USEFULNESS OF ESTRONE/CREATININE RATIO OF OVERNIGHT 12 HOUR URINE SAMPLES FOR MONITORING OVARIAN FUNCTION

by

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Introduction

It is generally accepted that chemical quantitation of urinary total estrogens or its individual fractions is a reliable index of ovarian function during a menstrual cycle. This parameter is largely used for diagnosing the degree of ovarian function in menstrual dysfunctions and infertility. Daily or frequent assessment of estrogens becomes of paramount importance in the monitoring of a patient with ovulation inducing drugs.

Although many laboratory obstacles have been overcome with the development of Brown's short method for estrone or total estrogens, the main disadvantage still lies in the problem of collecting 24 hours urine specimens. This is a tedious procedure and very much dependent on the motivation, honesty and reliability of patients.

Several investigators have used the constancy of the creatinine output to check the completeness of the 24 hours urine samples. (Wray and Russell, 1960; Welshman *et al*, 1969 and Shelley *et al*, 1970). Shelley and co-workers (1970) as well as Osofsky and colleagues (1970) have demonstrated that the curve for estrogen/creatinine ratio for single voided specimens throughout pregnancy closely approximate in shape and direction the curve for 24 hours urinary estriol assays, the correlation coefficient being 0.930. The expression of these results as a ratio offsets the problem of diurnal variation in steroid excretion and the problems associated with renal function.

Luther and co-workers (1973) have also reported that estrogen/creatinine ratio performed on single morning specimens during pregnancy correlated well with the 24 hours urine determinations (correlation coefficient being 0.78).

In this study comparative determinations of estrone and creatinine were made on overnight 12 hour urine samples in relation to 24 hour specimens on the same day of the cycle. The estrone/creatinine (E/C) ratios of these paired samples were compared to each other and also to 24 hour values in order to evaluate the usefulness of overnight samples.

To our knowledge such studies have not been reported in the non-pregnant urine samples probably because of the lower estrogen excretion levels. Nonetheless the practical advantages of an overnight collection of urine against 24 hour collection remains undisputed.

Material and Methods

One hundred and eighty urine samples were collected from 27 normally menstruating women for 33 cycles during

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different days of the menstrual cycle. Routinely these patients were collecting daily or on alternate day 24 hour urine specimens for estrogen and creatinine estimations. For this comparative study the urine collection routine was modified so that the overnight 12 hour specimen was collected separately and a small aliquot was removed from this specimen for estrone and creatinine determinations and the remaining urine was mixed with the previous day's sample (12 hours collection) and treated as a 24 hour specimen.

A total of 103 paired specimens were collected and evaluated. The creatinine value of all these samples was within the normal range accepted by this laboratory (0.8-1.3 gm/24 hours).

Estrone values (μg/24 hours) were determined by the Brown *et al* (1968) modified short method. The estrone values are considered a satisfactory index of the total estrogen excretion and of ovarian function during a menstrual cycle. The creatinine determinations were measured by a method based on Jaffe's reaction (1945). The results were expressed as gm/24 hours.

Results

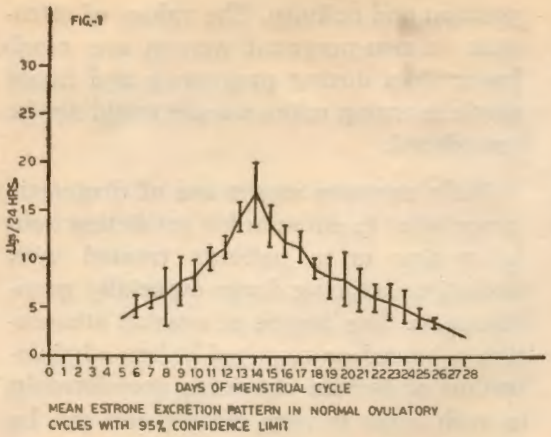
Table I describes the mean values of 24 hour urinary estrone excretion and 95% confidence limits on different days of the normal ovulatory cycles studied.

