Human Reproduction vol.11 no.10 pp.2090–2096, 1996

Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles

S.F.Lipson[1] and P.T.Ellison

Department of Anthropology, Harvard University, Cambridge, MA 02138, USA

[1]To whom correspondence should be addressed

Oestradiol and progesterone profiles from naturally occurring conception and exposed non-conception cycles were compared to assess the impact of natural variation in concentrations of ovarian steroid hormones on female fecundity. In a prospective, longitudinal study, 24 women collected saliva samples twice daily and recorded intercourse for up to 1 year or until a pregnancy was clinically confirmed. Oestradiol and progesterone concentrations were measured by a salivary radioimmunoassay. Average mid-follicular oestradiol concentrations were significantly higher in conception than in non-conception cycles (12.6 ± 1.7 versus 8.5 ± 0.6 pmol/l, $P < 0.01$). A separate analysis, including only cycles from those women who contributed both conception and non-conception cycles, demonstrated an even more pronounced difference in mid-follicular oestradiol concentrations, not just for conception and non-conception cycles as groups (14.5 ± 2.3 versus 6.5 ± 0.7 pmol/l, $P < 0.001$), but also between the conception and average non-conception concentrations of individual women. Among these women, relative mid-follicular oestradiol concentration was highly correlated with the probability of successful conception. In addition, relative body weight was significantly positively correlated with mid-follicular oestradiol concentration. These findings indicate that variation in follicular development, reflected in variation in follicular oestradiol concentrations, is an important indicator of fecundity.

Key words: conception/fecundity/follicular phase/oestradiol/salivary steroids

Introduction

The primary reproductive functions of the human ovary, the preparation of the oocyte for fertilization and the preparation of the uterus for implantation depend on the actions of the ovarian steroid hormones oestradiol and progesterone. With the development of sophisticated measurement techniques, ovarian function has now been monitored, not just within clinical populations (Lenton *et al.*, 1982; Walker *et al.*, 1984; Lewinthal *et al.*, 1986; Cedard *et al.*, 1987), but among non-clinical (Read *et al.*, 1984; Vuorento *et al.*, 1989; De Crée *et al.*, 1990) and field (Ellison *et al.*, 1989; Worthman *et al.*, 1993) populations as well. Such studies have revealed considerable variation in the concentrations of ovarian hormones among Western women (Bullen *et al.*, 1985; Pirke *et al.*, 1985; Lipson and Ellison, 1992; O'Rourke and Ellison, 1993) and between Western and non-Western populations (Danutra *et al.*, 1989; Panter-Brick *et al.*, 1993). They have also demonstrated that variation in ovarian function is associated with age, acute energetic stresses and possibly chronic environmental conditions (Ellison *et al.*, 1993). At the same time, in the course of the development of assisted reproductive techniques, there have been studies of the relationship between ovarian hormone concentrations and pregnancy rates (Zarutskie *et al.*, 1987) or proximate determinants of pregnancy such as follicle size (Lejeune *et al.*, 1986) and endometrial thickness (Noyes *et al.*, 1995). However, hormone concentrations in these cycles were achieved through exogenous supplementation and/or stimulation regimens. Thus, while the existence of significant natural variation in ovarian function has been documented, and the importance of ovarian hormones in controlling many of the processes required for the initiation of pregnancy has been demonstrated, the impact of variation in natural, as opposed to stimulated or exogenously supplemented, concentrations of ovarian hormones on female fecundity has not yet been established. We conducted a prospective, longitudinal study of women who were trying to become pregnant, measuring daily concentrations of oestradiol and progesterone using a salivary radioimmunoassay. Thus we were able to compare ovarian hormone concentrations in naturally occurring conception and non-conception cycles so as to investigate the relationship between variation in ovarian steroid profiles and the probability of successful conception.

Materials and methods

Subjects

Women aged 20–40 years who were trying to become pregnant were recruited by newspaper advertisement from the local community and screened to ensure good general health, normal weight for height, regular menstrual cycles, no history of, or previous treatment for, infertility, and no recent use of oral contraceptives or other steroid medication. Women who had been trying to conceive for ≥12 months were not accepted. The study was approved by the Committee on the Use of Human Subjects, Faculty of Arts and Sciences, Harvard University, MA, USA, and all subjects gave written informed consent.

During an initial interview, women provided information on menstrual history, previous pregnancies and regular exercise; their height and weight were also measured. Weight was monitored during the course of the woman's participation by periodic measurements made when she returned samples to the laboratory. Participants were instructed to collect samples of their own saliva at home twice daily (morning and evening) and to record daily whether they had had

intercourse or menstrual bleeding in the previous 24 h. Collection of saliva samples followed our previously established protocols (Lipson and Ellison, 1989).

The results reported here are based on samples from 129 cycles collected by 24 women who were enrolled in the study. At enrolment their ages ranged from 26 to 39 years (average 31.8), and all were within ±20% of their ideal body weight (Metropolitan Life Insurance Company, 1983). Of the 24 women, 17 became pregnant during the course of their participation in the study.

