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Endocrinology of Human Infertility: New Aspects

edited by
P.G. Crosignani and B.L. Rubin

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PREFACE

A meeting on *Endocrinology of Human Infertility: New Aspects* was held in Oxford, September 29 to October 1, 1980. It was organized by P.G. Crosignani and Betty L. Rubin, with support from Serono Symposia.

This volume, which contains the manuscripts presented by the invited speakers, is being published as Number 2 in the series called *Proceedings of the Serono Clinical Colloquia on Reproduction,* in which one new volume is expected to appear each year.

The editors wish to thank Serono Symposia for making this meeting possible, the participants and the Scientific Committee for making it so successful. They wish to extend particular thanks to Drs C. Ferrari and E. Reschini for their dedicated labour, which contributed immeasurably to the quality of the congress.

They are also grateful to Dr Stephen Franks for stepping, at very short notice, into the place of Dr Yen, who was unable to come, and thus avoiding a serious lacuna in the coverage of the topic.

April 1981

P.G. Crosignani
Betty L. Rubin

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HOW NORMAL IS THE NORMAL CYCLE?

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INTRODUCTION

Thanks to the development of sensitive radioimmunoassay techniques, during the past decade a large amount of information has been accumulated from normally menstruating women on the day-to-day hormonal changes in the peripheral levels of immunoreactive lutropin (LH), follitropin (FSH), estradiol, progesterone and a number of other steroids of ovarian and/or adrenal origin (cf. Diczfalusy and Landgren, 1977; Landgren *et al.*, 1980) and the general profile of these indices of pituitary-ovarian function during the menstrual cycle is well established.

On the other hand, there is still considerable uncertainty about the limits of normal variation of these indices and the ranges which can be considered with confidence to indicate a normal ovulatory cycle. With the exception of two studies (Johansson *et al.*, 1971; Lehmann *et al.*, 1976), the numbers of subjects investigated by the various authors have not been large enough for such an assessment and statistical appraisal of the limits of normal range in the published

literature was an exception (e.g. Aedo *et al.*, 1976; Guerrero *et al.*, 1976; Nuñez *et al.*, 1977) rather than the rule.

However, solid information on the limits of normal variation is important not only for the assessment of the hormonal effects of fertility-regulating agents (especially of those which invariably inhibit ovulation) but also for the improved diagnosis and treatment of certain forms of infertility. In addition, the study of various hormonal indices in relation to the length of the phases of the cycle might contribute to a better understanding of the hormonal mechanisms involved.

With these considerations in mind, we have initiated a number of investigations during the past five years to assess the "between subject" and "within subject" variation of peripheral hormone levels in normally menstruating women and in women using low-dose progestogen contraceptives.

PROGESTERONE LEVELS IN NORMALLY MENSTRUATING WOMEN

What are the progesterone levels in ovulatory cycles? In search of an answer, we have recently assessed the day-to-day changes in peripheral progesterone levels in 68 normally menstruating women (Landgren *et al.*, 1980). We have also re-analysed our previously published data on 32 normally menstruating women (Guerrero *et al.*, 1976; Aedo *et al.*, 1976). The combined analysis of these 100 subjects is presented in Table I.

Table I. Number of cycles with certain progesterone levels among one hundred normally menstruating women (modified from Landgren *et al.*, 1980).

Duration (days)	Progesterone level (nmol/l)				
	>13	>16	<26	>32	>48
6	95	92	85	69	19
5	98	95	90	79	32
4	99	98	91	84	44
3	100	100	94	88	57
2	100	100	96	92	75
1	100	100	96	94	77

The data of Table I indicate that 95 of 100 normally menstruating women exhibited plasma progesterone levels at or above 16 nmol/l for a minimum of five days. These progesterone levels are

considered to reflect a normal (presumably ovulatory) cycle in our local population. Obviously, for the practitioner it is not a very practical proposition to collect daily blood samples throughout a complete cycle and a question of practical importance is whether or not one can establish the diagnosis of adequate luteal function on the basis of a single progesterone assay. The recommendations of previous investigators are shown in Table II.

Table II. Recommended levels of a single progesterone assay to be considered to suggest an ovulatory cycle.

