Serum Kisspeptin-l at time of Pregnancy Diagnosis is superior to serum ßhCG for prediction of Early Pregnancy Loss

Basma E. Sakr MD & Amira MN Abdelrahman MD*
Departments of Obstetrics & Gynecology, and Clinical & Chemical Pathology', Faculty of Medicine, Benha University.
Obstetrics and Gynecology, Benha University Hospital
Mobile No; 01 128810122

Abstract

Objectives: Estimation of serum Kisspeptin-l (Kiss-I) and beta human chorionic gonadotropin (ßhCG) levels at time ofpregnancy diagnosis (Booking time) and at the 12th gestational week; Sl and S2 samples, in trial to evaluate the predictability of these levels for the possibility of early pregnancy loss (EPL).

Patients & Methods: 283 women gave S2 samples, 76 women (26.9%) developed EPL (EPL group) and 207 women had viable fetus (Control group). Blood samples were obtamed for ELISA estimation of serum Kiss-I and ßhCG. The study outcome was the predictability of Sl levels of both parameters for the possibility of oncoming pregnancy loss.

Results: Sl sample ßhCG levels showed non-significant differences between both groups, while serum Kiss-I levels were significantly lower in EPL than in control women. Serum levels of both parameters in S2 sample were signifi cantly lower m EPL than both control women and levels estimated in Sl samples of EPL women. Incidence ofEPL was negatively correlated with Sl sample serum levels of both parameters. Regression analysis defined 6500 IU/L and 318 pmol/L as cutoff points for ßhCG and Kiss-I to predict 50% hazard for EPL. These cutoff points defined 158 and 181 tme cases, respectively with significant difference m favor of Kiss-I value. ROC curve analysis assured the sensitivity of lower Sl levels of both pammeters for prediction of 50% hazard ofEPL, but area difference under ROC was significant in favor of low serum Kiss-I as a significant sensitive predictor for EPL.

Conclusion: Estimation of serum kiss-I at time of pregnancy diagnosis inversely correlates with incidence of EPL. Lower serum Kiss-I levels had significantly higher predictability for EPL than ßhCG. Lower serum Kiss-I at the 12th GW or development of EPL assured the predictability of the result obtained at booking time and spared the need for sequential estimations.

Corresponding author:
Basma Elsayed Sakr
Affiliation: Benha Faculty of Medicine, Benha University e-mail: basma.abdelhalim@ fmed.bu.edu .eg The study was done at department of

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Introduction

Peptide hormones, including kisspeptin (Kiss) play a prominent role in controlling energy homeostasis and metabolism, and are implicated in controlling the reproductive functions([l]).Kisspeptin is a potent stimulant of lutemizing hormone (LH) pulses and preoutlatory surge in females, which is essential for fertility ([2]). Kiss-stimulated LH release involves neurokinin B that acts through the hypothalamic neurokinin-3 receptor[(3)].

Early pregnancy loss (EPL) is defined as the spontaneous loss of a pregnancy before the 13th weeks of gestation ([4]). EPL is one of the most common pregnancy complications that is mostly encountered early during pregnancy with an incidence up to 15% of all clini cally recognized pregnancies A significant proportion of patients with EPL are unaware of their miscamage especially when it occurs in the early stages of pregnancy , and so may be misdiagnosed as an expected menstmal cycle[(7)].

Although the actual mechanism underlying the development of EPL is undefined, multiple attributions were claimed; decidualiza tion is essential for the successful pregnancy, and if it is dysregulated this may lead to EPL Regulation of nucleosome was found to be critical for the maintenance of genome stability and epigenetic Information, and lack of this may lead to EPL Overexpression of IL-7 by decidua Induces a proinflamma toy environment that may induce EPL ([9]).As another mechanism, postovulatory apoptosis leads to oocyte aging, which could be considered as one of the major causes for human EPL (10).

Objectives

Estimation of serum levels of Kiss-I and ßhCG in newly pregnant women at booking time and at the 12thgestational week (GW) in trial to evaluate the predictability of at booking levels for the possibility of EPL Departments of Obstetrics & Gynecology,
Faculty of Medicme, Benha University

Design

Prospective observational comparative study

Patients & Methods

Throughout the duration of the study smce March 2019 till Jan 2021, all newly pregnant women who attended the Antenatal Care Unit, Benha University Hospital for assurance of being pregnant were eligible to evaluation. At the 6th GW (Booking visit), general clinical evaluation including history
taking with special regardtoparity, previous miscaniage, missed abortion, pregnancy-mduced diseases during the previous complet-
ed pregnancies, hormonal disturbances, nu tritional deficiencies, or lifestyle initiating or promoting EPL. Then, clinical and ultra sonographic examinationswere performed. Blood samples were obtained for routine lab oratory investigations, ELISA estimation of serum Kiss-I and estimation of serum levels of ßhCG.