Assay methods

Evening and morning saliva samples were assayed for progesterone and oestradiol respectively by a radioimmunoassay (Ellison *et al.*, 1986; Ellison, 1988; O'Rourke and Ellison, 1993). Assays were run using specific antisera [anti-progesterone #337 (Gibori *et al.*, 1977) or anti-oestradiol #244 (Korenman *et al.*, 1974)] and four-position tritiated progesterone (Amersham, Arlington Heights, IL, USA) or oestradiol (New England Nuclear, Boston, MA, USA). Samples were extracted twice in diethyl ether prior to assay. Procedural losses during extraction were monitored by the addition of an internal standard to each sample. Linearity of the assay response in saliva samples with known amounts of added steroid was demonstrated by a correlation coefficient of >0.99 between amount added and amount measured for each hormone. Correlation coefficients of hormone concentrations in matched serum and saliva samples collected over the course of the menstrual cycle in three separate subjects ranged from 0.80 to 0.97 for progesterone and 0.79 to 0.89 for oestradiol. Hormone concentrations in serum and saliva are not, however, entirely equivalent; while serum measurements represent total (bound and free) steroid concentrations, salivary values directly reflect free, biologically active plasma concentrations (Ellison, 1988).

Assay sensitivity, the smallest amount of steroid distinguishable from 0 pmol with 95% confidence, averaged 10 ± 3 pmol/l for progesterone and 1.3 ± 0.4 pmol/l for oestradiol. Average intra-assay variability, estimated at the 50% binding point of the standard curve, was 9.3% for progesterone and 4.2% for oestradiol. For the progesterone assay, interassay variability, estimated from low (~100 pmol/l), medium (~375 pmol/l) and high (~650 pmol/l) pools, averaged 17.6, 9.2 and 10.6% respectively. Corresponding values for the oestradiol assay, estimated from low (~10 pmol/l), medium (~20 pmol/l) and high (~50 pmol/l) pools, averaged 34.3, 7.5 and 14.1% respectively. These values were calculated after averaging the two copies of each pool run with each assay. These relatively high values for the oestradiol assay, particularly for the low pool, reflect the low concentrations being measured; for values so near the sensitivity limit of the assay, extremely high precision measurements are not possible, even though the measurements are accurate reflections of the amounts of steroid present.

The effects of interassay variability on the analysis were reduced in two ways. First, each oestradiol assay included samples from three cycles, and cycles from an individual woman were preferentially run together in assays. In particular, each assay that contained a conception cycle also included at least one non-conception cycle (if available) from the same woman. In addition, individual sample values were never used for analysis. Rather, all analyses were based on data in which individual measurements were averaged; the SE of such averages are reduced relative to the SD of individual measurements.

Definition of variables

For each cycle, the evening saliva samples were assayed to determine daily progesterone concentrations. A preliminary designation of mid-cycle was made on the basis of the daily progesterone concentration (following the method of Walker *et al.*, 1985), and was used to define the 20 days spanning mid-cycle. The morning samples from these days were assayed to determine daily oestradiol concentrations. The 129 cycles included in this study represent 3793 potential daily values for progesterone and 2580 potential daily values for oestradiol. Of these, 131 (3.4%) progesterone and 81 (3.1%) oestradiol values were lost because of missed or improper collection or loss during the assay procedure.

Final alignment of the cycles for analysis was based on the identification of the day of the mid-cycle oestradiol drop, defined as the second of the two consecutive days (following the day of peak oestradiol) between which the greatest decrease in oestradiol occurred. As oestradiol concentrations are known to peak at around the time of onset of the luteinizing hormone (LH) surge, starting to drop sharply within a few hours and continuing to drop during the 12–48 h after the initiation of the surge, and as ovulation occurs ~36 h after onset of the LH surge (Hoff *et al.*, 1983), the day of the mid-cycle oestradiol drop provides a good estimate of the day of ovulation. To minimize misidentification of the day of the mid-cycle oestradiol drop, we required non-conception cycles to have no missing values for days −18 to −12 (relative to the next menstrual onset); 20 cycles with missing days in this interval were eliminated from the statistical analysis. Only one conception cycle had a missing value relevant to the determination of the day of the mid-cycle oestradiol drop. In this case, there was a potential uncertainty of 24 h in the identification of the day of the mid-cycle oestradiol drop because of a single missing value at mid-cycle. The criterion of concordance with the preliminary designation of mid-cycle was applied to assign one of the two possible days as the day of the mid-cycle oestradiol drop; there was no effect on the results of the statistical analysis depending on which of the days was chosen. Thus, 92 non-conception and 17 conception cycles could be aligned on the day of mid-cycle oestradiol drop.

To investigate the effect of ovarian steroid concentrations on the probability of successful conception, it was necessary to confirm that exposure to the risk of conception was the same in the non-conception cycles as it was in the conception cycles. After identification of the day of the mid-cycle oestradiol drop (day 0), the daily records for the conception cycles were examined. It was found that in each conception cycle intercourse occurred at least once between day −2 and day +2. Therefore we required that for a non-conception cycle to be included as an exposed cycle, intercourse must have taken place at least once in this interval. Of the non-conception cycles, 11 failed to meet this criterion and were eliminated from the group of exposed cycles. The remaining 98 cycles represented all of the original 24 women, 10 of whom contributed both a conception cycle and one or more (n = 1–13) exposed non-conception cycles, seven of whom contributed only a conception cycle, and seven of whom contributed only exposed non-conception cycles (n = 1–12).

Following identification of the day of the mid-cycle oestradiol drop, several indices of hormone concentration were calculated. A follicular index was defined as the average of the hormone concentrations on days −10 to −1; this interval was also divided to yield mid-follicular (days −10 to −6) and late follicular (days −5 to −1) indices. Similarly, a luteal index was defined as the average of the concentrations on days 0 to +5 only (to avoid including any days on which the hormone concentrations in conception cycles might have been influenced by the presence of an implanted embryo). Very early luteal (days 0 to +2) and early/mid-luteal (+3 to +5) indices were also calculated. An index of relative body weight was also calculated for each exposed cycle for as many of the women as possible. Relative weight was defined as the body weight for a cycle (most recent weight recorded prior to the start of the cycle) divided by the average weight for that woman over the course of her participation in the study.

