
Serum Kisspeptin-1 at time of Pregnancy Diagnosis is superior to serum β hCG for prediction of Early Pregnancy Loss

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Abstract

Objectives: Estimation of serum Kisspeptin-1 (Kiss-1) and beta human chorionic gonadotropin (β hCG) levels at time of pregnancy diagnosis (Booking time) and at the 12th gestational week; S1 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 obtained for ELISA estimation of serum Kiss-1 and β hCG. The study outcome was the predictability of S1 levels of both parameters for the possibility of oncoming pregnancy loss.

Results: S1 sample β hCG levels showed non-significant differences between both groups, while serum Kiss-1 levels were significantly lower in EPL than in control women. Serum levels of both parameters in S2 sample were significantly lower in EPL than both control women and levels estimated in S1 samples of EPL women. Incidence of EPL was negatively correlated with S1 sample serum levels of both parameters. Regression analysis defined 6500 IU/L and 318 pmol/L as cutoff points for β hCG and Kiss-1 to predict 50% hazard for EPL. These cutoff points defined 158 and 181 true cases, respectively with significant difference in favor of Kiss-1 value. ROC curve analysis assured the sensitivity of lower S1 levels of both parameters for prediction of 50% hazard of EPL, but area difference under ROC was significant in favor of low serum Kiss-1 as a significant sensitive predictor for EPL.

Conclusion: Estimation of serum kiss-1 at time of pregnancy diagnosis inversely correlates with incidence of EPL. Lower serum Kiss-1 levels had significantly higher predictability for EPL than β hCG. Lower serum Kiss-1 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.

Keywords: Early pregnancy loss, Early prediction, Kisspeptin-1, β hCG

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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⁽¹⁾. Kisspeptin is a potent stimulant of luteinizing hormone (LH) pulses and pre-ovulatory 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 clinically recognized pregnancies⁽⁵⁾. A significant proportion of patients with EPL are unaware of their miscarriage especially when it occurs in the early stages of pregnancy⁽⁶⁾, and so may be misdiagnosed as an expected menstrual cycle⁽⁷⁾.

Although the actual mechanism underlying the development of EPL is undefined, multiple attributions were claimed; decidualization is essential for the successful pregnancy, and if it is dysregulated this may lead to EPL⁽⁸⁾. 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⁽⁷⁾. Overexpression of IL-7 by decidua induces a proinflammatory 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-1 and β hCG in newly pregnant women at booking time and at the 12th gestational week (GW) in trial to evaluate the predictability of at booking levels for the possibility of EPL

Setting

Departments of Obstetrics & Gynecology, Faculty of Medicine, Benha University

Design

Prospective observational comparative study

Patients & Methods

Throughout the duration of the study since 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 regard to parity, previous miscarriage, missed abortion, pregnancy-induced diseases during the previous completed pregnancies, hormonal disturbances, nutritional deficiencies, or lifestyle initiating or promoting EPL. Then, clinical and ultrasonographic examinations were performed. Blood samples were obtained for routine laboratory investigations, ELISA estimation of serum Kiss-1 and estimation of serum levels of β hCG.

Exclusion criteria

Presence of an embryonic sac, multiple pregnancy, 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 development 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 were put in a plain tube, allowed to clot, centrifuged at $1500\times g$ for 15 min and the serum samples were divided into two parts:

1. The 1st part was used for immediate estimation of serum βhCG levels
2. The 2nd part was collected in clean Eppendorf tube and stored at $-20^{\circ}C$ for ELISA estimation of Kiss-1 serum levels using ELISA kit (catalogue no. ab19028 abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique.

Study outcome

1. The primary outcome was the predictability of at booking levels of βhCG and Kiss-1 for the possibility of oncoming pregnancy loss.
2. The secondary outcome was the extent of change in serum levels of βhCG and Kiss-1 at the 12th GW or development of EPL, in relation to booking levels.

Statistical analysis

The obtained data were presented as mean, standard deviation (SD), numbers, percentages, median and interquartile ranges (IQR).

The percentage of change was calculated as the level estimated in S2 minus the level estimated in S1 sample divided by the level estimated in S1 sample and multiplied by 100. Parametric data were compared using paired t-test and Mann-Whitney test. Non-parametric 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 Receiver 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 newly pregnant women; 21 were excluded for not fulfilling the inclusion criteria and 290 women were enrolled in the study and gave S1 sample. During follow-up, 7 women were missed and 283 women gave S2 sample. Unfortunately, 76 women developed EPL for an incidence 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. 1). Enrolment data of women of both groups showed non-significant differences (Table 1).

