of access to assisted reproductive techniques in India.

Premature Progesterone Rise Incidence (PPR): PPR incidence in the review was 55% overall, 44.8% (buserelin group) and 74.1% (cetrotide group). This is similar to the finding of Martinez (21) with PPR incidence of 52.3% for the agonist & flare agonist group using leuprolide though the cut off value used for the definition of PPR in Martinez study was >0.9ng/ml as opposed to >1.5ng/ml used in our study which could explain why their incidence was slightly higher. Our value was higher than that the finding of Huang et al that showed incidence of PPR to be 36%. The cut-off used by Huang (23) was P4> 1.0ng/ml, it however analyzed subjects that used three different protocols (long agonist, short agonist and antagonist down regulations) compared to ours where patients used two protocols (long agonist & antagonist down regulation), thus difference in values may be explained by difference in cut off values and protocols between the two studies. Venetis et al (24) had a PPR of 7.4% overall, 8.3% agonist cycles, 6.8% antagonist cycles but all cycles where gonadotrophin releasing hormone analogue agonist was used as a trigger instead of HCG to prevent OHSS were excluded. This may explain why they had extremely low PPR incidence as subjects with higher number of follicles in antagonist cycles with tendency to have high progesterone level were excluded though cut-off value used was > 1.5ng/ml to define PPR like our study .

The number of follicles, age, type of eggs used and type of down regulation method used was found to be associated with pre-HCG progesterone level on bivariate analysis with only number of follicles remaining significantly associated with progesterone levels after correcting for confounders using binary logistic regression. The result showed that association becomes significant once number of follicles retrieved is equal to or greater than fourteen. Sangisapu et al (22) found that serum progesterone level at pre-HCG & ovum pick up (OPU) to be significantly associated with number of follicles retrieved at OPU and number of follicles in turn to be positively associated with positive IVF outcome. Huang et al (23) found body weight, BMI, estrogen level & clinical pregnancy rate to be additionally associated with duration of progesterone elevation on bivariate analysis but only did multivariate analysis of determinants of clinical pregnancy rate. Ashmita et al (26) found human menopausal gonadotropin compared to recombinant FSH, high total dose of gonadotropin >2000IU/L in addition to estrogen level & number of follicles of more than nine (≥ 10mm) on HCG day to be associated with high progesterone levels. Our findings where incidence of PPR was significantly higher among antagonist down regulated cycles compared to GNRH-agonist down regulated cycle did not agree with results of Huang et al (23) & Venetis et al (24), it is generally accepted that PPR is higher in agonist cycle compared to antagonist cycle. This could be explained based on selection bias as majority of subjects in the cetrotide group in our study were donor egg subjects who had higher risk of having excessive oocytes with corresponding risk of higher estrogen levels and PPR. Many of the studies evaluating PPR

excluded polycystic ovarian syndrome patients or those with polycystic ovaries on ultrasound scanning. The second reason may be the fewer numbers of subjects in the cetrotide group with ratio 1:3 antagonist: agonist groups.

Pregnancy Outcome: In this study, pregnancy rate was lower (22.2%) among subjects with premature progesterone rise of >1.5ng/ml at pre-HCG trigger day compared to those with normal level of ≤ 1.5ng/ml and the odd of getting pregnant by subjects with PPR is 1.5 times lower than those with normal pre-HCG progesterone level. The difference in pregnancy rate was most marked when progesterone cut-off of ≥2.0 ng/ml was used 30.6% versus 18.2% with odds of almost 2 times lesser chance of pregnancy among the PPR subjects. The differences seen however were not statistically significant.