Table I. Comparison of indices of salivary oestradiol (pmol/l) between naturally occurring conception and non-conception cycles

	Conception cycles (n = 17)	Significance of difference	Exposed non-conception cycles (n = 81)
Follicular (days –10 to –1)	14.2 ± 1.5	[a]	10.9 ± 0.7
Luteal (days 0 to +5)	13.6 ± 1.3	[a]	10.7 ± 0.7
Mid-follicular (days –10 to –6)	12.6 ± 1.7	[b]	8.5 ± 0.6
Late follicular (days –5 to –1)	15.6 ± 1.5	NS	13.0 ± 0.8
Very early luteal (days 0 to +2)	12.8 ± 1.4	[a]	9.9 ± 0.7
Early/mid-luteal (days +3 to +5)	14.5 ± 1.6	NS	11.4 ± 0.9

Cycles aligned on the day of the mid-cycle oestradiol drop (day 0). Values are means ± SE. NS = not significant.
[a] $P < 0.05$; [b] $P < 0.01$; one-sided t-tests.

Table II. Comparison of indices of salivary oestradiol (pmol/l) in 10 naturally occurring conception cycles and 40 non-conception cycles from the same women

	Conception cycles (n = 10)	Significance of difference	Exposed non-conception cycles (n = 40)
Follicular (days –10 to –1)	15.7 ± 2.2	[a]	8.3 ± 0.8
Luteal (days 0 to +5)	14.5 ± 1.5	[b]	8.8 ± 0.9
Mid-follicular (days –10 to –6)	14.5 ± 2.3	[a]	6.5 ± 0.7
Late follicular (days –5 to –1)	16.8 ± 2.3	[b]	9.9 ± 0.9
Very early luteal (days 0 to +2)	13.7 ± 2.0	[b]	7.7 ± 0.8
Early/mid-luteal (days +3 to +5)	15.5 ± 1.4	[c]	9.9 ± 1.2

Cycles aligned on the day of the mid-cycle oestradiol drop (day 0). Values are means ± SE.
[a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$; one-sided t-tests.

Statistical analysis

Group differences in hormone concentrations were analysed using t-tests (P values one-sided). Within-woman differences between conception and average non-conception concentrations were analysed using paired t-tests (P values one-sided). The relationship between indices of hormonal concentration and the probability of successful conception was examined using logistic regression. Data for the logistic regression consisted of a hormonal index as the independent variable and the outcome of the cycle (non-conception or conception) as the dependent variable; a maximum likelihood curve was fitted specifying the effect of the independent variable on the probability of the outcome. The relationship between relative weight and indices of hormonal concentration was investigated using linear regression.

All of the analyses described in this report involved comparisons of values that represented averages of from three (for indices of luteal oestradiol) to 81 (for the profile of daily concentrations in non-conception cycles) samples. In all cases, the SE of these averages, which are used to judge the statistical significance of the comparisons, are reported; they reflect the increased precision of average over individual values. For example, the SE of the indices of follicular and luteal oestradiol compared in Tables I and II range, in absolute terms, from 0.6 to 2.3 pmol/l (average 1.3) or, in relative terms, from 6.2 to 15.7% (average 10.3) of index mean.

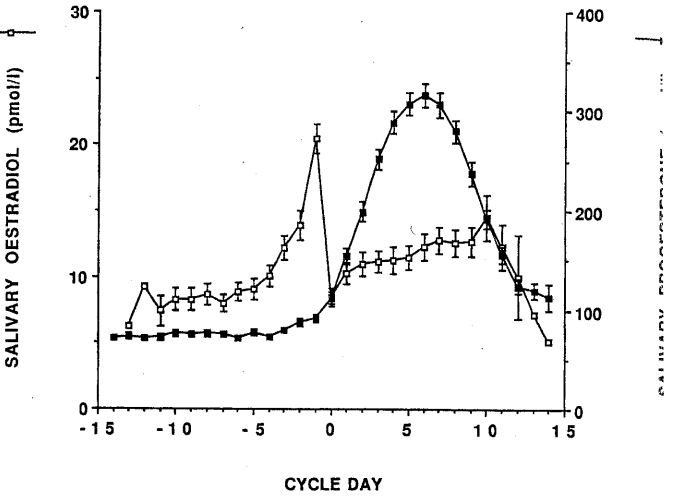

Figure 1. Profiles of average daily concentrations of salivary oestradiol and progesterone from 92 non-conception cycles. Vertical bars represent SE. Data have been aligned on the day of the mid-cycle oestradiol drop.

Results

The average daily profiles of progesterone and oestradiol for all non-conception cycles (n = 92), aligned on the day of the mid-cycle oestradiol drop, are shown in Figure 1. Although the total ranges of progesterone and oestradiol concentrations obtained in saliva were compressed compared with their ranges in serum, the pattern of the profiles was clearly comparable with profiles based on blood hormone concentrations (Yen, 1991).

Figure 2A shows the average daily oestradiol profile for the group of all exposed non-conception cycles (n = 81) compared with the profile for the group of all conception cycles. In Figure 2B, this comparison was further narrowed so that only cycles from those 10 women who contributed both conception and non-conception cycles were included. In both comparisons it was evident that the average oestradiol profiles from the conception cycles were consistently higher than the average profiles from the non-conception cycles, with the most pronounced difference being in the mid-follicular interval (days –10 to –6). When only cycles from the same women were included (Figure 2B), the contrast between the average oestradiol profiles from conception and non-conception cycles was even greater than when values from all women were combined. The analogous comparisons of average daily progesterone profiles (data not shown) displayed no differences between non-conception and conception cycles up to day +5. Divergence between conception and non-conception progesterone profiles was clear only after day +8, when progesterone concentrations in conception cycles began to rise, presumably in response to human chorionic gonadotrophin.

