Changing Estrogen and Progesterone Receptor Patterns in Breast Carcinoma during the Menstrual Cycle and Menopause

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BACKGROUND. Estrogen receptor (ER) and progesterone receptor (PgR) status at the time of breast carcinoma surgery is used as a marker of both prognosis and hormone dependency to guide adjuvant therapy. The authors studied the influence of hormonal milieu at the time of surgery on ER and PgR levels.

METHODS. A population of 2020 patients with breast carcinoma, including 575 premenopausal women, was analyzed. ER and PgR levels were determined by radioligand binding assays (cutoff values, 10 fmol/mg). Serum estradiol (E2), progesterone (Pg), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels obtained on the day of surgery were used to define the menstrual cycle phase in premenopause.

RESULTS. In premenopause, there was a higher proportion of ER positive (ER^+) tumors in the follicular phase (62%, n = 316) than in the ovulatory phase (51%, n = 59) and the luteal phase (53%, n = 200, $P = 0.03$). The mean ER level was also higher in the follicular phase (30 fmol/mg) than in the ovulatory phase (20 fmol/mg) and the luteal phase (25 fmol/mg, $P < 0.001$). The percentage of PgR positive (PgR^+) tumors tended to be higher in the ovulatory phase (85%) than in the follicular (78%) and luteal (72%) phases ($P = 0.11$). The mean PgR was also higher in the ovulatory phase (177 fmol/mg) than in the follicular and luteal phases (134 and 92 fmol/mg, respectively; $P < 0.001$). The percentage of ER^+ tumors was higher among menopausal women than among premenopausal women (67% vs. 59%, respectively; $P < 0.001$). Conversely, the percentage of PgR^+ tumors was lower among menopausal women than among premenopausal women (65% vs. 78%, respectively; $P < 0.001$). In premenopause, there was a weak negative correlation between ER and E2 levels. No correlations were found between levels of ER and Pg and levels of FSH and LH or among levels of PgR and E2, Pg, and FSH and LH in premenopausal and menopausal women.

CONCLUSIONS. Changes in ER and PgR levels in breast carcinoma during the menstrual cycle and menopause suggest that interpretations of hormone dependency on the basis of steroid receptor values should take into account hormonal status at the time of surgery. ***Cancer* 1998;83:698–705.**
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KEYWORDS: breast cancer, estrogen receptors, progesterone receptors, menstrual cycle, menopause.

Estrogen receptor (ER) and progesterone receptor (PgR) status can predict both the response to endocrine therapy[1,2] and the likelihood of recurrence.[3] Determinations of ER and PgR status at the time of surgery, thus, are widely used in clinical practice to guide adjuvant therapy.

There is currently considerable interest in the effect of surgical

timing according to the phase of the menstrual cycle in terms of prognosis[4–7] and tumor biology.[8–10] However, little is known about possible variations in steroid receptors relative to the phase of menstrual cycle. Several studies have shown that ER and PgR contents in normal human breast epithelium underwent regulation throughout the menstrual cycle. As observed in the endometrium,[11] ER levels have been found consistently to be higher in the follicular phase than in the luteal phase in normal breast epithelium.[12–15] PgR, which is decreased during the luteal phase in endometrium, appears to be unchanged in normal breast epithelium.[12,13] Only a few studies have been conducted on steroid receptor variations during the menstrual cycle in breast carcinoma,[16–21] but they did not reveal any overall conclusive differences between phases. However, the results are limited by the relatively small number of cases, or by the absence of hormonal measurements at the time of surgery.

Possible variations in steroid receptors according to the menstrual phase raise important questions, because the timing of breast cancer surgery could influence ER or PgR status and, thus, interpretations of hormone dependency. We studied the relation between steroid receptors and hormonal status according to levels of serum estradiol (E2), progesterone (Pg), and gonadotrophin in a large population of breast carcinoma patients.

PATIENTS AND METHODS

Patients

A total of 2359 patients with primary breast cancers were included in a monocentric prospective study from 1988 to 1994 (Montpellier Cancer Center, France). Exclusion criteria were preoperative radiotherapy, neoadjuvant chemotherapy or tamoxifen therapy, use of oral contraceptives less than 1 month before surgery, hormone replacement therapy, and pregnancy. Three hundred thirty-nine patients were excluded, and 2020 patients qualified for the study. According to French legislation at the time of the study, oral consent was obtained for all patients.

