SUMMARY

In a retrospective studies of spontaneous abortion, bias can arise if the investigator restricts attention to the outcome of the most recent pregnancy. Bias happens because couples tend to replace spontaneous abortions with other pregnancies until they achieve a live birth, after which they may avoid conception for a long time. Consequently, the proportion of most recent conceptions ending in loss might be very low compared to what would be seen in a prospective study. Also, time since conception is negatively correlated with apparent risk, because most pregnancies which occurred relatively long ago and ended in loss have been replaced by subsequent pregnancies. If the exposure of interest may also have changed over time, then time since conception should be considered a potential confounder. In addition, the consistency with which couples use birth control varies. Some couples are planners who use effective contraception and plan the growth of their family with deliberation; others have most of their pregnancies unintentionally. As a result, the most recent pregnancy is less likely to be a spontaneous abortion for a planner than for a nonplanner. If planners and nonplanners also differ in their exposures, then bias will result. Other factors which affect the nterpregnancy interval, such as fecundability, breastfeeding, and desired family size can distort the relative sampling fraction for spontaneous abortions compared to live births and, to the extent that such factors are associated with the exposure, can bias estimates of relative risks.

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Salivary Steroids and Natural Variation in Human Ovarian Function[a]

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INTRODUCTION

Measurements of salivary steroids were first made in the 1960s,[1] but it was not until Walker, Read, Riad-Fahmy and their colleagues at the Tenovus Cancer Institute began to develop and apply salivary steroid assays in clinical investigations in the late 1970s[2-9] that the potential utility of saliva as a sampling medium for steroid analyses became apparent. Assays for the major gonadal and adrenal steroids were developed in several laboratories in the ensuing years[10] and applied to a wide range of clinical problems and basic research areas such as screening for adrenal hyperplasia,[5] monitoring adolescent development,[11] monitoring gonadal function as an adjunct to the diagnosis and treatment of infertility,[12] monitoring the progress of pregnancy,[13] aiding the diagnosis of depression,[14] elucidating the mechanisms of aggressive behavior,[15] assessing non-specific stress,[16] and studying the origins of personality and temperament.[17]

Among the earliest and now most widely used applications has been the use of salivary progesterone measurements to assess female ovarian function.[18,19] Salivary progesterone assays are now ranked as "mature" assays according to criteria set forth by Read,[20] with good consistency across laboratories, well-defined reference values, and high correlations with reference methods such as mass spectroscopy.[19] At the same time the relatively non-invasive nature of sample collection facilitates the longitudinal monitoring necessary to adequately assess ovarian function.[4,6,8,21] Estradiol measurements in saliva are considerably more difficult, owing to the much lower circulating levels of this hormone combined with its high affinity for sex hormone binding globulin.[20]

This report describes my experience and that of my colleagues in using salivary progesterone assays to monitor human ovarian function in a variety of settings. Our principal motivation has been to understand patterns of normal variation in ovarian function as an important component of variation in female fecundity, as well as a contributing risk factor for breast and ovarian cancer and for osteoporosis. We conceive of ovarian function as occurring along a graded continuum from fully competent cycling at one extreme to frank amenorrhea at the other, separated by intermediate levels of follicular and luteal suppression, ovulatory failure, and oligomenorrhea[22,23] (Fig. 1). It is now apparent that many constitutional, behavioral, and ecological factors which are associated with menstrual disruption at extreme values (*e.g.*, aerobic exercise, weight loss, nutritional status, intensity of lactation, even age) are associated with more subtle suppression of ovarian activity at more moderate values, and that individual women often progress in one direction or another along the

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continuum of ovarian function as the influence of these factors in their lives waxes and wanes.[22]

