Evaluation of Reproductive Function in Turkana Women with Enzyme Immunoassays of Urinary Hormones in the Field

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Abstract The frequently reported observation that nomadic populations have lower fertility than their settled counterparts is often attributed to what are perceived as harsh, stressful conditions under which the nomads live. But the consequences of the hypothesized stresses for the reproductive biology or demography of these populations have been documented only a little. Traditionally, the Turkana of northwest Kenya are nomadic herders, but increasing numbers have settled on agricultural development schemes. We used an array of hormonal assays along with anthropometric indexes of nutritional status and interviews covering reproductive history, recent menstruation, diet, and health to compare reproductive function in nomadic and settled Turkana women. First morning urine samples were collected for three consecutive days during a series of surveys. Human choriogonadotropin (hCG; a marker for pregnancy), luteinizing hormone (LH; an indicator of ovulation), and pregnanediol glucuronide (PdG; an indicator of postovulatory luteal function) were assessed in the field with commercially available dipstick enzyme immunoassays. These assays along with the interview data allowed us to determine the reproductive status (e.g., pregnant or cycling, and if cycling, which phase of the ovarian cycle) of 166 nomadic and 194 settled Turkana women. The cross-sectional classifications allowed inferences of conception rates and normality of ovarian function. Follow-up surveys provided rates of pregnancy loss. Compared with the settled women, the nomadic women exhibited lower pregnancy rates and cycling nomadic women were less likely to show evidence of ovulation or luteal function. These results suggest that reproductive function of the nomadic women

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is diminished relative to the settled women. However, the settled women experienced a much higher rate of pregnancy loss, which may mean that their effective fecundability is in fact lower than that of the nomadic women. This study is the first to apply such a wide range of hormonal assays in the field. It demonstrates that field-based assays are feasible and robust and can play an important role in epidemiological and biodemographic studies, even in remote locations under conditions that would ordinarily be considered incompatible with on-site laboratory analysis.

The number of demographic studies of nomadic pastoralists is small but is increasing slowly. A conclusion commonly drawn from these studies is that nomadic populations generally exhibit low to moderate levels of fertility and with few exceptions low levels relative to neighboring sedentary populations (Muhsam 1950; Henin 1968, 1969; Ganon 1975; Hill 1985; Marriott 1994). But interpretation of these results is problematic. This relationship is often explained in terms of what is perceived to be the hard life (i.e., harsh environment, poor health) of nomads, and it is argued that sedentism mitigates certain environmental stresses faced by the nomads (Swift 1977). However, the consequences of the hypothesized stresses for reproductive biology and demography, not to mention the stresses themselves, have seldom been documented adequately for each separate population in a given comparison. Unraveling the causes of the fertility differentials is further complicated because in most cases the populations being compared differ in more than subsistence pattern—they are distinct ethnic groups.

Turkana District in northwest Kenya is inhabited by both nomadic and settled Turkana people. The traditional, nomadic Turkana subsist principally on products of their livestock (camels, cattle, goats, sheep) and move frequently in search of forage. Settled Turkana cultivate crops along the two major rivers that have water for a substantial part of the year. The two populations are culturally similar and can be considered to be drawn from the same gene pool. Both must cope with seasonal and longer-term droughts, high temperatures, serious human and livestock diseases, and other stresses. The major differences between them are those directly associated with their contrasting subsistence base: diet, activity pattern, and perhaps disease stress. Food resources for both populations are periodically insufficient. For further description of the Turkana and the larger project of which this study is a part, see Little and Leslie (1990), Dyson-Hudson and McCabe (1985), and Little et al. (1990).

One unusual feature of Turkana demography revealed by our earlier studies is that the nomadic population appears to have *higher* fertility than its settled neighbors (Leslie et al. 1988; Brainard 1986). This is just the opposite of what has been reported in studies of other populations. There is some evidence that infant mortality is higher among the nomadic Turkana than among the settled Turkana (Brainard 1991), which would truncate breast

feeding, shorten birth intervals, and contribute to higher fertility in the nomadic women.

To better understand the determinants of fertility and to explore reproductive function as an aspect of human variation, we initiated a study of the reproductive ecology of the Turkana. More specifically, we wanted to learn how differences in ovarian function, intrauterine mortality, and infant mortality are influenced by environmental characteristics and the extent to which they contribute to the fertility pattern. Level of reproductive function, as reflected in hormone profiles and early pregnancy loss, is a sensitive, demographically meaningful indicator of environmental stress. We have reported elsewhere on results of other aspects of this study: pregnancy loss (Leslie et al. 1993) and maternal depletion of energy reserves (Little et al. 1992). Here, we focus on our use of recently available urinary hormone assays that can be performed in the field to evaluate and compare reproductive function in nomadic and settled Turkana women. Preliminary results were presented by Leslie et al. (1994).

Sample and Methods

The nomadic women in this study all belong to the Ngisonyoka subsection of the Turkana-speaking people. The settled women live in Morulem, at the southern end of Ngisonyoka territory, where for at least part of most years there is water available from the Kerio River for irrigation. Most of these women are also Ngisonyoka; about one-fifth of the women in the Morulem sample are Ngibilae Turkana. Some have lived there all their lives; some moved there after loss of family herds to drought and intertribal livestock raiding, especially in the late 1970s and early 1980s.

We carried out two surveys of each population. The first survey of the nomadic Turkana (June 1989) came near the end of what was only a fair rainy season; the rains came when expected but did not last long. The second survey (July 1990) was a bit later in a year that saw the rains come earlier and last longer. Conditions were therefore somewhat better for the nomads during the 1990 survey but not markedly so. The first survey in Morulem (May 1989) fell about four months before the expected harvest, a time when the previous year's crop had been depleted but work on the new crop was required. It was a period of low food availability. Morulem experienced a crop failure in Fall 1989, and the bulk of the settled people's diet at the time of the second survey (January 1990) was obtained by foraging for wild foods. The sequence of surveys in the nomadic population represents, then, a modest improvement in conditions; the sequence for the settled population represents a deterioration of already poor conditions.

