Supplemental Material. Scenarios in which an endogenous pulse occurs shortly after the time of kisspeptin administration, and estimates of the effects of these scenarios on the measured pulse intervals.

Because there is no way to distinguish LH pulses that occur endogenously from those induced by kisspeptin, an endogenous pulse could either be obscured by the kisspeptin-induced pulse or mistaken for the kisspeptin-induced pulse:

Scenario 1. Kisspeptin does not induce an LH pulse, and an endogenous pulse occurs in the sampling interval (10 min) after kisspeptin administration. This endogenous pulse would be incorrectly labeled the kisspeptin-induced pulse, rather than the first endogenous pulse occurring immediately after kisspeptin. As a result, the Kiss-to-Post interval (B2) would appear to be longer than it actually was. The amount by which the observed interval would exceed the actual interval would be equal to the endogenous pulse interval, and the probability of this occurring is 10 min (the blood sampling interval) divided by the endogenous pulse interval. Multiplying these two factors, the effect of this scenario would be to lengthen interval B2, on average, by 10 min.

Scenario 2. Kisspeptin does induce an LH pulse, and an endogenous pulse occurs after kisspeptin administration but before the peak of the kisspeptin-induced pulse. This endogenous pulse would be obscured by the kisspeptin-induced pulse, and would not be labeled the first endogenous pulse after kisspeptin. As a result, the observed interval B2 would exceed the actual interval by an amount equal to the endogenous pulse interval. The probability of this occurring is the duration of the kisspeptin-induced LH pulse (time from nadir to peak, shown in Fig. 2C) plus 10 min, divided by the endogenous pulse interval. Multiplying these two factors, the effect of this scenario would be to lengthen interval B2, on average, by the time from nadir to peak of kisspeptin-induced LH pulses plus 10 min.

(Note: These scenarios 2A and 2B apply only if kisspeptin does not reset the GnRH pulse generator. If kisspeptin does reset the GnRH pulse generator, the next endogenous pulse is very unlikely to occur within these relatively short time intervals.)

The effect of these scenarios in the follicular phase would be:

Interval	A (min)	B1 (min)	B2 (min)	C (min)
Measured	71	60	78	73
Correction factor			10 (Scenario 1) 10 + 23 (Scenario 2)	
Corrected	71	60	68 (Scenario 1) 45 (Scenario 2)	73

Predictions of resetting are that B2 would be longer than B1, and that B2 would equal A. While the "uncorrected" measurements suggested that this might be the case, the "corrected" measurements are less suggestive.

In the luteal phase (where Scenario 2 applies, as kisspeptin induced an LH pulse in all subjects):

Interval	A (min)	B1 (min)	B2 (min)	C (min)
Measured	213	155	130	152
Correction factor		10	10 + 24	
Corrected	213	145	96	152

Predictions of resetting are that B2 would be longer than B1, and that B2 would equal A. These predictions were not supported by the observed intervals, and this remains true for the "corrected" intervals.

Note: Applying these corrections to the data obtained in our prior study in healthy men (1) does not change the pattern observed, which was consistent with resetting of the GnRH pulse generator by kisspeptin (data not shown).

Reference

1. **Chan YM, Butler JP, Pinnell NE, Pralong FP, Crowley WF, Jr., Ren C, Chan KK, Seminara SB** 2011 Kisspeptin resets the hypothalamic GnRH clock in men. J Clin Endocrinol Metab 96:E908-915