The differences between oestradiol concentrations in conception and non-conception cycles, shown by the average daily profiles, were confirmed when the indices of oestradiol concentration were analysed. Table I presents the average values of the oestradiol indices for all exposed non-conception cycles

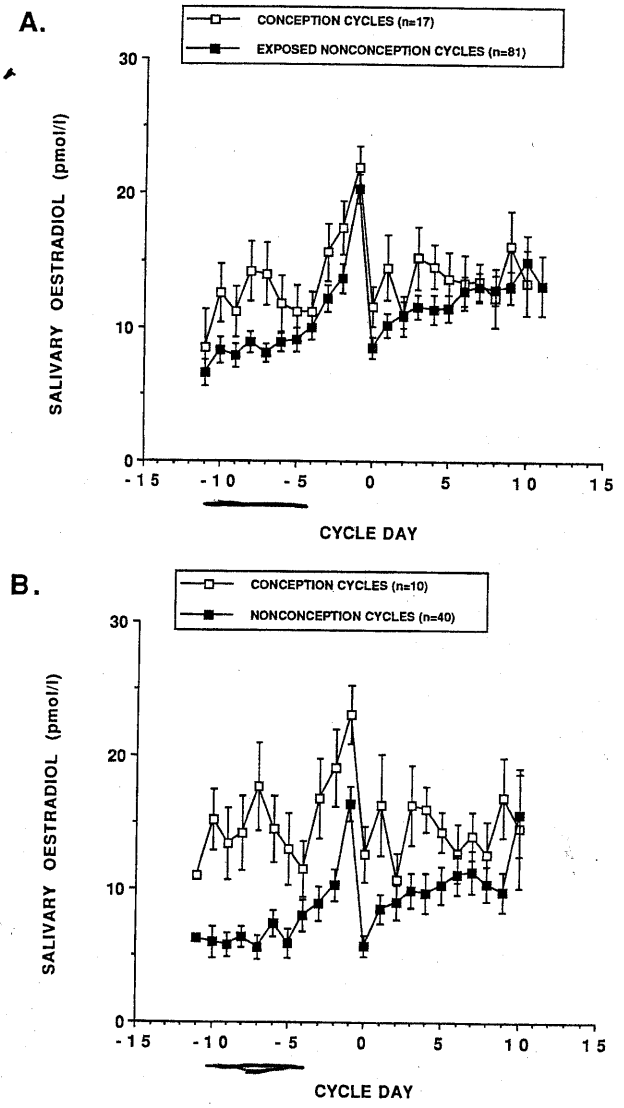

Figure 2. Comparison of average daily salivary oestradiol concentrations in naturally occurring conception and non-conception cycles. (**A**) Comparison of 17 conception cycles with 81 exposed non-conception cycles. (**B**) Comparison of the conception (n = 10) and the exposed non-conception (n = 40) cycles from 10 conceiving women. Vertical bars represent SE. Data have been aligned on the day of the mid-cycle oestradiol drop.

and for all conception cycles. Average values of all indices were higher for the conception cycles; these differences were statistically significant for the indices of average follicular and average luteal oestradiol and for the indices of mid-follicular and very early luteal oestradiol. Table II presents similar values for the cycles of the 10 women who contributed both conception and non-conception cycles. In this analysis, the differences between the average values in conception and non-conception cycles were statistically significant for all indices, with the indices of average follicular oestradiol and mid-follicular oestradiol achieving the greatest significance.

To investigate whether these average differences in oestradiol concentration between conception and non-conception cycles were also demonstrable at the level of the individual, we used paired t-tests to compare the conception and average non-conception values of the oestradiol indices for each of the 10

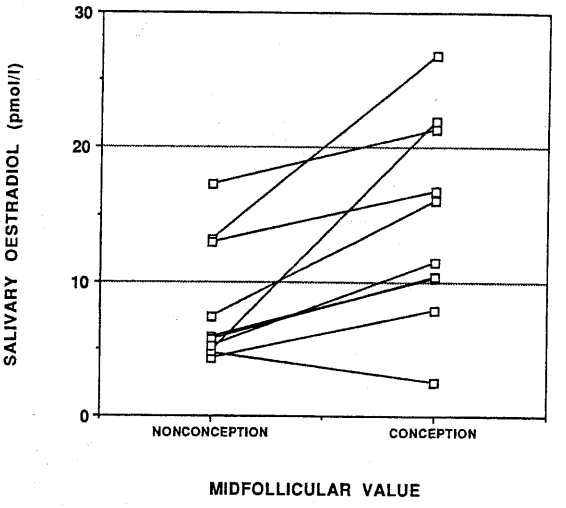

Figure 3. Comparison of average non-conception and conception cycle values of mid-follicular oestradiol from 10 conceiving women.

women for whom we had both conception and non-conception cycle data. This analysis showed that conception cycle values were significantly higher than non-conception cycle values for all three of the follicular oestradiol indices ($P < 0.01$). Figure 3 shows, for example, the difference between the conception and non-conception values of mid-follicular oestradiol for the 10 women. Conception cycle values of the same three oestradiol indices were also significantly higher than non-conception values when the analysis was restricted to pairwise comparisons of the values from each woman's conception cycle with those from her preceding (non-conception) cycle run in the same assay. These results demonstrated that enhanced concentrations of follicular oestradiol were characteristic of the conception cycles of individual women, as well as of conception cycles on average, and confirmed that this finding was not biased by interassay variability.

