HORMONAL PROFILE IN CASES OF FEMALE INFERTILITY-A STUDY BY RADIOIMMUNOASSAY

by

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Introduction

The estimation of Follicular stimulating hormone (FSH), Luteinizing hormone (LH) and prolactin have been found useful in the diagnosis of infertility and its management. Detection of presence or absence of ovulation and its timing in the menstrual cycle is the corner stone in the evaluation of an infertile woman.

Basal body temperature which coincides with ovulation is one of the traditional indications of ovulation but it is often unfeasible and unreliable. LH surge fairly coincides with the rise in BBT and may prove helpful in documenting ovulation precisely in infertile menstruating women (Friedman, 1973). Timing of endometrial biopsy for evaluation of luteal functions can be done accurately in relation to ovulation which coincides with the LH peak in serum. LH surge in serum could be helpful in predetermination of ovulation and can easily be measured by RIA (Gistch and Spena; 1973 and Abdol and Moon 1976).

Further, it is only lately that we have begun to understand the role of prolactin in normal and abnormal ovarian function and its relation to anovulatory cycles. In view of the problems encountered in the diagnosis and treatment of infertility and the recognition of increasing importance of endocrine and pituitary factors, especially prolactin, it was considered relevant to carry out the present study.

Material and Methods

Thirty females with established sterility were taken in the study along with 10 fertile and healthy females who served as controls. Out of 30 sterility patients, 4 had secondary amenorrhoea and 2 had primary amenorrhoea. The material for the study was made available from the Department of Obst. & Gynaecology and the RIA was performed in the Nuclear Medicine and RIA Unit, P.G. Deptt. of Medicine, S.N. Medical College, Agra.

Table I lists the demographic data of these 40 subjects.

The cases selected were subjected to detailed history taking, general examination

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TABLE I

S. No.	Age	Diagnosis	Age at Menar-che	Age at Marri-age	Type of Cycle	S/E Status	Cervix	Uterus		Fallopian Tube
								Positiion	Size	
1.	18	Pri. St.	13	13	Ovul.	II	Healthy	Retro.	Normal	Patent
2.	24	Pri. St.	12	18	Anovul.	III	Pinhole. OS	Ante.	Normal	Patent
3.	24	Pri. St.	14	17	Ovul.	III	Healthy	Retro.	Hypoplastic	Patent
4.	31	Pri. St.	15	19	Ovul.	I	Pinhoel. OS	Ante.	Normal	Patent
5.	23	Pri. St.	14	19	Anovul.	II	Healthy	Retro.	Normal	Patent
6.	17	Pri. St.	15	13	Anovul.	II	Erosion	Retro.	Normal	Blocked
7.	29	Sec. Amen.	14	21	Ovul.	I	Pinhole. OS	Ante.	Hypoplastic	Patent
8.	18	Pri. St.	14	14	Ovul.	III	Cervicitis	Retro.	Hypoplastic	Patent
9.	26	Pri. St.	14	23	Anovul.	IV	Erosion	Retro.	Normal	Patent
10.	20	Pri. St.	15	16	Ovul.	II	Healthy	Retro.	Normal	Blocked
11.	19	Pri. St.	14	13	Anovul.	III	Pinhole. OS	Ante.	Normal	Patent
12.	20	Pri. St.	17	16	Ovul.	III	Erosion	Retro.	Normal	Patent
13.	28	Pri. St.	14	22	Ovul.	II	Cervicitis	Ante.	Hypoplastic	Patent
14.	25	Pri. St.	14	15	Ovul.	IV	Pinhole. OS	Ante.	Normal	Patent
15.	25	Pri. St.	16	17	Ovul.	II	Cervicitis	Retro.	Normal	Patent
16.	26	Pri. St.	14	15	Anovul.	III	Pinhole. OS	Ante.	Hypoplastic	Patent
17.	27	Pri. St.	13	19	Ovul.	II	Pinhole. OS	Retro.	Normal	Patent
18.	27	Sec. Amen.	17	20	Anovul.	II	Erosion	Ante.	Normal	Blocked
19.	29	Sec. St.	14	20	Ovul.	III	Cervicitis	Retro.	Hypoplastic	Patent
20.	23	Sec. St.	13	16	Ovul.	IV	Erosion	Retro.	Normal	Patent
21.	24	Sec. St.	16	16	Ovul.	II	Cervicitis	Retro.	Normal	Patent
22.	29	Pri. St.	15	15	Anovul.	IV	Healthy	Ante.	Normal	Patent
23.	24	Pri. St.	13	18	Ovul.	II	Healthy	Ante.	Normal	Patent
24.	25	Pri. Amen.	14	14	Ovul.	IV	Healthy	Retro.	Hypoplastic	Patent
25.	32	Pri. St.	14	20	Ovul.	III	Erosion	Retro.	Normal	Patent
26.	35	Pri. St.	14	18	Anovul.	IV	Healthy	Erect.	Normal	Patent
27.	25	Pri. Amen.	14	20	Anovul.	II	Pinhole. OS	Retro.	Normal	Patent
28.	26	Sec. Amen.	12	19	Ovul.	II	Pinhole. OS	Ante.	Hypoplastic	Patent
29.	28	Sec. Amen.	13	16	Ovul.	III	Pinhole. OS	Retro.	Normal	Patent
30.	18	Pri. Amen.	14	14	Anovul.	I	Pinhole. OS	Retro.	Normal	Patent