Venetis et al (24) recorded similar findings to this study. It showed that live birth rates were significantly reduced if serum progesterone level (P4) was >1.5ng/ml on trigger day after correcting for cofounders. (O.R 0.68 95% CI: 0.48-0.97. The strongest confounder in their study was number of oocytes retrieved with the intermediate group of 6-18 oocytes having statistically detrimental effect compared to >18 & <6 oocytes groups. This study is at concordance with Venetis et al study regarding number of follicles versus PPR and live birth rate versus P4 cut off levels but our study did not relate follicular numbers to life birth rates. De Cesare et al (27) in agreeing with our study found out that serum progesterone level was inversely related to cumulative pregnancy rate (O.R 0.71 CI: 0.62-0.80) and live birth rate (O.R 0.73 CI: 0.63-0.84). The level became significant especially when progesterone was >1.75ng/ml and when day 3 cleavage embryos were transferred compared to blastocyst. We found most marked difference in pregnancy rates if cut-off value for progesterone level of 2.0ng/ml was used, this study only determined clinical pregnancy rate with fresh transfers not cumulative pregnancy rate in our study.

Ashmita et al (26) found clinical pregnancy rate to be significantly higher among subjects with progesterone level <1.5ng/ml (33%) compared to those with progesterone >1.5ng/ml(p=0.037). The difference with our study is that the difference the team got was more pronounced than ours and statistically significant. Merviel et al (28) in the same vein with our findings showed that serum progesterone level was significantly lower among pregnant women compared to non-pregnant (p <0.01) and that progesterone follicular index of >0.6 and progesterone oocyte index of >0.36/ng/ml/oocyte were more predictive of lower pregnancy rate, thus bringing to fore the correlating nature of both numbers of oocytes retrieved and numbers of follicles above 14mm on HCG day to progesterone level. It differed from our study in that cut-off value used for progesterone was 0.9ng/ml. Huang et al (23) found that it is duration of premature rise in progesterone>1.0ng/ml before HCG administration rather than one-off measurement of progesterone value on HCG trigger day is inversely associated with IVF outcome(OR 0.773, P<0.0001). Mio et al found significantly reduced implantation rate in the group that had subtle rise of progesterone level from 1.0 to 2.0ng/ml compared to group that did not have subtle rise of progesterone level.

Martinez et al & Ubaldi et al did not find adverse effect of premature rise of progesterone on IVF outcome though the cut off used for PPR was ≥1.0ng/ml as against 1.5ng/ml used in this study. Hoffman et al did not find any difference in pregnancy rate at cut of progesterone levels of < 0.9, 1.1 & ≤1.4ng/ml but did not evaluate at high cut off of >2.0ng/ml because of sample size.

It appears that the difference in whether adverse effect is seen with progesterone elevation lies with the cut-off of progesterone level used. Many authors who found significant adverse effects used cut off values ranging from 1.0 to 2.0ng/ml whereas majority of those who did not find difference used cut-off values ranging from 0.4 to 0.9ng/ml. The higher the level of progesterone level on the HCG trigger day, the higher the chances of gradual rise for some days before the HCG day and also the higher the number of follicles more than 14mm seen producing high level of estrogen with ultimate non-synchronous endometrial maturation resulting in missed endometrial receptivity and reduced implantation and eventually clinical pregnancy rates.

CONCLUSION

Elevated progesterone level on HCG trigger day as well as retrieval of oocytes in excess of 13 may be associated with reduction in clinical pregnancy rate in fresh cycles. Patients who have less than fourteen oocytes retrieved and or who have less than 1.5ng/ml of progesterone on HCG trigger day can have fresh embryo transfer if no other contraindication is present whereas those who have more than 13 follicles retrieved and or who have HCG day progesterone level>1.5ng/ml may consider freeze-all modality

and do frozen embryo transfer in next natural or medicated cycle as appropriate as it is abundantly documented that elevated progesterone level neither affect the egg nor embryo quality

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Table 1: Baseline Characteristics.