Logistic regression was used to quantify the relationship between indices of hormonal concentration and the probability of successful conception. The hormonal index with the greatest predictive power for successful conception was mid-follicular oestradiol. For the group of all exposed cycles (n = 98), the correlation between mid-follicular oestradiol concentration and the probability of successful conception was weak but statistically significant ($P < 0.05$); thus, while the probability of conception was <10% at the lowest values of mid-follicular oestradiol, it rose to ~50% at the highest values. A similar analysis, using only values from women who contributed both conception and non-conception cycles (n=50), revealed that mid-follicular oestradiol concentrations had a much more powerful predictive value for this group. When either absolute or relative (difference between the value for a cycle and the average value for that woman over all of her exposed cycles) values of mid-follicular oestradiol were used as the independent variable, the correlation with the probability of successful conception was highly statistically significant ($P < 0.001$). Figure 4 presents the distribution of observed values of relative mid-follicular oestradiol. For each value, the proportion of conception and non-conception cycles is indicated. The logistic

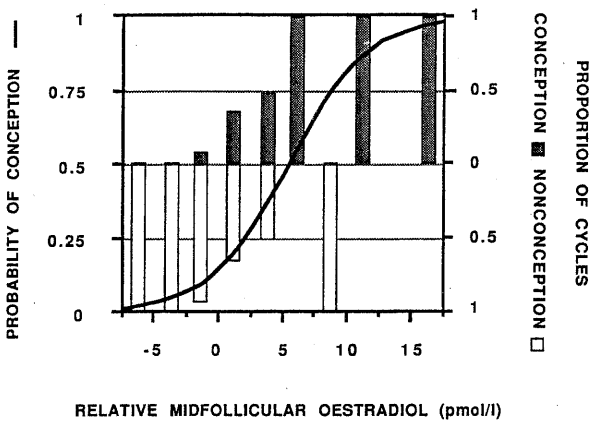

Figure 4. Relationship between relative mid-follicular oestradiol values and the probability of conception. The distribution of observed values (n = 50) is shown by the bars; for each value, the proportion of conception (shaded) and non-conception (open) cycles is indicated. The curve represents the form of the logistic regression, relating the probability of conception to the value of relative mid-follicular oestradiol, derived from these data ($P < 0.001$).

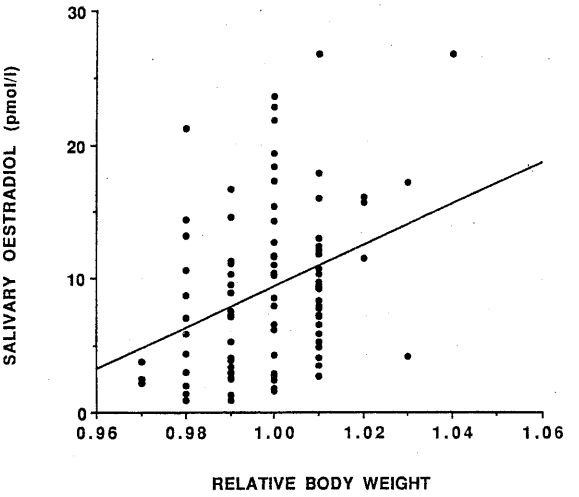

Figure 5. Correlation between relative body weight and mid-follicular oestradiol (r = 0.34, $P < 0.001$) in conception and non-conception cycles (n = 92). Relative body weight was defined as the most recent weight recorded prior to the start of a cycle divided by average weight for that woman over the course of her participation in the study.

regression curve derived from these data, which related the probability of conception to the value of relative mid-follicular oestradiol concentration, is also shown. From the curve, it can be seen that, whereas the probability of conception was ~15% in cycles where the concentration of mid-follicular oestradiol was average for a woman, it rose to >95% in cycles where mid-follicular oestradiol concentration was 15 pmol/l greater than average, and fell to <5% in cycles where mid-follicular oestradiol concentration was 5 pmol/l less than average. Although, as measured in saliva, the absolute magnitudes of these deviations were small, they represented significant relative changes compared with average values. For example, the deviations from average for the conception cycles corresponded to percentage increases ranging from 10 to 70% (two outliers excepted). These results demonstrated that, for this group, within-woman variance in mid-follicular oestradiol concentrations had a significant effect on the probability of successful conception.

The range of values of relative body weight observed in this study was rather narrow, i.e. the weights of our subjects did not vary greatly over the course of their participation in the study. Nevertheless, there was a statistically significant relationship, even in this study, between relative body weight and the oestradiol indices. In particular, as shown in Figure 5, mid-follicular oestradiol value was significantly positively correlated with relative body weight (r = 0.34, $P < 0.001$). Although relative weight accounted for only slightly over 10% of the variance in mid-follicular oestradiol values, this result suggested that cycles in which a woman was above her average body weight tended to have higher values of mid-follicular oestradiol than cycles in which she was below her average weight.

Discussion

In this study, we demonstrated a significant difference in follicular oestradiol concentration between naturally occurring conception and non-conception cycles. In contrast to many other studies, none of our data came from stimulated or treatment cycles, and exposure to intercourse in non-conception cycles was verified. Our subjects were healthy, well nourished, well educated women with no previous history of infertility. Thus they probably represent a relatively narrow sample of the total range of variability in female fecundity. Among these women, we found no difference between progesterone concentrations in conception and non-conception cycles until after the mid-luteal phase (the presumed time of implantation). In contrast, we found a significant difference in oestradiol concentration between conception and non-conception cycles, which was most pronounced during the mid-follicular phase, i.e. even before ovulation had occurred.