S. No.	Age	Diagnosis	Age at Menarche	Age at Marriage	Type of Cycle	S/E Status	Cervix	Uterus		Fallopian Tube
								Position	Size	
31.	22	Control	12	16	Ovul.	III	Cervicitis	Ante.	Normal	Patent
32.	31	Control	13	14	Ovul.	I	Healthy	Retro.	Normal	Patent
33.	24	Control	14	17	Ovul.	III	Cervicitis	Retro.	Normal	Patent
34.	29	Control	14	15	Ovul.	III	Cervicitis	Retro.	Normal	Patent
35.	24	Control	13	20	Ovul.	II	Healthy	Ante.	Normal	Patent
36.	28	Control	14	19	Ovul.	I	Healthy	Ante.	Normal	Patent
37.	24	Control	14	19	Ovul.	I	Healthy	Ante.	Normal	Patent
38.	27	Control	13	15	Ovul.	II	Cervicitis	Retro.	Normal	Patent
39.	22	Control	13	20	Ovul.	I	Healthy	Ante.	Normal	Patent
40.	25	Control	13	24	Ovul.	II	Healthy	Ante.	Normal	Patent

Pri. St.: Primary Sterility, Sec. St.: Secondary Sterility, Pri. Amen.: Primary Amenorrhoea, Ante.: Anteverted Sec. Amen.: Secondary Amenorrhoea, Ovul.: Ovulatory, Anovul.: Anovulatory Retro.: Retroverted.

and gynaecological examination. Blood samples were taken during proliferative, ovulatory and secretory phases of the menstrual cycle as determined by the study of cervical mucus. Blood samples were centrifuged immediately after one hour to separate the serum from blood and stored at -20°C, until assayed.

FSH, LH and prolactin were measured in each patient during all the three phases of the menstrual cycle by RIA using WHO Matched reagents for RIA. FSH, LH and prolactin labelled with I^{125} at the Swiss federal Institute for reactor research, Wurelinger, Switzerland by lactoperoxidase method were used. The antisera used for FSH and LH were provided by Prof. W. Butt (Birmingham, U.K.) and for prolactin by Dr. A. F. Parlow (Los Angeles, U.S.A.) respectively to the World Health Organization. The antisera for FSH, LH and prolactin used were at a final dilution of 1:28,00,000, 1:1,750,000, and 1:400,000 respectively. The FSH, LH and prolactin 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 anti-rabbit gamma globulin at a final dilution of 1:40.

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

The tubes containing the double antibody precipitate were counted. The unknown samples were estimated by interpolation from the standard curve parameters plotted on the basis of known hormone concentration.

Observations and Results

Figures 1, 2 and 3 are the representative standard curves for LH, FSH and prolactin.