Menopausal status was defined clinically as follows: premenopausal, patients with regular menstrual cycle (n = 575); postmenopausal, patients with the last regular menses occurring more than 2 years earlier (n = 1185); perimenopausal, patients with the last regular menses occurring less than 2 years earlier (n = 260). The hormonal phase of the menstrual cycle in premenopausal patients was determined according to levels of circulating hormones on the day of surgery, including E2 and Pg, follicle-stimulating hormone (FSH), and lutenizing hormone (LH). The ovulatory phase was defined on the basis of high LH (>10 IU/L) and estradiol (>100 pg/mL) values. Women with high progesterone values (exceeding 2.5 ng/mL) were considered to be in the luteal phase of the cycle. The remaining premenopausal women were classified in the follicular phase. The precise date of the last menstrual period was available in 341 of the 575 premenopausal patients.

Cytosolic Assays

ER and PgR were assayed by using the dextran-coated charcoal (DCC) method[22] with ^{3}H-estradiol and ^{3}H-progesterone (specific activity, 87 Ci/mmol; NEN, Paris, France). Protein concentration was assayed by using the Lowry technique.[23] Quality control included both internal controls and European Organization for Research and Treatment of Cancer (EORTC) standards. ER or PgR levels of 10 fmoL/mg cytosolic protein or more were considered positive.

Circulating Hormones

Circulating hormones were assessed in the routine laboratory of our Cancer Center from a blood sample obtained on the day of surgery. All patients in the study were analyzed. E2 and Pg were measured by using two commercially available radioimmunoassay kits (ESTR-US-CT and PROG-CTRIA; CIS, Gif-sur-Yvette, France). LH and FSH were assessed by using LH Coatria and FSH Coatria kits (Biomérieux, Crapone, France). Sera analyses were performed weekly and were blind to clinical information.

Statistical Analysis

The Mann–Whitney test or the Kruskall–Wallis test was used to compared nonparametric quantitative parameters. Chi-square or Fisher tests were used to compare frequencies of receptor positivity. Correlations between quantitative parameters were analyzed with Spearman's test. The significance level of *P* value was set at 0.05.

RESULTS

Characteristics of the Population

The study population included 575 patients in cycle (316 patients in the follicular phase, 59 patients in the ovulatory phase, 200 patients in the luteal phase) and 1445 patients in menopause (260 patients in perimenopause, 1185 patients in postmenopause). Mean levels of circulating hormones according to the hormonal status are given in Table 1. The percentage of ER positive (ER^{+}) tumors in the overall population was 64.1%, and the percentage of PgR positive (PgR^{+}) tumors was 68.7%.

TABLE 1
Hormonal Characteristics of the Population[a]

Hormonal status	E2 (pg/mL)	Pg (ng/mL)	FSH (IU/L)	LH (IU/L)
Follicular (n = 316)	48 (12–393)	1.3 (0.5–2.4)	6 (1–15)	5 (2–39)
Ovulatory (n = 59)	195 (107–561)	1 (0.1–2.3)	8.3 (3–41)	20 (12–140)
Luteal (n = 200)	58 (12–271)	5.3 (2.5–33)	3.5 (1–35)	3.3 (2–18)
Perimenopause (n = 260)	18 (12–65)	0.7 (0.5–2.5)	38 (10–139)	20 (3–62)
Postmenopause (n = 1185)	13 (12–16)	0.7 (0.7–1.4)	53 (10–184)	23 (3–102)

E2: serum estradiol; Pg: progesterone; FSH: follicle-stimulating hormone; LH: lutenizing hormone.
[a] The mean values are given. Numbers in parentheses indicate range.