Author	Level
Israel *et al.* (1972)	9.5 nmol/l (3.0 ng/ml)
Black *et al.* (1972)	16.0 nmol/l (5.0 ng/ml)
Abraham *et al.* (1974)	16.0 nmol/l *on 3 days*

Comparison of the data of Tables I and II reveals that the criteria adopted by us are more stringent than those advocated by previous investigators. Furthermore, the data of Table I indicate that in 96 of 100 normally menstruating women the highest progesterone level exceeded 26 nmol/l and in 94 of them 32 nmol/l. Hence a single progesterone value of 26 to 32 nmol/l could be used with great confidence to characterize normal luteal function. However, the chances of encountering such a value on an arbitrarily selected day of the cycle are rather slim, and the probability of obtaining false "negative" results is high. Therefore either one must increase the number of sampling occasions (days) in the same cycle, or maximize the chances of selecting a day with a high progesterone level by providing additional information, such as daily basal body temperature records, preferably from several cycles.

THE LEVELS OF 17-HYDROXYPROGESTERONE AND 20α-DIHYDROPROGESTERONE

In 37 of the 100 normally menstruating women in whom the daily progesterone levels were followed throughout a cycle, the levels of 17-hydroxyprogesterone and 20α-dihydroprogesterone were also estimated (Landgren, Lager and Diczfalusy, unpublished data). An analysis similar to that indicated in Table I revealed that in 36 of the 37 subjects the 17-hydroxyprogesterone levels of the luteal phase exceeded 4 nmol/l for a minimum of five days and those of

20α-dihydroprogesterone 4.5 nmol/l for a minimum of six days. Hence in our local population adequate luteal function is characterized as indicated by the data in Table III.

Table III. Minimum blood levels of steroids characterizing adequate luteal function in 95% of normally menstruating women followed by daily hormone assays throughout the cycle (Landgren *et al.*, 1980 and unpublished data).

Steroid	Critical level (nmol/l)	No. of days	No. of subjects studied
Progesterone	16	5	100
17-hydroxyprogesterone	4	5	37
20α-dihydroprogesterone	4.5	6	37

HORMONAL LEVELS ASSOCIATED WITH VARIOUS PHASES OF THE CYCLE

The limits of normal variation in the levels of follitropin (hereafter: FSH), lutropin (hereafter: LH), estradiol, 17-hydroxyprogesterone and 20α-dihydroprogesterone during the various phases of the cycle are indicated in Table IV.

The estradiol, 17-hydroxyprogesterone and 20α-dihydroprogesterone levels, together with those of LH, represent a useful diagnostic adjunct for the characterization of normal and abnormal hormonal profiles. At the same time, it should be borne in mind that the procedures used for the quantitation of circulating gonadotrophins are of limited value, not only because (like most methods for the assay of immunoreactive LH and FSH) of their shortcoming due to the inadequacy of the standard preparations (e.g. Robertson *et al.*, 1978; Balogh *et al.*, 1979; Marana *et al.*, 1979a; Zaidi *et al.*, in press; Strollo *et al.*, in press) and the iodination procedures (Suginami *et al.*, 1978; Marana *et al.*, 1979b) employed, but also because of the variation in the quantity of biologically inactive immunoreactive material present in different biological sources (Robertson *et al.*, 1979; Zaidi *et al.*, in press). Hence great caution is needed in the interpretation of numerical gonatrophin values reported from various laboratories.

PERIPHERAL HORMONE LEVELS AND CYCLE LENGTH

In a recent study of 68 cycles in as many subjects, significant correlations were found between peripheral hormone levels and the

Table IV. Critical levels of hormonal indices characterizing normal follicular and luteal function in more than 90% of the normally menstruating women studied (Landgren *et al.*, 1980 and unpublished data).

Hormonal index and no. of cycles studied	Characteristic mean level days 1 to 6		Mean level days LH-7 to LH3		Preovulatory peak		Luteal maximum		Mean level luteal phase	
FSH (68)[a]	> 0.9	< 4.0	> 0.6	< 5.2	> 2.5	< 16	–		> 1.5	< 3.3
LH (68)[a]	> 1.0	< 3.5	> 0.9	< 4.8	> 13	< 36	–		> 1.1	< 4.3
Estradiol[b] (68)	> 0.15	< 0.37	–		> 0.70	< 2.10	> 0.48	< 1.20	> 0.30	< 0.70
17-hydroxyprogesterone[b] (37)	> 0.60	< 1.80	–		> 3.2	< 6.3	> 5.5	< 11.8	> 3.2	< 6.4
Progesterone[b] (68)	> 1.2	< 4.4	–		–		> 32	< 96	> 15	< 42
20α-dihydroprogesterone[b] (37)	> 0.5	< 1.5	–		–		> 7.3	< 25	> 4.4	< 12

[a] I.U./l in terms of the 69/104 International Reference Preparation.
[b] nmol/l.

