CIRCADIAN VARIATIONS IN SERUM CONCENTRATIONS OF HUMAN CHORIONIC GONADOTROPIN, HUMAN CHORIONIC SOMATOMAMMOTROPIN, OESTRADIOL AND PROGESTERONE DURING PREGNANCY

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SUMMARY

Serum concentrations of oestradiol, progesterone, human chorionic somatomammotropin and human chorionic gonadotropin were measured three times daily (morning, afternoon and night) during early, middle and late pregnancy, in order to investigate the existence of a circadian secretion rhythm.

Twenty four women participated in the study. The results indicate the existence of a circadian secretion rhythm for oestradiol and human chorionic somatomammotropin during the second half of gestation.

There is contrasting evidence regarding daily physiological variations in the secretion or excretion of pregnancy hormones: human Chorionic Gonadotropin (hCG) serum concentration seems fairly constant, whereas urinary excretion is much more variable ([1, 2, 3]). Human Chorionic Somatomammotropin (hCS) also seems to be secreted in a continuous fashion ([4, 5]). On the other hand, progesterone (P) plasma concentrations show marked daily variations, with no regular secretion pattern ([6]).

Conflicting results are obtained for oestriol (E_3) depending on whether total ([7, 8]) or unbound ([9]) serum E_3 or urinary E_3 is measured ([10]).

Finally, there is some evidence that serum oestradiol (E_2) shows a morning-night secretion rhythm during late gestation ([11, 12]).

The present report summarizes data obtained examining the hormone secretion pattern of 24 women at various stages of gestation.

MATERIAL AND METHODS

Twenty four normal pregnant women participated in the study. Eight were in their first trimester, 8 in the second and 8 in the third; twelve were primigravidae and twelve multigravidae. All subjects had no family history of diabetes, no previous endocrine diseases, previous abortion, premature delivery or foetal macrosomia. No women had taken any medicine during the three months prior to the study and all pregnancies were uncomplicated.

Subject characteristics are shown in table one.

All subjects were hospitalized for a minimum of 3 days before initiation of the study.

All were kept on a 2,000 calories diet composed of 70.1 % carbohydrates, 12.5 % lipids and 17.4 % proteins.

Serum samples were taken at 08.00, 16.00 and 24.00 hours for three successive days following an hour rest in bed and 30 minutes after insertion in an antecubital vein of a no. 19 butterfly needle connected to a saline solution, transfused at a rate of 20 drops a minute.

The serum was separated by centrifugation and kept at — 20 °C until assays were performed.

Human Chorionic Gonadotropin was determined using anti-hCG (rabbit) serum and highly purified 2nd I.S. ^{125}I-hCG.

Table 1.

N. of subjects	Parity	Week of gestation	Age	Height (cm)	Weight (kg)	Age of menar.
8	0-7	6-13	21-40	155-168	50-92	11-14
8	0-4	18-25	18-39	154-170	42-97	11-15
8	0-4	26-40	21-37	150-172	48-95	11-14

Table 2. *Mean serum concentrations of hCG (mIU/ml) at various times during 3 consecutive days in early, middle and late gestation.*