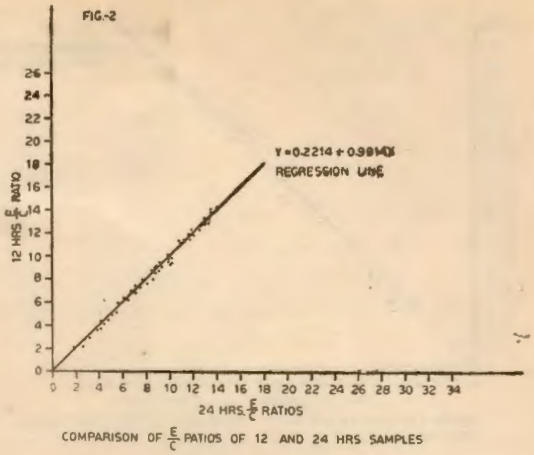
Fig. 1 shows a composite curve based on the mean of the values obtained on different days of the cycle. The levels are low during the first week, the mean on day 7 being 5.6 μg/24 hours. They tend to rise sharply during the second week reaching a peak (mean 16.68 μg/24 hours) around

TABLE I

Urinary Excretion of Estrone in ug/24 Hours at Different Days of Menstrual Cycle

Day	Mean	S.D.	S.E.	95% Confidence Limit
				Mean ± 2. S.E.
6	5.06	1.0	0.58	6.22– 3.9
7	5.6	0.88	0.39	6.39– 4.8
8	6.1	2.1	1.48	9.06– 3.14
9	7.7	3.51	1.24	10.19– 5.21
10	8.3	2.31	0.77	9.84– 6.76
11	10.0	1.99	0.61	11.22– 8.78
12	11.2	2.13	0.59	12.38–10.01
13	14.43	2.40	0.76	15.95–12.91
14	16.68	3.67	1.64	19.96–13.4
15	13.32	2.98	1.06	15.43–11.2
16	11.7	2.97	0.79	13.39–10.15
17	11.2	2.36	0.75	12.71– 9.68
18	8.9	1.89	0.51	9.91– 7.89
19	8.1	3.34	1.07	10.24– 5.96
20	7.97	0.39	1.38	10.73– 5.21
21	6.87	3.03	1.07	9.01– 4.72
22	6.10	1.74	0.71	7.44– 4.6
23	5.6	2.02	0.92	7.43– 3.77
24	5.1	1.58	0.79	6.68– 3.52
25	4.2	1.36	0.68	5.06– 2.84
26	3.7	0.36	0.21	4.12– 3.28





mid-cycle day 14-day 15. Thereafter, the levels drop with no definite rise during the mid-luteal phase as reported by Brown *et al* (1968). The mean value on day 21 being 6.87 µg/24 hours.

Fig. 2 shows the relationship between the E/C ratio of 24 hours to the E/C ratio of overnight 12 hours specimens. Most of the points seem to overlap on each other. The correlation coefficient is + 0.9719 ($P < 0.001$) and regression line is fitted according to the equation $Y = 0.2214 + 0.9914\ X$. The slope of the curve appears to bear a constant relationship between 12 hours and 24 hours specimens all through the menstrual cycle which is ideal. Shelly and co-workers (1970) have also shown excellent correlation between 24 hours E/C ratio to 12 hours E/C ratio with correlation coefficient of 0.996 in pregnancy samples.

Case example of constant relationship between E/C ratio of 12 hours against 24 hours in one subject throughout the menstrual cycle is shown in Fig. 3.

