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Short Communication

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® analyzer

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Abstract

During a normal menstrual cycle, serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, and progesterone can vary widely between cycles for the same woman, as well as between different woman. Reliable reference values based on the local population are important for correct interpretation of laboratory results. The purpose of our study was to determine detailed reference values for these hormones throughout the menstrual cycle using the Abbott ARCHITECT system. From 20 volunteers (age 20–36 years) with normal cycles and no use of oral contraceptives, samples were taken every day during their cycle. Volunteers received three vaginal ultrasound examinations (days 10 and 13, and 1 or 2 days after ovulation) to measure follicular and corpus luteum development. Hormone levels were measured using the corresponding ARCHITECT assay and were synchronized to the LH peak. Median, and 5th and 95th percentile values were determined for each day of the cycle, as well as for early follicular (days −15 to −6), late follicular (days −5 to −1), LH peak (day 0), early luteal (+1 to +4), mid-luteal (days +5 to +9), and late luteal (days +10 to +14) phases of the cycle. Based on our data, we were able to establish detailed reference values for LH, FSH, estradiol, and progesterone, which should aid in the interpretation of results for these reproductive hormones in a variety of circumstances.
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Keywords: ARCHITECT analyzer; estradiol; follicle-stimulating hormone; luteinizing hormone; progesterone; reference values.

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Normal reproductive endocrine function involves a wide variety of hormones controlled by a number of intricate feedback mechanisms (1). This is particularly true during the menstrual cycle, in which serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, and progesterone follow a cyclical pattern closely coordinated by the hypothalmic-pituitary-gonadal axis (1, 2). Because LH, FSH, estradiol, and progesterone play such a central role in female reproductive function, measurement of the serum concentration of these hormones is important in the diagnosis, treatment, and monitoring of many different conditions (1). According to the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standardization, NCCLS), ''for a decision making process to occur, reference values are needed for all tests in the clinical laboratory'' (3). Assay manufacturers typically provide reference range data for their assays. Ideally, laboratories should either verify these ranges or determine their own reference ranges, based on the populations they serve. However, performing detailed reference range studies can be logistically complex, and are beyond the capabilities of many clinical laboratories. This is especially true for reproductive hormones, for which serum concentrations vary significantly throughout the menstrual cycle, as well as from woman to woman, and collection of multiple samples across many days is required to establish reference values with any degree of resolution (4). The purpose of our study was to obtain detailed reference values for LH, FSH, estradiol, and progesterone for each day and phase of the menstrual cycle using the Abbott ARCHITECT *i*2000$_{SR}$ analyzer.

From 20 apparently healthy female volunteers (age range 20–36 years) with normal menstrual cycles and no use of oral contraceptives or other medications, serum samples were collected on every day of the cycle. Each sample was then assayed for LH, FSH, estradiol, and progesterone using the Abbott ARCHITECT *i*2000$_{SR}$ system (Abbott Diagnostics, Abbott Park, IL, USA). Reagent list numbers for these assays were: LH, 6C25; FSH, 6C24; estradiol, 2K25; and progesterone, 6C26. The ARCHITECT *i*2000$_{SR}$ is a high-throughput immunoassay analyzer that utilizes paramagnetic microparticles and chemiluminescent detection technology (5). Assays were performed according to the manufacturer's instructions. Analytical performance characteristics (e.g., imprecision,

Table 1A Reference range data for LH and FSH by day in normal cycling women.