length of the cycle or of its follicular phase (Landgren *et al.*, 1980). For instance, the length of the cycle and that of its follicular phase were positively correlated to the mean LH level of days LH-7 to LH-3 ($P < 0.001$) and negatively correlated to the mean estradiol level of the first six days of the cycle ($P < 0.001$). There was a positive correlation between the length of the cycle and the mean LH level throughout the cycle ($P < 0.01$), on the one hand or the height of the midcycle LH-surge ($P < 0.01$), on the other hand. A strong negative correlation was observed between cycle length and the mean estradiol level of the follicular phase ($P < 0.001$), or of the entire cycle ($P < 0.01$). Hence, the combination of high estradiol levels with low LH levels is associated with relatively short cycles and the combination of low estradiol levels with high LH levels with relatively long cycles. The LH and estradiol levels were strongly correlated with the length of the follicular phase, but not with that of the luteal phase. On the other hand, the FSH and progesterone levels were not correlated with the length of the cycle or of any particular cycle phase. Some of these relationships are indicated in Table V.

Table V. Selected correlation coefficients (r) between cycle length and peripheral hormone levels in 68 normally menstruating women (modified from Landgren *et al.*, 1980).

Characteristic (phase)	Hormonal index FSH	LH	Estradiol	Progesterone
Mean value Days 1–6	−0.11	−0.02	−0.44[c]	−0.02
Mean value Days LH-7 to LH-3	−0.01	0.45[c]	−0.20	−0.01
Mean value Follicular phase	0.00	0.29[a]	−0.41[c]	0.05
Peak level	−0.08	0.35[b]	−0.23	0.11
Mean value Luteal phase	−0.06	0.23	−0.23	0.05
Mean value Throughout the cycle	−0.06	0.35[b]	−0.35[b]	−0.13

[a] $P < 0.05$, [b] $P < 0.001$, [c] $P < 0.001$.

OVULATION AND CYCLE DAYS

The study of Landgren *et al.* (1980) also demonstrates that there is a high degree of correlation between cycle length and the length of the follicular phase ($P < 0.001$) but not of the luteal phase. This

paper also provides an indication of the extent of the variability in the occurrence of the preovulatory estradiol and LH surges at mid-cycle. As indicated by the data of Table VI, which are condensed from those originally reported, in one-third of the 68 cycles examined the estradiol and LH surges occurred before the 12th or after the 18th cycle day.

Table VI. Percentage of cycles exhibiting peak levels of estradiol and LH on different cycle days in 68 normally menstruating women (modified from Landgren *et al.*, 1980).

Hormonal index	Cycle days		
	8–11	12–18	19–23
Estradiol	20	66	14
LH	15	65	20

Furthermore, in one-third of the cycles the length of the luteal phase was shorter than 12 or longer than 15 days. These findings confirm the observations of previous investigators, which were obtained by more indirect methods, such as basal body temperature measurements (e.g. Hartman, 1962), and re-emphasize the difficulties in predicting the days of maximal fertility (e.g. Thorenycroft *et al.*, 1974; Landgren *et al.*, 1977). In the above paper (Landgren *et al.*, 1980) we conclude that "It is likely, however, that the within-subject variation in the length of the cycle and its luteal phase is considerably smaller than that between subjects. Studies in which several cycles are analysed in the same subjects may therefore be very helpful in assessing the extent of individual variability in the time of ovulation and length of the luteal phase". Unpublished data presented below indicate that this is indeed the case.

WITHIN SUBJECT VARIATION

In order to assess the relationship between within-subject and between-subject variations, blood samples were collected daily in every third cycle for a total of five cycles from each of 14 normally menstruating volunteers with an established record of fertility. Each collection cycle was followed by a recovery period of two cycles: hence the total duration of the study was 13 months. The objective was to find out whether or not there is any seasonal variation and to what extent are the hormonal profiles characteristic for the individual. Knowledge of these relationships may be of importance not

only in the management of the infertile patient, but also for those practicing various forms of so-called "natural" family planning methods.

The lengths of the follicular and luteal phases in the 70 cycles which were followed by daily hormone assays in the 14 subjects are indicated in Fig. 1.

