IMMULITE® FSH AND ESTRADIOL ASSAYS IN THE LUTEAL-FOLLICULAR TRANSITION PERIOD OF NORMAL OVULATORY CYCLES

Vankrieken L, El Shami AS (Diagnostic Products Corporation, Los Angeles, CA)

Abstract

During the follicular phase of the menstrual cycle, follitropin (FSH) is largely responsible for follicular development, and for inducing granulosa cells to develop and increase production of estradiol (E2). Measurement of FSH and E2 on days 2-3 of the follicular phase, combined with information on maternal age, may be useful for predicting a successful pregnancy. Accordingly, we undertook a multisite study aimed, in part, at elucidating the normal patterns and concentration levels of FSH and E2 during the final days of the luteal phase and the initial days of the follicular phase.

Subjects and Methods: Serum samples were obtained on a daily basis from 54 apparently normal cycling women (median age: 31 years, range: 16 to 44; median cycle length: 29 days, range: 23 to 35). Results were generated with the automated, chemiluminescent IMMULITE FSH and Estradiol assays (Diagnostic Products Corporation, Los Angeles, CA).

Results: Medians (and central 95% ranges) for FSH and E2 on days 2-3 of the follicular phase were 6.6 IU/L (3.0 – 14.4) and 114 pmol/L (ND – 308), respectively. A drop in E2 levels occurred towards the end of the luteal phase in all cycles. In Figure 2, results for one of the 54 subjects are displayed against a backdrop of the entire set of FSH results for the final eight days (coded –8 through –1) of the luteal phase and the first eight days (+1 through +8) of the follicular phase. On average, FSH levels were lowest near the end of the luteal phase, some 4-5 days before menstruation, and showed a progressive increase thereafter, reaching their highest levels by about day 4 of the early follicular phase. Medians (and central 95% ranges) for FSH on days –4 and +4 were 2.5 IU/L (1.1 – 5.8) and 6.9 IU/L (3.4 – 12.6), respectively.

Introduction

The pituitary hormone follitropin (FSH) is largely responsible for follicular development during the initial phase of the menstrual cycle, and for inducing granulosa cells to develop and increase production of the ovarian hormone estradiol (E2).

Measurement of FSH and estradiol in the very early days of the follicular phase (days 2–3) provides a basis for evaluating "ovarian reserve." Combined with information on maternal age, this may be useful for predicting a successful pregnancy.[Van99a]

As indexed by their ratio, relative levels of the pituitary hormones lutropin (LH) and FSH during the early follicular phase are also of clinical interest in these and related contexts.[Van99a]

Accordingly, with the goal of obtaining a more exact characterization of the values to be expected for these hormones and derived parameters during this critical segment of the menstrual cycle, we examined data from an international collaborative study based on a system of automated immunoassays for reproductive hormones on the IMMULITE® analyzer.

Subjects

In a study involving laboratories from five countries — Belgium, Germany, The Netherlands, the United Kingdom and the United States — serum samples were collected on a daily basis from 60 women in apparent good health, throughout one complete ovulatory cycle, beginning on the first day of menstruation and sometimes continuing one or even two days into the next cycle.[Van99b] All samples were assayed for follitropin (FSH), lutropin (LH), estradiol (E2), progesterone (P4) and prolactin (PRL). The 54 women (out of 60) with normal ovulatory cycles had a median age of 31 years (range: 16–44 years) and a median cycle length of 29 days (range: 23–35 days).

Methods

All results were obtained with a system of solid-phase, chemiluminescent, enzyme immunoassays, designed for use on the IMMULITE® automated analyzer (Diagnostic Products Corporation, Los Angeles, CA; www.dpcweb.com).

IMMULITE FSH and LH are immunometric enzyme assays based on specific monoclonal and polyclonal antibodies for capture and detection, respectively. IMMULITE FSH has a calibration range of up to 170 IU/L [2nd IRP 78/549], with a detection limit of approximately 0.1 IU/L. IMMULITE LH has a calibration range of up to 200 IU/L [1st IRP 68/40], with a detection limit of approximately 0.7 IU/L.

IMMULITE Estradiol is a competitive enzyme immunoassay based on enzyme-labeled analyte and an antibody highly specific for this steroid. The assay has a calibration range of 20 to 2,000 pg/mL (73 to 7,342 pmol/L).

Early Follicular Phase: IMMULITE Results

Analyte	Days	Median	Central 95%	Units
FSH	2 – 3	6.6	3.0 – 14.4	IU/L
Estradiol	2 – 3	31	ND – 84	pg/mL
		114	ND – 308	pmol/L
LH/FSH	2 – 5	0.60	0.15 – 1.5	(ratio)

ND denotes nondetectable.