Salivary steroid measurements are particularly useful for monitoring variation in ovarian function that is not associated with variation in the frequency of menstruation, variation that would not ordinarily be noticed by a woman or her physician. Although subtle, such variation can have real significance for individual fecundity. Estradiol levels have been associated positively with endometrial thickness and the probability of successful implantation,[24-27] as well as with the fertilizability of the oöcyte[28] and the density of progesterone receptors in the secretory phase endometrium.[29] Progesterone is necessary for the secretory transformation of the endometrium in preparation for implantation and for the maintenance of the endometrium throughout the pregnancy.[24,30-33] The crucial contribution of progesterone to female fecundity is made apparent by the effectiveness of RU486, a progesterone receptor antagonist, in terminating pregnancy and precipitating menstruation at anytime from the early luteal phase through mid-gestation,[34] and by the effectiveness of exogenous progesterone dministration in restoring the fecundity of women over 40.[35] Female fecundity may vary directly as a continuous function of steroid values, or as a stochastically variable step function, with similar cross-sectional results in either case. Variation in ovarian function, both acute and developmental, is also recognized as an important risk factor n ovarian and uterine cancer,[36] breast cancer,[37-39] and osteoporosis.[40]

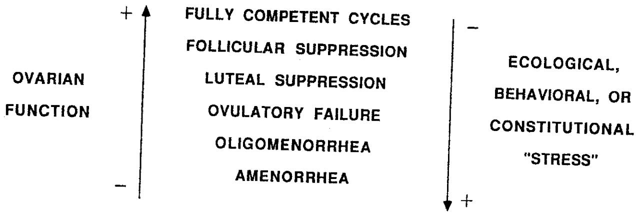

FIGURE 1. The continuum of ovarian function.[22,23]

We have developed a salivary progesterone assay that is both sensitive and pecific[41] together with protocols for the collection and handling of saliva samples in he field[42,43] that have allowed us to pursue a goal of gathering directly comparable ata on ovarian function from populations that differ substantially in their genetic ckground, ecology and geography, basic economy, social organization, and culture. t present we have information on populations from five continents representing a nge of levels of economic development, with further work in progress. The formation we have gathered in this way has allowed us to begin to gain a mparative perspective on human ovarian function that has previously been lacking d to distinguish patterns of variation that appear to be common across human pulations from aspects that appear to be locally specific. In this report I will review o particular correlates of variation in ovarian function that are of interest to students human reproductive ecology: age and energetics.

AGE AND OVARIAN FUNCTION

An older, general impression, reinforced by the eidetic representations of "the nstrual cycle" in textbooks of medicine and human physiology, has been that natural, non-pathological variation in ovarian function is not a significant phenomenon. This is akin to the Malthusian notion that there is no natural, non-pathological variation in human fecundity, that we would all reproduce like rabbits, or at least like Hutterites, if social and behavioral constraints did not intervene. In particular, there has been an impression that ovarian function varies little with female age between the perimenarcheal and perimenopausal years. This view was supported by the data of Treloar *et al.,*[44] showing little variation in menstrual cycle length or regularity between these two age extremes, even though contemporaneous data existed suggesting significant variation in hormonal patterns with age that was not reflected by menstrual variation.[45] Recently this older view of constant ovarian function across the middle decades of a woman's reproductive career has begun to give way under the weight of evidence from the success rates of various assisted reproductive technologies, including artificial insemination by donor,[46-48] *in vitro* fertilization,[49] ovulation induction,[27,50] and ovum donation,[35,51,52] which all show significant declines beginning as early as the mid-30s. At the same time, careful scrutiny of data on age-specific fertility in relation to coital frequency suggests that age-related declines in the frequency of intercourse are unlikely by themselves to account for declining fertility with increasing maternal age.[53] The general pattern of increasing fertility with age among married women under 25 has largely been ignored.

We set out to examine patterns of variation in ovarian function in Boston women as indexed by salivary progesterone across the very age interval that Treloar *et al.* had characterized as essentially constant.[54] One hundred twenty-four women were recruited for the study, evenly distributed in seven age groups between 18 and 44 years. All the subjects were regularly menstruating, of stable weight and normal weight for height. None exercised more than three hours a week or used any steroid contraception, none had been pregnant or lactating within six months, and none reported any history of menstrual or gynecological problems. All subjects collected daily saliva samples over the course of one menstrual cycle which were analyzed for their progesterone content.