Stage of gestation	Subjects	Week of gestation	Morning	Afternoon	Night	Average
Early	1 G. S.	6	230,000	180,000	234,000	244,670
	2 S. E.	6	108,400	159,000	114,600	127,533
	3 M. M.	7	33,600	37,440	25,596	32,212
	4 M. S.	8	62,780	56,560	50,480	56,606
	5 B. M.	9	79,120	85,360	85,680	83,386
	6 F. R.	10	107,600	87,800	not available	97,700
	7 D. I. M.	11	22,400	32,200	25,400	26,666
	8 S. M. D.	13	129,600	34,230	31,060	64,963
Middle	1 S. M. D.	18	6,746	5,310	6,800	6,285
	2 S. E.	18	39,400	34,420	41,100	38,306
	3 C. A.	20	10,068	11,608	8,860	10,178
	4 F. A.	22	10,152	10,480	7,158	9,263
	5 D. A. I.	24	9,030	6,810	4,023	6,621
	6 S. M. D.	24	8,461	20,460	13,980	14,300
	7 S. A.M.	25	15,740	14,488	12,900	14,376
	8 M. E.	25	17,332	24,264	18,110	19,902
Late	1 S. E.	26	13,020	10,460	16,600	13,360
	2 C. G.	29	23,500	17,998	34,300	25,266
	3 F. A.	30	25,080	50,000	44,000	43,293
	4 R. L.	30	47,100	24,560	28,528	33,396
	5 M. L.	32	21,540	25,500	30,488	25,842
	6 S. M.	33	9,100	7,120	5,400	7,206
	7 R. C.	36	33,096	46,076	43,300	40,824
	8 P. M. T.	40	12,070	11,750	7,840	10,553

Separation of bound from free hormone was achieved by the double antibody technique described by Midgley ([13]).

Human Chorionic Somatomammotropin was determined using the immunochemical reagents provided by the US N.I.H.

A double-antibody was used in order to separate free from bound hormone.

Oestradiol. Ether extractable E_2 was determined using an anti-7-E_2-BSA (Bovine Serum Albumin) serum ([14]) with the following characteristics: 1.4 % cross-reactivity with E_1 1.1 % cross-reactivity with E_3 and no cross-reactivity with cortisol.

The charcoal technique was used to separate free from bound hormone.

Table 3. *Mean serum concentration of hCS (γ/ml) at various times during 3 consecutive days in middle and late gestation.*

Stage of gestation	Subjects	Week of gestation	Morning	Afternoon	Night	Average
Middle	1 S. M. D.	18	2.85	3.45	2.40	2.9
	2 S. E.	18	2.1	2.2	2.5	2.26
	3 C. A.	20	2.85	2.4	2.75	2.66
	4 F. A.	22	2.6	2.6	2.1	2.43
	5 D. A. I.	24	3.5	2.5	2.5	2.83
	6 S. M. D.	24	4.9	5.2	4.3	4.8
	7 M. E.	25	4.3	3.6	4.8	4.23
	8 S. A. M.	25	2.4	2.7	2.9	2.6
Late	1 S. E.	26	1.8	1.0	1.1	1.3
	2 C. G.	29	7.7	6.0	6.0	6.56
	3 R. L.	30	8.9	5.7	6.8	7.1
	4 F. A.	30	8.1	8.4	8.1	8.2
	5 M. L.	32	9.1	7.2	7.7	8.0
	6 S. M. D.	33	12.2	11.2	12.8	12.06
	7 P. M. T.	40	9.7	6.8	6.3	7.6

Table 4. *Mean serum concentration of E_2 (ng/ml) at various times during 3 consevutive days in early, middle and late gestation.*

Stage of gestation	Subjects	Week of gestation	Morning	Afternoon	Night	Average
Early	1 G. S.	6	1.77	3.22	2.0	2.33
	2 S. E.	6	5.4	3.2	2.2	3.6
	3 M. M.	7	0.63	2.5	1.02	1.38
	4 M. S.	8	0.8	0.84	1.02	0.88
	5 B. M.	9	2.0	1.0	1.35	1.45
	6 F. R.	10	1.0	1.0	not available	1.0
	7 D. I. M.	11	2.5	2.5	2.7	2.56
	8 S. M. D.	13	4.19	4.33	3.64	4.05
Middle	1 S. M. D.	18	21.95	12.6	11.2	15.25
	2 S. E.	18	12.1	10.9	7.6	10.2
	3 C. A.	20	9.27	6.3	5.6	7.05
	4 F. A.	22	44.1	32.5	27.8	34.8
	5 D. A. I.	24	9.0	9.3	5.03	7.77
	6 S. M. D.	24	18.6	12.2	13.0	14.61
	7 M. E.	25	15.4	22.5	15.0	17.63
Late	1 S. E.	26	25.0	28.6	18.2	23.93
	2 C. G.	29	7.8	5.6	5.8	6.4
	3 F. A.	30	32.5	45.6	26.2	34.76
	4 R. L.	30	11.4	7.2	3.9	7.5
	5 M. L.	32	5.5	3.9	2.5	3.96
	6 S. M.	33	30.75	19.0	18.6	22.78
	7 R. C.	36	50.5	43.9	30.0	41.46
	8 P. M. T.	40	19.0	28.7	11.8	19.83