Data Analysis

S-PLUS v4.5.2 (www.mathsoft.com) was used for calculations and data visualization, and for the graphs in this presentation. The tabulated centiles were calculated using an S-PLUS implementation [Wil97] of the Harrell-Davis function, which is considered the nonparametric method of choice for univariate reference range analysis.[H&B95].

LFT Plots: Calculations of group-based reference limits in the early days of the follicular phase naturally require a representation in which results for the cycle days of interest are aligned across subjects. But this simply corresponds to the primary method of identifying the samples for each subject, counting from the first day of menstruation (+1, +2, etc.). The representation can be extended, in an obvious way, by stepping back through the luteal phase of the previous cycle, counting from the first day prior to menstruation (–1, –2, etc.). Graphing concentration levels against cycle days so numbered for the final days of one cycle and the initial days of the next cycle yields a luteal-follicular transition (LFT) plot.

Much as the conventional method of aligning cycle data via the midcycle LH peak focuses on the days surrounding ovulation, the LFT plot focuses on the days surrounding menstruation. It provides a window onto a particularly important segment of the ovulatory cycle, namely, the early follicular phase and the days leading up to it. These two representations yield complementary insights; they are not equivalent, due to variations in phase length from one subject to another.[Van99b]

Note: Because only one complete cycle was measured in this study, LFT plots were constructed largely from results obtained during the last days and the first days of the same rather than consecutive cycles.[Van99b]

Figure 1. IMMULITE FSH – Average Values

Figure 2. IMMULITE FSH – Subject Number 21

Figure 3. IMMULITE FSH – Subject Number 44

Figure 4. IMMULITE FSH – Subject Number 48

Results

Each of the seven LFT plots depicts the entire set of FSH or estradiol results obtained for the 54 normal subjects over the last 8 days of each woman's luteal phase and the first 9 days of her follicular phase.

In Figure 1, superimposed on the FSH results, is a connected series of points, in red, indicating the average FSH values for each of the eight central days of the luteal-follicular transition period. Figures 2–4 highlight the FSH results for each of three subjects.

On average, FSH levels were lowest near the end of the luteal phase, some 4-5 days before menstruation, and showed a progressive increase thereafter, reaching their highest levels by about day 4 of the early follicular phase. Medians (and central 95% ranges) for FSH on days –4 and +4 were 2.5 IU/L (1.1 – 5.8 IU/L) and 6.9 IU/L (3.4 – 12.6 IU/L), respectively.

Figures 5–7 highlight the estradiol results for the same three subjects. A drop in estradiol levels occurred towards the end of the luteal phase in all cycles.

Reference range limits for the very early days of the menstrual cycle are tabulated — as the 50th centile (median) and 2.5th–97.5th centiles (central 95%) — for FSH and estradiol, and also for the LH/FSH ratio.

Figure 5. IMMULITE Estradiol – Subject Number 21

Figure 6. IMMULITE Estradiol – Subject Number 44

Figure 7. IMMULITE Estradiol – Subject Number 48

Conclusions

A study of 54 normally ovulating women, based on IMMULITE® measurements of reproductive hormone levels in daily serum samples from one complete cycle, has yielded a precise delineation of FSH and estradiol levels in the clinically important luteal-follicular transition (LFT) period.

These and other reference limits (e.g. for the early follicular phase LH/FSH ratio) not only help to confirm the accuracy of the IMMULITE FSH, LH and Estradiol assays; they also contribute towards the practical interpretation of follicular phase reproductive hormone levels in routine clinical practice.

References

[H&B95] Harris EK, Boyd JC. Statistical bases of reference values in laboratory medicine. New York: Marcel Dekker, 1995.

[Van99a] Vankrieken L, Reuben H. Hormonal levels during the early follicular phase of the menstrual cycle. Los Angeles: Diagnostic Products Corporation, 1999. Technical report ZB182-A.

[Van99b] Vankrieken L, Van der Horst FAL, Castracane VD, et al. An international multicenter reference range study of normal menstrual cycles using IMMULITE" reproductive hormone assays [abstract 322]. Clin Chem 1999;45(S6):A92.

[Van99c] Vankrieken L. Choosing a window into the ovulatory cycle. News & Views (DPC) 1999 Winter;13(1):11.

[Wil97] Wilcox RR. Introduction to robust estimation and hypothesis testing. New York: Academic Press, 1997.

