Project REPA (Reproduction & Ecology in Provincía Aroma):
Sources of Intra- and Inter-Woman Variation in Progesterone Levels

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BACKGROUND & HYPOTHESES

How much do ovarian hormone levels vary from cycle to cycle?

Many studies assume low variation (i.e., high correlation) of ovarian steroid levels among a woman's ovarian cycle, but ***this assumption may not be justified.*** The few relevant studies have reported low[1,6] *and* high[4,5] inter-cycle variation in hormone levels.

Ovarian hormone levels vary with energy intake and expenditure[2] and lactation status,[9,10] but other than a study of 22 rural Polish women,[4] there has been little evaluation of the magnitude of the contribution of these factors to natural inter-cycle hormonal variation. In addition, the question of whether seasonal energetic stress affects only anovulation rates or also reduces hormone levels in ovulatory cycles *per se* has not been examined.

We tested 3 hypotheses regarding the extent and sources of progesterone [P] variation within and between individuals in a large sample of breastfeeding and non-breastfeeding Bolivians followed through the agricultural cycle in a single year.

HYPOTHESES:

Null Hypothesis 1: P levels in a woman's ovulatory cycles are not correlated.

Alternate Hypotheses: P levels in a woman's ovulatory cycles are significantly …

1a: **…** but only modestly correlated.

1b: **…** and highly correlated.

Null Hypothesis 2: P levels in ovulatory cycles do not vary with seasonal variation in energy intake and expenditure.

Alternate Hypothesis 2: Energetically stressful seasons are significantly associated with reduced P levels in ovulatory cycles.

Null Hypothesis 3: Among cycling women, breastfeeding status does not contribute to inter-woman variation in P levels in ovulatory cycles.

Alternate Hypothesis 3: Among cycling women, breastfeeding status is significantly associated with variation in P levels in ovulatory cycles.

STUDY DESIGN & METHODS

Study Population. Participants (n=316 women) in Project REPA, a longitudinal study of health and reproduction in rural agropastoral communities in the Bolivian *altiplano* (4000m altitude).

Data Collection. Menstruating women (n=191; 98 breastfeeding, 93 not breastfeeding) were visited every other day by a bilingual (Spanish/Aymara) *promotora* who recorded menstrual bleeding and lactation status, and collected a saliva sample later assayed for progesterone [P].[9,10]

Ascribing Ovulation. Progesterone levels are exceedingly low in anovulatory cycles. Inclusion of these cycles in analyses unavoidably obscures the issue of whether inter-cycle hormonal variation is principally a consequence of anovulation (i.e., hormone levels in *ovulatory* cycles of a given woman are highly correlated) or of anovulation plus significant hormonal variation in a woman's ovulatory cycles. We distinguished anovulatory cycles using previously described methods.[10]

Statistical Analyses (using SPSS *v19.0*) included only ovulatory cycles (n= 392 cycles, 122 women).

The **dependent variable, *mean peak-luteal-P,*** was defined as (the area-under-the-curve bounded by ± 2.5 days from the day of maximum observed P)/(5 days).

Predictor variables. *Season*: early harvesting and planting (shaded grey in Fig 1) are the most energetically demanding periods ("poor"), others being relatively less arduous ("good").
Breastfeeding status: Cycles were classified as "breastfeeding" or "not breastfeeding" based on women's recorded practices. ***Woman's age*** was based on several cross-verified sources.

RESULTS

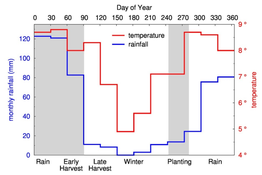

Figure 1. Agricultural cycle, rainfall, & temperature, during the year

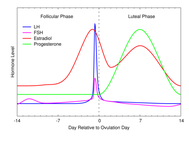

Figure 2. Area Under the Curve

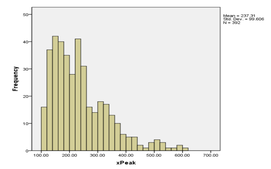

Figure 3. Frequencies of mid-luteal mean-peak P.

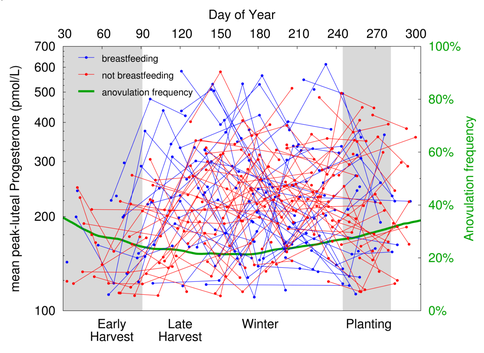

Figure 4. Fluctuations in mid-luteal mean-peak P across the days of the year in 1996.

