

First urinary reproductive hormone normograms referenced to independently determined ovulation day

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Introduction

- Reference ranges of reproductive hormones in women with natural menstrual cycles and no reported infertility, provide a valuable tool for understanding the normal range of hormones in relation to day of cycle
- However, due to variation in menstrual cycle length, alignment by cycle day obscures the endocrinology¹
- Other researchers have used the day of the luteinising hormone (LH) surge, i.e. when LH levels peak, to create reference ranges,² however this can still cause distortion of the hormonal profiles because:
 - There is intra-individual variation in time from peak LH to ovulation³
 - Different LH assays have different levels of sensitivity to the degradation products of LH, thus the timing of the LH peak is assay-dependent
- Therefore, the most appropriate way to present reproductive hormone reference ranges is as normograms referenced to the actual day of ovulation, as determined by an independent method
- Trans-vaginal ultrasound is an effective way to determine the day of ovulation if examinations are frequent enough
- This study sought to create new urinary reproductive hormone normograms in relation to the ultrasound-determined day of ovulation

Methods

A prospective study (NCT01802060) required women (aged 18–45 years) with natural menstrual cycles and no reported infertility to collect daily urine samples from the first day of their period (day 1 of their menstrual cycle) until the first day of their next period. Women attended the study site for trans-vaginal ultrasound to determine the day of ovulation. Where possible, visits were conducted every two days until the follicle size reached 16 mm, and then visits were conducted daily, with subsequent scans carried out on days 7 and 9 following ovulation. Where ovulation occurred between two scan days, the day of ovulation was considered as half a day following the last day where a dominant follicle was present. Serum samples were collected on scan days for measurement of serum LH, estradiol and progesterone (ADVIA Centaur XP).

Urinary LH, estrone-3-glucuronide (E3G, a metabolite of estradiol), follicle stimulating hormone (FSH) and pregnanediol-3-glucuronide (P3G) were measured using in-house assays on AutoDELFI (Perkin Elmer). Validation of these assays has previously been reported.⁴

Data was analysed using SAS version 9.2, to create normograms referenced to the day of ovulation.

Results

Of the total 41 volunteers, the day of ovulation was determined in 40 women; one woman had an anovular cycle. The mean age of the ovulatory volunteers was 28.9 years (SD 4.76, median 29.5, range 18–37); 95% and 5% of women were white and Asian, respectively. The menstrual cycle characteristics of the volunteers are described in Table 1.

Table 1: Characteristics of the menstrual cycles recorded in the study of volunteers whose data was used to produce the urinary hormone normograms.

	n	Median	Minimum	Maximum	5th centile	25th centile	75th centile	95th centile	Mean	SD
Cycle length*, days	37	27.00	22.00	37.00	22.00	26.00	29.00	34.00	27.70	3.37
Day of ovulation^	40	14.50	8.50	26.50	11.00	12.75	17.00	23.50	15.35	3.84

* Excluding pregnant volunteers

^ Excluding anovular cycles

Normograms consisting of the median hormone levels and 10th–90th centile ranges by day, relative to the day of ovulation observed by ultrasound, were derived for each urinary reproductive hormone measured (Figures 1–4).

