Journal of Assisted Reproduction and Genetics, Vol. 19, No. 8, August 2002 (© 2002)

Clinical Assisted Reproduction

Intraindividual Hormonal Variability in Ultrasonographically Timed Successive Ovulatory Menstrual Cycles is Detected Only in the Luteal Phase in Infertility Patients

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Submitted January 9, 2002; accepted March 8, 2002

***Objective*:** To assess intraindividual variation of follicle stimulating hormone, luteinising hormone, estradiol, progesterone, inhibin A, and inhibin B in three successive ovulatory cycles correlated with transvaginal ultrasound monitored morphological changes in the ovary.
***Methods*:** Serial transvaginal color and pulsed Doppler ultrasound and serum hormone analysis were performed during midfollicular, periovulatory, and midluteal phase for three consecutive cycles in 19 patients with normal menstrual cycles.
***Results*:** Luteinising hormone and progesterone showed significant differences in the midluteal phase between the 1st and 2nd cycle (luteinising hormone $p = 0.007$ and progesterone $p = 0.02$). Progesterone showed a similar significant change ($p = 0.013$) between the 2nd and 3rd cycle. No significant differences were seen in the midfollicular or periovulatory phases or between the 1st and 3rd cycle.
***Conclusions*:** Luteal phase progesterone and luteinising hormone concentrations showed individual variation in successive cycles suggesting early or late corpus luteolysis. Follicular and periovulatory hormone levels were similar in subsequent ovulatory cycles.

KEY WORDS: Intraindividual variation; ovulatory cycles; progesterone; serum gonadotrophins.

INTRODUCTION

The relationship between reproductive hormones, follicle stimulating hormone (FSH), luteinising hormone (LH), estradiol, and progesterone, in a normal menstrual cycle has been well documented (1–8). In two successive cycles the reproductive hormones show less variation within an individual than between individuals in normal menstrual cycle (9). The development of assays for dimeric inhibin A and inhibin B has similarly enabled studies of the role of these hormones in the regulation of the hypothalamo–pituitary–ovarian axis (10–14).

Rapid development of techniques for transvaginal ultrasonography with color and pulsed Doppler ultrasound allows the assessment of vascular changes in the ovary and the uterus during the menstrual cycle. Preovulatory follicular growth and corpus luteum formation can be assessed by ultrasound examination and the findings correlated with the biochemical assessment of estradiol and progesterone levels (15–17). A limitation in all these studies is the lack of independent correlation with follicular growth and development. Most of the above studies have been based on the day of urinary LH surge or the day of the menstrual cycle. There is no data on intraindividual variation of FSH, LH, estradiol, progesterone, dimeric inhibin A, and inhibin B in successive cycles

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1058-0468/02/0800-0363/0 © 2002 Plenum Publishing Corporation

by modern nonisotopic immunoassay methods correlated to follicular development. Serum hormone concentrations in successive normal menstrual cycles should be similar.

We assessed FSH, LH, estradiol, progesterone, inhibin A, and inhibin B at different phases of the menstrual cycle and correlated these with morphological changes observed in the ovary by transvaginal color and pulsed Doppler measurements.

MATERIAL AND METHODS

Subjects

This was a prospective observational study involving 19 patients with regular menstrual cycles (ranging 26–32 days) recruited from fertility clinics at St George's Hospital. The study was approved by the local Ethics Committee and was conducted between September 1999 and May 2000. Patients selected for the study had not been on any medication for the previous 6 months and received no medication during the study. Patients with ovarian endometriosis, uterine fibroids, hydrosalpinges, and ovarian cysts were excluded from the study. Of the 19 women in the study, 16 were followed for three consecutive cycles and 3 for two cycles. Patient demographics are summarized in Table I.

TECHNIQUE

Doppler Ultrasonography

Serial transvaginal ultrasound was performed during the midfollicular phase (Phase-I, Day 5–10), periovulatory phase (Phase-II, Day 11–14), and midluteal phase (Phase-III, Day 18–22). Six of the 19 patients were scanned during midfollicular phase and either periovulatory or midluteal phase.