Days from LH peak	n	LH, IU/L				n	FSH, IU/L			
		Mean	Median	Percentile			Mean	Median	Percentile	
				5th	95th				5th	95th
−15	9	2.59	2.28	0.37	5.79	9	4.44	4.59	1.95	5.86
−14	12	3.73	3.39	1.35	6.29	12	5.99	6.45	2.79	8.15
−13	15	4.10	3.47	2.35	7.36	14	6.29	6.34	4.24	7.79
−12	17	3.85	3.59	1.52	6.61	17	6.49	6.26	4.62	7.93
−11	19	4.27	3.82	2.50	7.81	19	6.87	6.61	5.50	9.01
−10	20	4.44	3.82	2.24	8.73	20	7.07	6.88	5.40	10.25
−9	20	4.46	4.26	2.43	7.39	20	6.69	6.65	5.04	8.80
−8	20	5.11	4.96	2.76	7.52	20	6.54	6.46	4.97	9.53
−7	20	5.13	4.54	3.57	7.90	20	6.74	6.63	4.66	8.59
−6	20	4.81	4.47	3.26	7.03	20	5.93	5.65	4.41	8.10
−5	20	4.42	4.18	3.11	6.68	20	5.33	5.45	4.19	7.33
−4	20	4.63	4.58	3.29	6.89	20	4.76	4.59	3.83	6.33
−3	20	5.23	5.12	3.31	7.35	20	4.61	4.35	3.55	6.62
−2	20	7.03	7.02	4.58	9.64	20	4.33	4.32	2.95	5.71
−1	20	12.55	12.36	6.36	19.08	20	5.26	5.15	3.20	8.56
0	20	42.65	41.19	21.16	73.63	20	11.54	12.82	5.30	15.07
1	20	14.54	14.92	7.47	21.38	20	8.44	8.01	5.20	15.43
2	19	7.92	7.75	3.93	13.24	19	6.20	5.19	3.78	9.51
3	20	6.66	6.26	3.15	11.00	20	5.30	4.73	3.05	8.18
4	20	6.38	6.46	3.42	9.98	20	4.72	4.36	2.71	7.10
5	19	4.91	4.32	2.53	9.19	20	4.03	3.88	2.22	5.97
6	20	5.39	4.50	1.32	10.75	19	3.64	3.34	2.01	5.53
7	20	4.14	4.29	1.04	7.20	20	3.24	3.31	1.47	5.34
8	20	3.87	3.89	1.58	5.90	20	2.97	2.97	1.53	4.88
9	20	3.19	3.07	0.73	6.51	20	2.58	2.73	1.26	4.09
10	19	2.63	1.75	0.97	6.34	19	2.40	2.55	1.12	3.57
11	20	2.85	2.52	0.49	6.05	20	2.42	2.54	1.09	3.26
12	20	3.59	3.42	0.42	7.29	20	2.84	3.08	1.13	4.37
13	16	3.13	2.55	1.19	6.05	16	3.94	3.73	1.55	7.58
14	9	3.11	3.23	0.85	6.20	8	3.80	3.81	1.54	5.78

sensitivity, method comparison) for these assays have previously been reported (6–9). Per the manufacturer, the assays are standardized accordingly: LH, WHO 2nd IS 80/552; FSH, WHO 2nd IRP 78/549; estradiol, gravimetric, ID-GCMS verified; progesterone, gravimetric, USP grade progesterone. For analysis of serum hormone concentrations, variations in the cycle length were normalized by defining ''day 0'' based on the peak LH value for each woman. To ensure a normal cycle was taking place, volunteers received three vaginal ultrasound examinations (days 10 and 13, and 1 or 2 days post-ovulation) to measure follicular and corpus luteum development. Ovulation occurred in all cycles used for data analysis. Owing to the highly specialized nature of the sample set required to perform this study, obtaining a large number of specimens from many different women representing each day of their menstrual cycle was logistically challenging. In recommendations for the determination of reference values, the CLSI recognizes that collecting large numbers of certain sample types may be ''difficult, if not impossible''; in such cases, their guidance is to report ''percentiles appropriate to the number of values obtained'' (3). In the present study, reference values were defined by calculating the median, and 5th and 95th percentiles in sample sets of $n \geq 19$, the minimum number needed to define these percentiles (3). For sample sets with $n < 19$, 5th and 95th percentile values are reported for illustrative purposes only, and these data should be interpreted with caution. Data were analyzed using Microsoft Excel 2000 (Microsoft, Redmond, WA, USA). The study protocol was reviewed and approved by the internal institutional Review Board.

