The human menstrual cycle: plasma concentrations of prolactin, LH, FSH, oestradiol and progesterone in conceiving and non-conceiving women

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Summary. Hormonal profiles were obtained throughout 26 conception cycles and 27 non-conception control cycles. The pregnancies followed treatment (clomiphene or bromocriptine) in 12 women but were spontaneous in the remaining 14. No sustained significant difference between the various types of conception cycle was found for LH, FSH, oestradiol or progesterone concentrations. Prolactin concentrations varied widely, suggesting that mean cycle prolactin concentrations ranging from 45 to 760 mi.u./l are compatible with conception. Although there were no significant differences in progesterone secretion within the conception cycles, there were highly significant differences between the conception cycles and the non-pregnant control cycles. Mean progesterone concentrations in the conception group were higher ($P < 0.005$) than those in the control women over Days 3–8 following the LH peak. This difference could only be partly accounted for by heterogeneity within the control group (15–20% of the control cycles had low progesterone concentrations and were probably subfertile). It is suggested that the higher conception cycle progesterone concentrations during the early part of the luteal phase may constitute a preimplantation component of the maternal recognition of pregnancy in women.

Introduction

In much of the published work on hormone profiles from women of varied reproductive status (particularly infertile women) comparisons are made with profiles of non-conception, but presumably ovulatory, cycles from volunteers (Sherman & Korenman, 1974; Lenton, Adams & Cooke, 1978). Two assumptions are repeatedly made about the control groups: firstly, that because they do not have a gynaecological complaint they are reproductively normal and, secondly, that a presumptively ovulatory cycle from a normal woman is likely to be fertile. Although hormone profiles from a number of conception cycles have been reported (Mishell, Thorneycroft, Nagata, Murata & Nakamura, 1973; Thomas, De Hertogh, Pizarro, Van Exter & Ferrin, 1973), sufficient data have not been provided to define the ranges of hormone concentrations compatible with fertility. This information is of some importance when assessing hormone profiles from infertile women, particularly those suspected of having defective luteal function. During long-term studies on the endocrine profiles of infertile and normal women we were fortunate to obtain blood samples in 26 cycles during which conception occurred. This study presents an analysis of the variability between individuals seen in the hormone profiles of these cycles, and compares them with those of a similar group of cyclic non-pregnant women.

Subjects and Methods

The 26 women who conceived have been divided into 4 groups according to the nature of their conception cycle. The control group consisted of 27 cycles from volunteer subjects who did not conceive. There were no significant differences between the groups with respect to age, which ranged from 22 to 35 years.

Group I: spontaneous conception cycles in normal women. This group contained 4 volunteer subjects who conceived spontaneously during their first cycle following removal of an intrauterine device.

Group II: spontaneous conception cycles in subfertile women. This group contained 10 women who conceived spontaneously while being investigated for infertility. The mean ($\pm$ s.d.) duration of their infertility was 3.7 ± 1.4 years. Five women had a history of primary infertility and 5 of secondary infertility; 4 of the latter had had first trimester abortions and only 2 had a living child. Of the 10 pregnancies, 8 went to term, one ended in an occult abortion at 35 days and the other in an abortion at 86 days. Both these patients have since successfully conceived again.

Group III: conception cycles following clomiphene treatment. There were 6 women who conceived while taking clomiphene citrate (50 mg daily for 5 days; Clomid: Merrell Division, Richardson Merrell Ltd, Slough, Berkshire, U.K.). Previously 4 of the women were amenorrhoeic ('clomiphene induced', Table 2), one was oligomenorrhoeic and one had been ovulating regularly ('clomiphene-treated', Table 2). Basal LH concentrations, androstenedione and testosterone concentrations were all slightly elevated in the 4 amenorrhoeic women. This was suggestive of polycystic ovarian disease but all 4 conceived before the diagnosis could be confirmed by laparoscopy.

Group IV: conception cycles during bromocriptine treatment. In this group there were 6 regularly ovulating but infertile women who, although they did not have gross hyperprolactinaemia, were being treated with bromocriptine (2.5–5.0 mg daily; Parlodel: Sandoz Products Ltd, Feltham, Middlesex, U.K.). Three women received clomiphene (as above) in addition to bromocriptine. Mean ($\pm$ s.d.) duration of infertility was 3.8 ± 2.1 years. Infertility was secondary in 4 women (who had all had first trimester abortions, there had in addition been one stillbirth). One of the 6 conceptions resulted in a ruptured tubal pregnancy and another ended in an occult abortion at 36 days. This patient conceived again in her next cycle but this subsequent pregnancy was ectopic.

Group V: non-pregnant control cycles. The 27 presumably ovulatory cycles in this group came from 19 women. All subjects were healthy volunteers who had not taken any oral contraception for at least 3 months. Seven cycles were from women attempting to conceive but who failed to do so in their first cycle of unprotected intercourse. In the remaining 20 cycles there was no exposure to risk of pregnancy.