	AGE BUS n= 84	**AGE CET** n=27	**AGE P4=1.5** n=50	**AGE P4>1.5** n=61	**BMI BUS** n=25	**BMI CT6** n=15	**BMI P4=1.5** n=22	**BMI P4>1.5** n=8
`Mean	27.43	32.19	31.08	26.54	26.12	27.35	27.01	25.36
SD	6.28	6.05	7.19	5.12	3.678	5.588	3.79	4.16
RANGE	23	23	27	18	18	16	12	13
p-value		0.001		0.000		0.447		0.313

BUS= buserelin; CET= cetrotide; P4= progesterone; BMI= body mass index

Table 2: PPR incidence by down regulation, types & numbers of eggs

	DRT BUS n= 84	**DRT CT6** n=18	**DRT CT5** n=9	**DRT BUS** n=84	**DRT CET** n=27	**EGN <5** n=20	**EGN 5-9** n=23	**EGN 10-18** n=37	**EGN >18** n=31	**EGT OWN** n=49	**EGT DONOR** n=62
P4=1.5(%)	51.2	27.8	22.2	51.2	25.9	75.0	65.2	40.5	16.1	61.2	32.3
P4> 1.5(%)	48.8	72.2	77.8	48.8	74.1	25.0	34.8	59.5	83.9	38.8	67.7
X^2		5.343			5.269		21.805				9.277
p-value		0.069			0.022		0.000				0.02

DRT=Down regulation group; BUS =Buserelin; CT6= Cetrotide day 6; CT5= Cetrotide day 5; CET= Cetrotide day 5 &6; EGN= Egg number. EGT= egg type

Table 3: Bivariate correlation demographic-biochemical parameters with pre-HCG progesterone level

PARAMETER	PEARSON CORRELATION	SIGNIFICANCE(2-tailed)
NUMBER OF FOLLICLES	0.441	0.000**
AGE	-0.399	0.000**
BMI	-0.375	0.000**
EGG OWNERSHIP	0.341	0.000**
DOWN REGULATION TYPE	0.228	0.016*

** correlation is significant at 0.01 level (2-tailed) * correlation significant at 0.05 level (2-tailed)

Table 4: Bivariate analysis of progesterone levels against demographic/clinico-chemical parameters

PARAMETER	CHI-SQUARE	RISK	P-VALUE
OWN EGG/DONOR EGG	9.2779	3.316	0.002
AGE 18-34/35-45	16.950	0.164	0.001
NO OF FOLLICLES 0-13/14-50	21.759	6.799	0.000
BUSERELIN/CETROTIDE	5.269	2.997	0.022

Table 5: Multivariate linear regression

VARIABLES	BETA	T-VALUE	P-VALUE
NO OF FOLLICLES RETREIVED	0.046	3.446	0.001
AGE	0.047	-2.196	0.030
DAYS OF STIMULATION	0.012	0.082	0.935
GRAVIDITY	0.054	0.383	0.702

Table 6: Pregnancy rates at different progesterone cut-off levels

CUT-OFF (ng/ml)	PREGNACY RATE NORMAL P4 (%)	PREGNANCY RATE PPR (%)	RISK	X2	P-VALUE
= 1.50	31	22.2	1.595	0.439	0.507
= 1.75	30	23.5	1.393	0.231	0.631
= 2.00	30.6	18.2	1.980	0.686	0.408
= 2.50	26.8	33.3	0.733	0.107	0.743
= 3.00	27.9	25.0	1.161	0.016	0.900
= 5.00	28.9	0	1.063	1.329	0.249