At the core of the study is an assessment of ovarian function through analysis of urinary hormones. Anthropometric assessment of body size and

composition and interviews covering reproductive histories, diet, health, recent menstrual function, and environmental conditions supplemented the hormonal data. Ages were estimated with the help of an event calendar developed for this part of Turkana District (Leslie and Lowoto 1990). Direct anthropometric measures included height, weight (corrected for clothing and ornaments), upper arm circumference, and skinfold thicknesses (triceps, subscapular, periumbilical, and midcalf for the nomadic Turkana; these skinfold measures plus suprailiac and midaxillary skinfold thicknesses for the settled women). Derived measures used in this report are body mass index and the sum of four skinfold thicknesses.

In all we conducted interviews with 208 nomadic women and 248 settled women. Women who were visibly pregnant or had given birth within the past three weeks or who were clearly old and reported themselves to be postmenopausal were not asked to provide urine samples. Unfortunately, not all those asked did agree to provide samples. There was little problem in the settled community, where cooperation was over 98%, but 18% of the reproductive-age nomadic women declined to give urine (all were willing to be interviewed and measured), and this raises the possibility of selection bias. The pattern of refusals, however, suggests that this risk is minimal.

Because of the low population density, the nomadic sample had to be accumulated by working for 5 to 10 days at each of a series of sites near different clusters of nomadic families. When the supply of subjects in a cluster was exhausted, we would move to a new location.

Before beginning the interviews in a particular place, we discussed our purposes and procedures with the families' elders (senior male herd owners) and women who knew us from previous field seasons. In general, we were able to allay fears, but in several locations one or more influential elders became suspicious that the urine collection might cause sterility or other problems. They would then discourage their wives and daughters from providing samples. With experience, we became better at avoiding this. Consequently, we had a pattern of either very good or very poor (even zero) cooperation at a given site. For example, seven of the ten refusals during the second nomadic survey occurred at one site. There is no reason to believe that the elders' suspicions about our motives or about the use of bodily fluids for witchcraft was influenced by the hormone profiles of the women in their families. Thus the reduced rate of cooperation among the nomads led to a reduction in sample size but is not likely to have biased the results. We attribute the greater cooperation among the Morulem women to their greater sophistication. Most had interacted with nurses at a nearby health clinic, and some had even given urine or blood samples there; our request did not strike them as odd.

Three hundred thirty-five nomadic and settled Turkana women provided first morning urine samples; most did so for three consecutive days. We performed assays in the field for human choriogonadotropin (hCG, a marker for pregnancy), for one protein hormone indicative of ovulation (luteinizing hor-

mone, LH), and for the major urinary steroid metabolite of progesterone, which is indicative of active postovulatory function of the corpus luteum of the ovary (pregnanediol glucuronide, PdG). LH and PdG provide information about ovarian cyclicity (phase of menstrual cycle).

The assays were all solid-phase enzyme immunoassays: Hybritech Inc.'s Tandem Icon II for hCG, Quidel's Ovulation Test for LH, and Monoclonal Antibodies Inc.'s RAMP ProgestURINE for PdG. The hCG assay shows no cross-reaction with LH, even at levels higher than expected during normal ovarian cycles. The LH assay does cross-react with hCG, but we can use this to our advantage here to confirm any positive hCG tests.

Test standards were run in parallel with the Turkana samples approximately every five days to ensure consistent performance of the assays. As a further check, female members of the field team donated daily urine samples, which were handled in the same way as the Turkana samples. They also maintained menstrual diaries, which were checked against the assay results. A portion of each sample was preserved with sodium azide and dried on filter paper or in Sephadex gel for later quantitative analysis. All urine samples and reagents were stored in a solar-powered refrigerator (on loan from Sandia National Laboratories) until used or transported to the United States.

Evaluation of Reproductive Status. Reproductive status of each woman was deduced from the results of the hormonal assays along with information from the reproductive and menstrual histories. The criteria used to assign reproductive status are summarized in Table 1. Taking samples for three days allows some assessment of trends and therefore yields a more accurate evaluation of reproductive status than do single spot samples. This is reflected in Table 1, where some of the hormonal criteria entries (three rightmost columns) include references to duration of positive assay outcomes; for example, pregnancy is indicated by a positive hCG test along with positive LH and/or positive PdG tests for one to three days. Some decisions also take into account the temporal sequence of results from the different assays. For example, a woman was classified as being in luteal phase if, in addition to the other criteria listed, her urine samples tested positive for PdG for one to three days and the first day of positive PdG was preceded by either zero or one day of positive LH (two or more days of positive LH would indicate midcycle).

Eighty of the women took part in both the first and second surveys of their subpopulation. Results for these women were checked for consistency; for example, if a woman was postmenopausal in the first survey, she should be the same in the second survey, and a woman who was pregnant during the first survey and then gave birth would be expected to be amenorrheic during the subsequent survey unless the infant died. No discrepancies of this sort were found. These criteria are designed to provide classifications that are useful for the specific analyses presented here, where we focus on the functioning of the mature reproductive system. Some criteria may not be appro-

Table 1. Criteria Used for Classification of Reproductive Status[a]