Table (1): Enrolment 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
Body mass index (kg/m ²)	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 interquartile 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

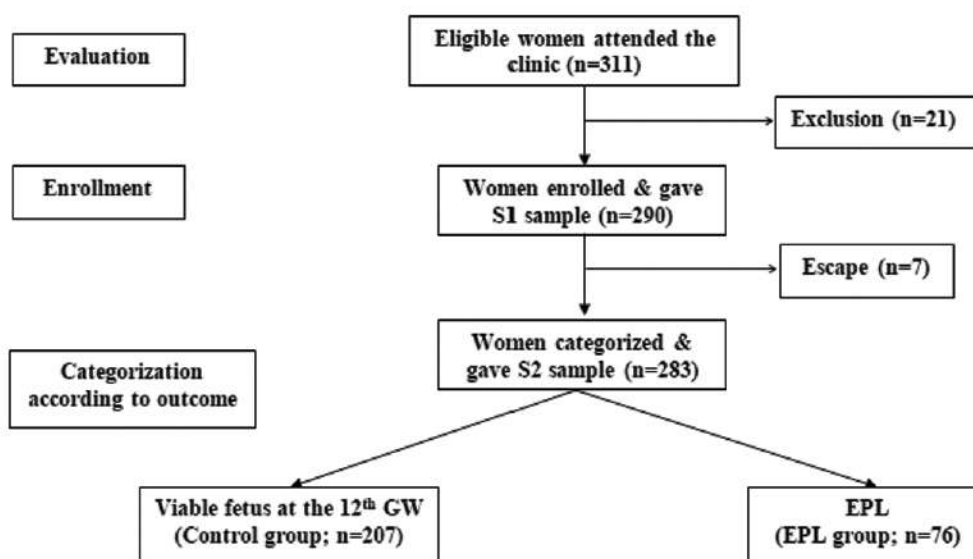


Fig. (1): Study Flow Chart

Estimated serum levels of β hCG at booking time (S1 samples) showed non-significant differences between samples of women of both groups. On contrary, at booking time mean serum levels of Kiss-1 were significantly ($p=0.0057$) lower in samples women of EPL group in comparison to levels estimated in samples of control women. On the other hand, mean serum levels of both β hCG and Kiss-1 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 β hCG and Kiss-1 in S2 sample of EPL women were significantly ($p<0.0001$) lower in comparison to levels estimated in their S1 samples with a median percentage of decrease by 47.1% and 45% for β hCG and Kiss-1, respectively. However, serum levels of β hCG and Kiss-1 estimated in S2 samples of control women were significantly ($p<0.0001$) higher in comparison to levels estimated in their S1 samples with median percentage of increase of 53.3% and 93% for β hCG and Kiss-1, respectively (Table 2, Fig. 3).

Table (2): Serum levels of β hCG and Kiss-1 estimated in S1 and S2 samples of patients of both groups

Data	Group	Control (n=207)	EPL (n=76)	P value
β hCG (IU/L)	Mean S1 level	6854.6 (1462.3)	7023.7 (1432)	0.387
	Mean S2 level	10541 (1964.3)	3909.2 (1331)	<0.0001
	P1	<0.0001	<0.0001	
	Percentage of change*	53.3 [44-66.1]	-47.1 [35.8-55]	
Kiss-1 (pmol/L)	Mean S1 level	341.4 (156.5)	282 (164.5)	0.0057
	Mean S2 level	655.3 (221.1)	142.7 (62)	<0.0001
	P1	<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 interquartile range (IQR); P value indicates the significance of difference between both groups; P1 value indicates the significance of difference between levels estimated in S1 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 correlated with serum β hCG (Rho=-0.157, p=0.008) and Kiss-1 (Rho= -0.375, p<0.001) levels estimated at booking time. Kaplan-Meier regression analysis defined median level of β hCG that predict 50% cumulative hazard for getting EPL at 6500 with 95% CI of 6218-6782 IU/L (Fig. 4) and the median level of Kiss-1 for prediction of 50% cumulative hazard of EPL at 318 with SE of 15.7 and 95% CI of 287-349 pmol/L (Fig. 4). At the cutoff point for β hCG (6500 IU/L), there were 158 true cases and 125 false cases, while at cutoff point for Kiss-1, there were 181 true and 102 false cases with significant difference (p=0.0485) in favor of Kiss-1 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 β hCG alone (p=0.0002) or Kiss-1 alone (p<0.0001), (Table).