Figure 1. Urinary LH normogram

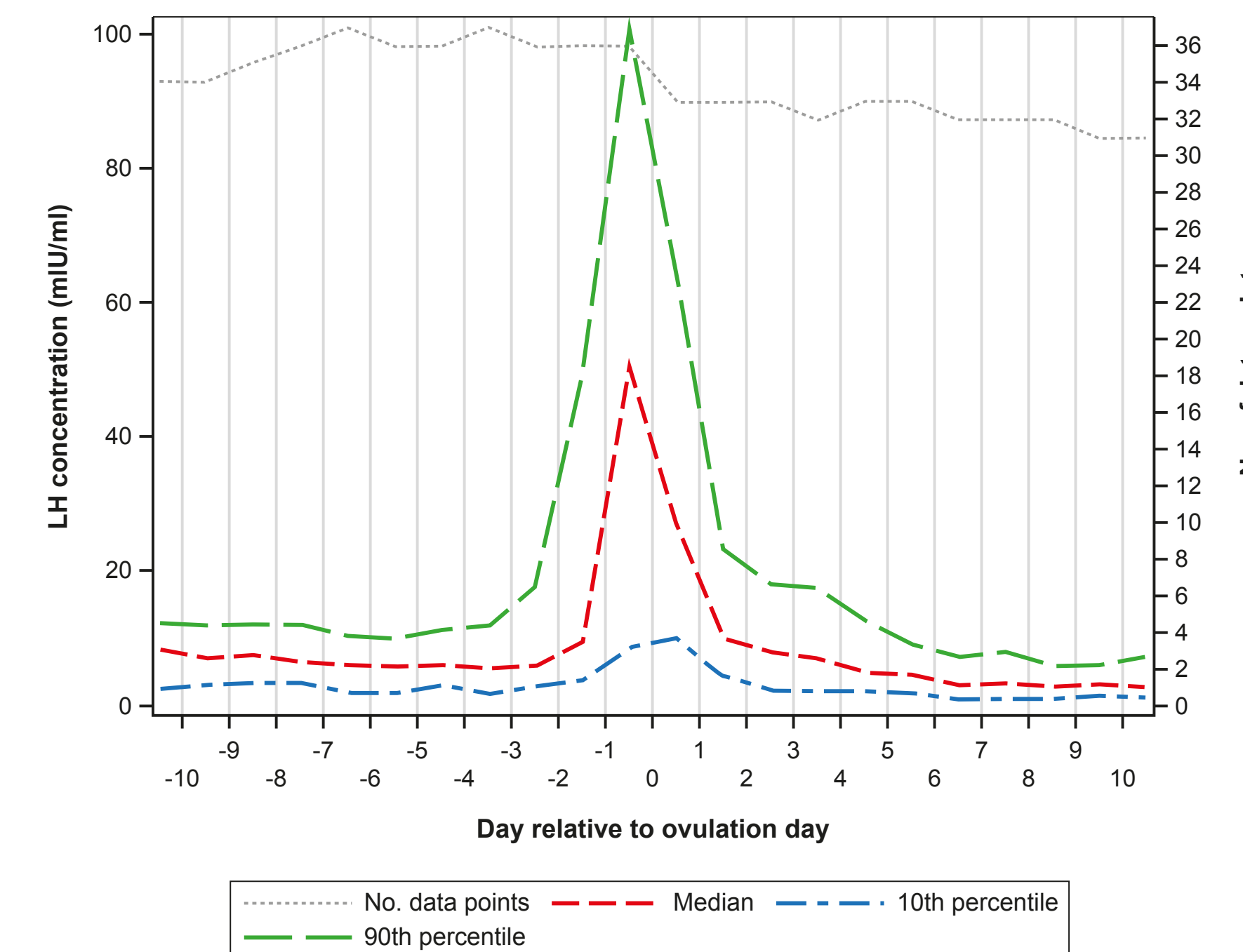


Figure 2. Urinary