Null Hypothesis 1: Rejected. Mean-peak-P levels in an individual's ovulatory cycles **are correlated** (Figures 5 and 6, Table 1).

Alternate Hypothesis 1a: Supported. These mean-peak-P levels **are only modestly correlated.** Alternate Hypothesis 1b is not supported.

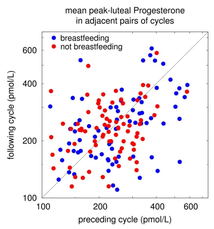

Figure 5. LAG 1 Correlations.

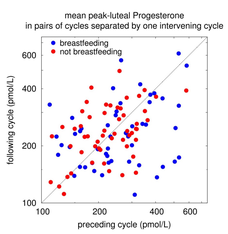

Figure 6. LAG 2 Correlations.

Table 1. Correlations between mean-peak luteal P (log-transformed) from different cycles in Good seasons (* p<0.001; ** p ≤ 0.05).

Cycles Compared	Reproductive Status	N	r
LAG 1: P Levels from all possible pairs of two consecutive cycles (ex: AB, BC, CE)	BF & Menstruating	73	0.0415*
	Menstruating	80	0.313**
	Combined	153	0.376*
LAG 2: Referent Cycle P levels compared to P levels two cycles later (ex: AC, CE)	BF & Menstruating	51	0.303**
	Menstruating	55	0.464*
	Combined	106	0.363*

Alternate Hypothesis 2: Supported. Mean-peak-P levels in ovulatory cycles **are significantly lower** in the poor season (Figure 7, Table 2). However, seasonality accounted for only 4% of the total variation in mean-peak-P, indicating that the source of most of the hormonal variation is unexplained.

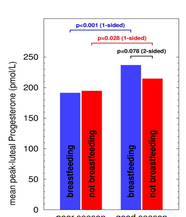

Figure 7. Differences in mean-peak P levels by Breastfeeding Status and Season.

Table 2. Model With Reproductive Status, Season, RS*Season

Model Parameter or Interaction	β	p	95% Confidence Interval
Intercept (good season, not breastfeeding)	2.33	0.000 (2-sided)	[2.30 , 2.36]
Poor season × not breastfeeding	−0.0425	0.028 (1-sided)	[−0.0862,+0.0012]
Good season × breastfeeding	+0.0429	0.078 (2-sided)	[−0.0049,+0.0906]
Intercept (good season, breastfeeding)	2.37	0.000 (2-sided)	
Poor season × breastfeeding	−0.092	0.001 (2-sided)	[−0.146 ,−0.039]

Null Hypothesis 3: NOT Rejected. (i.e., alternate hypothesis 3 was NOT supported). Breastfeeding status does not contribute to inter-woman variation in P levels in ovulatory cycles. (Figure 7, Table 2).

CONCLUSIONS

-High levels of both intra- and inter-woman variation
  -ICC= 0.32. The intra-class correlation coefficient (ICC) is defined as the ratio of the between-subject variance divided by the total variance in the sample.
  -Thus, 68% of the total variation in our sample is due to intra-woman variation.

-Seasonality significantly influences mean luteal peak progesterone levels.
  - Mean-peak P levels higher in Good seasons.
  -But, not the only factor, explains only 4% of the variance ($p < 0.001$).

-Breastfeeding status was not significantly related to inter-woman variation in mean-luteal peak progesterone levels.
  -Findings are surprising, given our expectations based on physiological understandings of lactation and suppression of reproductive hormones.
  -Future analyses will account for **other important factors** that mediate the impact of breastfeeding on progesterone levels:
    -Age of child being breastfed
      -Younger toddlers and infants tend to consume more breast milk than older toddlers.[7,8]
    -Age and anthropometrics of the mother; either of these variables might mediate maternal hormonal response to breast feeding.

-Despite concluding that seasonality significantly influences mean luteal peak P levels, **high levels of variation remain unexplained.**
  -This variation has **major implications** for both **clinical** and **field** studies.
  -Our results **challenge the notion** of a simple "baseline" hormone level for a given woman, as P levels across cycles were only moderately correlated.

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