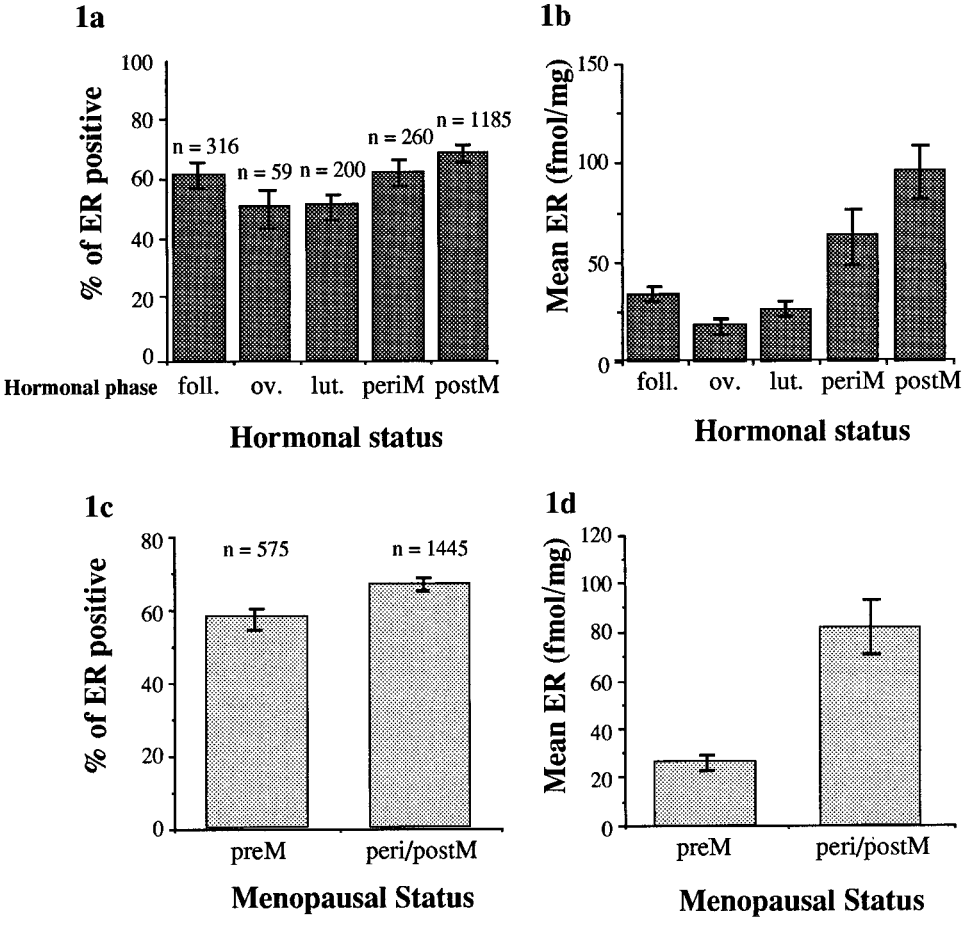

FIGURE 1. Change in estrogen receptor (ER) according to the hormonal status. (a) Percent of ER positive (ER^+) tumors. (b) Mean ER level. Bars represent confidence intervals. Abbreviations for hormonal phases are as follows: foll., follicular phase; ov., ovulatory period; lut., luteal phase; PeriM, perimenopause; PostM, postmenopause. (c) Percent of ER^+ tumors in premenopausal and menopausal women. (d) Mean ER level in premenopausal and menopausal women.

Estrogen Receptor According to Hormonal Status

In premenopausal women, there was a higher proportion of ER^+ tumors in the follicular phase (62%, n = 316) than in the ovulatory (51%, n = 59) and luteal (53%, n = 200) phases ($P = 0.03$; Fig. 1a). There was also a higher mean ER level in the follicular phase (30 fmol/mg) than in the ovulatory period (20 fmol/mg) and the luteal phase (25 fmol/mg, $P < 0.001$; Fig. 1b).

Patients in the menopausal group, including both peri- and postmenopausal women, were more likely to be ER^+ than patients in the premenopausal group (66.7% and 58.6%, respectively; Fig. 1c; $P < 0.0001$) Menopausal patients also had a higher mean ER level than postmenopausal patients (80 fmol/mg and 27.2 fmol/mg, respectively; Fig. 1d; $P < 0.0001$).

Estrogen Receptor and Circulating Hormones

In the overall population, ER was correlated slightly negatively with E2 and Pg, (r = −0.16 and −0.08 respectively; $P < 0.05$) and was correlated weakly positively with FSH and LH (r = 0.18 and 0.13, respectively; $P = 0.05$; Table 2). Because ER was related to