Table III describes the mean levels of

TABLE II

Protocol of Radioimmunoassay of FSH, LH and Prolactin

Test tube number	1-3 53-55	4-21	22-24 56-38	25-28 59-62	29-52 63-98	99-100
Description	Zero	Standard	NSB	INT. QC.	Un-knowns	Tc-TUBES
Tracer	Standard *100	100	100	100	100	100
Standard solution	—	100	—	—	—	—
Unknown and Int. Q.C.	—	—	—	100	100	—
Antiserum	100	100	—	100	100	—
Assay buffer	500	400	600	400	400	—
	Incubation at 4°C for 48 Hours					
Second antibody	100	100	100	100	100	—

Incubation at 4°C for 20 Hours. Centifugation at 4°C for 45 Mts. at 1500 g. and then Counting.

* All Volumes Are In Microlitres.

Fig. No. 1

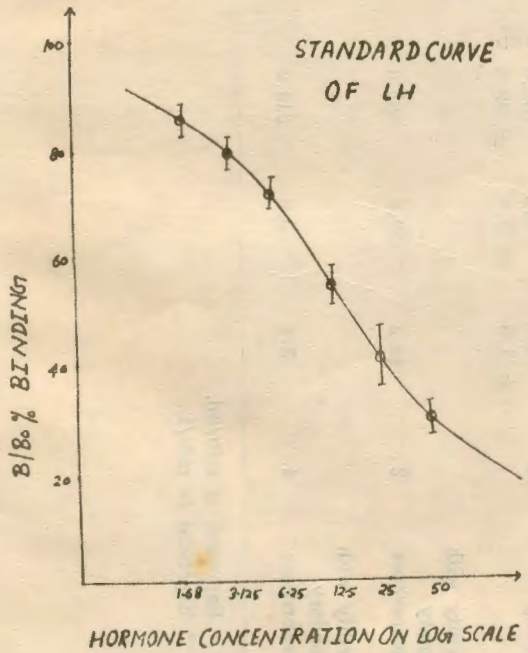


Fig. No. 2

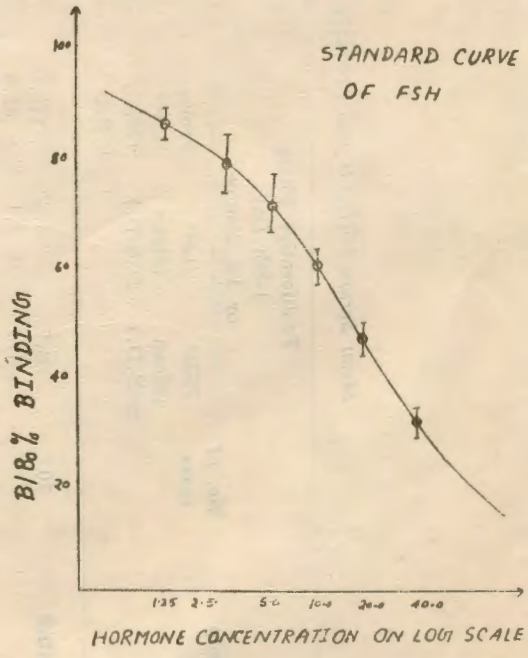


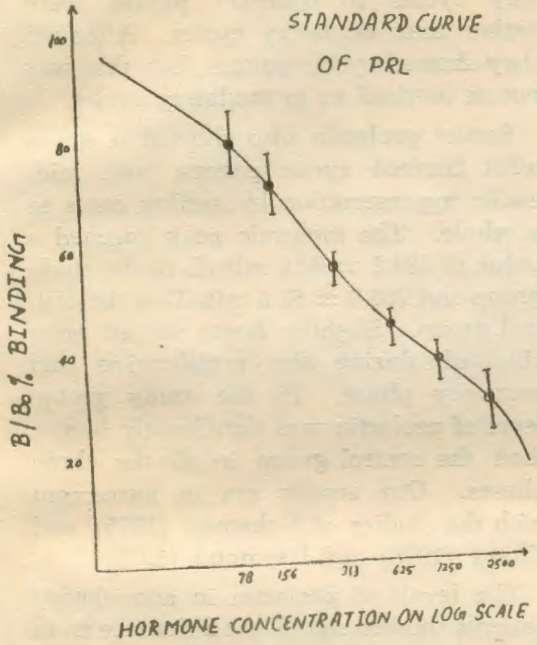
TABLE III

Mean Serum FSH, LH and Prolactin Concentrations in Control and Sterility Cases

Group	No. of cases	Proliferative Phase 1-8th Day or 1st Sample			Ovulatory Phase 9-16th Day or 2nd Sample			Secretory Phase 17-28/35 Days or 3rd Sample		
		FSH* (Mean ± S.D.)	LH* (Mean ± S.D.)	Prolac-tin** (Mean ± S.D.)	FSH* (Mean ± S.D.)	LH (Mean ± S.D.)	Pro-lactin (Mean ± S.D.)	FSH (Mean ± S.D.)	LH (Mean ± S.D.)	Pro-lactin (Mean ± S.D.)
Control	10	6.7 ± 1.4	4.6 ± 1.1	176.1 ± 42.8	24.6 ± 5.6	44.1 ± 12.4	230.6 ± 62.5	11.2 ± 3.2	3.3 ± 0.9	196.4 ± 58.4
Sterility	24	4.8 ± 1.5	9.6 ± 3.4	278.6 ± 89.9	33.7 ± 9.8	74.1 ± 15.0	284.3 ± 92.6	5.9 ± 1.5	6.3 ± 1.5	238.4 ± 78.8
Sterility with Primary Amenorrhoea	2	40.2	120.6	251.3	48.8	108.4	176.7	44.6	118.4	214.0
Sterility with Secondary Amenorrhoea	4	3.4	3.0	318.0	3.5	3.8	302.0	3.1	4.8	310.0

* Expressed in mIu/ml.
** Expressed in mIu/L.

Fig. No. 3



serum FSH, LH and prolactin in control and sterility cases.

Table IV represents the mean FSH, LH and prolactin levels in ovulatory and anovulatory patients during different phases of the menstrual cycle.

Discussion