Despite the outward manifestation of regular menses, significant age-variation in salivary progesterone levels was apparent in this sample. The average menstrual profiles for each age group showed significant differences by repeated measures analysis of variance, while indices derived from the daily progesterone values, including ovulatory frequency, average luteal progesterone, average midluteal progesterone, and day of peak progesterone secretion, all showed significant parabolic trajectories by age, rising until the mid-20s and falling by the late-30s.[54] Significant age variation in progesterone indices remained even after anovulatory cycles were excluded from the analysis.

A fuller appreciation of the degree and nature of age variation and its relationship to individual variation can be gained from a plot of individual values for average midluteal progesterone plotted against age (FIG. 2A). (This plot includes data from an additional 12 women between 45 and 48 years who also met our subject selection criteria.) In this case the best fitting regression line is a second order polynomial with highly significant linear and quadratic terms. The overall regression is highly significant ($p < 0.001$), and residual analysis indicates no remaining age variation. However, the regression explains only about 10% of the total variance. The high degree of individual variability in levels of ovarian function does not, however, negate the significance of the average age trajectory any more than significant individual variation in height among same-aged children negates the significant relationship of height with age.

The relationship between age and ovarian function among Boston women can be directly compared with similar values obtained from 144 cycles from regularly cycling

Lese women living in the Ituri Forest of northeastern Zaïre.[55] The Lese are slash-and-burn horticulturalists of Sudanic descent who cultivate subsistence crops of manioc, maize, dry rice, and peanuts with extremely limited access to market goods.[56] We have been involved in a longitudinal study of the ecology, demography, and behavior of the Lese for over a decade, in the course of which ovarian function has been assessed on three separate occasions, in 1983, 1984, and 1989. The sample of 144 cycles thus includes repeated observations of some women at different ages.

The Lese also show significant age variation in midluteal progesterone indices (FIG. 2B), again best represented by a second order polynomial regression with highly significant linear and quadratic terms and again explaining approximately 10% of the total variance. Residual analysis indicates no remaining age-associated variance, and a pattern and range of residual distribution virtually identical to that of the Boston sample. The shape of the relationship between midluteal progesterone and age is also nearly identical to that in Boston, although the average levels are lower at every age. A two way analysis of variance of the data from both populations indicates a highly significant age effect ($p < 0.0001$), a highly significant population effect ($p < 0.0001$), and the absence of any significant interaction term ($p = 0.984$).

