STUDY OF LUTEINIZING HORMONE, FOLLICULAR STIMULATING HORMONE & PROLACTIN LEVELS AT DIFFERENT PHASES OF NORMAL MENSTRUAL CYCLE

by

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Introduction

Investigation of gonadotrophin hormones produced by human pituitary i.e., luteinizing hormone, follicular stimulating hormone and prolactin form an essential part of the study of the reproductive physiology of the female. With the advent of radioimmunoassay techniques for measuring these hormones instead of the older techniques of bioassay, the levels of the hormones in various phases of menstrual cycle have been worked out in the Western literature. Since very few studies have been reported from this country, it was considered desirable to determine the normal values of these hormones in various phases of the menstrual cycle in normal healthy women in our country to serve as an adequate control group for various reproductive physiological studies. Further the earlier radioimmunoassay determinations of LH, FSH, PRL were by a variety of radioimmunoassays using different assay protocol. It has been pointed out that for high molecular weight substances which are not available in pure form or completely characterized chemically, absolute estimations can not be made. In order to overcome the variabilities introduced in gonadotrophin estimations because of different antisera, different standards, different reaction condition with different misclassification errors, World Health Organization (WHO) has recommended the use of Matched reagents and the same assay protocol. Results obtained in such a manner are alone comparable internationally or from one study to another. The present communication describes the results of our determination of LH, FSH, and PRL using the WHO protocol and matched reagents in healthy women.

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Material and Methods

Thirty healthy and fertile females of reproductive age group i.e. between 22-35 years were selected for this study. The

TABLE I *Demographic Data of Thirty Patients*

No. of patients	Age	Age of Menarche	Age of Marriage	Social Economic Status	Condition of Cervix	Position of Uterus	Size of Uterus	Tubal Patency	
1.	21	12	13	Medium	Healthy	Retroverted	Normal	Bilaterally	Patent
2.	20	13	14	Medium	Healthy	Anteverted	" "	" "	"
3.	22	13	17	Low	Mild Cervicitis	Anteverted	" "	" "	"
4.	24	12	18	Low	Healthy	Anteverted	" "	" "	"
5.	25	13	21	Medium	Mild Cervicitis	Anteverted	" "	" "	"
6.	25	13	19	Medium	Healthy	Retroverted	" "	" "	"
7.	29	14	18	Low	Healthy	Retroverted	" "	" "	"
8.	27	14	17	Low	Healthy	Anteverted	" "	" "	"
9.	30	13	15	Low	Healthy	Anteverted	" "	" "	"
10.	35	14	16	Medium	Mild Cervicitis	Anteverted	" "	" "	"
11.	25	13	17	Medium	Healthy	Retroverted	" "	" "	"
12.	32	14	20	Medium	Healthy	Retroverted	" "	" "	"
13.	26	13	19	Medium	Healthy	Anteverted	" "	" "	"
14.	29	12	21	Low	Mild Cervicitis	Anteverted	" "	" "	"
15.	34	14	20	Medium	Healthy	Retroverted	" "	" "	"
16.	25	13	17	Medium	Healthy	Retroverted	" "	" "	"
17.	31	13	18	Medium	Healthy	Anteverted	" "	" "	"
18.	18	13	15	Low	Healthy	Retroverted	" "	" "	"
19.	27	14	17	Medium	Healthy	Anteverted	" "	" "	"
20.	17	13	15	Low	Mild Cervicitis	Retroverted	" "	" "	"
21.	20	12	14	Medium	Healthy	Anteverted	" "	" "	"
22.	28	13	17	Medium	Healthy	Anteverted	" "	" "	"
23.	26	14	16	Medium	Mild Cervicitis	Anteverted	" "	" "	"
24.	20	12	15	Low	Healthy	Retroverted	" "	" "	"
25.	35	14	16	Low	Healthy	Anteverted	" "	" "	"
26.	24	14	18	Medium	Healthy	Anteverted	" "	" "	"
27.	25	13	17	Low	Healthy	Anteverted	" "	" "	"
28.	23	14	17	Low	Healthy	Retroverted	" "	" "	"
29.	20	15	16	Medium	Healthy	Retroverted	" "	" "	"
30.	18	13	15	Medium	Healthy				

material for the study was drawn from the Department of Gynaecology and Obstetrics and the radioimmunoassay was performed in the Nuclear Medicine and Radioimmunoassay Unit of the Post Graduate Department of Medicine, S.N. Medical College, Agra.