	Decision Parameters								
Status	*Age (Years)*	*Time Since Last Menses*	*Time Since Last Birth*	*Nursing*	*hCG (Days)*[b]		*LH (Days)*[b]		*PdG (Days)*[b]
Premenarcheal	<10[c]								
	<20	>2–3 months			–		–		–
Pregnant[d]	10–45				1–3 +	and	(+	or	+)
Postreproductive	>45[c]	long	long						
	>40	not recent	not recent	not recent	–		1–3 +		–
	>40	not recent	not recent	not recent	–		–		–
Follicular phase	10–45	<1 week[c]							
	10–45	<1 week			–		–		–
	10–45	<2 weeks		no	–		–		–
Midcycle	10–45	>1 week			–		1–3 +		→ ±1
Luteal phase	10–45	>10 days			–		±1 →		1–3 +
Cycling (phase not known)	10–45	< 1 month[c]							
	10–45	<2 months			–		–		–
Lactational amenorrhea	10–45	before pregnancy	<1.5 years	yes[c]					
	10–45	before pregnancy	<2 years	yes	–		–		–
Other amenorrhea	10–45	irregular, none recent	>1.5 years		–		–		–
Undefined[e]	10–45								

a. Based on interviews and evaluations of three consecutive early morning urine samples using semi-quantitative solid phase enzyme immunoassays for hCG (Icon II, Hybritech; field detectability ≤50 mIU/ml), LH (Ovulation Test, Quidel; field detectability ≤40 mIU LH/ml, cross-reactive with hCG), and PdG (Ramp ProgestURINE, Monoclonal Antibodies; detection limit 2–2.5 μg/ml). Standards in urine from a male child were run routinely for assay verification. Status assignments will be refined when quantitative laboratory tests are completed.

b. +, positive test result; −, negative test result; →, then or preceding; ±, with or without. See text for further explanation.

c. Used when only interview information was available.

d. For advanced pregnancies, visual assessment provided an obvious and decisive criterion.

e. Not classifiable with the available information.

priate for some other purposes. For example, women classified as premenarcheal by these criteria may include some who have recently reached menses but who are still cycling irregularly (period of adolescent subfecundity). This is appropriate here because the goal is to identify women who have reached reproductive maturity, not to estimate age at menarche. Further discussion of our procedures for deducing reproductive status are given by Campbell (1994).

The hCG tests used in the field are capable of detecting pregnancies as early as 7 to 14 days after fertilization. They provided the basis for a prospective study of intrauterine mortality. During each of the cross-sectional surveys, the hCG tests revealed a number of pregnancies, the outcomes of which we were able to determine by interviewing the women during subsequent fieldwork 8 to 12 months later [see Leslie et al. (1993) for details concerning procedures].

Our method of determining reproductive status has several limitations. Perhaps the most important is that the abbreviated hormone profiles do not by themselves distinguish women who are amenorrheic from women who are cycling and in the follicular phase. The menstrual histories help, but there may still be some ambiguity for women who have recently changed states (i.e., recently become amenorrheic or recently returned to menses). Anticipated results of our assays for estrone-3-glucuronide (a major estradiol metabolite in urine) should resolve this problem.

Another potential problem is that the dipstick assays have positive-negative cutoffs engineered to have their most meaningful interpretation in Western clinical settings. If a population has hormone profiles that differ from Western profiles, classification may be thrown off. Misclassification arising from this source is most likely between the follicular and luteal phases in cycling women. More specifically, the PdG test has a cutoff that may be too high for the Turkana women. Composite progesterone profiles that are low compared with Western standards have been found in Lese women (Zaire) (Ellison et al. 1989) and in Tamang women (Nepal) (Panter-Brick et al. 1993); it is not known whether other non-Western populations, including Turkana, exhibit similar profiles. Note that this problem would not affect classification of women as cycling or amenorrheic.

Results

Here, we compare women from the nomadic and settled subpopulations with regard to three aspects of reproductive function: (1) reproductive status of reproductive-age women (i.e., the proportions who are cycling, amenorrheic, and pregnant), (2) among cycling women the proportions who are in the various phases of the ovarian cycle, and (3) among pregnant women the

Table 2. Sample Sizes for Assessment of Cycle Status[a]

Number of Women Classifiable According to:	*Nomadic Women*			*Settled Women*		
	1989	*1990*	*Combined*	*1989*	*1990*	*Combined*
Reproductive status[b]	92	74	166	97	97	194
Cycle phase[c]	29	28	57	36	30	66

a. Excludes women determined to be postmenopausal or premenarcheal.
b. Includes reproductive-age women who were cycling, pregnant, or amenorrheic (not cycling and not pregnant).
c. Includes cycling women who could be further classified as being in follicular, midcycle, or luteal phases of the ovarian cycle.

ability to carry pregnancies to term. The sample sizes on which these comparisons are based appear in Table 2.

Reproductive Status. Figure 1 shows the proportion of reproductive-age women who were cycling, amenorrheic but not pregnant, and pregnant. Amenorrhea is further categorized as lactational or of other origin, according

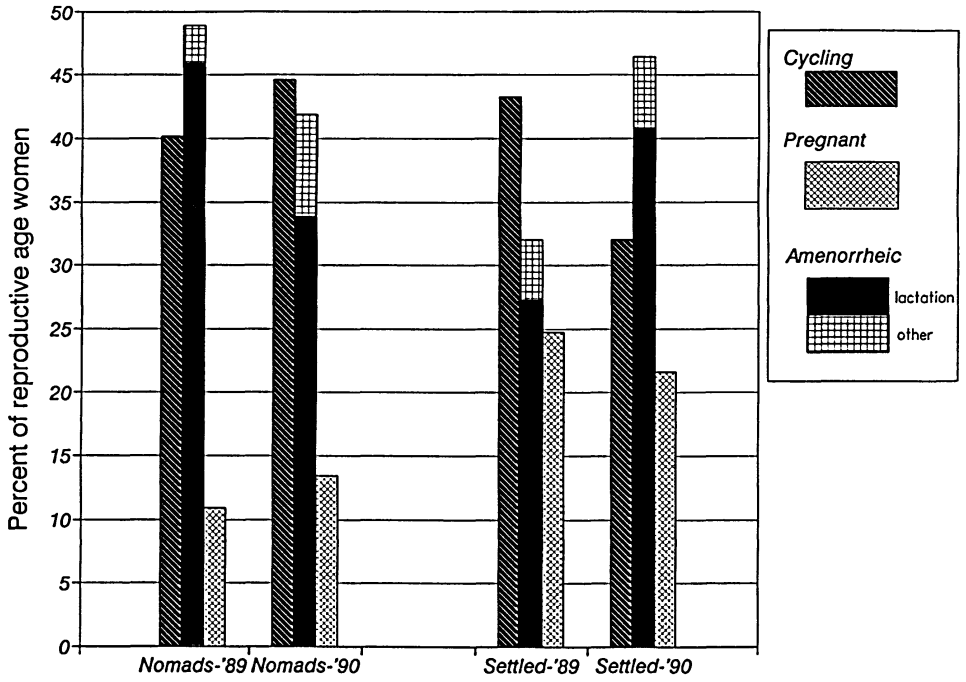

Figure 1. Reproductive status of reproductive age women: proportions of cycling, amenorrheic but not pregnant, and pregnant women.