Figure 1

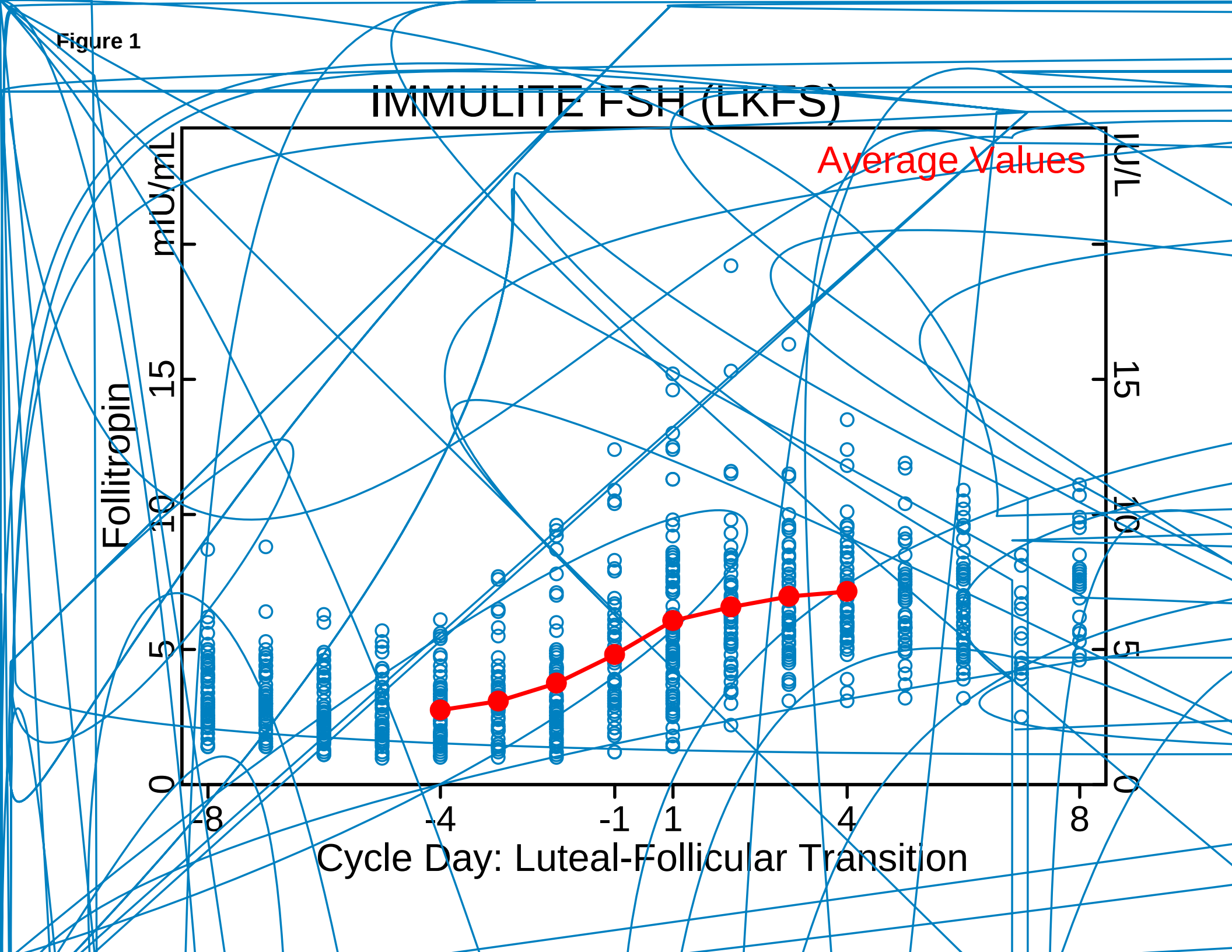

IMMULITE FSH (LKFS)
Average Values
Follitropin
mIU/mL
IU/L
0
5
10
15
-8
-4
-1
1
4
8
Cycle Day: Luteal-Follicular Transition