Table I lists the demographic data of these 30 subjects:—

The cases selected were subjected to detailed history, general examination and gynaecological examination. Blood samples were taken in every individual case during proliferative, ovulatory and secretory phases of the menstrual cycle as determined by the study of cervical mucus and were centrifuged immediately after one hour to separate the serum from blood and was stored at -20°C until assayed. Daily blood samples unfortunately could not be obtained.

FSH, LH and Prolactin were measured in each patient during all the three phases of the menstrual cycle by radioimmunassay using WHO Matched reagents for radioimmnassay. FSH, LH and PRL labelled with 125 I at the Swiss Federal Institute for Reactor Research, Wurenlinger Switzerland by lactoperoxidase method were used. The antisera for FSH and LH used were provided by Prof. W. Butt (Birmingham, U.K.) and for PRL by Dr. A. F. Parlow (Los Angeles, U.S.A.) respectively to the World Health Organisation. The antisera for FSH, LH and PRL were used at a final dilution of 1:2,800,000, 1:1,750,000 and 1:400,000 respectively. The FSH, LH and PRL standards having a standard code no. 69/104, 68/40 and 75/504 respectively were used. Separation of bound and free hormone was performed using a second antibody donkey Antirabbit gamma globulin at a dilution of 1:40.

A typical 100 tube assay design for FSH, LH and PRL is shown in Table II.

The tubes containing the double antibody precipitate were counted and interpolated from the standard curve parameters plotted on the basis of known hormone concentration.

TABLE II

Protocol of RIA of FSH, LH and PRL

Description	Test tube number	Tracer (ul)	Standard Solution (ul)	Anti-serum (ul)	Assay buffer (ul)		Second Anti-body	
Total Count Tubes	99-100	100	—	—	—	Incubation at 4°C for 48 hours.	—	Incubation at 4°C for 18-20 hours. Then centrifugation for 45 minutes at 1500 g and then counting.
Standards	4-6	100	100	100	400		100	
	7-9							
	10-12							
	13-15							
	16-18							
	19-21							
Unknown Samples	25-98	100	100	100	400		100	
Non-specific	32-24	100	—	—	600		100	
Nonspecific binding tubes (NSB)								
Zero Standard	1-3	100	—	100	500		100	

Observations and Results

Figs. 1, 2 and 3 are the representative standard curves for each of the LH, FSH and PRL assays.

TABLE III

ASSAYS	NSB	B°	Mean C.V.
F S H	3.6%	19.1%	1.6
L H	50%	13.2%	3.6
P R L	8.1%	31.0%	2.5

Table III describes the NSB, B° and Mean coefficient of variation of the assays for each of these hormones.

Table IV describes the mean levels of the three hormones observed.

Discussion

LH, FSH and PRL levels from normal women have been estimated using WHO protocol in the present study.