Table (3): Patients' distribution as true and false cases for oncoming EPL as the suggested cutoff points for β hCG and Kiss-1, and both

Variable	Cutoff point	True cases					
		Positive	Negative	Total	Positive	Negative	Total
β hCG	6500 (IU/L)	48 (16.9%)	110 (38.9%)	158 (55.8%)	97 (34.3%)	28 (9.9%)	125 (44.2%)
Kiss-1	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%)

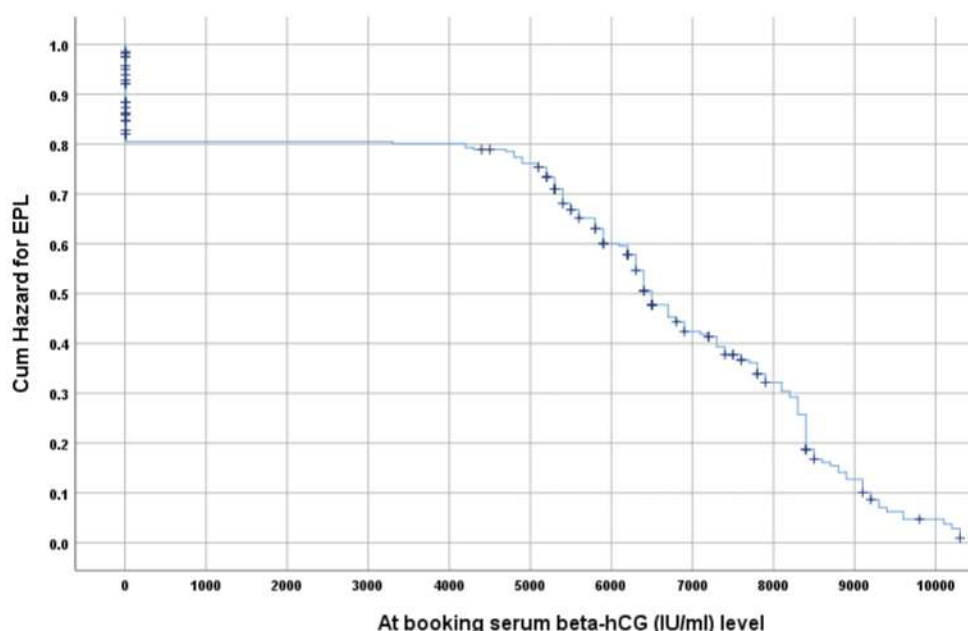


Fig. (4): Kaplan-Meier regression analysis of serum β hCG as predictor for the cumulative hazard for getting EPL

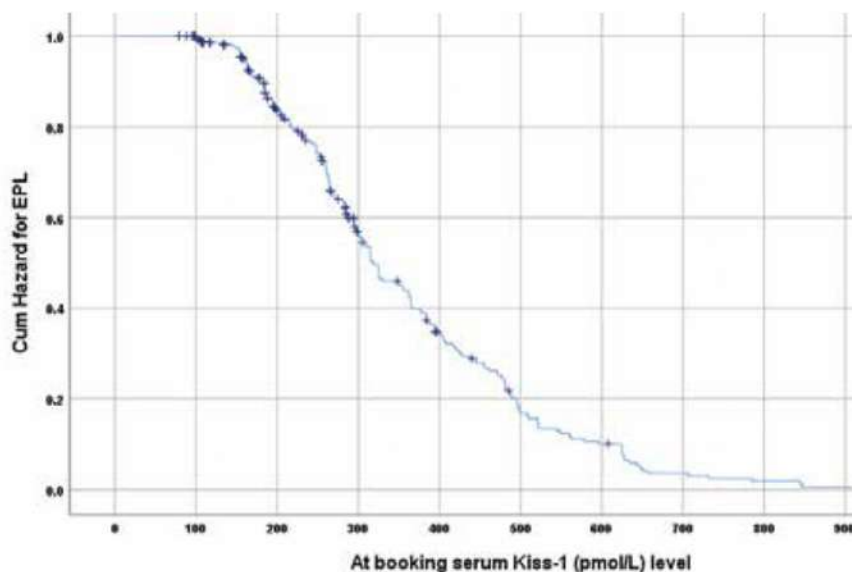


Fig. (5): Kaplan-Meier regression analysis of serum Kiss-1 as predictor for the cumulative hazard for getting EPL

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-1 as a more significant sensitive predictor for EPL than β hCG with significant ($p < 0.001$) difference in AUC ($= 0.164 \pm 0.262$; CI= 0.072-0.257).