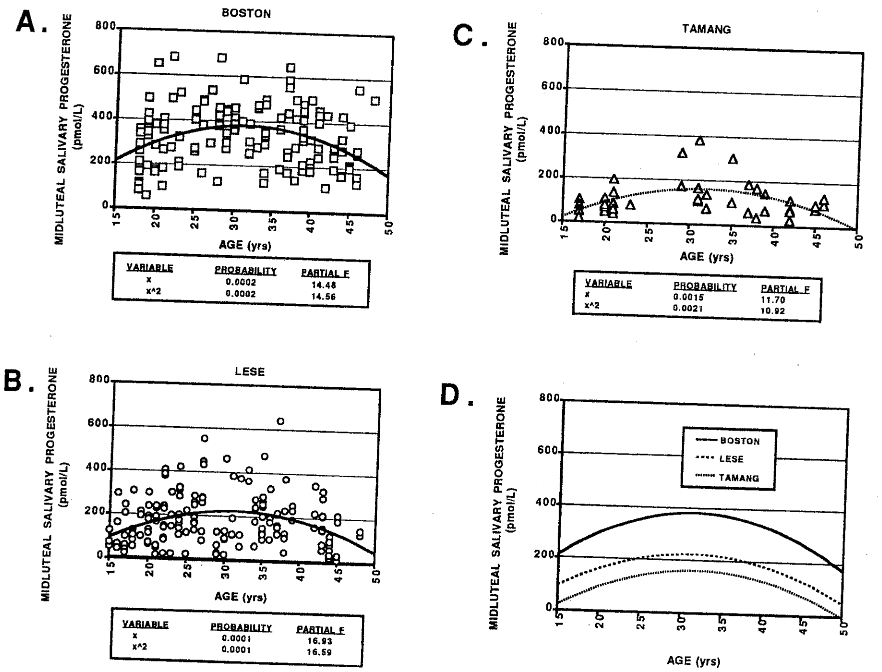

FIGURE 2. Age variation in values of midluteal salivary progesterone in three populations. A) Boston; B) Lese of Zaïre; C) Tamang of Nepal; D) a comparison of the three regression lines without individual data points.[78]

A similar analysis may be conducted of average midluteal progesterone values obtained from 45 Tamang women from the central highlands of Nepal.[55,57] The Tamang are agro-pastoralists who subsist on cereal crops and animal husbandry in the Himalayan foothills. Once again the relationship of midluteal progesterone to age is best fit by a second order polynomial with highly significant linear and quadratic terms (FIG. 2C). The average levels among the Tamang are lower even than among the Lese, and yet the shape of the relationship is extremely similar to that of both the Boston and Zaïre samples. Two way analysis of variance again confirms this impression, indicating significant age ($p < 0.0004$) and population ($p < 0.0001$) effects but no significant interaction term ($p = 0.980$).

These are the first data I am aware of which allow a direct comparison of patterns of age variation in ovarian function between different populations. The results are strikingly similar to the patterns of variation in natural fertility first described by Henry:[58] while considerable variation exists in the average levels of ovarian function between populations, the relative patterning by age is highly consistent (FIG. 2D). The demonstration of such a consistent pattern across populations of such different genetic, geographical, ecological, and cultural backgrounds indicates that age variation in this component of female reproductive physiology is a general feature of human reproductive biology and not one that is shaped by local circumstances. In addition, although ovarian function must be considered only one component of female fecundity, these results added to other evidence strongly suggest that age variation in female fecundity may well underlie general age patterns of natural human fertility.

The striking parallelism in the average trajectories of ovarian function by age may conceivably result from either of two mechanisms. Either acute effects are operating to modulate ovarian function among women of various ages that happen to be constant in their effects across all ages, or some more chronic, developmental effect results in a general resetting of the trajectory as a whole, akin to the resetting of growth trajectories that can result from chronic nutritional stress. Certain evidence on the relationship of menarcheal age to indices of ovarian function in the years after menarche suggest that developmental effects may indeed be involved.[59] Research on this issue will require a more extended longitudinal design capable of distinguishing the trajectories of individuals from cross-sectional averages for populations. A similar design will be necessary to determine whether ovarian function in individual women declines gradually with advancing age in a manner similar to the cross-sectional trends depicted here, or whether the decline in individual women is more abrupt but occurs at different ages for different women.

ENERGETICS AND OVARIAN FUNCTION

The relationship of various aspects of energetics to ovarian function, female fecundity, and fertility has been a matter of debate for some time.[23,60-63] The term energetics is used here to refer to the potentially separable variables of energy intake, energy expenditure, and net energy balance. Abundant evidence now indicates that extreme levels of energetic stress, such as those associated with vigorous endurance exercise, extremely low weight for height, or precipitous weight loss, are often associated with disruption of regular menstruation.[64-68] Using salivary progesterone measurements, we have been able to show in Boston subjects that moderate levels of exercise (*e.g.,* jogging an average of 12–15 mile a week) and moderate rates of weight loss (*e.g.,* an average loss of 1 kg/month), even in women of normal weight for height with regular menses, are also associated with suppressed ovarian function relative to age-matched controls.[41,69,70] Yet questions remain concerning the appropriateness of extrapolating from observations of women subject to specific forms of voluntary energetic stress to the potentially different forms of energetic stress experienced by women in other populations.[71]

We have undertaken investigations of variation in ovarian function related to energetic stress in three populations, the Lese of Zaïre,[72-74] the Tamang of Nepal,[75] and rural farm women in southern Poland.[76] The stresses faced by each population are different, yet the ovarian responses are quite similar.