Several pathways exist through which follicular oestradiol concentrations may affect cycle fecundity. Studies have shown that suppressed follicular development may lead to defective luteal function (DiZerega and Hodgen, 1981). Effects of follicular phase oestradiol concentrations have also been shown on cervical mucus and uterine perfusion; lowered penetrability of the cervical mucus and poor uterine perfusion, both related to lower oestradiol concentrations, have each been related to lowered fertility (Roumen *et al.*, 1982; Goswamy *et al.*, 1988). Oestradiol concentrations have also been related to endometrial morphology (Johannisson *et al.*, 1982; Li *et al.*, 1992) and endometrial thickness (Adams *et al.*, 1988; Noyes *et al.*, 1995); indeed Dickey *et al.* (1993) found that endometrial thickness was predictive of fecundity in ovulation induction cycles.

A correlation has also been demonstrated between circulating oestradiol concentrations during the follicular phase and follicle size (Hacklöer *et al.*, 1979; Eissa *et al.*, 1986; Lejeune *et al.*, 1986; Apter *et al.*, 1987; Adams *et al.*, 1988); specifically, ultrasound evidence suggests that circulating oestradiol concentrations are produced mainly by the dominant follicle (Hodgen, 1982; Adashi, 1994; van Santbrink *et al.*, 1995). Thus, a higher follicular oestradiol concentration is associated with the

presence of a larger dominant follicle. Oestradiol concentration and follicular size, in turn, have been correlated with oocyte quality. Low oestradiol concentrations have been shown to decrease oocyte fertilizability (Yoshimura and Wallach, 1987; Zelinski-Wooten *et al.*, 1994), while small oocyte and follicle size have been related to impaired oocyte maturation (Durzini *et al.*, 1995) and reduced pregnancy rates in in-vitro fertilization (IVF; Lejeune *et al.*, 1986). Conversely, in IVF programmes, increasing follicular volume has been correlated with an increased likelihood of successful egg retrieval, normal fertilization and good embryo quality (Arnot *et al.*, 1995), and a pattern of robust follicular growth (particularly rapid early growth) has been shown to be highly correlated with successful pregnancy outcome (Nayudu, 1991).

In this study we showed that (i) average oestradiol concentrations during the follicular phase are higher in naturally occurring conception cycles compared with exposed non-conception cycles, (ii) this difference is most significant during the mid-follicular phase (6–10 days before the mid-cycle oestradiol drop), and (iii) this difference exists not just for conception and non-conception cycles as a group but also between the conception and non-conception cycles of individual women. These findings are consistent with studies of follicular development which show that a significant increase in circulating oestradiol concentrations occurs ~1 week before the LH surge, this increase is associated with the appearance of the dominant follicle, the production of oestradiol is correlated with follicular surface area, and large, oestrogen-rich follicles are the source of ova most likely to undergo successful fertilization and ongoing pregnancy. Thus, it may be that the higher follicular, and particularly mid-follicular, oestradiol concentrations we observed in conception cycles were associated with the selection and development, in those cycles, of dominant follicles with enhanced growth profiles and superior capacities to synthesize oestradiol.

It could be that the development of a 'good' dominant follicle is merely the result of random selection. Alternatively, environmental factors, such as energy balance, may influence levels of ovarian steroid production, which, in turn, may affect follicular and endometrial development and oocyte quality. Our observation in this study of a correlation between relative body weight and mid-follicular oestradiol concentration supports this hypothesis, as does the finding by Bates *et al.* (1982) that fertility is improved by 5–10% increases in body weight among women who practice weight control, and the demonstration by Bailey *et al.* (1992) that periods of female weight loss were correlated with decreased ovulatory frequency and fewer conceptions among Lese agriculturalists in Zaire. Ellison *et al.* (1993) have proposed that the sensitivity of ovarian function to energy balance provides a means of adjusting female fecundity to ecological context; recent studies showing that insulin has a stimulatory effect on ovarian steroid production (Samoto *et al.*, 1993; Nahum *et al.*, 1995; Willis and Franks, 1995; Willis *et al.*, 1996) suggest a mechanism by which weight gain could influence oestradiol concentrations.

This demonstration of higher follicular oestradiol concentrations in cycles that go on to become conception cycles, compared with non-conception cycles from the same women and non-conception cycles as a group, emphasizes the important relationship between variation in follicular development and fecundity, even in the absence of significant luteal variability. Our findings also support the observation that not all women can be characterized as having either uniformly high or uniformly reduced fecundity. Rather, at least for some women, there is significant between-cycle variation in the pattern of follicular development, which may be associated with significant between-cycle variability in fecundity. Recognition of such variability as a normal aspect of female ovarian function, along with increased knowledge of the causes and patterns of occurrence of higher and lower fecundity cycles, may have implications for the counselling and treatment of women who are trying to become pregnant.

Acknowledgements

We thank Sara Sukalich for technical assistance, and Dr Gordon D.Niswender, Colorado State University, Fort Collins, CO, USA for supplying progesterone and oestradiol antisera. This research was supported by the National Science Foundation, grant BNS-91-18074.

References

Adams, J.M., Tan, S.L., Wheeler, M.J. *et al.* (1988) Uterine growth in the follicular phase of spontaneous ovulatory cycles and during luteinizing hormone-releasing hormone-induced cycles in women with normal or polycystic ovaries. *Fertil. Steril.*, **49,** 52–55.

Adashi, E.Y. (1994) Endocrinology of the ovary. *Hum. Reprod.*, **9,** 815–827.