Data for normal cycling women by day of cycle, with median, and 5th and 95th percentiles, are shown in Table 1 and represented graphically in Figure 1. Reference values for different phases of the menstrual cycle are shown in Table 2. During the early follicular phase (days −15 to −6) median, and 5th and 95th percentile values were: LH, 3.96 (2.01 and 7.80) IU/L; FSH, 6.42 (3.91 and 8.76) IU/L; estradiol, 149.74 (77.99 and 266.08) pmol/L; and progesterone, 0.64 (0.32 and 1.91) nmol/L. For the late follicular phase (days − 5 to −1), median, and 5th and 95th percentile values were: LH, 5.68 (3.24 and 14.68) IU/L; FSH, 4.66 (2.97 and 6.99) IU/L; estradiol, 450.49 (195.43 and 1146.91) pmol/L; and progesterone, 0.64 (0.32 and 1.59) nmol/L. During the LH peak (defined as day 0), median, and 5th and 95th percentile values were: LH, 41.19 (21.16 and 73.63) IU/L; FSH, 12.82 (5.30 and 15.07) IU/L; estradiol, 671.06 (482.00 and 1425.39) pmol/L; and progesterone, 2.54 (1.24 and 4.13) nmol/L. For the early luteal phase (days +1 to +4), median, and 5th and 95th percentile values were: LH, 7.86 (3.65 and 18.03) IU/L; FSH, 5.62 (3.02 and 11.09) IU/L; estradiol,

Table 1B Reference range data for estradiol and progesterone by day in normal cycling women.

Days from LH peak	n	Estradiol, pmol/L				n	Progesterone, nmol/L			
		Mean	Median	Percentile			Mean	Median	Percentile	
				5th	95th				5th	95th
−15	9	118.42	139.46	57.55	194.44	9	1.66	1.27	0.32	3.75
−14	12	133.01	149.00	66.06	187.67	12	1.27	1.27	0.32	2.73
−13	14	133.17	137.63	75.20	189.34	14	1.02	0.95	0.52	1.59
−12	17	125.95	128.45	79.42	200.97	17	0.82	0.64	0.32	1.65
−11	19	134.65	130.29	82.98	196.75	19	0.74	0.64	0.32	1.30
−10	20	151.33	145.15	87.60	209.56	20	0.94	0.64	0.32	2.32
−9	20	159.48	154.14	93.40	224.68	20	0.72	0.64	0.32	1.29
−8	20	170.34	162.76	112.49	220.86	20	0.67	0.48	0.32	1.61
−7	20	195.90	196.16	124.67	267.23	20	0.73	0.64	0.32	1.30
−6	20	228.20	215.06	157.66	313.36	20	0.51	0.32	0.32	1.00
−5	20	269.07	262.22	190.47	346.76	20	0.57	0.48	0.32	1.02
−4	20	343.68	363.15	186.47	490.81	19	0.59	0.64	0.32	0.95
−3	20	477.27	485.72	241.52	803.56	20	0.51	0.32	0.32	0.97
−2	20	661.19	651.06	337.35	1153.76	20	0.59	0.64	0.32	1.27
−1	20	914.84	939.34	503.85	1517.67	20	1.02	0.95	0.62	1.62
0	20	780.76	671.06	482.00	1425.39	20	2.66	2.54	1.24	4.13
1	20	320.59	312.87	159.87	514.35	20	5.02	4.93	2.21	8.95
2	19	261.32	260.57	154.62	398.38	19	12.13	12.72	4.74	18.67
3	20	338.94	322.96	208.64	495.82	20	20.84	20.51	11.34	29.05
4	20	454.07	403.52	269.80	665.99	20	29.75	30.85	11.38	45.51
5	20	499.49	450.68	292.57	703.85	20	36.08	34.03	23.72	46.46
6	19	497.07	486.64	267.07	719.72	19	36.52	35.30	22.36	48.18
7	20	531.14	551.78	291.71	695.47	20	40.32	42.45	25.98	54.17
8	20	504.39	491.23	281.91	718.20	20	39.65	37.05	25.06	60.39
9	20	499.18	503.52	279.18	764.41	20	33.76	32.44	18.25	46.65
10	19	526.68	495.45	294.48	806.70	19	34.11	32.75	17.74	55.94
11	20	350.65	340.41	210.22	640.16	20	18.24	14.47	6.74	38.21
12	20	322.24	314.52	174.82	546.15	20	16.90	13.04	3.67	41.58
13	16	229.70	170.10	101.93	497.74	16	10.73	5.25	2.15	33.15
14	8	249.28	151.20	93.97	693.54	9	9.50	4.13	1.91	33.39