In the present study, out of 30 sterility cases 18 were ovulatory and 12 were anovulatory. Out of 18 ovulatory cases 4 had secondary amenorrhoea and 2 anovulatory cases had primary amenorrhoea. Out of 18 ovulatory patients, 3 had tubal blockage, 1 had acutely anteverted uterus and 14 had unexplained infertility.

In the control group, serum LH levels showed a fluctuating pattern with a single sharp and significant LH peak (44.1 ± 12.4 mIu/ml) during the midcycle, coinciding with ovulation and relatively lower values in the proliferative and secretory phases being 4.6 ± 1.1 mIu/ml and 3.3 ± 0.9 mIu/ml respectively. The difference in serum LH concentration during the different phases of the menstrual cycle was statistically significant.

In the sterility group as a whole, similar phasic variation was observed in the LH

TABLE IV

Mean FSH, LH and Prolactin Concentrations in Serum During Different Phases of Ovulatory and Anovulatory Cycles in Cases of Sterility

Phases of cycle	Ovulatory Cycle (18)			Anovulatory Cycle (12)		
	FSH* (Mean ± S.D.)	LH* (Mean ± S.D.)	Prolactin** (Mean ± S.D.)	FSH* (Mean ± S.D.)	LH (Mean ± S.D.)	Prolactin (Mean ± S.D.)
Proliferative	3.77 ± 0.83	6.57 ± 2.33	258.91 ± 77.39	11.8 ± 14.2	30.45 ± 42.33	321.7 ± 62.8
Midcycle	31.42 ± 17.04	65.66 ± 35.44	259.75 ± 66.9	29.5 ± 11.7	69.16 ± 21.08	326.9 ± 64.86
Secretory	4.55 ± 1.09	5.27 ± 1.29	225.14 ± 69.3	12.7 ± 15.2	26.04 ± 43.77	258.33 ± 30.94

* Expressed in mIu/ml.
** Expressed in mIu/L.

pattern. Serum LH was 9.6 ± 3.4 mIu/ml in the proliferative, 74.1 ± 15.0 mIu/ml in the ovulatory and 6.3 ± 1.5 mIu/ml in the secretory phase respectively. LH level was thus found raised in all the three phases of the menstrual cycle. Our results are in good agreement with other investigators namely Midgley and Jaffe (1966), Rosselin and Dolais (1967), Taymor *et al* (1968), Cargille *et al* (1969), Boon *et al* (1972), Thorneycroft *et al* (1974) and Dodson *et al* (1975).