Figure 2

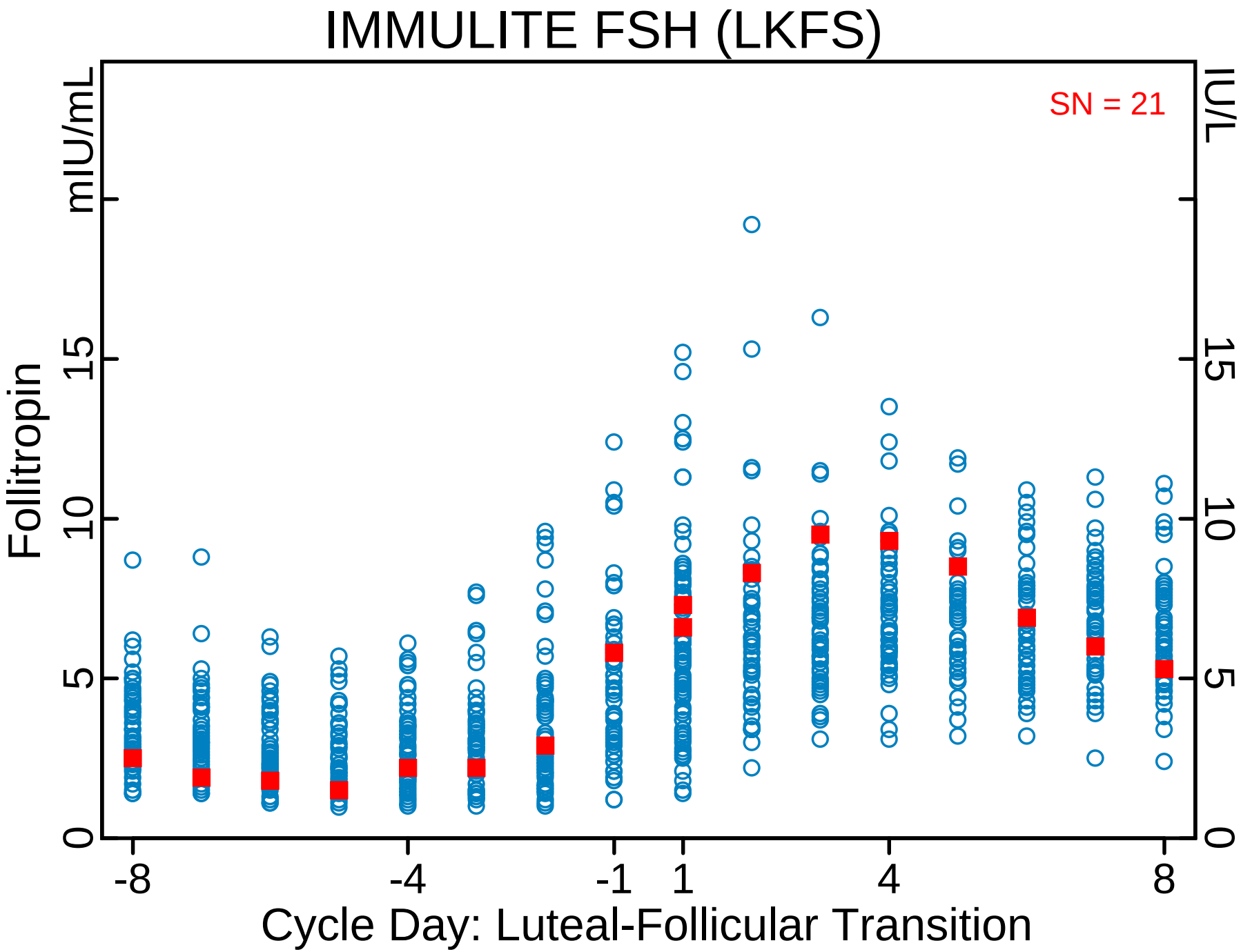

IMMULITE FSH (LKFS)
SN = 21
Follitropin
mIU/mL
IU/L
0
5
10
15
-8
-4
-1
1
4
8
Cycle Day: Luteal-Follicular Transition