Table 5. *Mean serum concentration of Progesterone (ng/ml) at various times during 3 consecutive days in early, middle and late gestation.*

Stage of gestation	Subjects	Week of gestation	Morning	Afternoon	Night	Average
Early	1 G. S.	6	14.4	5.8	10.2	10.1
	2 S. E.	6	18.7	23.2	14.5	18.8
	3 M. M.	7	4.8	8.2	4.7	5.9
	4 M. S.	8	8.2	11.4	10.2	9.93
	5 B. M.	9	14.0	15.2	15.2	14.8
	6 F. R.	10	30.0	12.6	not available	21.3
	7 D. I. M.	11	12.0	19.6	19.6	17.06
	8 S. M. D	13	13.0	14.2	10.6	12.6
Middle	1 S. M. D.	18	65.7	32.4	26.4	41.35
	2 S. E	18	55.5	41.4	54.0	50.3
	3 C. A.	20	32.8	35.5	44.1	37.46
	4 F. A.	22	40.9	53.5	36.4	43.6
	5 D. A. I.	24	27.2	33.5	18.2	26.3
	6 S. M. D.	24	127.0	106.7	135.8	123.16
	7 S. A. M.	25	28.4	47.9	28.8	35.03
	8 M. E.	25	29.1	46.3	32.0	35.8
Late	1 S. E.	26	70.5	65.6	52.8	62.96
	2 C. G.	29	72.6	67.7	75.0	71.76
	3 F. A.	30	131.2	122.5	158.1	137.26
	4 R. L.	30	110.0	71.6	64.2	81.93
	5 M. L.	32	91.0	96.2	35.5	74.23
	6 S. M.	32	312.0	273.7	278.7	288.13
	7 R. C.	36	171.2	291.8	214.9	225.96
	8 P. M. T.	40	156.2	181.2	141.2	159.53

Progesterone. An 11-α-hydroxy-hemisuccinate-BSA anti-serum was used for assay. Also in this case the charcoal technique was utilized to separate free from bound hormone.

RESULTS

Serum concentrations of hCG observed at 8.00, 16.00 and 24.00 hours during early, middle and late pregnancy are presented in table 2.

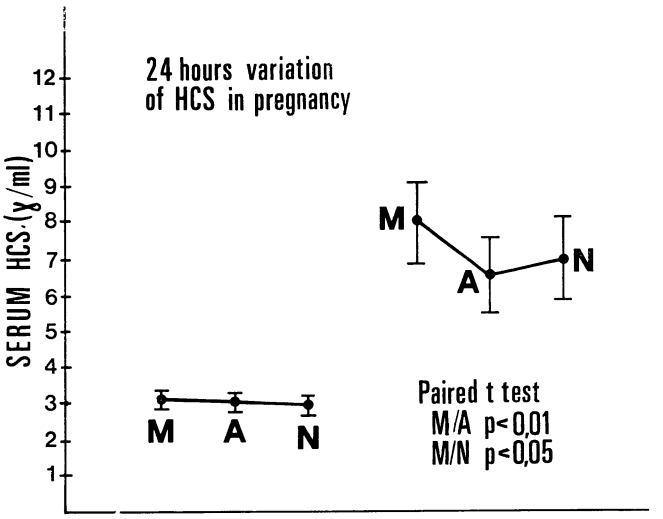
Serum concentrations of hCS at various time and gestation periods are presented in table 3.