to the criteria set out in Table 1. Overall, 88% of the amenorrheic cases were associated with lactation. The two populations did not differ in this regard. The apparent shift from cycling to amenorrheic status that occurred between the two settled population surveys is not statistically significant (log-likelihood ratio test, $G = 4.46$, 2 d.f., $p = 0.11$) but is in the direction expected, given the more stressful conditions during the second survey. Among other things those conditions may have prolonged lactational amenorrhea. The difference between the two nomadic samples is also in the direction expected on the basis of environmental conditions (slightly improved) but again is not significant ($G = 0.87$, 2 d.f., $p = 0.65$).

There are some striking contrasts between the two populations. First and most obvious is that the proportion of nomadic women who were pregnant is only half that found in the settled samples. Second, a much larger proportion of nomadic women in the first survey were amenorrheic than was the case for settled women at about the same time of year (May–June 1989). The difference between the combined nomadic and combined settled samples is significant ($G = 7.75$, 2 d.f., $p = 0.02$).

Cycling Women. We can look a little more closely at differences in ovarian function, apart from pregnancy, by comparing the proportions of cycling women who are in the various phases of the ovarian cycle (see Figure 2). Again, the most obvious difference is the contrast between the nomadic and the settled samples: A much greater proportion of nomadic women appeared to be in follicular phase. The difference between the two populations is statistically significant ($G = 6.70$, 2 d.f., $p = 0.03$); those between the two surveys for either population are not. Based on studies of Western populations (Treloar et al. 1967; Vollman 1977), we would expect 41–61% of cycling women to be in follicular phase, 5–11% to be midcycle, and 34–48% to be in luteal phase. Taken together, the settled samples show a fairly close match to this, but the nomadic women exhibit an unexpectedly low proportion in luteal phase.

Pregnancy Loss. We were able to determine the outcomes of 50 of the 60 pregnancies detected during the surveys [see Leslie et al. (1993) for further details]. Figure 3 shows the number of pregnancies carried to term and the number lost for each survey. Once again the successive surveys of each population do not differ significantly. And once again there is a dramatic difference between the nomadic and settled populations: None of the nomadic women's pregnancies were lost; 45% of the settled women's pregnancies detected at any gestational age were lost. The 95% confidence limits for the proportions lost in the two populations do not overlap (Leslie et al. 1993).

The risk of pregnancy loss is known to decrease rapidly during the first weeks of pregnancy, so these cross-sectional samples include pregnancies that have already survived the period of maximum risk. Focusing on those preg-

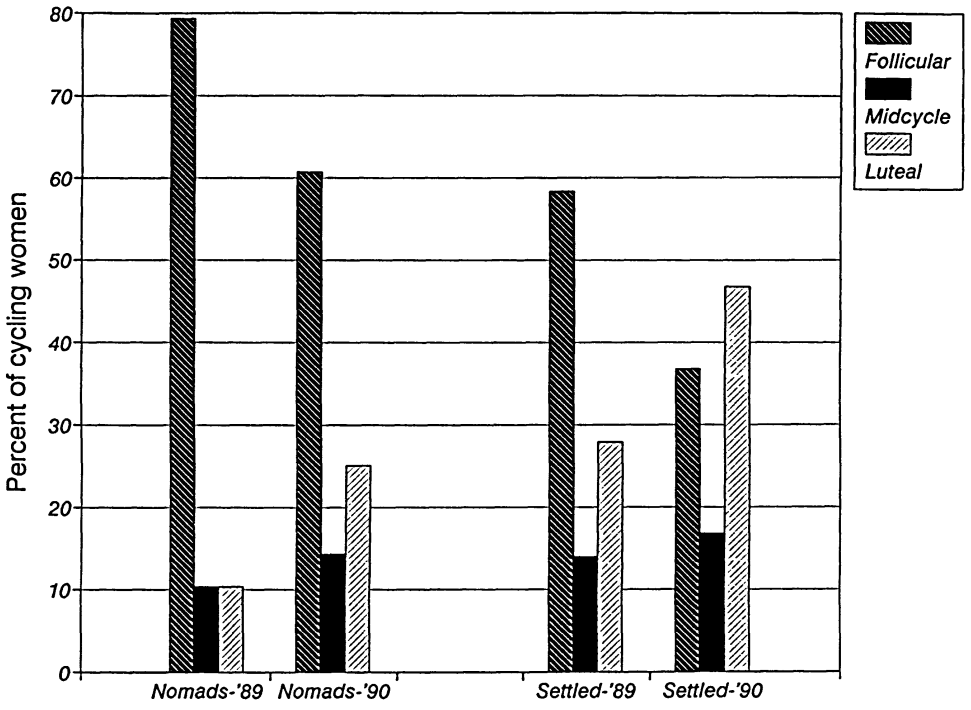

Figure 2. Distribution of cycling women according to phase of ovarian cycle.

nancies that were detected early gives a better idea of the overall risk of intrauterine loss. To this end Figure 3 also shows the number of pregnancies carried to term and the number lost for pregnancies detected in the first trimester. Nearly 70% of the first-trimester pregnancies in settled women were not carried to term. Even this may underestimate the actual loss, because it is likely that some of the pregnancies in this subsample were well into the first trimester at the time of detection and were therefore at lower risk.

Characteristics of the Nomadic and Settled Samples

Age. There is evidence that fecundability and specific aspects of ovarian function change with age [see Wood (1989) and Ellison et al. (1993) for useful reviews]. The differences in ovarian function apparent in the results therefore might simply be a function of the demographic composition of the samples.