Blood samples were obtained daily at the same time of day for each individual patient (08:00–09:30 or 12:00–14:00), except in 2 women (one each in Groups III and IV) for whom sampling was less frequent. Plasma was separated by centrifugation and the samples stored at -20°C .

Hormone concentrations were measured by radioimmunoassay (Lenton *et al.*, 1978; Lenton, Brook, Sobowale & Cooke, 1979) as previously described; the characteristics of the assays are summarized in Table 1. The Second International Reference Preparation of human menopausal gonadotrophin (2IRP-HMG, in i.u./l) and the First International Reference Preparation for prolactin (75/504 in m.i.u./l) were used, both supplied by the World Health Organisation through the National Institute for Biological Standards and Control, London. The gonadotrophin standard, although now superseded for general use, was employed to permit detailed comparisons with the results of earlier studies.

Calculation of a mean ($\pm$ s.d.) is only valid when applied to normally distributed data and it

has been shown (Lenton *et al.*, 1979, for prolactin; Kletzky, Nakamura, Thorneycroft & Mishell, 1975, for the other hormones) that the frequency distribution of individual hormone concentrations does not follow a normal (Gaussian) curve. The distribution can generally be

Table 1. Performance characteristics of the radioimmunoassays for prolactin, LH, FSH, oestradiol and progesterone

	Prolactin	LH	FSH	Oestradiol	Progesterone
Sensitivity*	25	4	1.5	40	0.6
	mi.u./l	i.u./l	i.u./l	pmol/l	nmol/l
Intra-assay coefficient of variation† (%)	4.0	5.2	6.1	5.2	7.0
Inter-assay coefficient of variation† (%)	9.3	10.3	11.3	12.9	8.8
Efficiency of extraction‡ (%)	n.a.	n.a.	n.a.	87.1 ± 5.5	74.2 ± 4.0

* The least concentration consistently detectable from zero.

† Calculated from repeated assay of a plasma pool in a single assay and in separate assays. The plasma pool fell approximately on the mid-point of the standard curve.

‡ Determined by the recovery of ³H-labelled steroid added to a pool of plasma; n.a. = not applicable.

Table 2. Details of the age and reproductive status (past obstetrical and gynaecological history, and duration of infertility, when relevant) of the 26 women who conceived

Group	Age (years)	Previous menstrual history*	Infertility history† (years)	Type of conception	Pregnancy outcome‡
I	25	Reg. ov.	None	Spontaneous, normal	FTD
	30	Reg. ov.	None	Spontaneous, normal	FTD
	27	Reg. ov.	None	Spontaneous, normal	FTD
	28	Reg. ov.	None	Spontaneous, normal	FTD
II	30	Reg. ov.	5 (0 + 0)	Spontaneous, subfertile	Occult abortion 35 days
	26	Reg. ov.	3 (0 + 0)	Spontaneous, subfertile	FTD
	30	Reg. ov.	3 (0 + 0)	Spontaneous, subfertile	FTD
	29	Reg. ov.	7 (0 + 0)	Spontaneous, subfertile	FTD
	32	Reg. ov.	4 (0 + 0)	Spontaneous, subfertile	FTD-some vaginal bleeding
	33	Reg. ov.	3 (1 + 2)	Spontaneous, subfertile	FTD
	35	Reg. ov.	4 (0 + 0)	Spontaneous, subfertile	Abortion at 86 days
	31	Reg. ov.	2 (2 + 0)	Spontaneous, subfertile	FTD
	30	Reg. ov.	3 (0 + 2)	Spontaneous, subfertile	FTD
	30	Reg. ov.	3 (0 + 0)	Spontaneous, subfertile	FTD
III	29	Anov.	—	Clomiphene-induced	FTD
	27	Anov.	—	Clomiphene-induced	FTD
	28	Anov.	—	Clomiphene-induced	FTD
	29	Reg. ov.	1 (1 + 0)	Clomiphene-induced	FTD
	22	Oligo. ov.	—	Clomiphene-induced	FTD
	24	Anov.	—	Clomiphene-induced	FTD, some vaginal bleeding
IV	29	Reg. ov.	4 (0 + 1)	Bromocriptine and clomiphene-treated	Occult abortion at 36 days
	25	Reg. ov.	3 (0 + 0)	Bromocriptine and clomiphene-treated	Ectopic
	29	Reg. ov.	3 (0 + 0)	Bromocriptine and clomiphene-treated	FTD
	32	Reg. ov.	2 (0 + 1)	Bromocriptine-treated	FTD
	33	Reg. ov.	8 (1 + 2)	Bromocriptine-treated	FTD
	25	Reg. ov.	3 (0 + 1)	Bromocriptine-treated	FTD

* Reg. ov. = regular ovulatory cycles; Anov. = anovulatory cycles; Oligo. ov. = oligo-ovulatory cycles.