E3G normogram

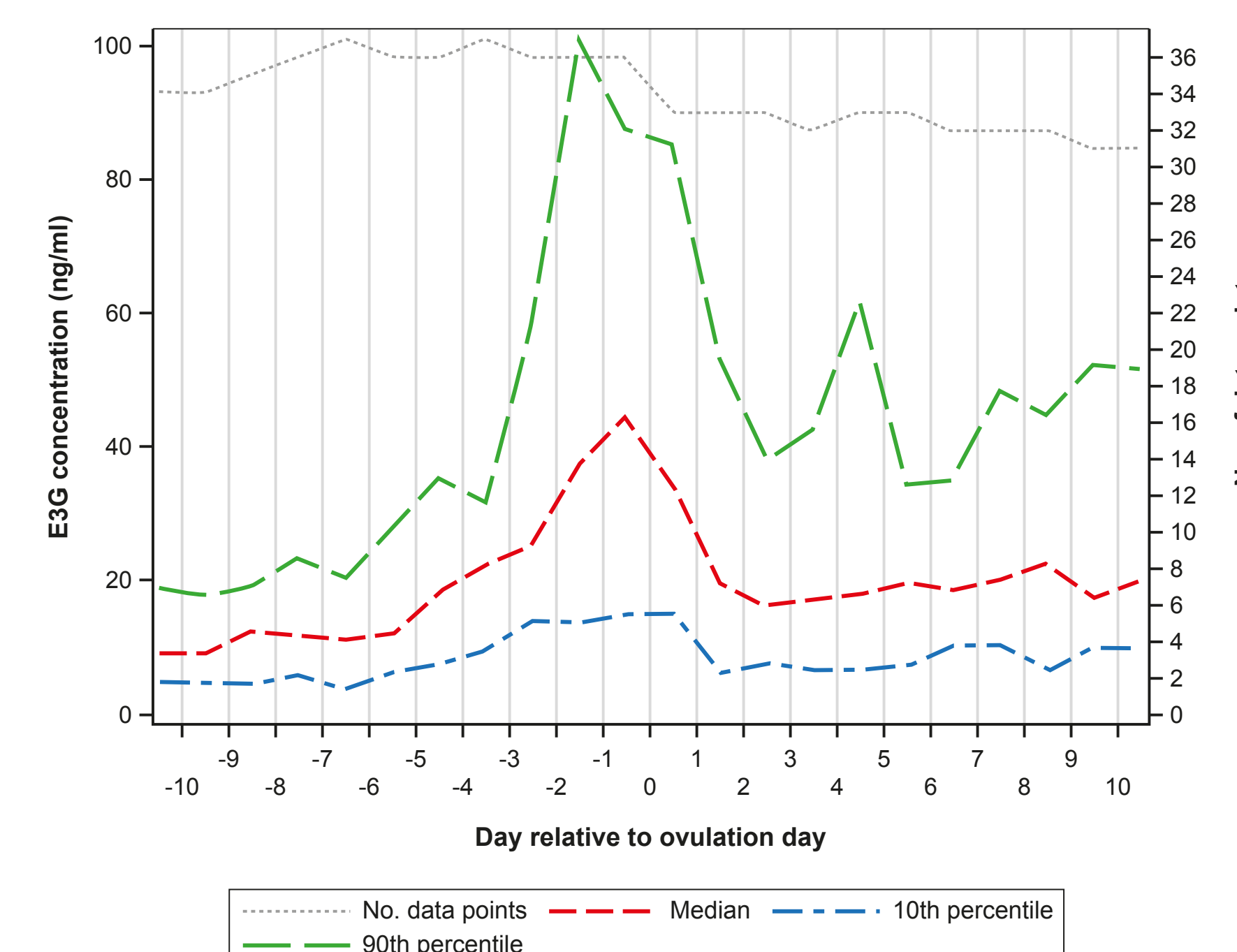


Figure 3. Urinary FSH normogram