Exclusion criteria

Presence ofanembryonic sac, multiple preg nancy, renal, cardiac or hepatic disorders.

Inclusion criteria

Pregnant women with singleton fetus, free of exclusion criteria and accepted to sign the written fully informed consent to attend the follow-up visit at the 12th GW or on devel opment of manifestations of EPL were enrolled in the study.

Investigations

At booking time, blood sample was drawn under complete aseptic conditions from the antecubital vein. Blood samples wereput m a plain tube, allowed to clot, centrifuged at 1500xg for 15 min and the serum samples were divided Into two palts:

l. The 1st palt was used for immediate estimation of serum ßhCG levels

2. The 2nd part was collected in clean Eppindorff tube and stored at -200C forE LISA estimation of Kiss-I serum levels using ELISA kit (catalogue no. ab19028 abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique.

Study outcome

l. The pr1ma1Y outcome was the predictability of at booking levels of ßhCG and Kiss-I for the possibility of oncoming pregnancy loss.

2. The secondary outcome was the extent of change in serum levels of ßhCG and Kiss-I at the 12 th GW or development of EPL, in relation to booking levels.

Statistical analysis

The obtained data were presented as mean, standard deviation (SD), numbers, percent ages, median and interquartile ranges (IQR).
The percentage of change was calculated as the level estimated in S2 minus the level estunated m Sl sample divided by the level es - tlmated m Sl sample and multiplied by 100. Parametric data were compared using paired t-test and Mann-Whitney test. Non-paramet ric data were compared using Chi-square test. Kaplan-Meier regression analysis was used to determine the cutoff point that can predict 50% of getting EPL. Predictability of cutoff points was assured by the Receiv er characteristic curve analysis. Statistical analysis was performed using SPSS software package, 2015. P value of <0.05 was considered significant.

Results

The study included 31 Inewly pregnant women; 21 were excluded for not fulfilling the inclusion criteria and 290 women were enrolled in the study and gave Sl sample. During follow-up, 7 women were missed and 283 women gave S2 sample. Unfortunately, 76 women developed EPL for an inci dence of 26.9% and were categorized as EPL group, 207 women had viable fetus at the 12th GW visit and were grouped as Control group (Fig. l). Enrolment data of women of both groups showed non-significant differences (Table l).