Figure 3

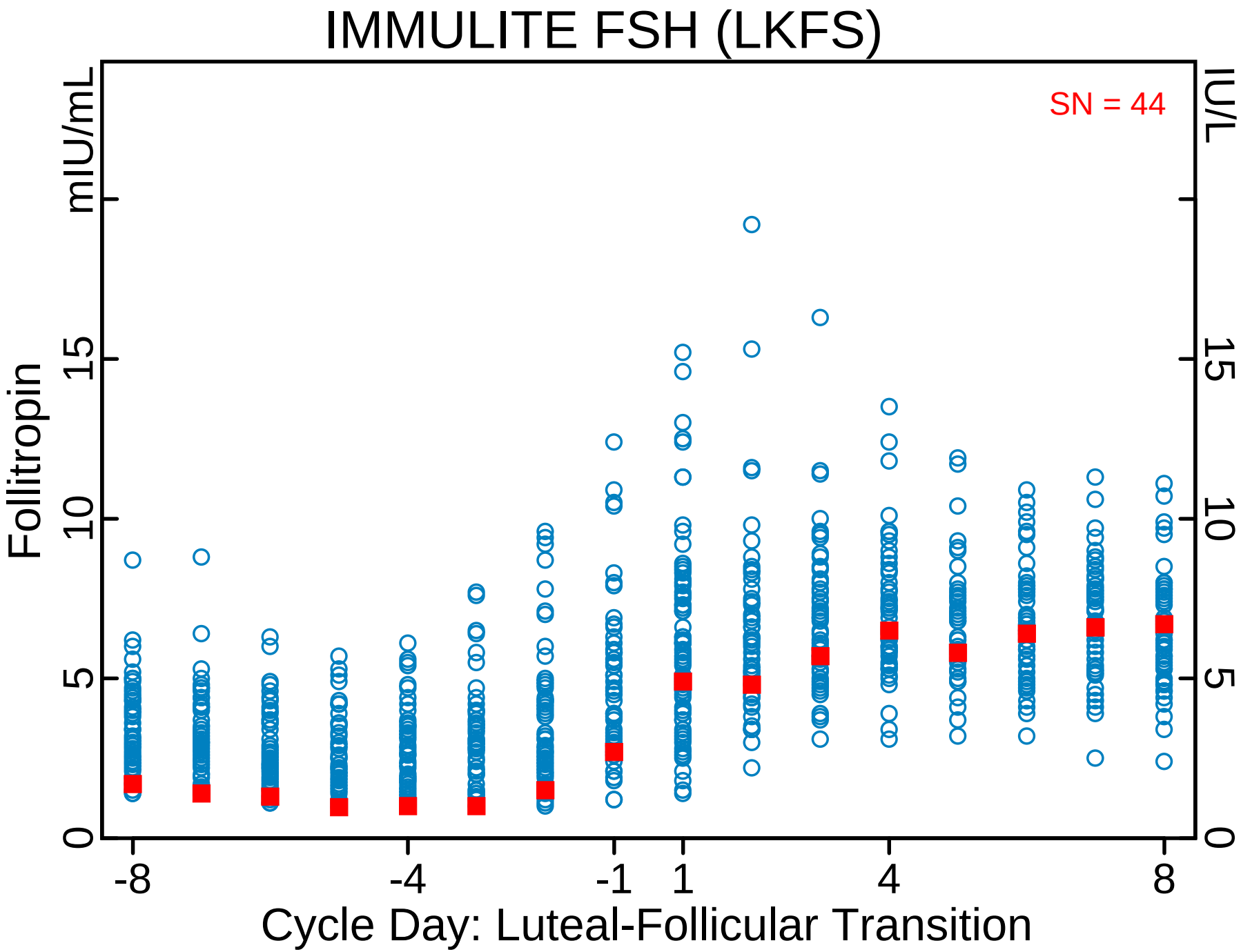

IMMULITE FSH (LKFS)
SN = 44
Follitropin
mIU/mL
IU/L
0
5
10
15
-8
-4
-1
1
4
8
Cycle Day: Luteal-Follicular Transition