† Duration of infertility includes unsuccessful pregnancies with the number of full-term pregnancies and abortions respectively indicated within parentheses.

‡ FTD = delivery of a full-term infant.

Text-fig. 1. Mean ($\pm$ s.d.) log concentrations of hormones in Groups I, II, III and IV (see Table 1). The standard deviations represent logarithmically distributed data and so are unequal in size.

‘normalized’ by converting all hormone concentrations to their logarithms before commencing the analysis, and this procedure has been followed here. However, presentation of results in logarithmic units is difficult to assimilate and so the data have been converted back to arithmetic units for graphic display, thus producing values for the standard deviations above and below the mean which are unequal, with the standard deviation above mean being greater and more

Text-fig. 1—cont.

accurately describing the original distribution of the data (see Text-fig. 1). Furthermore, the value for two standard deviations below the mean should not result in negative hormone concentrations as can occur when untransformed data are used to calculate the mean ($\pm$ s.d.). The significance of differences between mean values was determined by applying Student's *t* test to the logarithmically transformed data.

Results

Details of the menstrual and obstetric histories of the subjects who conceived are given in Table 2. Each of the full-term deliveries resulted in a single living infant. Four pregnancies did not go to term but as there were no obvious differences between these cycles and the remainder, up to the 14th day after ovulation, they have not been excluded from the analysis.

To permit comparison of the hormone profiles in the different types of conception cycle (Text-fig. 1), the day of the LH peak was defined as Day 0 in each cycle and daily mean ($\pm$ s.d.) log concentrations for each hormone were calculated for all conception groups.

Prolactin profiles differed greatly with the type of conception cycle (Text-fig. 1). As expected, concentrations were lowest in women taking bromocriptine (Group IV) and highest in the subfertile women (Group II): Group IV levels were significantly lower than those of any other groups throughout the cycle ($P < 0.01$) while prolactin values in Group II women were significantly higher ($P < 0.04$) than those in Group I on most days of the cycle. Prolactin levels in Group III were also raised particularly from the late follicular phase. In this group elevations in prolactin and oestradiol concentrations tended to occur simultaneously and the resultant profiles were similar.

LH concentration profiles were similar in all groups. The only significant difference was seen during the follicular phase when values for Group III women were significantly higher ($P < 0.02$) than those of any other group. An apparent secondary rise in LH, due to cross-reaction with hCG, was seen from about Day 11 or 12 in all cycles. FSH profiles also varied little: the only significant differences were between Group III and Group II ($P < 0.04$) during the mid-follicular phase. The late luteal rise in FSH seen in non-conception types of menstrual cycles was absent.

Text-fig. 2. Mean ($\pm$ s.d.) log concentrations of LH, FSH, oestradiol, progesterone and prolactin in 27 control (non-conception cycles) (○—○) and 26 conception cycles (●—●).

Oestradiol concentrations were particularly high and variable in Group III. Because of this Group III oestradiol levels were intermittently significantly greater ($P < 0.05$) than those in the other groups. In each conception group a secondary rise in oestradiol, presumably due to 'rescue' of the corpus luteum by hCG, was observed between Days 9 and 11. Progesterone concentrations were very uniform throughout the conception groups and there were no significant differences. As with oestradiol a secondary rise in progesterone, probably due to corpus luteum 'rescue', occurred between Days 9 and 11.

With the exception of prolactin the endocrine differences between the groups of conception cycles were small and of limited duration. Therefore all conception cycles were grouped together for comparison with the control non-pregnant cycles (Group V) (Text-fig. 2). There were no significant differences in follicular-phase LH, preovulatory oestradiol and luteal-phase FSH concentrations, but the differences in progesterone profiles were pronounced. Values in Group V were significantly lower than those of Groups I–IV ($P < 0.005$) from Day 3 to Day 8 (the last day that was tested). It was not strictly valid to average the values of prolactin concentrations for Groups I–IV because of the significant differences between these groups, and in particular between Groups II and IV. However, comparison of the mean values with Group V mean prolactin values did not show any marked divergence (Text-fig. 2).

The mean progesterone concentration on Days 6, 7 and 8 following the LH peak (the 'progesterone index') was calculated for the individual cycles. The distribution of the progesterone index values between the conception and control groups is shown in Text-fig. 3. The distribution of the conception group values appeared to be normal (suggesting an homogeneous population) but the control group showed two peaks, the smaller of which consisted of 5 (18.5%) values that were below the range of the conception group. These particular cycles might have been infertile, but even when they were excluded, the distribution of the progesterone index for the control group was still not equivalent to that of the conception group.