Table 3 shows the age distributions of the women in the four surveys. The age distribution of the combined nomadic sample does not differ significantly from that of the combined settled sample ($G = 2.52$, 3 d.f., $p = 0.47$). The two nomadic samples do have different age distributions ($G = 11.13$, 3 d.f., $p = 0.01$) yet do not differ significantly with regard to reproductive

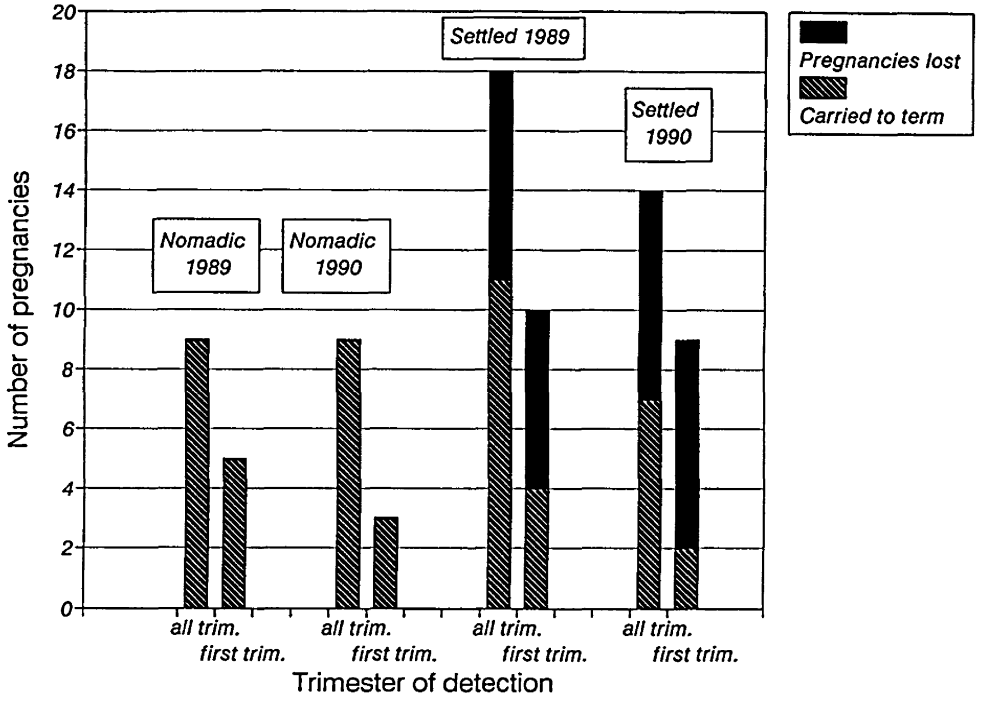

Figure 3. Pregnancy outcomes for pregnancies detected in the first trimester and for pregnancies detected at any stage.

status (see Figures 1 and 2). It is therefore of interest to learn whether our samples exhibit any age-related changes in reproductive function.

As shown at the top of Table 4, reproductive status does vary by age group ($G = 20.44$, 4 d.f., $p < 0.001$; because of the small number of subjects younger than 20, the youngest age group was combined with the 20–29-year-old group for this and the following statistical tests). Women in the oldest age group (40–49) were less likely to be pregnant or amenorrheic (recall that postmenopausal women were excluded from this sample and that most of the

Table 3. Age Distributions of Reproductive-Age Women in the Four Surveys

Age Group	*Nomadic Women, 1989*	*Nomadic Women, 1990*	*Settled Women, 1989*	*Settled Women, 1990*
<20	3	4	4	1
20–29	26	36	33	38
30–39	37	26	44	43
40–49	25	8	16	15
All	91	74	97	97

Table 4. Reproductive Status of Nomadic Women by Age Group

Age Group	Number of Reproductive-Age Women	Percentage of Reproductive-Age Women Who Were: Cycling	Amenorrheic	Pregnant	Total
<20	7	85.7	14.3	0.0	100
20–29	62	38.7	48.4	12.9	100
30–39	63	30.2	57.1	12.7	100
40–49	33	63.6	27.3	9.1	100

	Number of Cycling Women	Percentage of Cycling Women Who Were: Follicular	Midcycle	Luteal	Total
<20	3	66.7	0.0	33.3	100
20–29	21	71.4	14.3	14.3	100
30–39	16	75.0	6.3	18.7	100
40–49	17	64.7	17.7	17.6	100

amenorrhea was associated with lactation). Among cycling women there is no difference among the age groups in the proportion who were in each phase of the ovarian cycle (bottom part of Table 4; $G = 1.13$, 4 d.f., $p = 0.89$). Examining age effects for each survey sample separately gave similar results.

Body Composition. Table 5 shows three measures of body composition for the reproductive-age women in the two surveys of each population. Pregnant

Table 5. Indicators of Body Composition of Women in the Four Surveys

	Nomadic Women, 1989 ($n = 70$)		Nomadic Women, 1990 ($n = 57$)		Settled Women, 1989 ($n = 71$)		Settled Women, 1990 ($n = 61$)[a]	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sum of 4 skinfold thicknesses (mm)[b]	28.2	9.9	38.2	13.7	39.2	14.0	28.8	11.9
Body mass index (kg/m^2)	17.7	2.3	18.1	1.8	16.5	1.9	17.4	1.7
Upper arm circumference (cm)	24.8	2.0	24.5	2.0	23,8	2.4	23.7	2.0

a. *n* for sum of skinfold thicknesses in this sample is 39 rather than 61. Interobserver comparisons suggested that some of the skinfold measurements were unreliable; these have been excluded.
b. Triceps, subscapular, periumbilical, midcalf.