Table (3): ROC curve analysis for serum levels of β hCG and Kiss-1 as predictors for oncoming EPL

ROC curve analysis	Parameter	AUC	SE	P	95% CI
	β hCG	0.396	0.037	$= 0.008$	0.323-0.470
	Kiss-1	0.232	0.031	< 0.001	0.170-0.293
Paired-sample area difference under ROC analysis	AUC difference	SE difference	p	95% CI	
	0.164	0.262	< 0.001	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

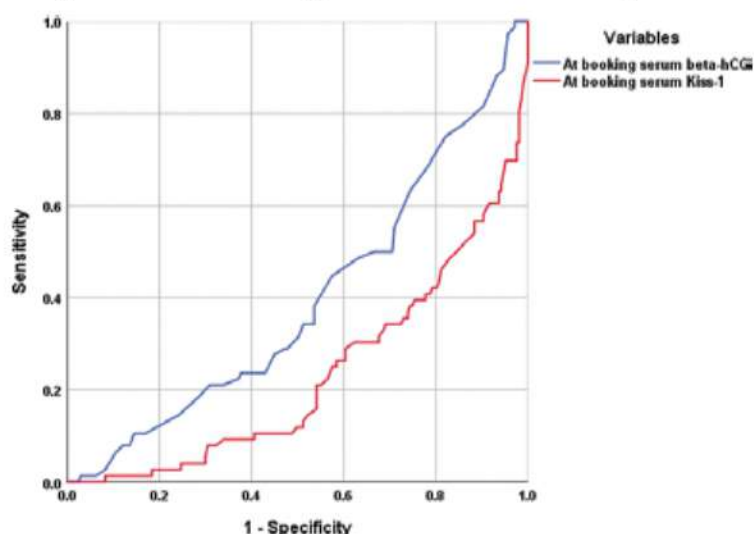


Fig. (6): ROC curve analysis of serum β hCG and Kiss-1 as predictors for getting EPL

Discussion

Control women showed significant increases of estimated serum levels of Kiss-1 in samples obtained at the 12th GW (S2 samples) in comparison to levels estimated in their S1 samples that were obtained at booking time. These data indicated source-related increases of Kiss-1 with progress of pregnancy and a possible relation between maintenance of pregnancy and serum Kiss-1 either as a factor for or as indicator of pregnancy progress and maintenance. In line with these findings, multiple previous in-vitro studies detected expression of Kiss gene in placental tissues of normal pregnancies^(11,12,13) and with the afterward 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-1 levels estimated in S1 sample of women of EPL group were significantly lower than that of S1 sample 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 assured 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 possibility 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 miscarriage.

In support of the role of increased Kiss levels for maintenance of pregnancy, Bódis et al.,⁽¹⁷⁾ reported that in women undergoing IVF, serum kiss levels significantly increased in 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 significant correlation between serum levels of Kiss and β hCG and a negative significant correlation between S1 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 true cases at cutoff points determined by regression analysis was significantly higher with Kiss than with β hCG. These findings point to the superior predictability of serum levels of Kiss at booking time over that of β hCG and the possibility of dependence on single estimation of Kiss without the need for sequential estimations.

These findings support the previously reported by Jayasena et al.,⁽²⁰⁾ who found Kiss had a higher diagnostic performance for miscarriage than hCG with significant AUC difference. Thereafter, Sullivan-Pyke et al.,⁽¹⁶⁾ detected that serum Kiss levels were positively 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 β hCG in samples from

women with miscarriages than in healthy pregnancies by 79% and 70% and the AUC for identifying 1st trimester miscarriage was 0.874 and 0.859, respectively, and detected improved performance of Kiss but worsened performance of β hCG 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 correlations 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 β hCG concentrations were 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 determining pregnancy outcome.

In trial to explain the relation between low serum Kiss and EPL, Wu et al.,⁽²³⁾ detected a positive correlation between expression of Kiss and progesterone-induced blocking factor 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 primary placental cotyledon cell lines isolated at the 1st trimester detected involvement of the Kiss-1R/Kps system in the regulation of cell proliferation of bovine placenta but, it may not be involved in modulating placental progesterone secretion. Thereafter, Li et al.,⁽²⁴⁾ using immunohistochemistry 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 miscarriage.

Conclusion

Estimation of serum kisspeptin at time of pregnancy diagnosis correlates with pregnancy outcome concerning viability. Lower serum Kiss-1 at booking time had significantly higher predictability for EPL than β hCG. Lower serum Kiss-1 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

Limitation

The small sample size limited the assurance of the suggested cutoff point for at booking serum Kiss-1 level as early predictor for EPL

Recommendations

Wider scale studies for assurance of the proposed cutoff point of serum Kiss-1 prior to defining its clinical utility.

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