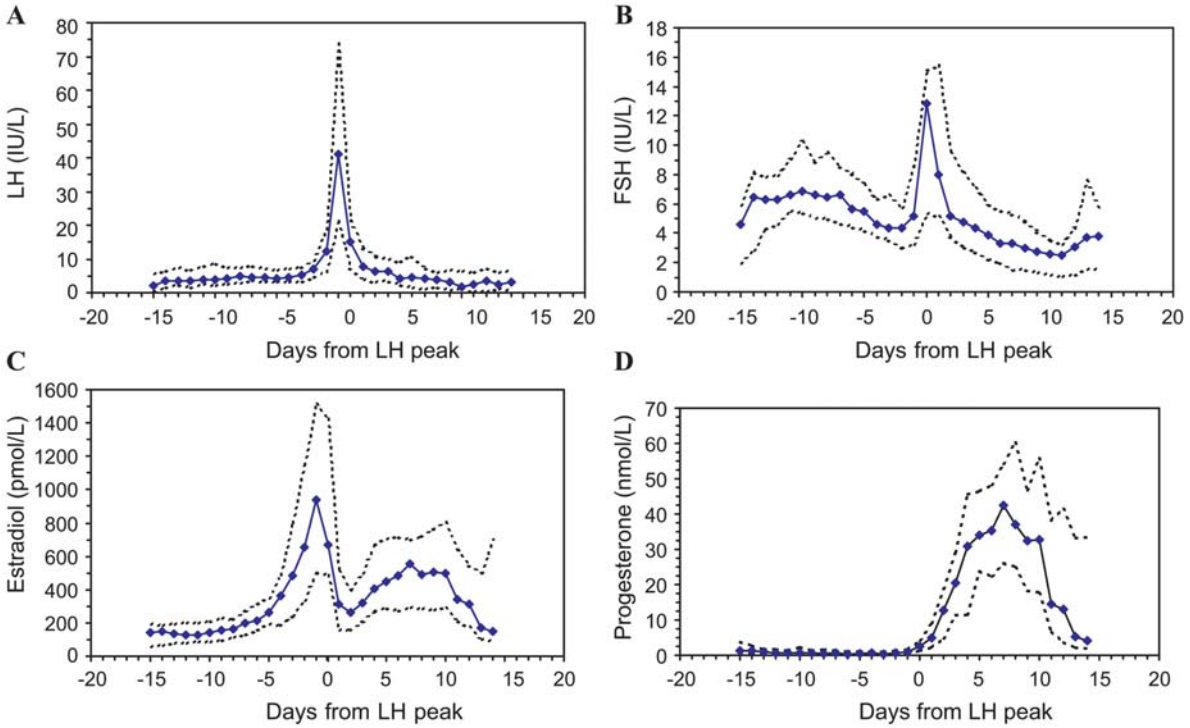

Figure 1 Hormone values in daily serum samples across the menstrual cycle for normal women: (A) luteinizing hormone; (B) follicle-stimulating hormone; (C) estradiol; and (D) progesterone. Solid lines represent median values; dotted lines represent 5th and 95th percentiles.

Table 2 Reference values during different phases of the menstrual cycle.