Table 6. Pairwise Comparison Probabilities for Measures of Body Composition in the Four Surveys[a]

	Nomadic Women, 1989	*Nomadic Women, 1990*	*Settled Women, 1989*
Nomadic women, 1990	SSF[b]	–	–
Settled women, 1989	SSF[b] BMI[c] UAC[d]	BMI[b]	–
Settled women, 1990	–	SSF[c]	SSF[b]

SSF, Sum of four skinfold thicknesses; BMI, body mass index; UAC, upper arm circumference.

a. Measures listed in each cell of the matrix indicate significant difference, according to Tukey HSD (honestly significant difference) post hoc test, with Tukey-Kramer adjustment for unequal sample sizes, as implemented in SYSTAT 5.0 (Wilkinson 1990).

b. $p < 0.001$.

c. $p < 0.01$.

d. $p < 0.05$.

women were excluded from these calculations. The differences among the four survey samples are summarized in Table 6.

The changes in adiposity (measured as the sum of four skinfold thicknesses) between the first and second surveys of each population are statistically significant. The nomadic women gained body fat between the two surveys, upholding our impression that conditions for them had improved somewhat in 1990. The increase in body mass index is consistent with the change in fatness among the nomadic women but is not statistically significant. The settled women show a marked loss of body fat between the two surveys, again in accordance with our picture of environmental conditions and reported food shortages during the second survey.

Because the surveys were conducted at nearly the same time of year (May–June 1989), it is of special interest to compare the results of the 1989 surveys of the two populations. In 1989 the nomadic women were leaner than the settled women. As reported, these nomadic women were both less likely to be cycling and, if cycling, less likely to exhibit luteal function than their settled counterparts. By the second survey the nomadic women had reached the level of fatness seen in the first settled sample (which was by no means a time of plenty in Morulem); comparison of the second nomadic sample with the first settled sample shows no significant difference between them in either proportion of cycling women or proportion of women in each phase of the ovarian cycle.

Fatness, Reproductive Status, and Parity. Elsewhere we have reported parity-related losses in fat stores in both nomadic and settled Turkana, with the relationship being stronger in the nomadic women (Little et al. 1992). If

Table 7. Body Composition of Cycling and Amenorrheic Women[a]

	Nomadic Women			*Settled Women*		
	Mean	*SD*	*n*	*Mean*	*SD*	*n*
Sum of four skinfold thicknesses (mm)						
Cycling	35.5	14.8	65	37.2	14.5	57
Amenorrheic	29.7[b]	9.3	62	33.7	13.6	53
Body mass index (kg/m^2)						
Cycling	17.7	1.9	65	16.9	1.7	67
Amenorrheic	18.0	2.3	62	17.0	2.0	66
Upper arm circumference (cm)						
Cycling	24.8	2.2	65	24.0	2.1	67
Amenorrheic	24.5	1.8	62	23.4	2.3	66

a. Excludes pregnant women.
b. $t = 2.6$, 108.4 d.f., $p = 0.01$, separate variances t test. For other differences, $p > 0.15$.

pregnancy and lactation constitute stress sufficient to deplete maternal energy reserves, it might be expected that women experiencing lactational amenorrhea (who therefore had sustained both a recent pregnancy and ongoing lactation) would be leaner than cycling women. This is indeed the case for the nomadic women (see Table 7). The difference in the sum of four skinfold thicknesses between cycling and amenorrheic women in the settled population is in the expected direction but is not statistically significant.

Cycling and amenorrheic women did not differ significantly in body mass index or in upper arm circumference in either population. It might be expected that anthropometric differences between cycling and amenorrheic women would increase with parity under the stress of repeated pregnancies. In fact, the fatness differential noted among the nomadic women disappears when higher parity women (>4 births) are considered separately. This result is surprising, but the relationship between parity and reproductive status may be obscured here by selection bias; that is, the more robust women may be more likely to reach higher parities. Also, even low-parity Turkana women are quite lean. Because fatness declines with parity for both the cycling and amenorrheic subsamples, it may be that higher parity women have become so lean that there is simply little room for a discernible difference between the two groups.

Discussion

Reproductive Status. The difference between the nomadic and settled samples in the proportions of cycling, pregnant, or amenorrheic women (Figure 1) can be attributed to the difference in the proportion of pregnant women. Indeed, the picture of reproductive function afforded by this classification is

largely a reflection of pregnancy and its sequelae. The proportion of pregnant women is a function of fecundability and rates of pregnancy loss during the past nine months. The proportion of women classified as amenorrheic (but not pregnant) is strongly influenced by the proportion of women who are breast feeding (recall that 88% of those so classified were nursing), which is itself a function of pregnancy rates in the recent past and may also reflect recent pregnancy loss.

Nomadic Turkana births are highly seasonal, and the rate of conception apparently peaks in July and August (Leslie and Fry 1989). The surveys of the nomadic population were carried out shortly before this expected peak, so the low prevalence of nomadic pregnancies may reflect low conception rates during the preceding months. The distribution in Figure 1 may indicate a backlog of fecund and potentially fecund women, and we might expect the proportion of pregnant women to increase in subsequent months. Alternatively, it is possible that the dearth of nomadic pregnancies is due to losses occurring too early to be detected by the assay used, but we defer discussion of pregnancy loss to a later section.

Ovarian Cyclicity. Among cycling nomadic women the proportions found to be in each phase of the ovarian cycle deviate from expectations based on normally cycling Western women. Specifically, the nomadic women show a particularly low incidence of luteal function. These results may be due at least in part to limitations of the classifications possible with the field assays. As discussed earlier, if the normal progesterone profile for the nomadic women is lower than Western standards, as has been reported for two non-Western populations (Ellison et al. 1989; Panter-Brick et al. 1993), the cutoff points of the qualitative assays may be too high to detect luteal phases reliably. But this would not explain the difference between the Turkana populations, unless the nomadic and settled women have different progesterone profiles. The likelihood of such a difference is unknown; there have been few studies of the relationship between dietary intake and steroid levels, except in extreme situations (Cumming et al. 1994), and the roles of other factors, including heredity, remain poorly explored.