Tables V and VI compare the values

Fig. No. 2

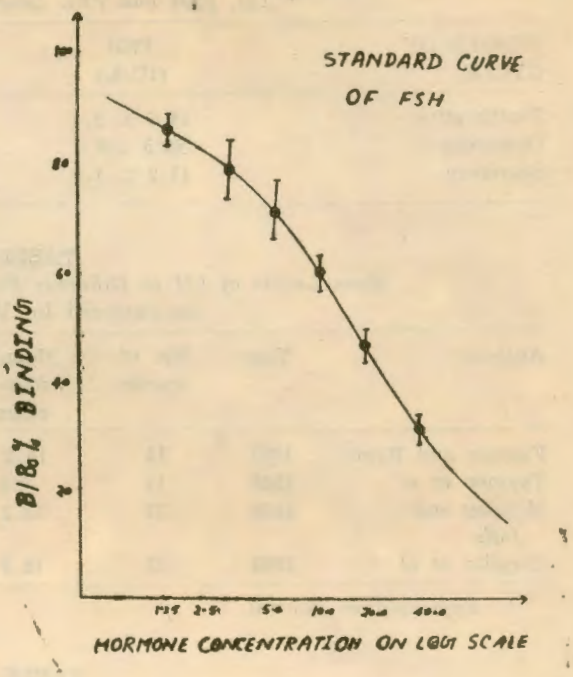

Fig. No. 1

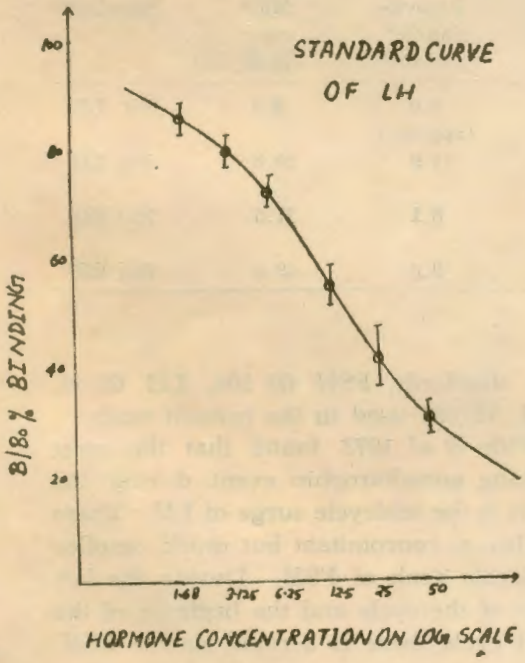

Fig. No. 3

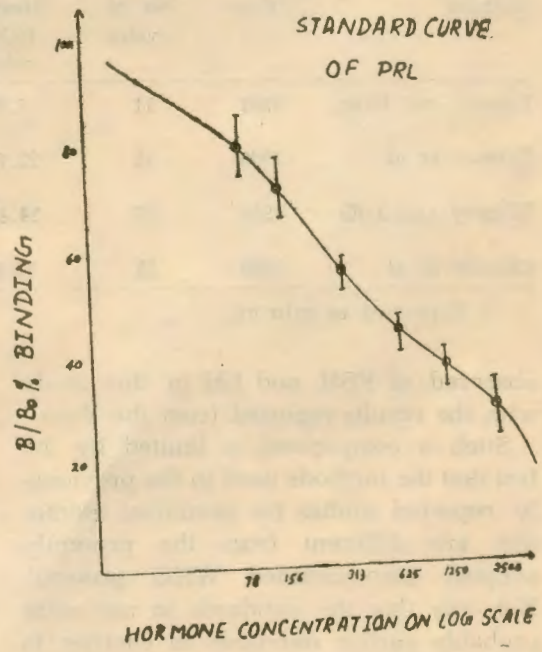

TABLE IV
LH, FSH and PRL Levels in Normal Females

PHASES OF CYCLE	FSH (IU/L)	LH (IU/L)	PRL (mU/L)
Proliferative	14.6 ± 2.4	14.7 ± 2.3	176.1 ± 42.8
Ovulatory	26.5 ±3.6	79.6 ± 6.9	230.6 ± 62.3
Secretory	11.2 ± 1.9	13.5 ± 2.0	196.4 ± 58.4

TABLE V
Mean Levels of LH at Different Phases of Normal Menstrual Cycle as observed by Various Authors

Authors	Year	No. of cycles	Mean* follicular	Mean* luteal	Mid* cycle peak	Standard
Faiman and Ryan	1967	11	16.2	15.0	45.2	Pit. LH
Taymer *et al*	1968	11	7.3	6.0	29.3	Pit. LH
Midgley and Jaffe	1968	37	13.2	12.0	47.8	2nd IRP
Cargille *et al*	1969	21	18.2	13.7	76.3	2nd IRP

* Expressed as mlu/ml.