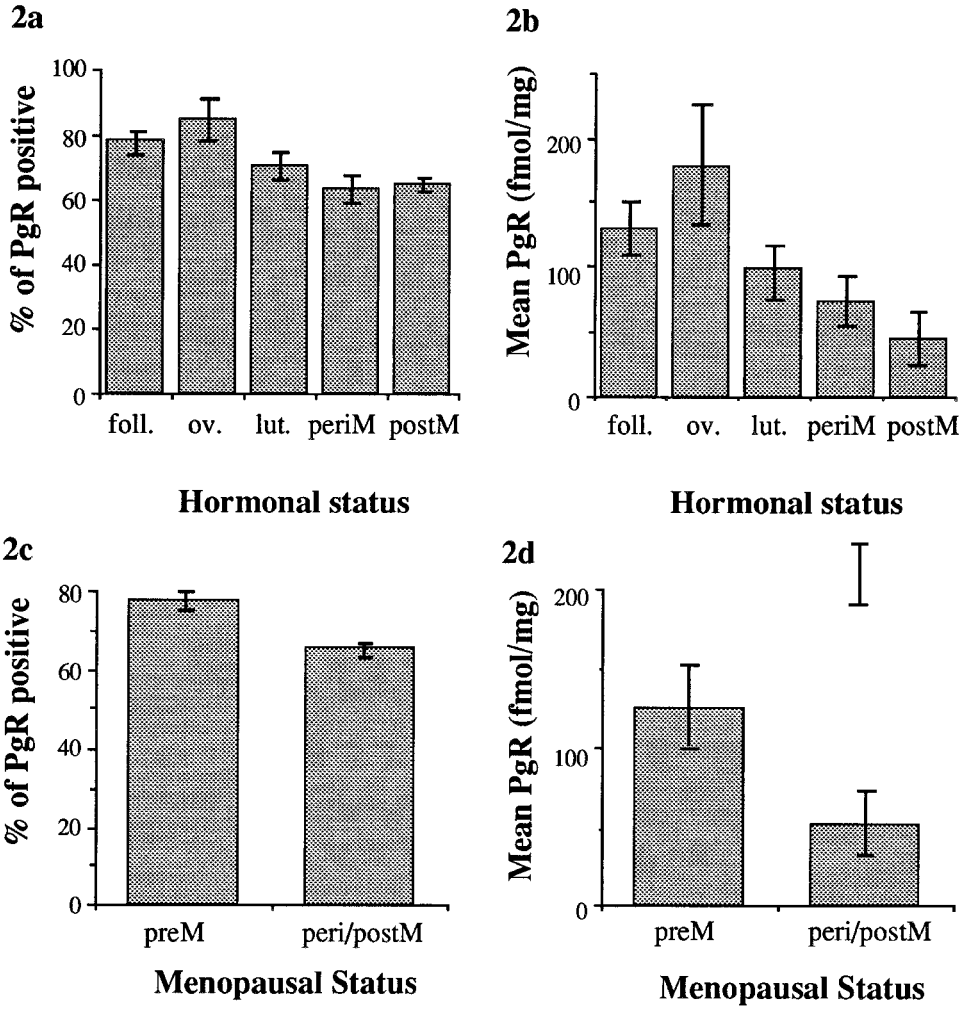

FIGURE 2. Change in progesterone receptor (PgR) according to the hormonal status. (a) Percent of PgR^+ tumors. (b) Mean PgR level. (c) Percent of PgR^+ tumors in premenopausal and menopausal women. (d) Mean PgR level in premenopausal and menopausal women.

menopausal status, we analyzed the subpopulation of women in cycle. In premenopause, there was also a weak trend that showed a lower level of ER in women with a high E2 level ($r = -0.10$, $P = 0.07$). The mean level of E2 was 70 pg/mL in ER^+ tumors compared with 82 pg/mL in ER negative (ER^-) tumors ($P = 0.02$). No association was observed between ER and Pg, FSH or LH levels or age at diagnosis (by year) in premenopause.

Progesterone Receptor According to Hormonal Status

The percentage of PgR^+ tumors tends to be higher during the ovulatory phase (85%) than during the follicular (78%) and luteal (72%) phases ($P = 0.11$; Fig. 2a). The mean PgR value was higher in the ovulatory phase (177 fmol/mg) than in the follicular phase (134 fmol/mg) and the luteal phase (92 fmol/mg, $P < 0.001$; Fig. 2b).

PgR percentage was higher in premenopausal women compared with menopausal women (77.8% and 65.2%, respectively; $P < 0.0001$; Fig. 2c). There was also a decrease in mean PgR of women in cycle compared with menopausal women (123.7 vs. 48.6 fmol/mg, respectively; $P < 0.001$; Fig. 2d).