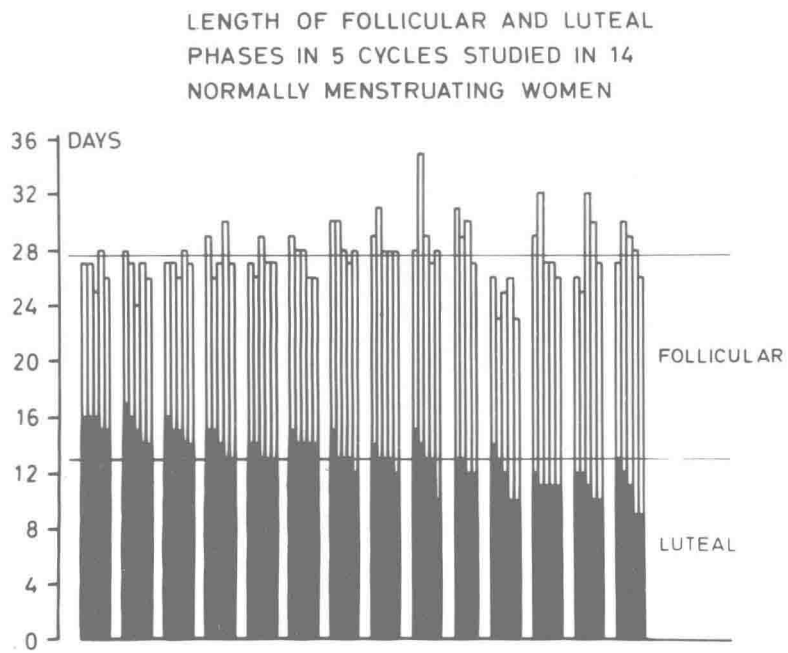

Fig. 1. The length of follicular and luteal phases in five cycles studied in each of 14 normally menstruating women by daily hormone assays (unpublished data).

Inspection of the data of Fig. 1 reveals considerable individual differences in the lengths of follicular and luteal phases of the cycle. Indeed, statistical analysis of the data indicates that the average cycle length was 27.7 days, with a variation between 24 and 35 days, with no seasonal variation, but with significant difference ($P < 0.01$) between subjects in the length of the entire cycle. Furthermore, there was no seasonal variation within the length of the follicular and luteal phases, but again, the variation between subjects in both instances was highly significant ($P < 0.001$). The average individual lengths of the follicular phase varied between 10.5 and 17.8 days and those of the luteal phase between 10.8 and 16.0 days.

It can also be seen from the data of Fig. 1 that in individual subjects long follicular phases were associated with short luteal phases and short follicular phases with long luteal phases. This association was highly significant, as indicated by the strong negative correlation between follicular phase and luteal phase lengths ($r = -0.760$; $P < 0.001$). Hence, there is a typical individual ratio characterizing the relative duration of the follicular and luteal phases; in the present study the mean individual follicular to luteal phase (F/L) ratios varied between 0.66 and 1.65.

Are these individual cycle characteristics associated with definite

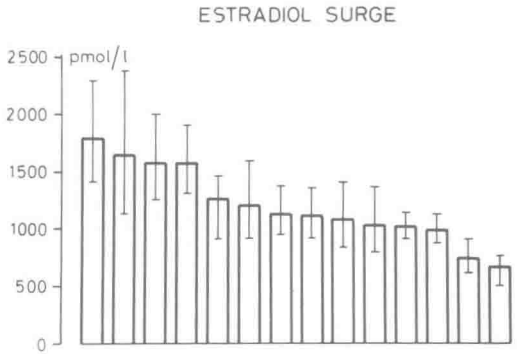

Fig. 2. Geometric mean levels (with 95% confidence limits) of the preovulatory estradiol surge in five cycles in each of 14 normally menstruating women (unpublished data).

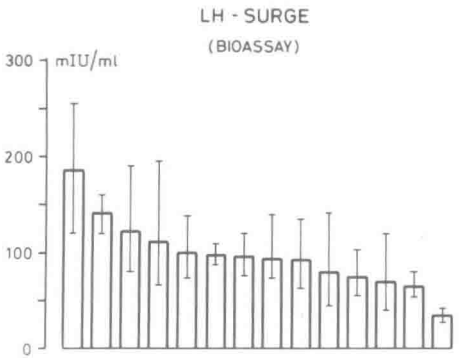

Fig. 3. Geometric mean levels (with 95% confidence limits) of the preovulatory lutropin (LH) peak in five cycles in each of 14 normally menstruating women. The results of the *in vitro* bioassays (Van Damme *et al.*, 1974) are expressed in terms of the 69/104 International Reference Preparation. The results obtained in the 14 subjects are presented in decreasing order, which is different from that shown in Fig. 2. (unpublished data).

hormonal profiles? The data of Fig. 2 indicate the individual (geometric) mean values and 95% confidence limits of the preovulatory estradiol peak and the data of Fig. 3 indicate similar data for the LH-surge in the 14 subjects.