Table (1): Enmlment data of patients of both groups

Data	Group A (n—107)	Group B (n—116)	P value

Age (years)	25 (3.4)	24.6 (4)	0.441

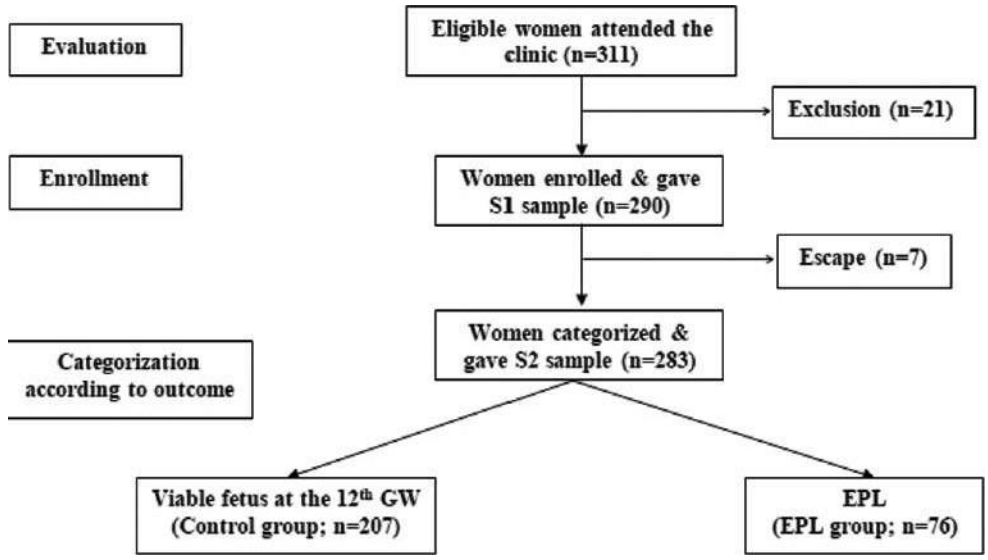

Fig. (I): Study Flow Chart

Body mass index (kg/m2)	28.7 (2.1)	28.1 (2.8)	0.119
Gravidity*	2 [1-2]	2 [1-2]	0.184
Parity*	1 [0-1]	1 [0-1]	0.116
Systolic blood pressure (mmHg)	115 (6.6)	114 (5.8)	0.217
Diastolic blood pressure (mmHg)	75.6 (4.9)	77 (5.1)	0.098
Random blood glucose (mg/dl)	116.8 (6.9)	115.8 (7.7)	0.297

Data are presented as mean; standard deviation (SD); *median and interqualtile range (IQR); P value indicates the significance of difference between both groups; P<0.05 indicates significant difference; P>0.05 indicates non-significant difference

Categorization according to outcome

Estimated serum levels of ßhCG at booking time (Sl samples) showed non-significant differ ences between samples of women of both groups. On contray, at booking time mean serum levels of Kiss-I were significantly (p=0.0057) lower in samples women of EPL group in com panson to levels estimated m samples of control women. On the other hand, mean serum levels of both ßhCG and Kiss-I estimated at the 12th GW (S2 samples) were significantly (p<0.0001) lower in samples of EPL women in comparison to samples of control women (Table 2, Fig. 2). Serum levels of ßhCG and Kiss-I in S2 sample ofEPL women were significantly (p<0.0001) lower in comparison to levels estimated in their Sl samples with a median percentage of decrease by 47. I % and 45% for ßhCG and Kiss-I,

respectively. However, serum levels of ßhCG and Kiss-I estimated in S2 samples of control women were significantly (p<0.0001) higher In comparison to levels estimated in their Sl samples With median percentage of increase of
53.3% and 93% for ßhCG and Kiss-I, respectively (Table 2, Fig. 3).

Table (2): Serum levels of ßhCGand Kiss-I estimated in Sl and S2 samples of patients of both groups

Data	Group	Control (n=207)	EPL (11-76)	P value
ßhCG (IU/L)	Mean Sl level	6854.6 (1462.3)	7023.7 (1432)	0.387
	Mean S2 level	10541 (1964.3)	3909.2 (1331)	<0.0001
		<0.0001	<0.0001	
	Percentage of change*	53.3 [44-66.1]	-47.1 [35.8-55]	
Kiss-I (pmol/L)	Mean Sl level	341.4 (156.5)	282 (164.5)	0.0057
	Mean S2 level	655.3 (221.1)	142.7 (62)	<0.0001
		<0.0001	<0.0001	
	Percentage of change*	93 [64.9-123.6]	-45 [37.3-54.1]	

Data are presented as mean; standard deviation (SD); *median and interqualtile range (IQR); P value indicates the significance of difference between both groups; PI value indicates the significance of difference between levels estimated m Sl and S2 samples of each group; P<0.05 indicates significant difference; P>0.05 indicates non-significant difference

The incidence of EPL was negatively conelated With serum ßhCG (Rho—-0.157, p 0.008) and Kiss-I (Rho -0.375, p<0.001) levels estimated at booking time. Kaplan-Meier regression analysis defined median level of ßhCG that predict 50% cumulative hazard for getting EPL at 6500 wlth 95% Cl of 6218-6782 IU/L (Fig. 4) and the median level of Kiss-I for prediction of 50% cumulative hazard ofEPL at 318 with SE of 15.7 and 95% CI of 287-349 pmol/L (Fig. 4). At the cutoff point for ßhCG (6500 IU/L), there were 158 hue cases and 125 false cases, while at cutoff point for Kiss-I, there were 181 tlue and 102 false cases with significant difference (p—0.0485) in favor of Kiss-I value as determinant of 50% cumulative hazard for EPL. Using both variables at the same cutoff points, there were 114 true and 169 false cases with significantly lower predictability for the 50% cumulative hazard of EPL in comparison to ßhCG alone (p 0.0002) or Kiss-I alone (p<0.0001), (Table).