Another possibility is that the reduced incidence of nomadic women in luteal phase may indicate luteal insufficiency on the part of the women rather than inadequacy of the assays. There is accumulating evidence that environmental factors and activity patterns, acting through nutritional status and energy balance, affect various aspects of reproductive function. Ellison in particular has argued that ecological stresses can produce a continuum of responses in ovarian function and that luteal suppression is a major type of response (Ellison 1990; Ellison et al. 1993). Our results are consistent with this view. Some women may be cycling and may even be ovulating, but progesterone output may be brief and/or low—not just too low to be detected by the assays but insufficient to maintain pregnancy even if conception oc-

curs. That is, the low proportion of women exhibiting luteal activity may indicate reduced fecundity.

A third possibility is differences in ovarian cycle length parameters. The follicular phase is the most variable of the phases; perhaps it is lengthened in the nomadic women, as is the case in at least one other population, the Gainj of Papua New Guinea (Johnson et al. 1987). Like Turkana women, Gainj women subsist on a calorically marginal diet and expend considerable energy in food production and household activities. A lengthened ovarian cycle would also suggest reduced fecundity. The changes in cycle length would have to be dramatic to account fully for the differences depicted in Figure 2.

We cannot now distinguish among these explanations, which are not mutually exclusive. The reduced fecundity implied by luteal insufficiency and increased cycle length is consistent with the lower pregnancy rate in the nomadic women but not necessarily with the realized fertility differential. In any event, ovarian cyclicity does seem to differ between the two populations, with the difference manifest in an excess of women in follicular phase among the nomadic population compared with the settled population. The immediate source of this difference is not clear. It may arise from a relatively long follicular phase in the nomadic women or lower luteal phase progesterone levels or both. It is possible that quantitative analysis of the preserved urine samples will show that some of the women classified in the follicular phase are really in a state of secondary amenorrhea. Reclassification could either increase or decrease the difference.

Much of the evidence presented is suggestive of diminished reproductive function in the nomadic population relative to the settled population. The nomadic women show lower rates of pregnancy than the settled women; among nonpregnant women more of the nomadic Turkana are amenorrheic; and the hormone profiles among cycling women suggest reduced luteal function in the nomadic population. The third aspect of reproduction examined here, however, stands in apparent contradiction to this picture, that is, reproductive function as reflected in rates of early pregnancy loss.

Pregnancy Loss. Not only is the rate of loss in the settled population high relative to that in the nomadic population, but, it is also surprisingly high compared with rates estimated for other populations. Because the risk of intrauterine death decreases with gestational age and because methods of ascertaining pregnancy differ with regard to how early they can detect pregnancy, it is not surprising that estimates of the overall risk of pregnancy loss vary widely (Kline et al. 1989). The best estimate of the probability of intrauterine mortality per conception from a study that is comparable to ours in terms of the assay used (a urinary hCG assay) is that done by Wilcox et al. (1988). They reported that 31% ($\pm$3%) of 198 pregnancies to women in North Carolina were eventually lost. Even with the large degree of uncertainty associated with an estimate based on a sample the size of ours, the observed

rate of loss in the settled group is significantly higher than in the North Carolina study (Leslie et al. 1993). This result is even more remarkable in light of the fact that some of the pregnancies in this subsample were most likely well into the first trimester at the time of detection and thus had survived the period of maximum risk. The hCG assay we used in Turkana is less sensitive than that used by Wilcox and will not detect pregnancies as early. Both the cross-sectional nature of the Turkana samples and the lower sensitivity of the assay produce a bias toward later ascertainment of pregnancies relative to the North Carolina study and should result in *lower* apparent rates of intrauterine mortality if the underlying rate of loss is the same in both populations.

A satisfactory explanation for the pregnancy loss results is not at hand. The results are not likely to be due to technical problems with the hCG assays or to induced abortion. The results are not explained by the age composition of the samples, and there is no clear relationship with body composition. Greater prevalence of infectious diseases that can induce pregnancy loss, especially malaria, might produce the observed differential in risk. The people of Morulem live closer to more standing water and shade than most nomadic families usually do, so it is reasonable to expect malaria to be a more severe problem there, but our data on disease prevalence are not now sufficient to evaluate this possibility. It does seem that higher parity women in the settled sample were at greater risk of experiencing pregnancy loss [see Leslie et al. (1993) for a discussion of these points and other considerations]. It may be that losses in the nomadic population tend to occur too early for the Icon II assay to detect them, whereas losses in the settled population occur later and are detected. The apparent differential between the groups would then change drastically, but we would still be left with the question of the etiology of the differential timing of the losses.

The rate of early pregnancy loss among the settled Turkana women appears to be higher than the rate seen in Western populations. This and the difference between the nomadic and settled women point to substantial variation among populations in intrauterine mortality. Few studies of pregnancy loss have been done outside the context of modern Western populations, but it is commonly assumed that different populations experience similar rates of overall intrauterine mortality [e.g., Bongaarts (1993)]. An expanded range of rates of loss suggests that pregnancy loss may have a greater impact on fecundability than has been suspected previously.

In the context of the evidence that we have presented concerning other aspects of reproductive function, the pregnancy loss results introduce some confusion. It is certainly possible that the pregnancy loss differential is a function of factors that are not related to ovarian cyclicity (e.g., specific infections). Nevertheless the differences in ovarian function suggested by the hormonal profiles of the two populations should translate into lower fecundability in the nomadic women relative to the settled women, yet the settled Turkana seem to have greater difficulty in carrying pregnancies to term. The

higher rate of early pregnancy loss in the settled women may mean that their *effective* fecundability (i.e., the probability that they will not only conceive but also carry the pregnancy to term) is actually lower than that of the nomadic women.

Reproductive Function, Body Composition, and Stress. The most obvious explanation of differences between two populations in reproductive function would lie in current or recent physiological stress associated with diet, disease, and activity patterns. Differences arising from developmental effects of environmental stresses or from genetic differences are possible, but little is known about these sources of variation in reproductive function in humans. In the present case neither a genetic nor a developmental explanation is likely, because many of the settled women grew up as nomads and were thus drawn from the same gene pool and experienced much the same lifestyle and environment as the nomadic sample. Even the lifelong residents of Morulem have relatives among the nomadic families.