Figure 4

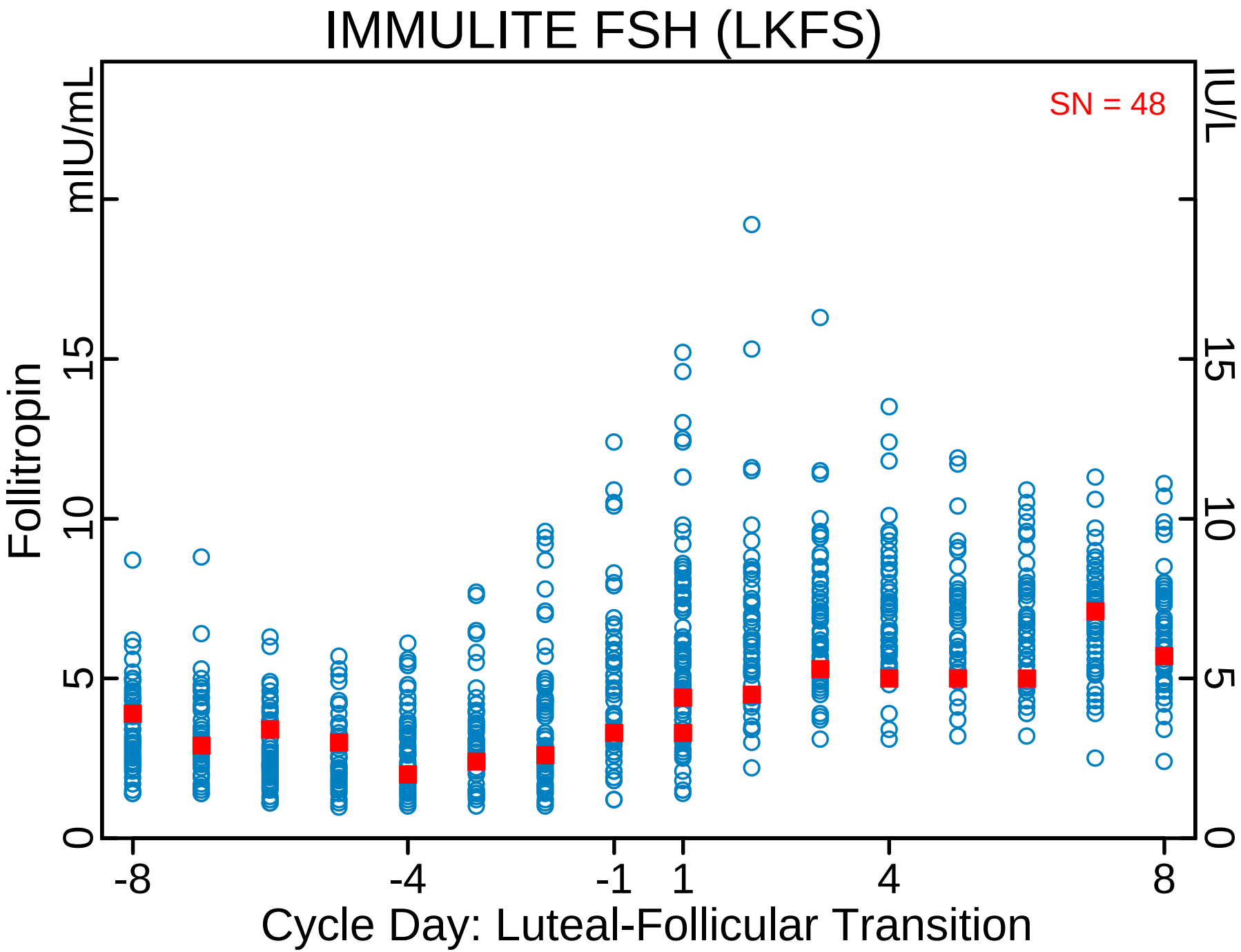

IMMULITE FSH (LKFS)
SN = 48
Follitropin
mIU/mL
IU/L
0
5
10
15
-8
-4
-1
1
4
8
Cycle Day: Luteal-Follicular Transition