TABLE VI
Mean Levels of FSH at Different Phases of Normal Menstrual Cycle as observed by Various Workers

Authors	Year	No. of cycles	Mean* follicular	Preovulatory* nadir	Mid* cycle peak	Standard
Faiman and Ryan	1967	11	7.6	2.0 (approx.)	8.0	Pit. LH
Taymer *et al*	1968	11	22.7	14.9	20.8	Pit. LH
Midgley and Jaffe	1968	37	14.4	8.1	21.5	2nd IRP
Cargille *et al*	1969	21	15.6	9.0	19.6	2nd IRP

* Expressed as mlu/ml.

observed of FSH and LH in this study with the results reported from the West.

Such a comparison is limited by the fact that the methods used in the previously reported studies for hormonal estimation are different from the presently adopted recommended WHO protocol. Not only that the standards in use were probably earlier standards in contrast to the standards, FSH 69/104, LH 68/40, PRL 75/504 used in the present study.

Wide *et al* 1973 found that the most striking gonadotrophin event during the cycle is the midcycle surge of LH. There is also a concomitant but much smaller midcycle peak of FSH. During the last days of the cycle and the begining of the next cycle there is a rapid rise of FSH.

At this time the increase in the LH level is less pronounced. Ovulation is likely to occur 24 to 48 hours after the start of the LH surge.

In the present study the magnitude of the ovulatory peaks of LH and to a lesser extent of FSH represent an increament of 66 and 15 mIu/ml, over the minimal values seen in the secretory phase, 13.5 and 11.2 mIu/ml. It is possible that the so called ovulatory value may have missed the true hormonal peak by an interval of 12 to 36 hours, since daily blood sampling as is ideally desirable could not be performed. In the present study, the preovulatory nadir of FSH could not be determined.

Although most reports have failed to demonstrate any significant change in the pattern of prolactin secretion throughout the menstrual cycle, some investigators (Robin and Vekeman, 1972) have found that, its level is highest at mid-cycle. During luteal phase they fluctuate around a higher level than during the follicular phase. It is not yet elucidated whether these variations during the menstrual cycle are of physiological significance. These changes have been related to the circulating levels of oestrogens during the menstural cycle. A greater prolactin response to TRH at midcycle, compared to that seen in the follicular phase, suggests that pituitary store of prolactin may be increased near the time of ovulation (Raymond, *et al* 1976).

In gonadotrophin estimation interlaboratory variability arises because of number of factors. Firstly, hormone free serum has to be included in the standard tubes to ensure a comparable milieu to the test serum samples (Ekins, 1974) and this has been obtained in various ways—hypopituitary patients (Jacobs, 1969), heterologous sera (Odell *et al*, 1968) Charcoal stripping (Albano 1972) and physiological manipulations such as sera from spontaneous or T3 induced thyrotoxicosis for TSH assay from oestrogen treated males for gonadotrophin assay, none of which are free from criticism. Secondly, use of different antisera may change the results despite otherwise identical RIA protocols as has been observed for FSH (Cargille *et al* 1968, Amin and Hunter 1970 and Franchimont, 1971). Thirdly, iodination damage of the labelled antigen altering its immunoreactivity is a third source of variability as evidenced by different TSH values obtained using assays with different sephadex Iodination fractions (Lawton, quoted by Jacobs and Lawton 1974); labelled antigen eluates may contain subunits with different immunoreactivity as reported for rat LH by Nabarro (1973).

In view of the above the only method of avoiding such interlaboratory variability is the use of Matched reagents as advocated by Ekins *et al* WHO matched reagent scheme and external quality control is a step in this direction. It is hoped that even when a national quality control and Matched reagents scheme is set up in India, it will be possible to interdigitate it with an international scheme as suggested earlier (Hazra *et al* 1977) through a system of primary and secondary standards, so as to ensure intercountry comparability. It is also recommended that all subsequent studies in this country involving gonadotrophine measurement should also utilize the same protocol so that meaningful results are obtained.

Summary

Using the WHO protocol and Matched reagents and a double antibody separation system norms for the FSH, LH and

PRL have been determined in 30 normal Indian women. The importance of using a standardized assay protocol and Matched reagents for the hormones which has not been completely characterized chemically has been emphasized.

Acknowledgement

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