Figures 1 and 2 suggest that the nomadic women's fecundity may be reduced compared with that of the settled women. It might then be expected that anthropometric assessment would indicate poorer nutritional status or general physiological condition in the nomadic women. There is some support for this, but the results are neither consistent nor compelling. For example, the difference in fat stores is congruent with detected differences in ovarian function between the 1989 nomadic and settled samples but not between the 1990 samples. And differences in adiposity are not in all cases paralleled by differences in body mass index.

Recent research suggests that ovarian function is responsive to energy *balance* apart from energy reserves at a given point in time (Ellison 1990; Panter-Brick et al. 1993); that is, *trend* in body composition may be more important than state. Thus the nomadic-settled differences in reproductive function may arise from differences in *trajectories* of physiological status. Cross-sectional anthropometric data would then yield only limited insight into the differences in reproductive function. Nor can relevant trajectories be reliably inferred from a knowledge of either body composition or environmental conditions during each of a sequence of cross-sectional surveys. For example, even though we have reason to believe that conditions during the second survey in Morulem were worse than those during the first survey, it is likely that food was becoming scarcer during the months preceding the first survey (because the previous harvest was depleted), and it is possible that food supply had stabilized or was even improving at the time of the second survey. Expectations based on trajectories would then be at odds with expectations based on static indicators of environmental conditions. The potential disparity between trend and state could explain the inconclusive results concerning the relationship between ovarian function and body composition.

Another complication is that we do not know how activity levels of the Morulem women compare with those of the nomadic women. This is potentially important because the effects of diet on ovarian function may to a great extent be tied up with workload: Energy balance and energy turnover may be crucial (Lunn 1994). Furthermore, high levels of energy expenditure (exercise) can affect ovarian function independently of diet and energy balance (Beitins et al. 1991). We have a considerable amount of information about the activity patterns of nomadic Turkana [e.g., Galvin (1985) and Little et al. (1988)], but at this time we have no quantitative data about workloads in Morulem.

Use of Field Assays. To our knowledge, this is the first study to use an array of hormonal assays, as opposed to pregnancy tests alone, in the field to evaluate reproductive function. Several observations about this experience are in order.

Use of the assays under adverse field conditions is feasible and can produce useful results. Consistency of results and extensive use of controls indicate that the assays we used are stable under less than ideal storage conditions. Phillips et al. (1991) arrived at the same conclusion after testing essentially the same hCG and LH kits as the ones we used under simulated and actual field conditions.

The ability to obtain results in the field shortly after collection of samples has several advantages. First, the risk of damage to or loss of hard-won samples during transport or shipment is eliminated (although preservation and retention of samples for later analysis is highly recommended). Second, the immediate results may enhance the efficiency of sampling. For example, being able to identify women who are in a particular reproductive state (e.g., cycling) would facilitate identifying subjects for a study of how dietary or activity patterns are related to reproductive status or function.

But there are limitations. Clearly, the qualitative (or at best semiquantitative) nature of these tests results in some ambiguities that quantitative assays can resolve. This problem would be reduced but not eliminated by research designs that are longitudinal rather than cross-sectional. Although additional tests (e.g., one for estrone glucuronide) would be useful and may be available in the near future, the range of hormones that can be tracked is always limited. Other parameters may prove useful during field or post hoc analyses, and these will be accessible only from preserved specimens. These are the main reasons for preserving samples for later quantitative analysis. Finally, if laboratory analyses of stored samples are going to be done anyway, the field assays are an additional cost in both time and materials. However, if funds for laboratory analysis are limited, the qualitative assays may help identify those samples for which analysis would be most informative or would provide provisional results until quantitative analyses can be completed.

Summary and Conclusions

Nomadic and settled Turkana women appear to differ with regard to pregnancy rates, incidence of amenorrhea, and hormonal profiles among cycling women. If the hormonal profiles reflect luteal insufficiency, fecundability of the nomadic women would be significantly affected. Results presented elsewhere (Little et al. 1992) indicate that parity-related loss of fat stores (maternal depletion) is greater in nomadic women than in settled Turkana women. All these differences suggest that reproductive function in the nomadic women is diminished relative to the settled women. But such a conclusion is countered by the high rate of pregnancy loss in Morulem, which may mean that *effective* fecundability is lower among the settled women than among the nomadic women. That, in turn, would help explain the fertility differential between the two populations (higher completed fertility among the nomadic women).

Some of the ambiguities in the results presented here will be difficult to resolve without longitudinal studies of ovarian function in Turkana. However, more detailed analysis is possible with the data and materials we have now. A portion of each urine specimen collected in Kenya was preserved and saved for laboratory analysis. This will enable us to perform quantitative assays and to determine parameters other than those permitted by the dipstick field assays (e.g., estrone glucuronide). Further research, in progress or planned, will enhance our knowledge of breast-feeding patterns, pregnancy loss, disease patterns, and temporal changes in diet, activity, and health in both populations. These field studies along with the more thorough laboratory analyses should help clarify our understanding of reproductive ecology in Turkana and provide important clues to interpopulation variability elsewhere.

The research described here prompts three suggestions relevant to the more general study of reproductive ecology: (1) Pregnancy loss may vary more among populations and may therefore be a more important proximate determinant of fecundability than has been recognized; (2) getting pregnant and remaining pregnant are two different things; different aspects of reproductive function may compensate for one another in their effects on fecundability and realized fertility; and (3) easily performed enzyme immunoassays of urinary hormones provide a feasible means of assessing certain aspects of female reproductive function in the field and are a valuable tool in the study of human reproductive ecology. The first two points contribute to a growing appreciation on the part of anthropologists, human biologists, and others of the complexity of human reproduction. The biological and ecological determinants of reproductive performance at the individual level and of fertility at the population level are many, are sometimes subtle, and exhibit substantial variability.

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