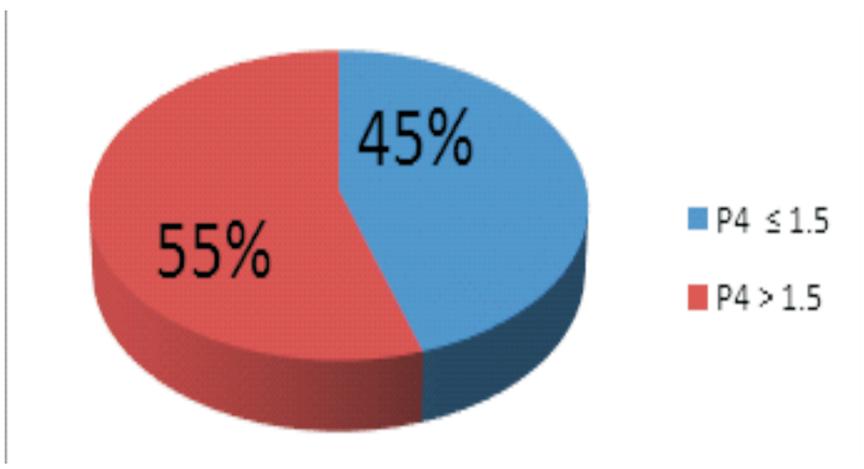

Figure 1: Pre-HCG Progesterone Level

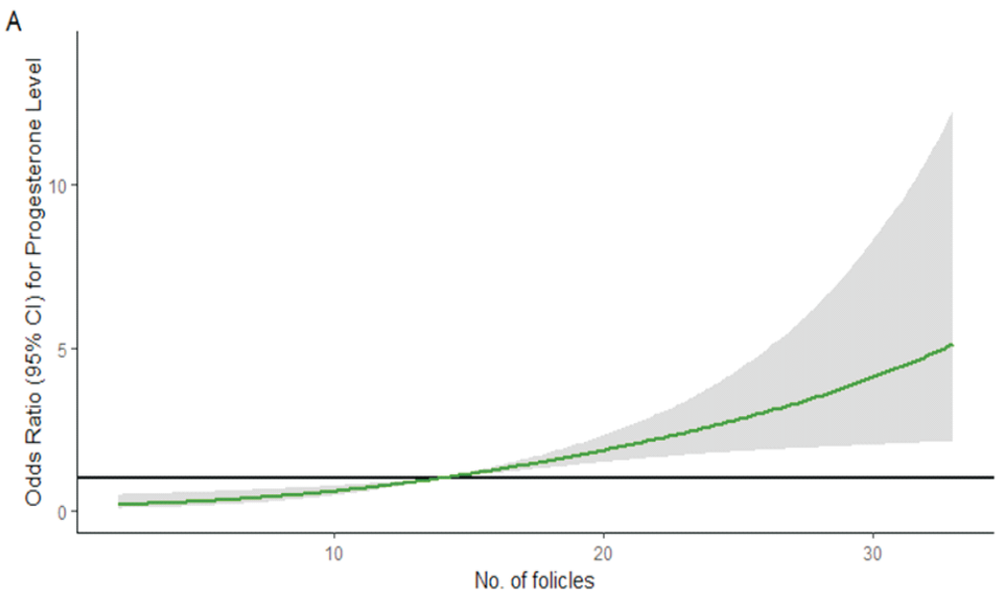

Figure 2: Dose response relationship between the number of follicles & progesterone levels.

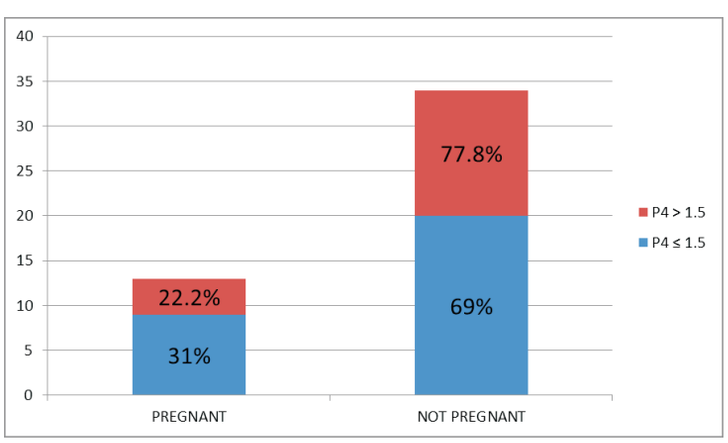

Figure 3: Pregnancy rates by progesterone (P4) levels
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