The Lese subsist by shifting horticulture in a tropical rain forest where the timing and duration of the brief dry season in December and January are crucial to the successful clearing, burning, and planting of a new garden each year.[74] When the rains recommence too soon, clearing and burning cannot be completed, resulting in either smaller gardens or replanting of the previous year's plots. When the rains are late, germination of newly planted crops is poor. The result is large variations in annual garden yields across the population as a whole. This fact, together with generally poor conditions for long term food storage result in a "hunger season" preceding each year's major harvest in June and July that varies in intensity depending on the size of the previous year's harvest. These hunger seasons are reflected in alternating cycles of weight gain and weight loss experienced by the whole adult population with a nadir at the end of the hunger season. As women lose weight during the hunger season menstrual cycles lengthen, the average duration of menstrual flow shortens, and indices of ovarian function decline.[73,74] A ten-year accumulation of vital event data also indicates a significant dearth of conceptions at the height of the hunger season, with year-to-year variation that parallels the severity of the average weight loss.[74] Although other factors cannot be absolutely ruled out, there is no evidence of an increased incidence of spousal separation or a decline in the frequency of intercourse that might otherwise account for the birth seasonality in this population. Nor do the Efe pygmies, who cohabit the same part of the Ituri Forest but who are buffered by their hunting and gathering lifestyle from the seasonal weight fluctuations experienced by the Lese, exhibit any significant birth seasonality. Rather it seems likely that declining ovarian function associated with negative energy balance contributes to lower female fecundity and hence to the observed birth seasonality.

Catherine Panter-Brick has conducted a similar study of seasonal variation in ovarian function among the Tamang of Nepal.[75] The Tamang are subject to heavy workloads throughout the year associated with travel between fields and pastures located at different elevations in the Himalayan foothills. Significant seasonal variation in workloads occurs, however, occasioned by the shift in subsistence activities during the summer monsoon when long hours must be spent transplanting rice.[77] Average progesterone profiles are lower in the monsoon than in the winter, measured in the same women ($p < 0.001$, repeated measures ANOVA). In this case, however, despite comparable increases in workload, changes in energy balance and ovarian function are not equally distributed across women. Those women who lose weight show significant suppression in progesterone profiles during the monsoon ($p < 0.01$), while those who maintain or gain weight do not. Young women in particular, who may still be growing, continue to gain weight even during the monsoon and show no suppression of ovarian function. The Tamang also show seasonal variation in conceptions with a local nadir during the monsoon.

Grazyna Jasienska has initiated a study of energetics and ovarian function using salivary progesterone assays among rural Polish farm women.[76] This study is motivated by a desire to separate short-term variation in energetic stress from chronic energetic stress. The subjects in this study live in a community where most agricultural labor is still performed manually or with draft animals with minimal mechanization. Workloads during the agricultural season are substantial. During the summer haying season, for example, entire hay fields must be cut by hand, turned by hand daily, and collected by hand. During the winter, in contrast, most activities are confined to

animal care and household chores. Energy intake is sustained at high levels year round, however, with little variation in diet.

Twenty women, all of proven fertility, were recruited in a pilot study of ovarian function. Although all participated in agricultural work, relative workloads varied between women due to the size and location of fields and the availability of other help. The subjects were divided into high and moderate workload groups on the basis of the average number of hours spent in agricultural labor per day as a rough index of the duration of sustained energy expenditure. The two groups did not vary in average age, weight, weight for height, or menstrual cycle length. Yet significant differences were apparent in the average progesterone profiles ($p < 0.0001$, repeated measures ANOVA) with the high workload group suppressed relative to the moderate workload group. A follow-up study is now underway of seasonal variation in ovarian function within women between winter and summer in association with changes in energy expenditure, energy intake, and energy balance.