Those of E_2 are shown in table 4; whereas the values for Progesterone are indicated in table 5.

The morning, afternoon and night results for each hormone were analyzed by the paired Student t test.

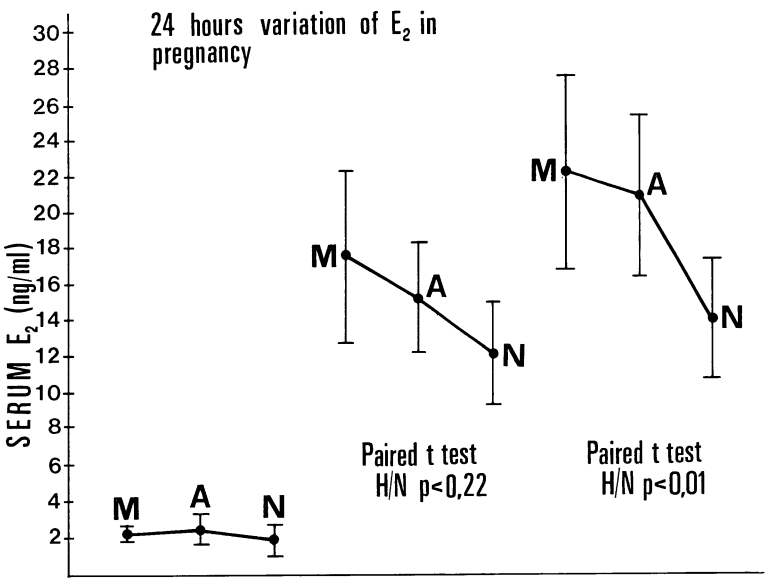
There were no statistically significant differences for hCG and for Progesterone, while in the case of hCS there were significant differences between morning (M) and afternoon (A) values and between morning and night (N) values in the third trimester of pregnancy (M/A $p < 0.01$ and M/N $p < 0.05$); also in the case of Oestradiol significant differences were observed between morning and night values during the second ($p < 0.02$) and in the third trimester ($p < 0.01$).

Figures 1 and 2 show hCS and E_2 variations in a graphic fashion.



	I trimester			II trimester		
	M	A	N	M	A	N
x̄:	3.19	3.13	3.03	8.21	6.61	6.97
SE:	0.34	0.34	0.34	1.20	1.17	1.31

Fig. 1.



	I trimester			II trimester			III trimester		
	M	A	N	M	A	N	M	A	N
x̄:	22.9	2.32	1.99	17.64	15.19	12.18	22.15	20.18	14.1
SE:	0.6	0.45	0.36	4.61	3.45	2.96	5.26	5.20	3,3

Fig. 2.

The same data, were also subjected to an analysis of variance according to Fischer-Snedecor after logarithmic transformation, in order to ensure that the error of the estimate be indipendent of the hormonal levels.

No statistically significant difference was found for any of the hormones, not even in the cases where the Student test gave significant results.

DISCUSSION

Of the four hormones analyzed, hCS seems the one secreted in a more constant way. The absence of quick rhythmic variations in hCS secretion was observed also when sampling was carried out at 10 min. intervals ([15]). For this reason when considering all cases as an homogeneous group as in the t test, a circadian rhythm could be evidenced during the third trimester. However when an analysis of variance was carried, which takes into account also inter subject variability, no significant differences remained.

By the paired Student t test also oestradiol showed a circadian secretion pattern during the second and third stage of pregnancy. Although no such pattern had been observed before during the second trimester, Townsley ([11]) and Munson ([12]) observed similar variations during the third trimester. Even in this case though, no significant variation could be retained in the analysis of variance, due to inter subject variation.

It is possible that more significant results could be obtained by selecting a more homogeneous study group, comprising women in the same week of pregnancy.

The present results support the suggestion of Goebel and Kuss ([9]) to obtain two serum samples from each subject to be examined for hormonal secretions in pregnancy.

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