Figure 5

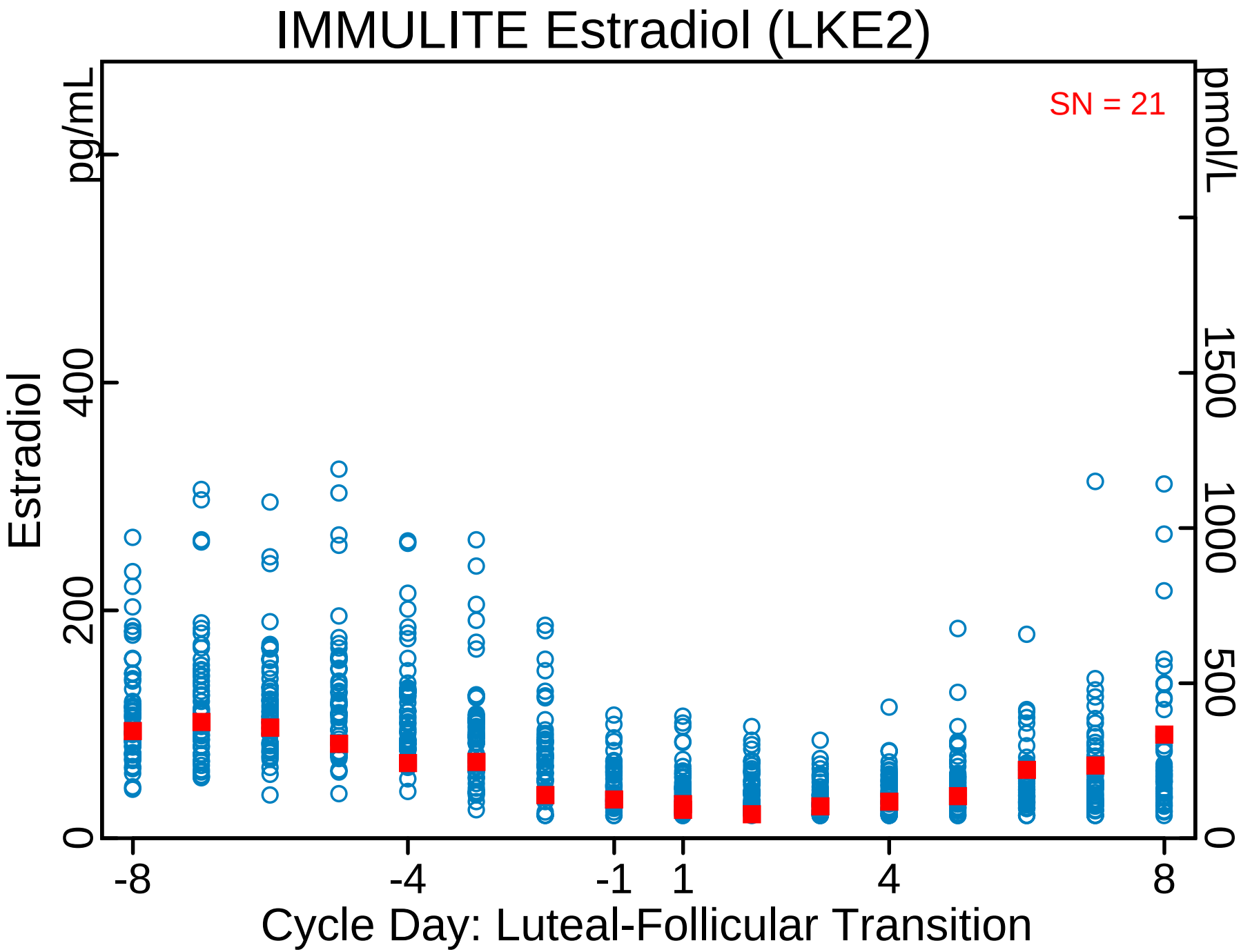

IMMULITE Estradiol (LKE2)
SN = 21
Estradiol
pg/mL
pmol/L
0
200
400
0
500
1000
1500
-8
-4
-1
1
4
8
Cycle Day: Luteal-Follicular Transition