Apter, D., Räisänen, I., Ylöstalo, P. and Vihko, R. (1987) Follicular growth in relation to serum hormonal patterns in adolescent compared with adult menstrual cycles. *Fertil. Steril.*, **47,** 82–88.

Arnot, A.M., Vandekerckhove, P., DeBono, M.A. and Rutherford, A.J. (1995) Follicular volume and number during in-vitro fertilization: association with oocyte developmental capacity and pregnancy rate. *Hum. Reprod.*, **10,** 256–261.

Bailey, R.C., Jenike, M.R., Ellison, P.T. *et al.* (1992) The ecology of birth seasonality among agriculturalists in central Africa. *J. Biosoc. Sci.*, **24,** 393–412.

Bates, G.W., Bates, S.R. and Whitworth, N.S. (1982) Reproductive failure in women who practice weight control. *Fertil. Steril.*, **37,** 373–378.

Bullen, B.A., Skrinar, G.S., Beitins, I.Z. *et al.* (1985) Induction of menstrual disorders by strenuous exercise in untrained women. *N. Engl. J. Med.*, **312,** 1349–1353.

Cedard, L., Guichard, A., Janssens, Y. *et al.* (1987) Progesterone and estradiol in saliva after in vitro fertilization and embryo transfer. *Fertil. Steril.*, **47,** 278–283.

Danutra, V., Turkes, A., Read, G.F. *et al.* (1989) Progesterone concentrations in samples of saliva from adolescent girls living in Britain and Thailand, two countries where women are at widely differing risk of breast cancer. *J. Endocrinol.*, **121,** 375–381.

De Crée, C., Lewin, R. and Ostyn, M. (1990) The monitoring of the menstrual status of female athletes by salivary steroid determination and ultrasonography. *Eur. J. Appl. Physiol.*, **60,** 472–477.

Dickey, R.P., Olar, T.T., Taylor, S.N. *et al.* (1993) Relationship of endometrial thickness and pattern to fecundity in ovulation induction cycles: effect of clomiphene citrate alone and with human menopausal gonadotropin. *Fertil. Steril.*, **59,** 756–760.

DiZerega, G.S. and Hodgen, G.D. (1981) Luteal phase dysfunction infertility: a sequel to aberrant folliculogenesis. *Fertil. Steril.*, **35,** 489–499.

Durzini, K.L., Saniga, E.M. and Lanzendorf, S.E. (1995) The relationship between size and maturation *in vitro* in the unstimulated human oocyte. *Fertil. Steril.*, **63,** 404–406.

Eissa, M.K., Obhrai, M.S., Docker, M.F. *et al.* (1986) Follicular growth and endocrine profiles in spontaneous and induced conception cycles. *Fertil. Steril.*, **45,** 191–195.

Ellison, P.T. (1988) Human salivary steroids: methodological considerations and applications in physical anthropology. *Yearb. Phys. Anthropol.*, **31,** 115–142.

Ellison, P.T., Peacock, N.R. and Lager, C. (1986) Salivary progesterone and luteal function in two low-fertility populations of northeast Zaire. *Hum. Biol.*, **58,** 473–483.

Ellison, P.T., Peacock, N.R. and Lager, C. (1989) Ecology and ovarian function among Lese women of the Ituri forest, Zaire. *Am. J. Phys. Anthropol.*, **78,** 519–526.

Ellison, P.T., Panter-Brick, C., Lipson, S.F. and O'Rourke, M.T. (1993) The ecological context of human ovarian function. *Hum. Reprod.*, **8,** 2248–2258.

Gibori, G., Antczak, E. and Rothchild, I. (1977) The role of estrogen in the regulation of luteal progesterone secretion in the rat after day 12 of pregnancy. *Endocrinology*, **100,** 1483–1495.

Goswamy, R.K., Williams, G. and Steptoe, P.C. (1988) Decreased uterine perfusion — a cause of infertility. *Hum. Reprod.*, **3,** 955–959.

Hacklöer, B.J., Fleming, R., Robinson, H.P. *et al.* (1979) Correlation of ultrasonic and endocrinologic assessment of human follicular development. *Am. J. Obstet. Gynecol.*, **135,** 122–128.

Hodgen, G.D. (1982) The dominant ovarian follicle. *Fertil. Steril.*, **38,** 281–299.

Hoff, J.D., Quigley, M.E. and Yen, S.S.C. (1983) Hormonal dynamics at mid-cycle: a reevaluation. *J. Clin. Endocrinol. Metab.*, **57,** 792–796.

Johannisson, E., Parker, R.A., Landgren, B.-M. and Diczfalusy, E. (1982) Morphometric analysis of the human endometrium in relation to peripheral hormone levels. *Fertil. Steril.*, **38,** 564–571.

Korenman, S.G., Stevens, R.H., Carpenter, L.A. *et al.* (1974) Estradiol radioimmunoassay without chromatography: procedure, validation and normal values. *J. Clin. Endocrinol. Metab.*, **38,** 718–720.

Lejeune, B., Degueldre, M., Camus, M. *et al.* (1986) In vitro fertilization and embryo transfer as related to endogenous luteinizing hormone rise or human chorionic gonadotropin administration. *Fertil. Steril.*, **45,** 377–383.

Lenton, E.A., Sulaiman, R., Sobowale, O. and Cooke, I.D. (1982) The human menstrual cycle: plasma concentrations of prolactin, LH, FSH, oestradiol and progesterone in conceiving and non-conceiving women. *J. Reprod. Fertil.*, **65,** 131–139.

Lewinthal, D., Furman, A., Blankstein, J. *et al.* (1986) Subtle abnormalities in follicular development and hormonal profile in women with unexplained infertility. *Fertil. Steril.*, **46,** 833–839.