In three quite different populations, then, directly comparable measures of ovarian function have revealed similar responses to energetic stress, whether occasioned by changes in energy intake or energy expenditure. In all three cases higher levels of energetic stress are associated with significant suppression of ovarian function even in women who continue to menstruate regularly. In all three cases the variation in energetic stress is related to the subsistence ecology of the population and not to a self-imposed regime of dieting or exercise. Finally, the suppression of ovarian function associated with energetic stress appears to occur in a qualitatively similar fashion in populations with different levels of chronic energetic stress and different average levels of ovarian function. Thus while energetic suppression of ovarian function is a manifestation of local ecological conditions, the general responsiveness of the human ovary to energetic stress appears, like age variation, to be a general feature of human reproductive biology.

CONCLUSIONS

The data reviewed here provide a unique opportunity to directly compare patterns of variation in ovarian function across human populations. The use of salivary steroid assays has allowed the collection of serial samples from a large number of women outside the traditional confines of the clinic. Perhaps the single most important observation to be made is that significant natural variation in human ovarian function does indeed occur, even when menstrual cycles remain regular. Average profiles of salivary progesterone vary considerably between different populations, with levels in Boston women representing, if anything, one extreme of the range of observed variation (FIG. 3). Such high levels of ovarian function may be associated with higher fecundity, but they may also be associated with higher rates of ovarian and breast cancer.[36-39] Certainly, there seems little reason to enshrine the hormonal profiles typical of populations like Boston as eidetically "normal," against which other profiles are judged to be "pathological." Rather we should accept the notion of natural variation as applicable to ovarian function, as to other aspects of physiology, and seek to understand the structure of that variation.[79]

Two elements of that structure have been demonstrated in the studies reviewed here. Ovarian function appears to vary with age in a pattern that is consistent across different populations, rising steadily on average through the first decade of reproductive life and declining steadily through the last. The average level of ovarian function along which this trajectory is traced can vary considerably between populations, however. It remains to be seen whether these differences in level are the consequence of chronic or acute effects, and whether individuals follow trajectories comparable to the cross-sectional averages of populations.

Ovarian function also appears to vary with the energetic stresses imposed by local ecologies. The ovarian response to energetic stress does not, however, appear to be confined to populations under chronic stress, nor to individuals of sub-standard nutritional status. Modulation of ovarian function in response to energetic stress may well represent an evolved feature of the female reproductive system designed to lower fecundity in association with diminished probabilities of successful reproductive outcome, or a diminished probability of maintaining adequate energy balance during pregnancy.[22,23] To this extent such modulation of ovarian function may represent a functional response and not a failure of homeostatic mechanisms and thus should perhaps not be referred to as "dysfunction."[64,71]

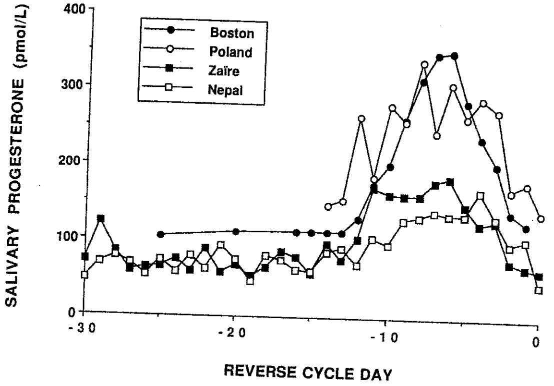

FIGURE 3. A comparison of the average salivary progesterone profiles from four populations, Boston, Poland, Zaïre, and Nepal.

Advancing our understanding of human reproductive ecology will depend on our ability to build a comparative perspective on human ovarian function, and on other aspects of human reproductive biology and behavior. Salivary steroid assays represent one of many methods by which such a perspective may be achieved. While we are still in the process of working toward such a perspective we would do well to avoid premature assumptions about the nature of and significance of variation, not only in ovarian function, but in all aspects of human reproductive ecology.

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