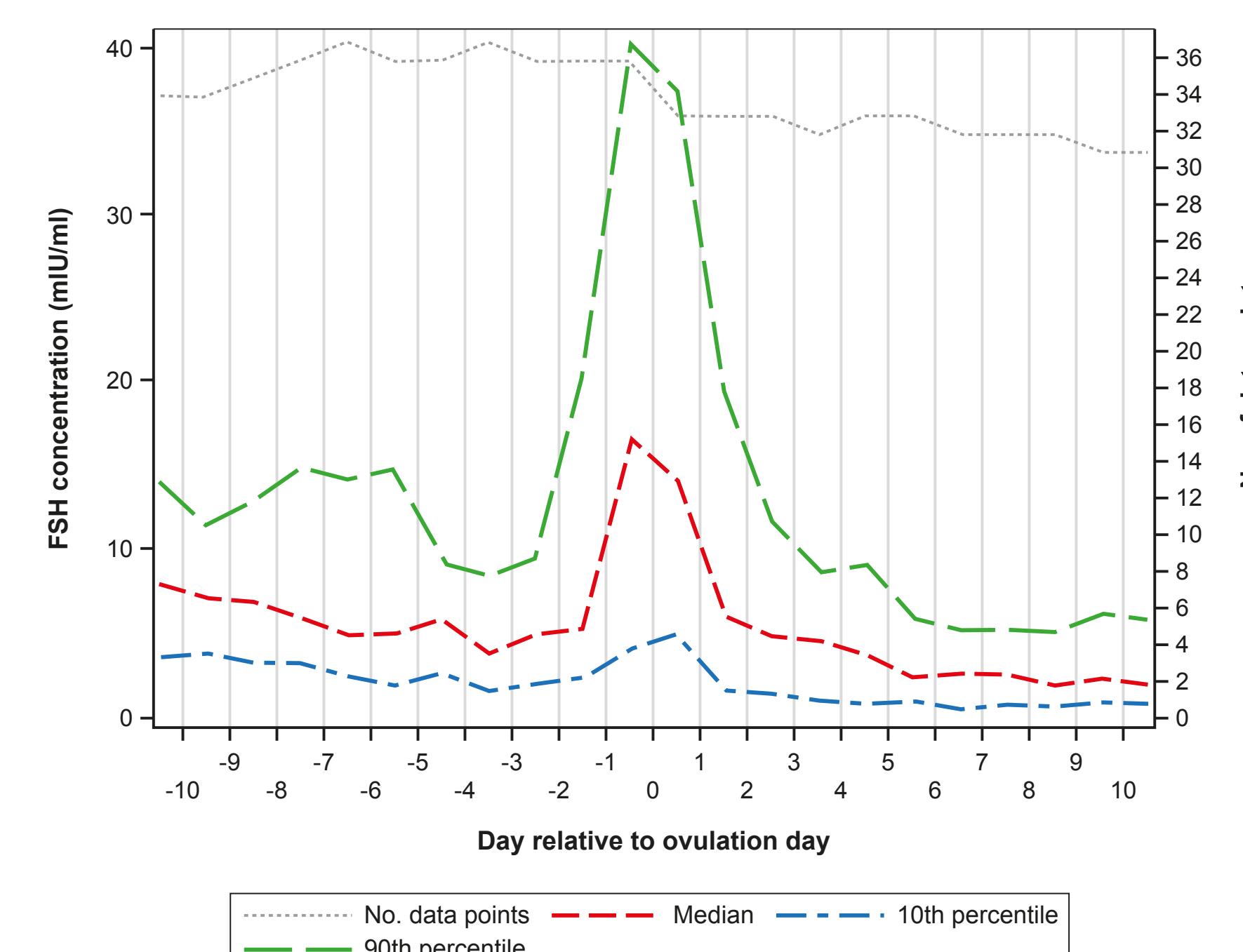
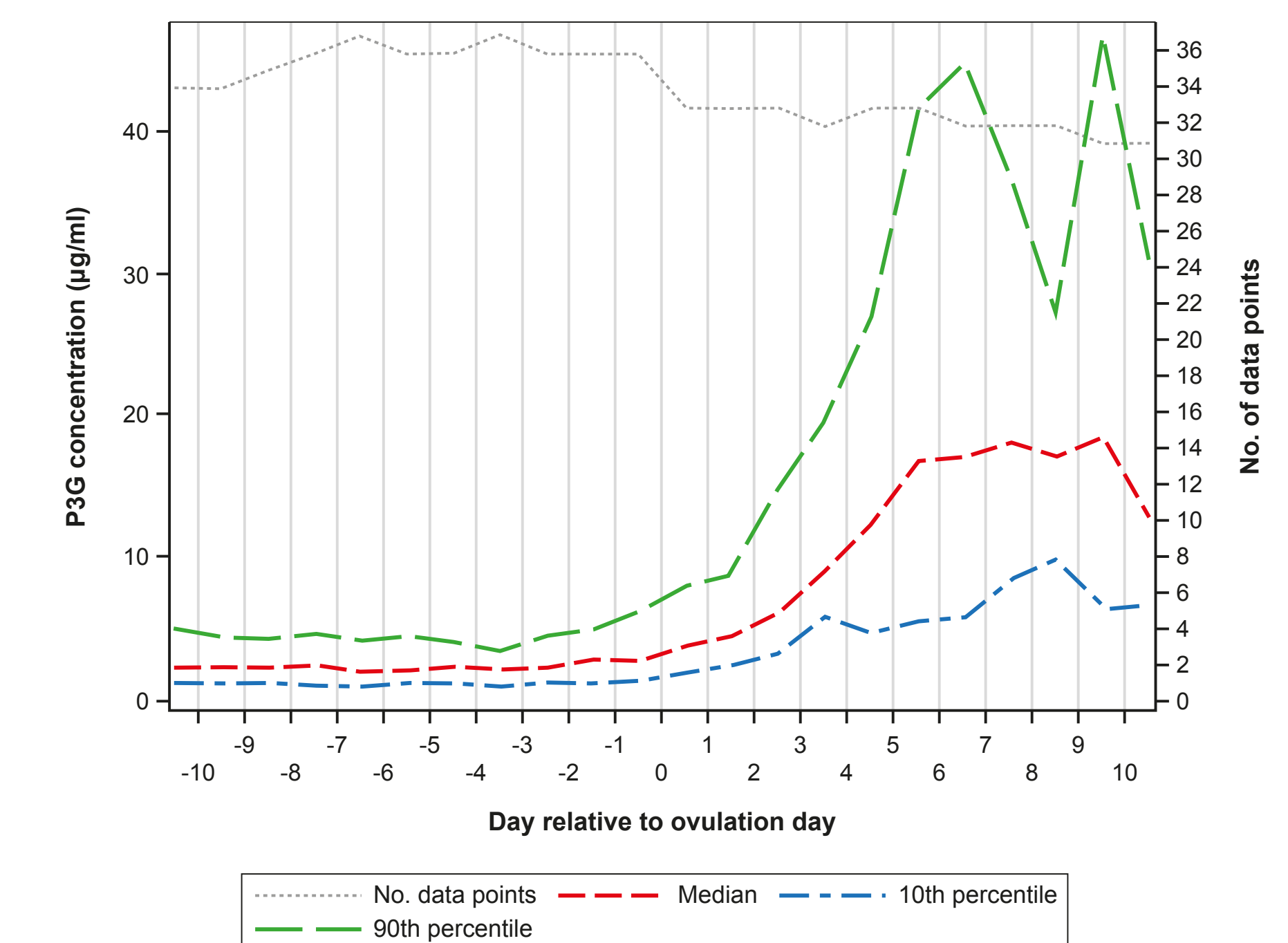