Text-fig. 3. Distribution of progesterone indices (mean progesterone for Days 6–8, see text) for (a) normal conception cycles and (b) control cycles.

Discussion

Because the majority of women in the conception group had a history of infertility or gynaecological problems, their hormone profiles may not be typical of a normal conception cycle. Despite this, mean concentrations of LH, FSH and oestradiol were generally very similar to those of 'normal' control cycles (Text-fig. 2), suggesting that a conception cycle even in a previously infertile woman is likely to conform closely with 'normal' conception cycles.

The large range of prolactin concentrations (Text-fig. 1) compatible with conception was somewhat surprising. It is possible that the higher prolactin levels in Group II could have been due largely to increased amounts of biologically inactive hormone (big prolactin; Farkouh, Packer & Frantz, 1979). The elevated prolactin concentrations in Group III are probably secondary to the raised levels of oestradiol, and the suppressed concentrations in Group IV are clearly the result of bromocriptine therapy (Ylikorkala, Huhtaniemi, Tuimala & Seppala, 1979).

The follicular-phase elevations in LH and FSH in Group III are consistent with the indication that at least 4 women from this group showed some of the features of polycystic ovary syndrome (raised androgens and LH despite low/normal FSH concentrations (Yen, Vela & Rankin, 1970)). Such patients are generally sensitive to clomiphene therapy (Boon, Schalch, Lee & Reichlin, 1972) which significantly raises follicular-phase FSH levels (Lenton *et al.*, 1978), although concomitant clomiphene treatment in 3/6 bromocriptine-treated cycles in Group IV had no effect on follicular-phase values of LH or FSH. The clomiphene-induced elevations in follicular-phase gonadotrophin concentrations in Group III were associated with large and variable increases in preovulatory and luteal oestradiol secretion. Despite this, none of these conceptions resulted in an overt multiple pregnancy.

Progesterone concentration profiles were similar in all four conception groups. It would appear that the amount of progesterone secreted during a conception cycle resulting in a single fetus is a reproducible and quantifiable phenomenon. This interpretation is supported by the narrow range and normal distribution of the progesterone index in conception cycles (Text-fig. 3).

Comparison of all the conception cycles with equivalent numbers of control cycles showed only small and insignificant differences in the profiles of LH, FSH and oestradiol but major differences in progesterone concentration (Text-fig. 2). The small difference in mid-follicular LH values was entirely attributable to the influence of the Group III women who were considered to have polycystic ovarian syndrome. Re-calculation of the group mean profiles of conception cycles omitting all Group III cycles entirely normalized LH concentrations, and greatly reduced the range of oestradiol concentrations. FSH concentrations between the conception and control groups during the first half of the cycle were indistinguishable (despite the influence of clomiphene therapy in 6/26 cycles) but during the second half of the cycle, mean conception cycle FSH concentrations were slightly lower than the control cycle from about Day 2 onwards. This difference did not, however, reach significance when *t* tests were applied.

The highly significant differences in mean progesterone concentration from Days 3 to 8 were unexpected. From Day 9, the clear divergence in progesterone concentrations is due to 'rescue' of the corpus luteum (E. A. Lenton, L. M. Neal & R. Sulaiman, unpublished observations) after implantation on about Day 7 (Hertig, Rock & Adams, 1956). It is unlikely that treatment (such as clomiphene) had a marked effect on progesterone levels because there were no differences in mean concentration between the individual conception groups, and the frequency distribution of conception cycle progesterone index values (Text-fig. 3) indicates a homogeneous population. There are a number of possible explanations for this observation. The control group is heterogeneous (Text-fig. 3) and contains a small number (15–20%) of cycles in which the progesterone index is clearly outside the range of the main group. These particular cycles may not have been fertile. However, eliminating them from the control group did not yield a median progesterone index value equivalent to that of the conception group; progesterone con-

centrations were still higher during the mid-luteal phase of conception cycles than in the control cycles. It is possible that many more than 15–20% of the control cycles may have been infertile. Certainly it is known that only about 30% of nulliparous women will conceive in the first cycle (Lenton, Weston & Cooke, 1977), although it is likely that mistimed coitus accounts for a significant number of these failures.

Another explanation, which is also difficult to prove, is that the presence within the reproductive tract of the preimplantation embryo has already influenced corpus luteum progesterone production in the conception cycles. The only other evidence that a preimplantation human embryo may influence the maternal organism is the demonstration of immunosuppression by the rosette inhibition test (Morton, Rolfe & Clunie, 1977). The signals involved in such a mechanism in man are, however, unknown and further work is clearly needed to extend and confirm these observations.

It is hoped that better understanding of the specific differences between fertile cycles and ovulatory cycles will eventually assist the diagnosis and management of infertile patients, particularly for the large proportion for whom no gynaecological abnormality can be demonstrated.

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