The LH levels showed a cyclic pattern in ovulatory cycles with a well marked midcycle peak (65.66 ± 35.44 mIu/ml). In contrast comparatively higher LH values were recorded in anovulatory patients without a well defined cyclicity. Elevated LH levels in anovulatory cycles were also reported by Abraham (1972) and Root *et al* (1972).

FSH also showed a cyclic pattern during the different phases of the menstrual cycle. Although FSH levels showed large individual variations, serum FSH showed a distinct midcycle peak, the values being 33.7 ± 9.8 mIu/ml and 24.6 ± 5.6 mIu/ml, in the study and control groups respectively. The FSH peak coincided with the LH peak; similar values were reported in the proliferative and secretory phases of the cycle in control as well as in sterility group. The finding of cyclic pattern of FSH is in close consonance with that reported by Midgley and Jaffe (1968), Taymor *et al* (1968), Abraham *et al* (1972), Wide *et al* (1973) and Thorneycroft (1974).

Serum FSH values showed variations from phase to phase in ovulatory cycles with definite midcycle peak coinciding with the LH surge. The serum FSH being 3.77 ± 0.837 mIu/ml during the proliferative phase, rose significantly to 31.4 ± 17.04 mIu/ml during the midcycle and then declined to 4.59 ± 1.09 mIu/ml. The FSH levels in patients with anovulatory cycles in different phases were higher than ovulatory cycles. Although they showed cyclic pattern but this was not as marked as in ovulatory cycles.

Serum prolactin also showed a somewhat blunted cyclic pattern with midcyclic hypersecretion in sterility cases as a whole. The midcycle peak reached a value of 284.2 ± 92.6 mIu/L in the study group and 230.6 ± 62.3 mIu/L in the control group. Slightly lower values were obtained during the proliferative and secretory phase. In the study group, level of prolactin was significantly higher than the control group in all the three phases. Our results are in agreement with the studies of Vekeman (1975) and Chang (1978) and Raymond (1976).

The levels of prolactin in anovulatory patients showed significant difference from ovulatory patients. In anovulatory cycles the levels of prolactin were constantly raised as compared to the ovulatory cycles. The values being 321.7 ± 62.8 mIu/L, 326.9 ± 64.86 mIu/L and 258.3 ± 30.94 mIu/L during the proliferative, ovulatory and secretory phases. Our findings are in agreement with the observations of Besser and Thorner (1977), that hyperprolactinaemia appears to be common in infertile patients.

In the 2 cases of primary amenorrhoea studied the LH levels were found consistently raised, of the three samples analysed, the levels ranged from 108.4 mIu/ml to 120.6 mIu/ml. FSH was also raised in these cases ranging from 40.2 mIu/ml to 48.8 mIu/ml. Serum prolactin however was well within the normal range of 176.7 mIu/L to 251.3 mIu/L. High FSH and LH values indicated an absence of ovarian negative feed back on the hypothalamopituitary unit, suggesting

either gonadal dysgenesis or ovarian failure as a cause of the primary amenorrhoea.

Of the cases in the series who presented with secondary amenorrhoea, low levels of both FSH and LH were recorded, being 3.1 mIu/ml to 3.4 mIu/ml and 3.0 to 4.8 mIu/ml respectively. Since these cases were not in the age group for menopause they were originally suspected to be cases of hypothalamic amenorrhoea rather than primary ovarian failure. Serum prolactin was found slightly elevated in these cases ranging from 302 mIu/L to 318 mIu/L suggesting that hyperprolactinaemia may have a small contribution to the secondary amenorrhoea.

Summary

Out of 30 sterility cases, 12 cases had anovulatory cycle with primary ovarian failure with higher levels of serum FSH and LH and Prolactin. Out of the 18 ovulatory patients, 3 cases with blocked tubes and one case with acute anteversion of the uterus had almost normal levels of FSH, LH and Prolactin. 14 cases had no explainable cause of infertility.

In the cases of sterility associated with primary amenorrhoea, levels of FSH and LH were consistently raised being 44.6 mIu/ml and 118.4 mIu/ml, respectively suggesting either gonadal dysgenesis or ovarian failure. Serum prolactin levels in this group was within the normal range. In contrast to this the cases of secondary amenorrhoea had low FSH and LH levels being 3.1 mIu/ml and 3.4 mIu/ml respectively and these are suspected to be cases of hypothalamic amenorrhoea.

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