Li, T.C., Cooke, I.D., Warren, M. *et al.* (1992) Endometrial responses in artificial cycles: a prospective study comparing four different oestrogen dosages. *Br. J. Obstet. Gynaecol.*, **99,** 751–756.

Lipson, S.F. and Ellison, P.T. (1989) Development of protocols for the application of salivary steroid analyses to field conditions. *Am. J. Hum. Biol.*, **1,** 249–255.

Lipson, S.F. and Ellison, P.T. (1992) Normative study of age variation in salivary progesterone profiles. J. *Biosoc. Sci.*, **24,** 233–244.

Metropolitan Life Insurance Company (1983) 1983 Metropolitan height and weight tables. *Stat. Bull. Metrop. Insur. Co.*, **64,** 2–9.

Nahum, R., Thong, K.J. and Hillier, S.G. (1995) Metabolic regulation of androgen production by human thecal cells *in vitro*. *Hum. Reprod.*, **10,** 75–81.

Nayudu, P.L. (1991) Relationship of constructed follicular growth patterns in stimulated cycles to outcome after IVF. *Hum. Reprod.*, **6,** 465–471.

Noyes, N., Liu, H.-C., Sultan, K. *et al.* (1995) Endometrial thickness appears to be a significant factor in embryo implantation in in-vitro fertilization. *Hum. Reprod.*, **10,** 919–922.

O'Rourke, M.T. and Ellison, P.T. (1993) Salivary estradiol levels decrease with age in healthy, regularly cycling women. *Endocr. J.*, **1,** 487–494.

Panter-Brick, C., Lotstein, D.S. and Ellison, P.T. (1993) Seasonality of reproductive function and weight loss in rural Nepali women. *Hum. Reprod.*, **8,** 684–690.

Pirke, K.M., Schweiger, U., Lemmel, W. *et al.* (1985) The influence of dieting on the menstrual cycle of healthy young women. *J. Clin. Endocrinol. Metab.*, **60,** 1174–1179.

Read, G.F., Wilson, D.W., Hughes, I.A. and Griffiths, K. (1984) The use of salivary progesterone assays in the assessment of ovarian function in postmenarcheal girls. *J. Endocrinol.*, **102,** 265–268.

Roumen, F.J.M.E., Doesburg, W.H. and Rolland, R. (1982) Hormonal patterns in infertile women with a deficient postcoital test. *Fertil. Steril.*, **38,** 42–47.

Samoto, T., Maruo, T., Ladines-Llave, C.A. *et al.* (1993) Insulin receptor expression in follicular and stromal compartments of the human ovary over the course of follicular growth, regression and atresia. *Endocr. J.*, **40,** 715–726.

van Santbrink, E.J.P., Hop, W.C., van Dessel, T.J.H.M. *et al.* (1995) Decremental follicle-stimulating hormone and dominant follicle development during the normal menstrual cycle. *Fertil. Steril.*, **64,** 37–43.

Vuorento, T., Lahti, A., Hovatta, O. and Huhtaniemi, I. (1989) Daily measurements of salivary progesterone reveal a high rate of anovulation in healthy students. *Scand. J. Clin. Lab. Invest.*, **49,** 395–401.

Walker, S.M., Walker, R.F. and Riad-Fahmy, D. (1984) Longitudinal studies of luteal function by salivary progesterone determinations. *Horm. Res.*, **20,** 231–240.

Walker, R.F., Wilson, D.W., Truran, P.L. *et al.* (1985) Characterization of profiles of salivary progesterone concentrations during the luteal phase of fertile and subfertile women. *J. Endocrinol.*, **104,** 441–446.

Willis, D. and Franks, S. (1995) Insulin action in human granulosa cells from normal and polycystic ovaries is mediated by the insulin receptor and not the type-I insulin-like growth factor receptor. *J. Clin. Endocrinol. Metab.*, **80,** 3788–3790.

Willis, D., Mason, H., Gilling-Smith, C. and Franks, S. (1996) Modulation by insulin of follicle-stimulating hormone and luteinizing hormone actions in human granulosa cells of normal and polycystic ovaries. *J. Clin. Endocrinol. Metab.*, **81,** 302–309.

Worthman, C.M., Jenkins, C.J., Stallings, J.F. and Lai, D. (1993) Attenuation of nursing-related ovarian suppression and high fertility in well-nourished, intensively breast-feeding Amele women of lowland Papua New Guinea. *J. Biosoc. Sci.*, **25,** 425–443.

Yen, S.S.C. (1991) The human menstrual cycle: neuroendocrine regulation. In Yen, S.S.C. and Jaffe, R.B. (eds), *Reproductive Endocrinology.* W.B.Saunders Company, Philadelphia, PA, USA, pp. 273–308.

Yoshimura, Y. and Wallach, E.E. (1987) Studies of the mechanism(s) of mammalian ovulation. *Fertil. Steril.*, **47,** 22–34.

Zarutskie, P.W., Kuzan, F.B., Dixon, L. and Soules, M.R. (1987) Endocrine changes in the late-follicular and postovulatory intervals as determinants of the in vitro fertilization pregnancy rate. *Fertil. Steril.*, **47,** 137–143.

Zelinski-Wooten, M.B., Hess, D.L., Wolf, D.P. and Stouffer, R.L. (1994) Steroid reduction during ovarian stimulation impairs oocyte fertilization, but not folliculogenesis, in rhesus monkeys. *Fertil. Steril.*, **61,** 1147–1155.

Received on November 24, 1995; accepted on July 24, 1996