Figure 4. Urinary P3G normogram



- Peak LH levels were seen a median of 0.5 days prior to ovulation (5th–95th centile: -1.5 to +0.5 days).
- Median peak E3G levels were also observed 0.5 days prior to ovulation (5th–95th centile: -2.5 to +9.5 days)
- The same median peak day was seen for FSH levels (-0.5 days, 5th–95th centile: -2.5 to +0.5 days).
- P3G levels peaked a median of 7.5 days following ovulation, (5th–95th centile range: +4.5 to +10.5 days).

Conclusion

These are the first urinary reproductive hormone normograms referenced to the actual day of ovulation, and therefore provide the most robust reference ranges to examine menstrual cycle endocrinology.

References

- Johnson SR, Miro F, Barrett S, Ellis J. (2009) Levels of urinary human chorionic gonadotrophin (hCG) following conception and variability of the menstrual cycle in a cohort of women attempting to conceive. *CMRO* 25: 741–748
- Stricker R, Eberhart R, Chevailer M, Quinn F, Bischof P, Stricker R. (2006) Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT analyser. *Clin Chem Lab Med* 44: 883–887
- Seibel M. (1986) Luteinizing hormone and ovulation timing. *Journal of Reproductive Medicine* 31: 754–759
- Miro F, Coley J, Gani M, Perry P, Talbot D, Aspinall L. (2004) Comparison between creatinine and pregnanediol adjustments in the retrospective analysis of urinary hormone profiles during the human menstrual cycle. *Clin Chem Lab Med* 42: 1043–1050

Declaration of interest

Sarah Johnson, Sarah Weddell and Sonya Godbert are employees of SPD Development Company Ltd., a fully owned subsidiary of SPD Swiss Precision Diagnostics GmbH., the manufacturers of Clearblue Pregnancy and Fertility tests. This study was funded by SPD Development Company Ltd.

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