Table (3): Patients' distribution as true and false cases for oncoming EPL as the suggested cutoff points for ßhCG and Kiss-I, and both

Variable	Cutoff point	True cases					
		Positive	Negative	Total	Positive	Negative	Total

ßhCG	6500 (IU/L)	48 (16.9%)	110 (38.9%)	158 (55.8%)	97 (34.3%)	28	125 (44.2%)
Kiss-I	318 (pmol/L)	56 (19.8%)	125 (44.2%)	181 (64%)	83 (29.3%)	19 (6.7%)	102 (36%)
Both				114 (40.3			169 (59.7%)

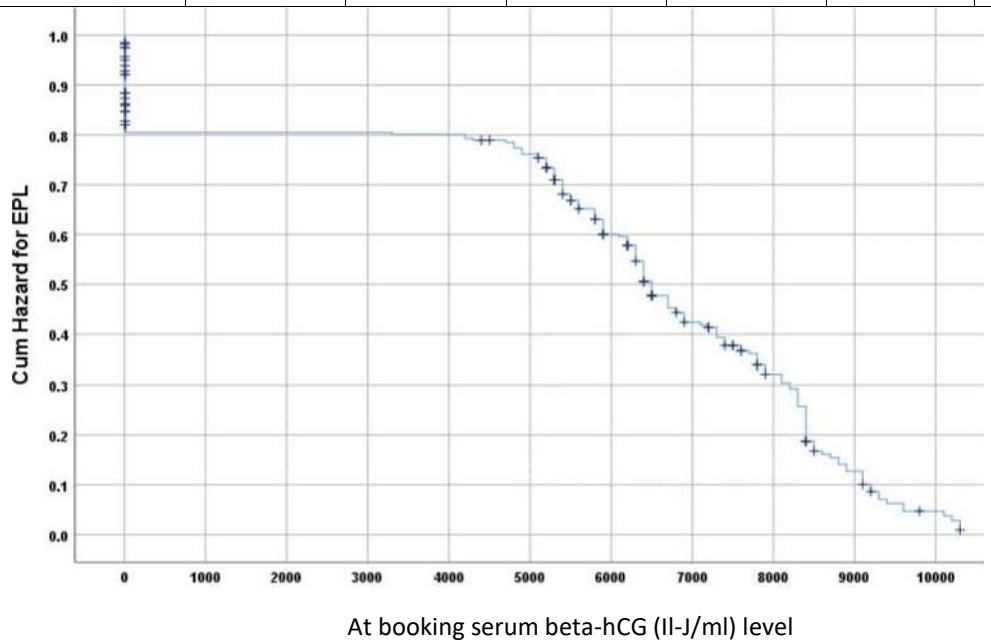

Fig. (4): Kaplan-Meier regression analysis of serum ßhCG as predictor for the cumulative hazard for getting EPL

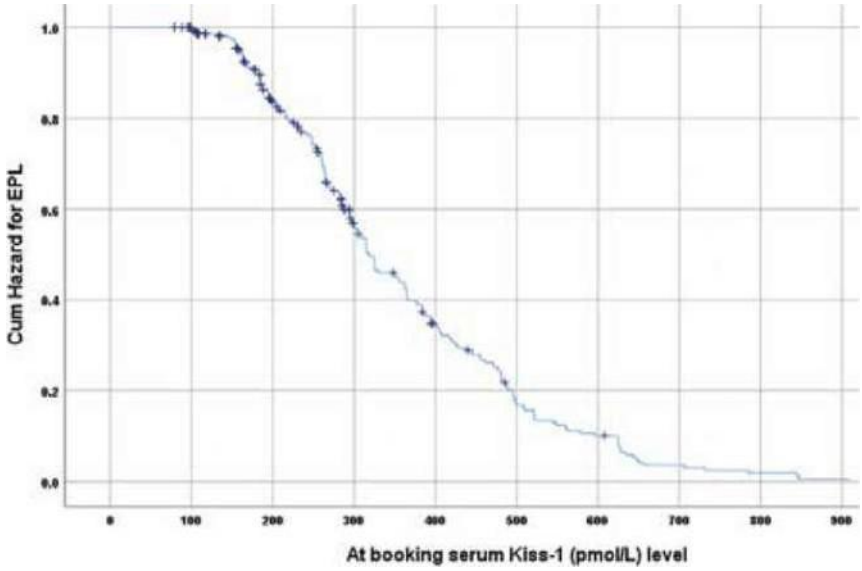

Fig. (5): Kaplan-Meier regression analysis of serum Kiss-I as predictor for the cumulative hazard for getting EPL

ROC curve analysis assured the sensitivity of lower at booking levels of both parameters for prediction of 50% cumulative hazard of EPL (Fig. 2). However, paired-sample area difference under ROC analysis defined low serum Kiss-I as a more significant sensitive predictor for EPL than ßhCG with significant p<0.001) difference in AUC (0.164±0.262; 0.072-0.257).