Phase of cycle	Days from LH peak	LH, IU/L					FSH, IU/L				
		Median	Min	Max	Percentile		Median	Min	Max	Percentile	
					5th	95th				5th	95th
Early follicular	−15 to −6	3.96	0.19	10.17	2.01	7.80	6.42	1.29	10.81	3.91	8.76
Late follicular	−5 to −1	5.68	2.80	22.62	3.24	14.68	4.66	2.14	9.79	2.97	6.99
LH peak	0	41.19	20.48	74.83	21.16	73.63	12.82	4.65	16.28	5.30	15.07
Early luteal	+1 to +4	7.86	2.03	26.17	3.65	18.03	5.62	2.22	17.04	3.02	11.09
Mid-luteal	+5 to +9	3.88	0.68	12.12	1.05	9.73	3.14	0.92	7.34	1.48	5.31
Late luteal	+10 to +14	2.75	0.21	8.02	0.51	6.98	2.85	0.89	7.67	1.14	5.64
		Estradiol, pmol/L					Progesterone, nmol/L				
		Median	Min	Max	Percentile		Median	Min	Max	Percentile	
					5th	95th				5th	95th
Early follicular	−15 to −6	149.74	49.91	358.93	77.99	266.08	0.64	0.32	4.77	0.32	1.91
Late follicular	−5 to −1	450.49	152.31	1568.93	195.43	1146.91	0.64	0.32	2.23	0.32	1.59
LH peak	0	671.06	372.87	1884.91	482.00	1425.39	2.54	0.64	4.13	1.24	4.13
Early luteal	+1 to +4	313.42	101.29	1068.34	178.14	566.43	13.67	1.91	46.11	3.15	39.65
Mid-luteal	+5 to +9	495.82	252.13	1163.02	275.95	761.67	36.25	14.63	71.87	21.21	54.28
Late luteal	+10 to +14	327.36	51.75	962.27	100.52	787.14	13.99	0.95	69.96	1.96	49.18

313.42 (178.14 and 566.43) pmol/L; and progesterone, 13.67 (3.15 and 39.65) nmol/L. During the mid-luteal phase (days +5 to +9), median, and 5th and 95th percentile values were: LH, 3.88 (1.05 and 9.73) IU/L; FSH, 3.14 (1.48 and 5.31) IU/L; estradiol, 495.82 (275.95 and 761.67) pmol/L; and progesterone, 36.25 (21.21 and 54.28) nmol/L. For the late-luteal phase (days +10 to +14), median, and 5th and 95th percentile values were: LH, 2.75 (0.51 and 6.98) IU/L; FSH, 2.85 (1.14 and 5.64) IU/L; estradiol, 327.36 (100.52 and 787.14) pmol/L; and progesterone, 13.99 (1.96 and 49.18) nmol/L.

In general, our data are in agreement with those provided by the assay manufacturer. However, direct comparison is difficult, as our data were analyzed to a higher degree of resolution, allowing establishment of reference values in greater detail throughout the menstrual cycle. It should be noted that gonadotropin hormones are known to be highly heterogeneous (10, 11). This heterogeneity is presumably one of the factors that leads to assay differences reported in the literature (12). These differences can, in turn, affect interpretation of results from different assay manufacturers or laboratories. For example, FSH concentration on day 3 of the menstrual cycle (along with estradiol and inhibin B) is commonly used by physicians to evaluate ovarian reserve. Taieb and colleagues, in their study of six different immunoassays for FSH, noted statistically significant differences for day-3 FSH values, depending on which immunoassay was used. Their data prompted them to recommend ''that it is advisable to refer patients to selected laboratories using analytical methods for which they have defined reference values …'' (12). Similarly, measurement of serum estradiol is important in evaluating a variety of conditions, including abnormal menstrual cycles. However, measurement of steroid hormones (such as estradiol) by immunoassay is notoriously difficult, and differences in assay performance significant enough to affect clinical utility have been reported (6). For these reasons, it is especially important for physicians and laboratories to be aware of the potential sources of differences that exist between immunoassay methodologies, and confirm that the reference values they use for reproductive hormones are valid for the populations they serve. Based on our study, we were able to develop detailed reference ranges for LH, FSH, estradiol, and progesterone on the Abbott ARCHITECT analyzer during the normal menstrual cycle. These data should aid laboratories and physicians in the interpretation of results for these reproductive hormones in a wide variety of clinical settings.

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