It should be noted that all the LH assays indicated in Figs 3–6

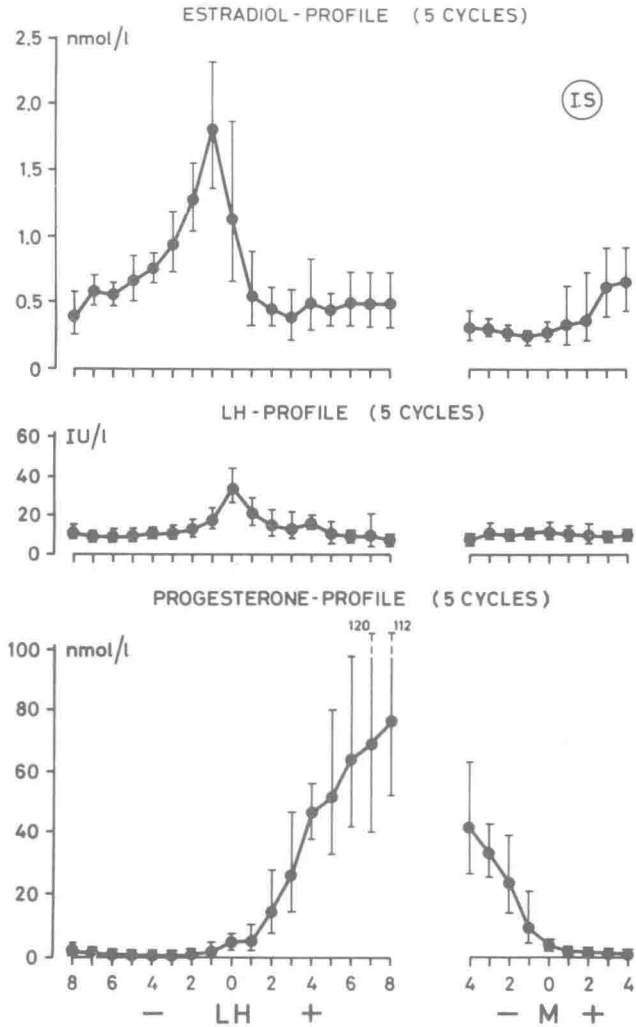

Fig. 4. Geometric mean estradiol, lutropin (LH) and progesterone profiles (with 95% confidence limits) estimated during five cycles in a woman with proven fertility. The sign LH indicates the day of the LH surge and M the day of the onset of menstruation (unpublished data).

were carried out by the *in vitro* bioassay procedure of Van Damme *et al.* (1974) as modified for assays in plasma by Romani *et al.* (1977) and Rajalakshmi *et al.* (1979). Because of the presence of varying quantities of biologically inactive, immunoreactive material in the standard preparations used, *in vitro* bioassay methods for LH yield considerably higher numerical values than radioimmunoassay procedures (cf. Bàrtfai *et al.*, 1979; Robertson *et al.*, 1979). It should also be noted that in Figs 2 and 3 the individual estradiol and LH values were arranged in simple decreasing orders which were different in the two figures. Indeed, so far no correlation has been found between the estradiol, LH and progesterone profiles, as indicated by three representative examples presented in Figs 4–6.

The estradiol, LH and progesterone profiles of a woman of proven fertility (two children) are shown in Fig. 4.

In each cycle studied, the marked preovulatory estradiol peak was followed by an LH surge of very limited amplitude. Nevertheless, the low LH levels were associated with very high luteal phase progesterone levels. It is also of considerable interest to note that this subject did not show any secondary rise in estradiol levels during the luteal phase, which is usually found in the great majority of normally menstruating women (cf. Guerrero *et al.*, 1976; Nuñez *et al.*, 1977; Landgren *et al.*, 1980). [Parenthetically, this luteal rise in estradiol levels, which is believed to be characteristic for the human and chimpanzee (Hobson *et al.*, 1974), does not occur in rhesus monkeys (Hotchkiss *et al.*, 1971; Hess and Resko, 1973), or baboons (Goncharov *et al.*, 1976).]

The individual hormonal profile shown in Fig. 5 is significantly different from that presented in Fig. 4; there was hardly any preovulatory estradiol peak in this subject and the progesterone levels of the luteal phase were also low. On the other hand, in each cycle there was a marked LH surge. This subject is also the mother of two living children (and is again pregnant). Whereas it might be tempting to speculate that in this case there could have been a correlation between the low estradiol and progesterone levels, such an assumption seems to be contradicted by the hormonal profiles found in a third subject (again a mother with two children) which are presented in Fig. 6.

The data of Fig. 6 strongly suggest that a relatively poor preovulatory estradiol peak is compatible with relatively high LH and progesterone levels.

A number of conclusions may be drawn from the data shown in Figs 1–6. It appears that fertility is compatible with considerable