All scans were performed with a Sequoia Acuson using a 7.5 MHz transvaginal probe for B-mode, color and pulsed Doppler examinations (Acuson Corp., Mountain View, CA). The spatial peak temporal average intensity for B-mode and Doppler examinations was <50 mW/cm^2, well within the safety limits recommended by the Bioeffects Committee of the American Institute of Ultrasound in Medicine. Each ultrasound was performed by one of the two observers.

In all cases, Doppler ultrasound confirmed the presence of a developing follicle in the midfollicular phase, a follicle of at least 18 mm in the periovulatory phase, and a corpus luteum in the midluteal phase. These findings were correlated with the day of the cycle as calculated from the first day of menstruation.

Table I. The Characteristics of the Women Selected for the Study

	Mean ± sem	Range
Age	36.4	28–44
Body mass index	21.1 ± 3.0	19.8–33.3
FSH concentration (IU/L – menstrual)	7.15 ± 2.64	5.0–11.4
Cycle length (days)	28	26–32

Endocrine Assessment

Blood samples for measurement of LH, FSH, estradiol, progesterone, inhibin A, and inhibin B were drawn from an antecubital vein and centrifuged. Serum was stored at -20°C until analysis was performed.

LH, FSH, estradiol, and progesterone were determined using an AutoDELFIA™ (Perkin Elmer Life Sciences, MA). The LH assay (detection limit 0.05 U/L, upper measuring limit 250 U/L, c.v. 3.3% at 1.54 U/L, 3.0% at 17.96 U/L, 3.5% at 64.06 U/L) uses immobilized and europium-labeled mouse monoclonal antibodies against LH in an immunometric "sandwich" assay. The FSH assay (detection limit 0.05 U/L, upper measuring limit 256 U/L, c.v. 2.6% at 7.64 U/L, 3.3% at 13.26 U/L, 4.7% at 39.42 U/L) uses the same principle as the LH assay. The estradiol assay (detection limit 30 pmol/L, upper measuring limit 15000 pmol/L, c.v. 4.2% at 258.9 pmol/L, 3.0% at 2198.7 pmol/L, 3.6% at 2932.7 pmol/L) and progesterone assay (detection limit 1 nmol/L, upper measuring limit 120 nmol/L, c.v. 10.3% at 2.9 nmol/L, 7.4% at 32.3 nmol/L, 7.4% at 99.4 nmol/L) are competitive immunoassays. Both the analyte in the serum and a europium-labeled analyte compete for immobilized rabbit polyclonal antibodies. In all assays, the signal is detected by time-resolved fluorescence.

Inhibin A and inhibin B were determined by an ELISA assay (Oxford Bio-Innovation Ltd, Oxford, U.K.). The inhibin A assay detection limit is 3.9 ng/L with interassay CV $< 10\%$ as quoted by the manufacturer. The quoted detection limit for the inhibin B assay is 15 ng/L with interassay CV $< 7\%$.

Statistics

The data was analyzed using the Statistical Package for the Social Sciences (Release 10.0 SPSS Inc.,

Chicago, IL). Nonparametric Wilcoxon signed ranks test was used to compare LH, FSH, estradiol, progesterone, inhibin A, and inhibin B during midfollicular, periovulatory, and midluteal phase between the 1st and 2nd cycle, 2nd and 3rd cycle, and 1st and 3rd cycle.

RESULTS

In all cases, the development of a dominant follicle and the presence of corpus luteum was confirmed by Doppler ultrasound. The median values and interquartile range for each hormone are given for the three stages of the menstrual cycle for the three successive cycles in Tables II and III. There were no statistically significant differences in hormone concentrations between the three cycles in the midfollicular and periovulatory phases. The LH and progesterone showed significant differences between midluteal phase in the 1st and 2nd cycle (LH, $p = 0.007$ and progesterone $p = 0.02$). Only progesterone showed a similar significant change in the midluteal phase between 2nd and 3rd cycle (progesterone $p = 0.013$). LH showed a difference; however this was not significantly different. No significant differences were found between the 1st and 3rd cycle for any of the hormones measured.