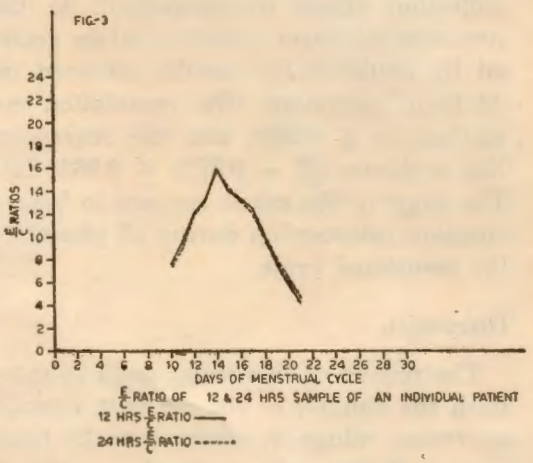


Fig. 4 shows the close relation between 24 hours estrone value to E/C ratio of 24 hours along with creatinine excretion values during the different days of a menstrual cycle in one subject.

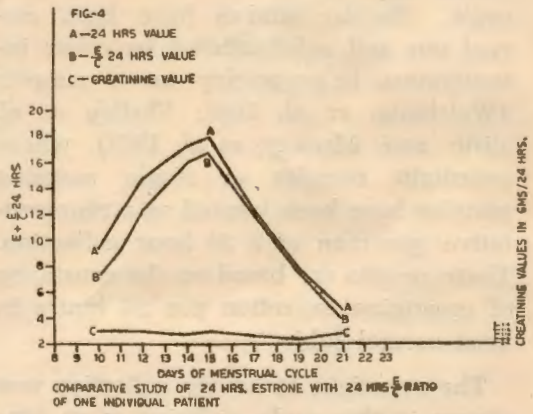
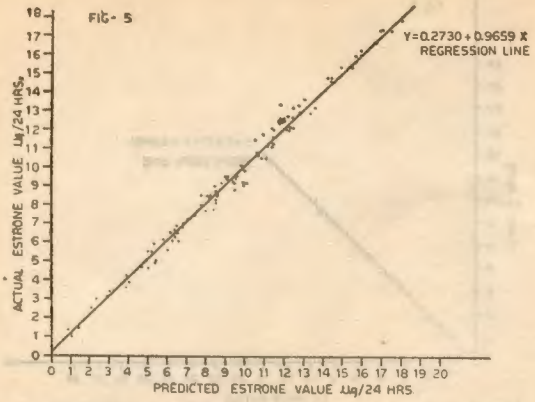


Fig. 5 graphically demonstrates the close correlation of the actual 24 hours



24 HRS ESTRONE EXCRETION IN COMPARISON TO PREDICTED 24 HRS. EXCRETION DERIVED BY DOUBLING THE OVERNIGHT 12 HRS SAMPLE

collection values in comparison to the predicted 24 hours excretion values derived by doubling the results obtained on 12 hour specimens. The correlation coefficient is + 0.9481 and the regression line is shown (Y = 0.2730 + 0.9659 X). The slope of the curve appears to bear a constant relationship during all phases of the menstrual cycle.

Discussion

The results of this study seem to establish the validity of the 12 hour estrone excretion values in contrast to 24 hour values during the normal menstrual cycle. Similar studies have been carried out and substantiated by other investigators in pregnancy urine samples (Welshman *et al*, 1969; *Shelley et al*, 1970; and Mackay, *et al*, 1968) where overnight samples or single morning samples have been treated as a representative specimen of a 24 hour collection. These results are based on the constancy of creatinine excretion per 24 hours in most normal subjects.

The overnight 12 hourly collection was chosen in this study as being more convenient and possibly more consistent since renal clearance may be affected by position and activity. The values of estrogens in non-pregnant women are much lower than during pregnancy and hence single morning urine sample could not be considered.

Daily estrogen assays are of diagnostic importance in patients for predicting ovulation time or in patients treated with ovulation inducing drugs especially gonadotropins. The degree of ovarian stimulation achieved or required before administration of human chorionic gonadotropin in such cases is very critical and can be gauged with a fair degree of accuracy provided daily assays are quickly available to the clinician. Thus the dosages of Human Menopausal Gonadotropin and timing of Human Chorionic Gonadotropin administration is to great extent dependation the evaluation of these assays by the clinician depending on his past experience with these assays.

The collection of 12 hour urine samples instead of a 24 hour sample would eliminate a lot of physical and mental inconvenience to such patients.

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