Table (3): ROC curve analysis for serum levels of ßhCG and Kiss-I as predictors for oncoming
EPL

ROC curve analysis	Parameter	AUC	SE		95% Cl
	ßhCG	0 396	0.037	=o.008	0.323-0 470
	Kiss-I	0.232	0.031	<O.OOI	o. 170-0.293
Paired-sample area difference under ROC analysis		AUC difference	SE difference		95% Cl
		0.164	0.262	<O.OOI	0.072-0.257

ROC: Receiver characteristic curve; AUC: Area under curve; P indicates the significance of the results; P<0.05 indicates significant difference; p>0.05 indicates non-significant difference

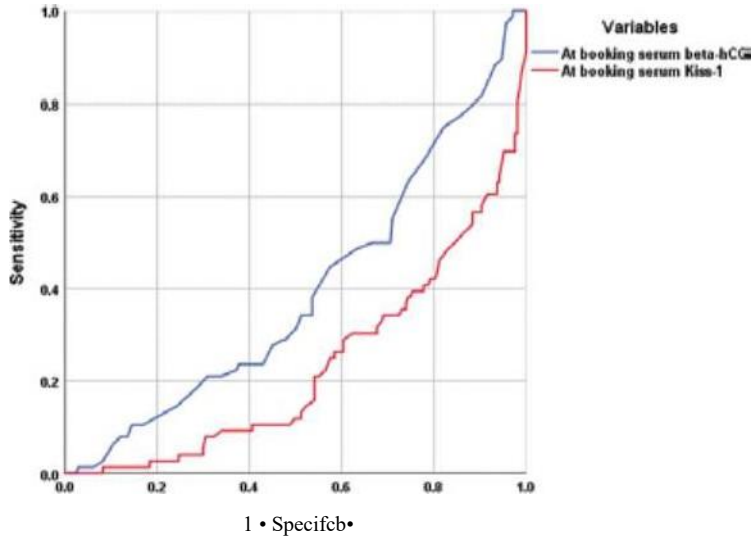

Fig. (6): ROC curve analysis of serum ßhCG and Kiss-I as predictors for getting EPL

Discussion

Control women showed significant increases of estimated serum levels of Kiss-I in sam ples obtained at the 12 th GW (S2 samples) m comparison to levels estimated in their Sl samples that were obtained at booking time. These data indicated source-related increases of Kiss-I with progress of pregnancy and a possible relation between maintenance of pregnancy and serum Kiss-I either as a factor for or as indictor of pregnancy progress and maintenance. In line with these findings, multiple previous in-vitro studies detected expression of Kiss gene in placental tissues ofnonnal pregnancies and with the af terward studies that assured placental source of plasma Kiss ([14]). Moreover, the obtained
results and the suggested assumption assured that previously detected by Hu et al., ([15]) who concluded that plasma KISS levels significantly increase across pregnancy.

On the other side, serum Kiss-I levels esti mated in Sl sample of women of EPL group were significantly lower than that of Sl sam ple of control women. Considering the previously mentioned of the placental source of Kiss, these low serum levels of Kiss detected early on pregnancy diagnosis (Booking time) indicated defective placentation and was as sured by the detected subsequent decreased Kiss levels. These results allow suggestion that estimated serum Kiss levels could be used as an early predictor for the possibili ty of oncoming pregnancy loss during the
first trimester. In line with these findings, Sullivan-Pyke et al., (16) reported that serum Kiss levels differ between the pregnant and

non-pregnant state and by viability, and Hu et al., ([15])documented that plasma kiss levels
could be used as a potential biomarker for the detection of miscamage.