Figure 1. Early Follicular Phase

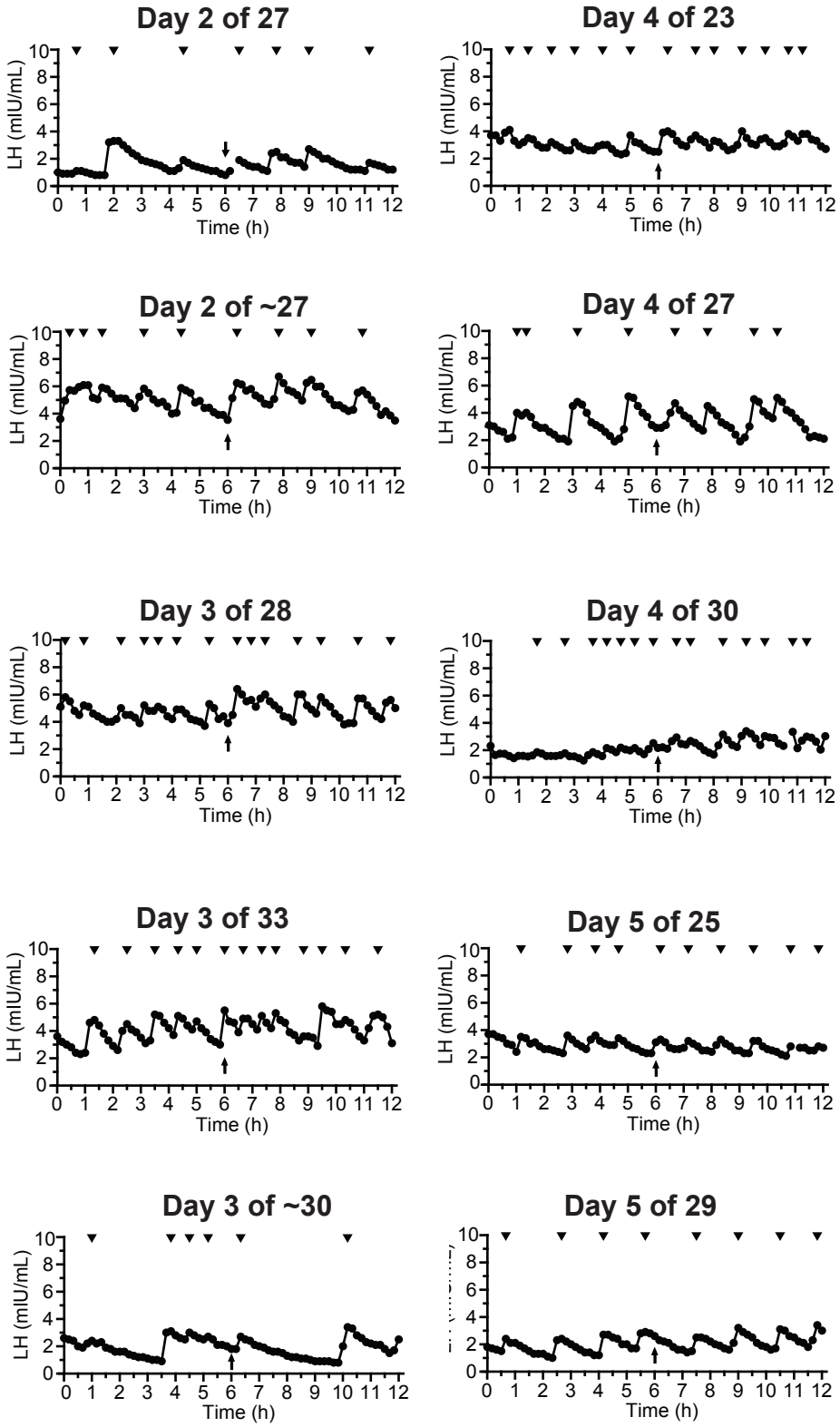

Figure 2. Follicular Phase (higher dose kisspeptin)

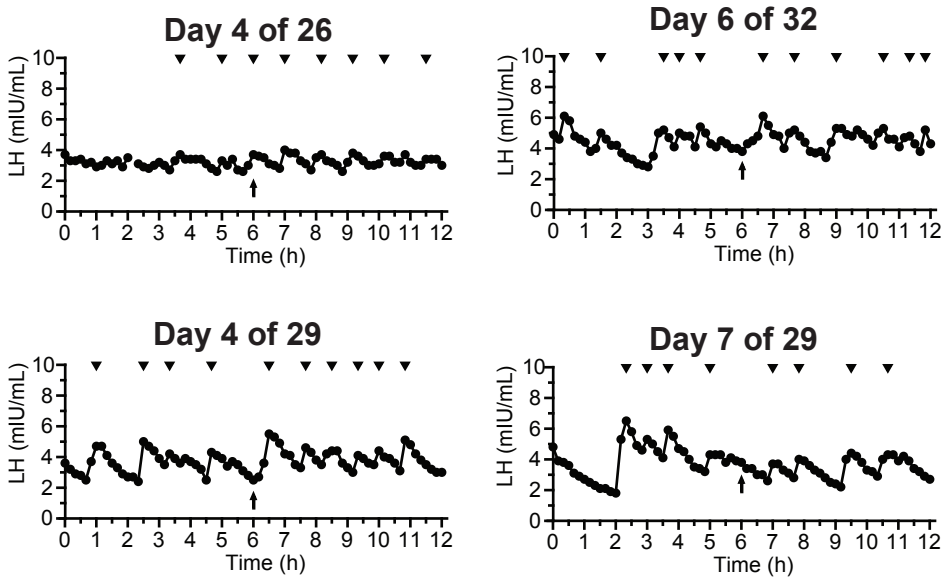

Figure 3. Pre-Ovulatory

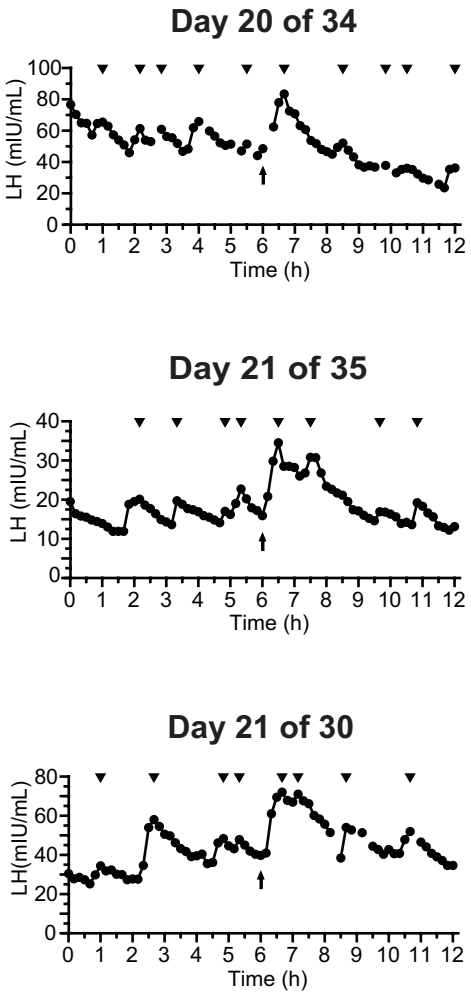

Figure 4. Mid-Luteal Phase