

Parallel comparison study of estradiol and FSH between Elecsys® and Access 2® analyzers

in relationship to number of oocytes and pregnancy outcome.

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Abstract

Day 3 FSH and estradiol levels are used as a prognostic indicator for women undergoing assisted reproduction. This study was a retrospective analysis to determine the relationship between Elecsys and Access2® assay values for basal estradiol and FSH, and compare the two assay methodologies for a successful pregnancy outcome predictor.

The subjects for this study (n=100) were 25 to 50 years of age. Serum levels of estradiol and FSH were collected on cycle day 3 and measured using an electrochemiluminescent enzyme immunoassay (Roche Elecsys) and a paramagnetic chemiluminescent enzyme immunoassay (Beckman Coulter Access2®). The number of oocytes retrieved from IVF with controlled ovarian hyperstimulation was recorded.

The two methods appear to be equivalent when compared using regression analysis by both the Deming (parametric) and Passing-Bablok (non-parametric) methods. The cumsum test for linearity shows a non-linear relationship between the two-assay methods for the estradiol assay. (P<0.01). Whereas, the FSH assay shows a proportional bias: slope of 0.882 (95%CI: 0.84 to 0.92) with Elecsys (predicate) and Access2® (test). Bland-Altman bias plot analysis shows a negative trend when FSH is greater than 10 mIU/ml between the two-assay methods as observed by trend lines. A Receiver Operating Curve (ROC) analysis of the FSH assays for successful pregnancy compared area under the curve (AUC) for Elecsys and Access2®. There was no statistical difference. Both the Access2® and Elecsys assays demonstrated comparable sensitivity of 0.892 and 0.912, at their respective cutoffs. However, specificity was substantially lower for the Elecsys assay, 0.145, compared to the Access2® at 0.306, at their respective cut-off. The Fisher exact test was used for the proportion of successful pregnancies. There is no statistical difference with Elecsys (p=0.8022) with the published FSH cutoff of 15 mIU/ml. Access2® shows statistical difference. (P<0.0328) with the published FSH cut-off of 10.6 mIU/ml. The Fisher exact test for the proportion of oocyte shows statistical difference for both Elecsys and Access2®, with p values of 0.0210 and 0.0054 respectively for their published FSH cutoffs.

Access2® FSH assay at day 3 is a better predictor for number of oocytes retrieved and successful pregnancy outcome.

Introduction

Depending on the laboratory methodology which is used, the physiologic range for the specific hormones being measured will differ between the commonly used serum analyzers. Establishing the cut off levels for serum FSH and estradiol on menstrual day 3 may assist the Clinician in the management of an Assisted Reproduction Cycle.

In this study, serum FSH and estradiol levels are compared using both the Roche Elecsys 2010 and the Beckman-Coulter Access 2 analyzers. This parallel study was performed during the implementation of a new laboratory methodology at a private fertility center.

Thus, serum FSH and estradiol hormone levels combined with the patients' age may be useful during the management of an IVF treatment cycle.

Methods & Materials

Method 1

Roche Elecsys 2010 analyzer:

Serum Estradiol and FSH

2 point calibration every two weeks. In addition, 6-point calibration check every 6 months.

Electrochemiluminescence immunoassay ('ECLIA') using polyclonal antibody in the reagent kit.

The product is measured by a photomultiplier.

Method 2

Beckman-Coulter Access 2 analyzer:

Serum Estradiol and FSH

6 point calibration every two weeks.

Paramagnetic chemiluminescence enzyme enhanced immunoassay, using monoclonal antibody in the reagent kit.

The product is measured by a luminometer

Procedure

Sera were collected from 100 subjects, ranging in age from 25 to 50 years, on day 3 of their menstrual cycle. The samples were analyzed and compared for estradiol and FSH using Elecsys 2010 and Access 2 analyzers. 50 subjects chose to go through in vitro fertilization (IVF). The serum concentration of FSH and estradiol for each subject was compared with the number of oocytes retrieved at In Vitro Fertilization. A pregnancy was defined by quantitative bHCG greater than 5 mIU/ml.

The FSH and estradiol concentration from Access 2 and Elecsys 2010 were plotted on the 'y' axis and the 'x' axis respectively for linearity of each assay.

The Fisher exact test showed proportion of population above and below published cut off concentration for FSH when compared with successfully achieved pregnancy and oocyte numbers after assisted reproduction.

The ROC analysis showed the highest concentration for true positive value on the 'y' axis and lowest possible concentration for false positive value on the 'x' axis.

The accuracy and precision were established by determining the standard of deviation and the coefficient of variation.

Results

There was no significant difference for:

- Regression analysis by Deming and Passing-Bablok methods for both estradiol and FSH.
- Receiver Operating Curve (ROC) analysis for sensitivity of the FSH assays for successful pregnancy comparing area under the curve (AUC) at each method's respective cut off values.

A significant difference was observed for:

- Bland-Altman bias plot analysis, when FSH value is >10.0 mIU/ml a negative trend is observed.
- Specificity and sensitivity with Fisher exact test for FSH (both methods) comparing the number of oocytes retrieved.
- ROC analysis for each FSH assay at their cut off values, for optimal specificity comparison for oocyte number.
- Statistical analyses of FSH level and successful pregnancy using the Fisher exact test.

(Table 1) Fisher exact test for FSH and pregnancy outcome.

Access 2 FSH cut off	Unsuccessful Pregnancy	Successful Pregnancy
<10.6	56%	44%
>10.6	83%	17%
Elecsys FSH cut off		
<15	61%	39%
>15	69%	31%

FSH cut off values are as published by Beckman and Roche respectively.