In support of the role of increased KISS levels for maintenance of pregnancy, Bödis et al.,

(17) reported that in women undergoing IVF , serum kiss levels significantly increased m successful cases and regression analyses showed that these increases improved IVF outcome. Also, Rehman et al., ([18])documented that increased levels of Kiss and estradiol In serum and Kiss in follicular fluid resulted in an optimum endometrial thickness, probability of fertilization of oocytes and chances of clinical pregnancy in ICSI cycles of unexplained infertile females. Recently, Qin et al., ([19]) found serum kiss levels estimated at the beginning of gonadotropin stimulation, 8-d thereafter and on the day of ovum pick-up in

IVF/ICSI-treated Infertile women were comparable in women had successful outcome, while in women had unsuccessful outcome serum Kiss levels on day of retrieval were significantly lower than levels estimated at the beginning of stimulation.

Statistical analyses detected positive signif icant correlation between serum levels of Kiss and ßhCG and a negative significant correlation between Sl serum levels of both biomarkers and incidence of EPL. The ROC curve analysis defined both biomarkers as sensitive early predictors for EPL, but area under curve (AUC) difference was significant for Kiss. Moreover, detection of hue cases at cutoff points determined by regression analysis was significantly higher With Kiss than with ßhCG. These findings point to the superior predictability of serum levels of Kiss at booking time over that of ßhCG and the possibility of dependence on smgle estimation of Kiss without the need for sequential estimations.

These findings support the previously report ed by Jayasena et al., ([20])who found KISS had a higher diagnostic perfonnance for miscarriage than hCG with significant AUC difference. Thereafter, Sullivan-Pyke et al., ([16]) detected that serum Kiss levels were posi tively associated with gestational age and hCG especially in spontaneous abortion and documented that stability of kisspeptin assay in serum and its potential clinical utility as a biomarker for early pregnancy viability. Recently, Abbara et al., ([21]) detected lower circulating Kiss and ßhCG in samples from women with miscamages than in healthy pregnancies by 79% and 70% and the AUC for identifying 1st trimester miscaniage was 0.874 and 0.859, respectively, and detected improved performance of Kiss but worsened performance of ßhCG in identifying miscarriage with increased length of gestation. Moreover, Qin et al., ([19])found serum kiss estimated 8-d after gonadotropin stimulation and on retrieval day had positive correla tions with serum E2 and hCG estimated at the same time and with the outcomes of IVF/ ICSI treatment.

In line with the dependence on single estimation of Kiss early in pregnancy as predictor for EPL, Jayasena et al. ([20])documented that single plasma kiss measurement during the booking visit could identify asymptomatic pregnant women at increased risk of miscarriage or EPL. Thereafter, Yu et al., ([22])found single serum estimation of Kiss and ßhCG concentrationswere correlated with different pregnancy outcomes and lower Kiss levels were detected in women who experienced biochemical pregnancy loss, but sequential measurements of serum Kiss levels are not effective in detenninmg pregnancy outcome. In trial to explain the relation between low serum Kiss and EPL, Wu et al., ([23])detected a positive conelation between expression of Kiss and progesterone-induced blocking fac tor in syncytiotrophoblasts, cytotrophoblasts and deciduas and concluded that decreased kiss and progesterone-induced blocking factor are associated With recurrent spontaneous abortion. Also, Martino et al., ([11])after in vitro proliferation of bovine pnmary placental cotyledon cell lines isolated at the 1st trimes ter detected Involvement of the Kiss-IR/Kps system in the regulation of cell proliferation of bovine placenta but, it may not be involved In modulatmg placental progesterone secretion. Thereafter, Li et al., (24)using immuno histochemist1Y for detection of Kiss gene in placentas of women had EPL versus controls indicated that down-regulation of kisspeptin expression acts as an invasion-inhibitor gene with subsequent Interference with normal homeostasis of trophoblast regulation, ultimately resulting in miscaniage.

Conclusion

Estimation of serum kisspeptin at time of pregnancy diagnosis correlates With pregnancy outcome concermng viability.

Lower serum Kiss-I at booking time had significantly higher predictibity for EPL than ßhCG. Lower serum Kiss-I at the 12th GW or development of EPL assured the predictivbili ty of the result obtained at booking time and spared the need for sequential estimations

Limitation

The small sample size limited the assurance of the suggested cutoff point for at booking serum Kiss-I level as early predictor for EPL

Recommendations

Wider scale studies for assurance of the pro posed cutoff point of serum Kiss-I prior to defining its clinical utility.

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