DISCUSSION

The LH and progesterone showed significant differences between midluteal phase in the 1st and 2nd cycle. Progesterone showed similar significant changes in the midluteal phase between 2nd and 3rd cycle.

Table II. FSH, LH, Inhibin A, Inhibin B, Progesterone, and Estradiol Comparing Midfollicular, Periovulatory, and Midluteal Phase in the 1st and 2nd Cycle Using the Wilcoxon Signed Ranks Test

Hormone parameter: Phase of cycle	Cycle	*N*	Median (interquartile range)	Significance (2-tailed)
FSH: midfollicular	1st	18	5.02 (4.32–6.07)	0.99
	2nd	18	5.39 (4.39–7.18)	
LH: midfollicular	1st	18	6.25 (5.80–6.07)	0.1
	2nd	18	6.14 (4.37–6.53)	
Inhibin A: midfollicular	1st	17	0.0 (0.0–5.84)	0.14
	2nd	17	0.0 (0.0–7.85)	
Inhibin B: midfollicular	1st	17	106.7 (88.74–125.66)	0.94
	2nd	17	102.7 (82.79–118.87)	
Estradiol: midfollicular	1st	18	266.5 (210.5–406.87)	0.59
	2nd	18	265.25 (201.88–412.62)	
Progesterone: midfollicular	1st	18	1.4 (1.0–2.12)	0.38
	2nd	18	1.1 (1.0–1.61)	
LH: periovulatory	1st	14	12.7 (5.6–22.36)	0.98
	2nd	14	9.61 (8.29–14.07)	
FSH: periovulatory	1st	14	5.45 (4.12–9.63)	0.95
	2nd	14	6.45 (3.96–7.1)	
Inhibin A: periovulatory	1st	13	18.3 (11.24–30.02)	0.53
	2nd	13	18.28 (9.38–30.77)	
Inhibin B: periovulatory	1st	13	69.25 (51.86–127.09)	0.92
	2nd	13	92.68 (67.48–108.11)	
Estradiol: periovulatory	1st	14	452.25 (373.25–649.67)	0.22
	2nd	14	457.25 (265.0–913.37)	
Progesterone: periovulatory	1st	14	4.05 (1.99–13.09)	0.7
	2nd	14	2.61 (1.40–30.77)	
LH: midluteal	1st	16	5.51 (2.71–8.14)	0.007*
	2nd	16	7.76 (4.91–9.17)	
FSH: midluteal	1st	16	3.04 (2.28–3.83)	0.08
	2nd	16	3.05 (2.74–5.15)	
Inhibin A: midluteal	1st	15	17.7 (14.16–29.46)	0.73
	2nd	15	23.3 (11.23–31.45)	
Inhibin B: midluteal	1st	15	0.0 (0.0–26.09)	0.24
	2nd	15	0.0 (0.0–44.51)	
Estradiol: midluteal	1st	16	465.25 (320.75–698.37)	0.72
	2nd	16	476.75 (384.13–828.62)	
Progesterone: midluteal	1st	16	47.53 (37.50–52.09)	0.02*
	2nd	16	36.28 (22.94–46.16)	

* $p < 0.05$.

Table III. FSH, LH, Inhibin A, Inhibin B, Progesterone, and Estradiol Comparing Midfollicular, Periovulatory, and Midluteal Phase in the 2nd and 3rd Cycle Using Wilcoxon Signed Ranks Test