The fisher analysis of the null hypothesis that the proportion of successful pregnancies as defined as bHCG >5 or oocyte number as defined >3 are equal above and below the respective FSH assay.

For Elecsys, there is no significant difference in the proportion of pregnancies above and below the cutoff with p value of 0.8022. The Access shows significant difference in the proportion of successful pregnancies with p value of 0.0328. This is the test of the respective cutoff for FSH to the proportion of pregnancies.

Fisher exact test for oocyte number. (Table 2)

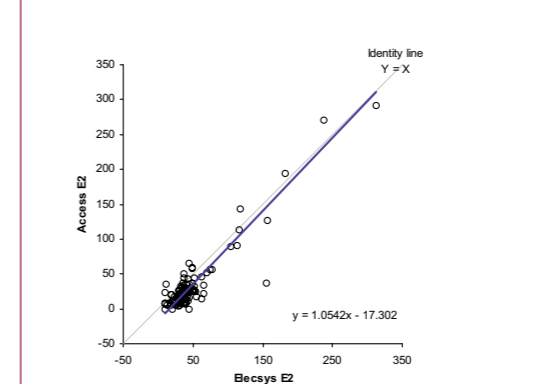
Elecsys FSH Cutoff	oocyte Cutoff		Total
	> 3	< 3	
< 15	37	8	45
>= 15	1	3	4
Total	38	11	49

Access FSH Cutoff	oocyte Cutoff		Total
	> 3	< 3	
< 10.6	35	8	43
>= 10.6	3	3	6
Total	38	11	49

Deming graph data for Estradiol. (Table 3)

	Coefficient	SE	95% CI
Intercept	-17.3019	2.9039	-23.0647 to -11.5392
Slope	1.0542	0.0443	0.9662 to 1.1422

Deming graph for Estradiol. (Fig. 1)

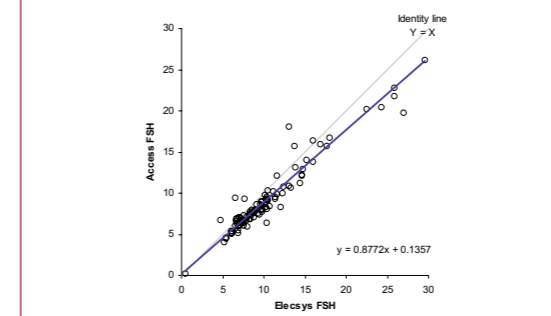


The Deming graph reports the analytical performance of two assay methods, to remove assay error in the analysis and focuses on the trend in the data.

(Table 4) Deming graph data for FSH.

	Coefficient	SE	95% CI
Intercept	0.1357	0.2989	-0.4574 to 0.7289
Slope	0.8772	0.0258	0.8259 to 0.9285

Deming graph for FSH (Fig. 2)



ROC analysis. (Fig. 3) This analysis defines highest possible FSH assay value with optimal sensitivity and specificity.

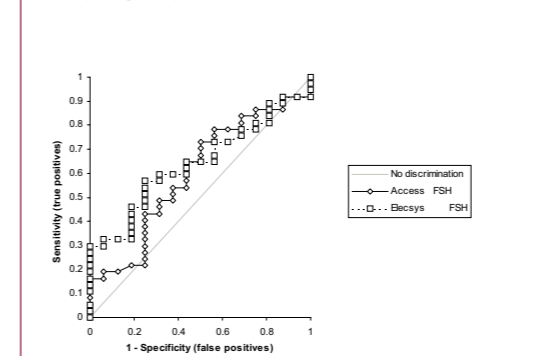


Table 5

Precision: Bio-Rad immunoplus tri level quality control material used for comparison.

	Estradiol	
	Roche Elecsys	Beckman-Coulter Access 2
Level one		
Mean	83.74	120.85
SD	16.12	13.56
%CV	19.3	11
Level two		
Mean	228.69	297.17
SD	47.38	20.33
%CV	20.7	6.8
Level three		
Mean	528.41	657.18
SD	97.92	37.55
%CV	18.5	5.7

	FSH	
	Roche Elecsys	Beckman-Coulter Access 2
Level one		
Mean	6.207	5.642
SD	0.801	0.410
%CV	12.9	7.3
Level two		
Mean	20.138	17.747
SD	3.324	1.255
%CV	16.5	7.1
Level three		
Mean	58.184	53.168
SD	7.602	2.546
%CV	13.1	4.8

The above tables show the accuracy and precision of the assay. A lower standard of deviation shows better accuracy. The % coefficient of variation less than or equal to 10 shows better precision.

Conclusions

- Both Estradiol and FSH results show greater precision using Beckman-Coulter Access 2 as determined by comparing SD and %CV with Bio-Rad quality control material data.
- Estradiol alone was not a good indicator of oocyte number or pregnancy outcome as measured by either method.
- FSH was a better predictor than estradiol of oocyte number and pregnancy outcome.
- For the Access 2, a FSH cut off 10.6 mIU/ml provides the optimal specificity and sensitivity. However, from our data analysis 9.5 mIU/ml would provide better optimal specificity for a successful pregnancy.
- For the Elecsys 2010, the published FSH cut off 15 mIU/ml lacks specificity. From our data analysis, a cut off of 12 mIU/ml would provide optimal specificity.

Accurate and precise day 3 estradiol and FSH measurements may assist Clinician in patient management for ovarian stimulation during assisted reproductive treatment that may lead to a successful pregnancy.