Figure 6

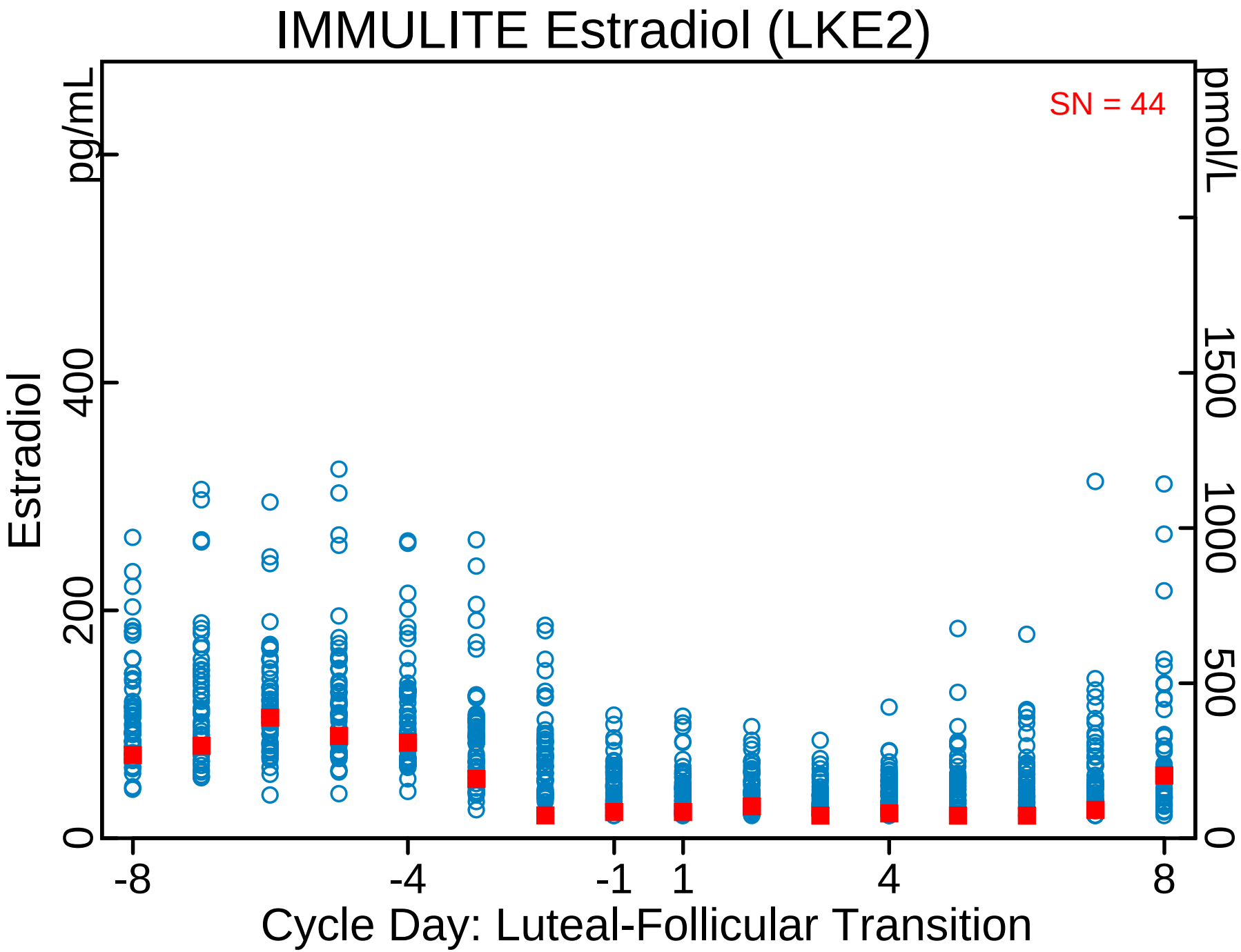
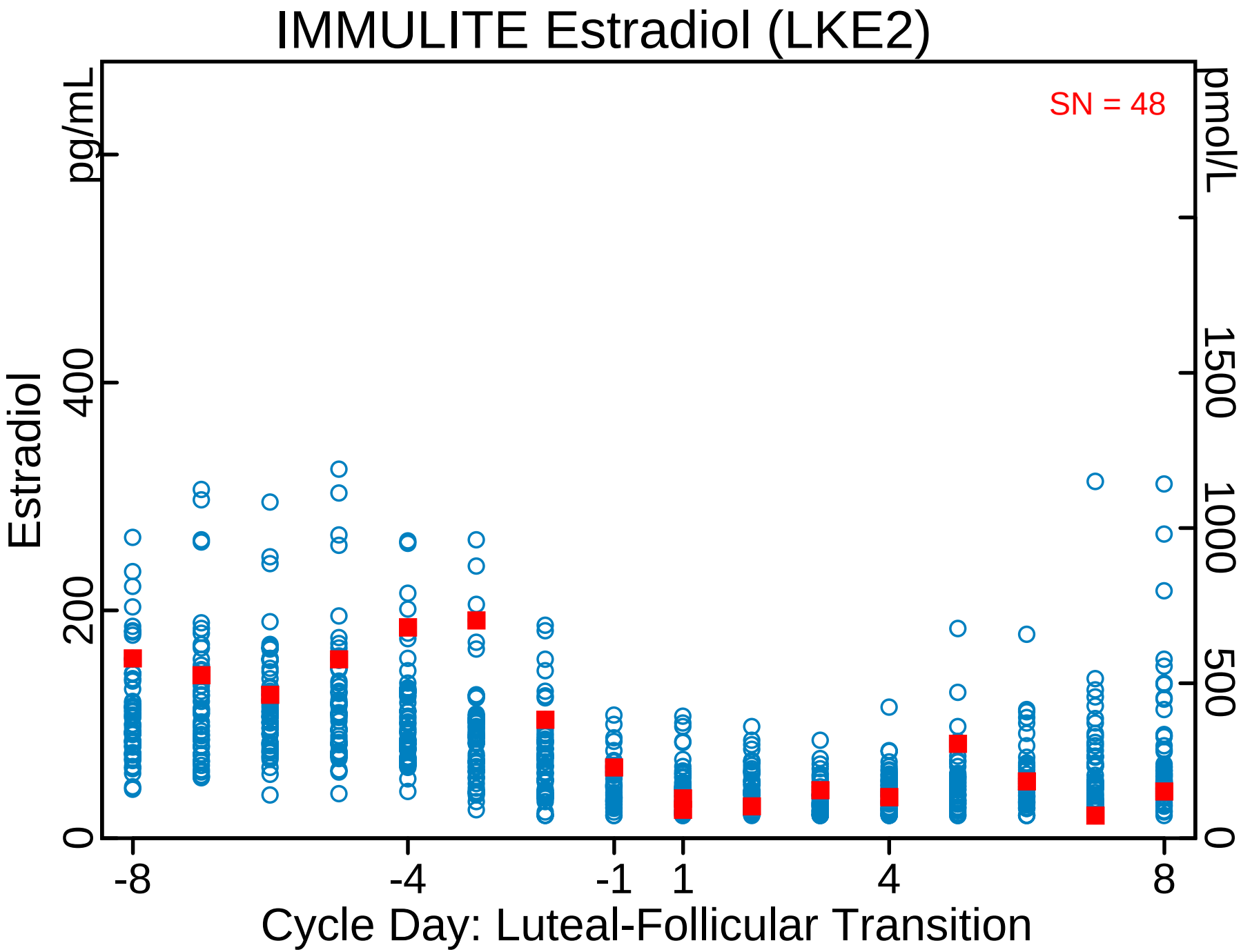

IMMULITE Estradiol (LKE2)
SN = 44
Estradiol
pg/mL
pmol/L
0
200
400
0
500
1000
1500
-8
-4
-1
1
4
8
Cycle Day: Luteal-Follicular Transition

Figure 7


IMMULITE Estradiol (LKE2)
SN = 48
Estradiol
pg/mL
pmol/L
0
200
400
0
500
1000
1500
-8
-4
-1
1
4
8
Cycle Day: Luteal-Follicular Transition