Hormone parameter: Phase of Cycle	Cycle	*N*	Median (interquartile range)	Significance (2-tailed test)
FSH: midfollicular	2nd	15	5.39 (4.39–6.53)	0.69
	3rd	15	4.93 (4.19–5.91)	
LH: midfollicular	2nd	15	6.14 (4.37–7.18)	0.69
	3rd	15	4.97 (4.31–9.05)	
Inhibin A: midfollicular	2nd	16	0.0 (0.0–7.85)	0.59
	3rd	16	0.0 (0.0–8.44)	
Inhibin B: midfollicular	2nd	16	102.70 (82.79–118.87)	0.32
	3rd	16	115.53 (68.21–144.79)	
Estradiol: midfollicular	2nd	15	265.25 (201.88–412.62)	0.43
	3rd	15	290.75 (188.38–704.50)	
Progesterone: midfollicular	2nd	15	1.10 (1.0–1.61)	0.48
	3rd	15	1.30 (1.0–1.45)	
LH: periovulatory	2nd	15	9.61 (8.29–14.07)	0.61
	3rd	15	7.70 (5.27–17.44)	
FSH: periovulatory	2nd	15	6.45 (3.96–7.10)	0.23
	3rd	15	4.48 (3.40–7.03)	
Inhibin A: periovulatory	2nd	16	18.28 (9.38–30.77)	0.35
	3rd	16	13.90 (11.74–18.39)	
Inhibin B: periovulatory	2nd	16	92.68 (67.48–108.11)	0.61
	3rd	16	91.08 (60.26–146.26)	
Estradiol: periovulatory	2nd	15	457.25 (265.0–913.37)	0.86
	3rd	15	636.25 (304.38–803.37)	
Progesterone: periovulatory	2nd	15	2.61 (1.4–10.39)	0.51
	3rd	15	2.63 (1.36–9.77)	
LH: midluteal	2nd	13	7.76 (4.91–9.17)	0.17
	3rd	13	4.74 (3.97–9.69)	
FSH: midluteal	2nd	13	3.05 (2.74–5.15)	0.48
	3rd	13	2.99 (2.39–3.93)	
Inhibin A: midluteal	2nd	12	23.30 (11.23–31.45)	0.35
	3rd	12	21.93 (18.76–42.53)	
Inhibin B: midluteal	2nd	12	0.00 (0.00–44.51)	0.23
	3rd	12	0.00 (0.00–20.50)	
Estradiol: midluteal	2nd	13	476.75 (384.13–828.62)	0.46
	3rd	13	508.75 (297.88–709.50)	
Progesterone: midluteal	2nd	13	36.28 (22.94–46.16)	0.01*
	3rd	13	44.38 (29.88–55.55)	

* $p < 0.05$.

LH showed a difference; however this was not significantly different. Regression of the corpus luteum may vary between cycles and this may explain the differences in the LH and progesterone levels. Luteal phase defects have shown that lower LH and progesterone concentrations are associated with early luteolysis (5,8,18).

In ovulatory cycles, FSH, estradiol, inhibin A, and inhibin B concentrations during the midfollicular, periovulatory, and midluteal phase of the menstrual cycle are similar for three successive cycles. All the reproductive hormones showed less variation within an individual than between individuals in two cycles (9). These findings question the rationale of testing the hormonal profile in infertile women with normal menstrual cycles prior to treatment. This study also shows that repeating test for FSH, estradiol, inhibin A, and inhibin B concentrations in successive cycles is not beneficial. Thus reliance on the menstrual history provides more information than a thorough hormonal evaluation of a single cycle prior to treatment for infertility.

Most previous studies of serial gonadotrophins, inhibin A, and inhibin B in the different phases of the menstrual cycle have been based on the first day of the menstrual cycle or the day of LH surge. This is the first study looking at intraindividual changes in the peripheral hormones for successive cycles correlating with morphological changes observed in the ovary and uterus with high resolution transvaginal Doppler ultrasound.

In conclusion the luteal phase LH and progesterone levels show individual variation in successive cycles suggesting early or late corpus luteal regression. The follicular and periovulatory hormones' levels do not show significant intraindividual variation in

subsequent ovulatory cycles. A more detailed study of the luteal phase in women with normal menstrual cycles identifying luteofollicular transition by transvaginal ultrasound and peripheral hormones is required.

ACKNOWLEDGMENT

We are grateful to Perkin Elmer for the supply of reagents for analysis of LH, FSH, progesterone, and estradiol.

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