Progesterone Receptor and Circulating Hormones

In the overall population, PgR was correlated slightly positively with E2 and Pg and was correlated slightly negatively with FSH and LH (Table 2). No correlations were found between PgR and circulating hormones in the subgroup of premenopausal women (Table 2). There was no difference in mean E2 or Pg levels between PgR^- or PgR^+ tumors in premenopausal women or in the overall population (data not shown).

Receptor Status Distributions According to Menopause

The distribution of patients according to stratification by steroid receptor status is shown in premenopausal women and in menopausal women (Fig. 3). The percentage of ER^+PgR^+ tumors was similar in premeno-

TABLE 2
Correlation between Steroid Receptors and Circulating Hormones[a]

	E2	Pg	FSH	LH	ER	Age
Overall population						
ER	−0.16*	−0.08*	0.18*	0.13*		0.28*
PgR	0.12*	0.10*	−0.11*	−0.08*	0.53*	0.03
Premenopausal women						
ER	−0.10*	−0.07	0.06	0.008		0.05
PgR	0.03	−0.09	0.06	0.09	0.60*	0.19*

ER: estrogen receptor; PgR: progesterone receptor.
[a] Spearman rank coefficient.
* $P < 0.005$.

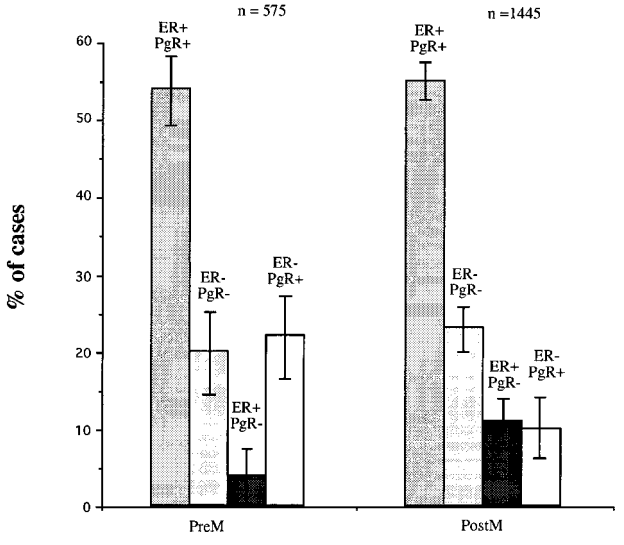

FIGURE 3. Distribution of receptor status according to menopausal status.

pause and menopause (54% and 55%, respectively). The percentage of ER^-PgR^- was also similar between women in cycle and women in menopause (20% and 23%, respectively). The percentage of ER^+PgR^- tumors was higher in menopause than in premenopause (13% and 4%, respectively; $P < 0.0001$). In contrast, the percentage of ER^-PgR^+ tumors was lower in menopause than in cycle (9% and 22%, respectively; $P < 0.0001$).

DISCUSSION

The present study investigates the relation between steroid receptors and hormonal status according to levels of circulating hormones in a large population of breast carcinoma patients. We found a higher ER level in the follicular phase and a higher PgR level in the ovulatory phase of the menstrual cycle.

The fact that the phase of the menstrual cycle influences the ER status is of clinical importance, because it can affect directly the prediction of hormone dependency and, finally, the choice of adjuvant therapy. The increased ER level in the follicular phase of breast carcinoma could reflect physiologic regulation that has been observed in normal breast epithelial cells.[12–15] It could be hypothesized that tumors that became ER^- in the luteal phase are likely to undergo such regulation throughout the menstrual cycle. Therefore, these tumors could be considered falsely as hormone-resistant when they are operated in the luteal period.

In the present study, the possibility of misclassification of menstrual cycle status because of misreported data from the last menstrual phase was minimized by the measurement of circulating hormones. Moreover, steroid and gonadotrophin hormone assessments were performed at the time of surgery, thus avoiding methodological problems with stored serum. However, anovulatory or atypical cycles cannot be ruled out, although a recent study showed that hormonal characteristics of the menstrual cycle normally are preserved in women undergoing breast carcinoma surgery.[9]

Some investigators have examined the question of the effect of the menstrual cycle on ER and PgR in breast carcinoma with various results. Several authors found significantly higher ER levels during the proliferative phase than during the secretory phase.[16,17,20,21] However, Coradini et al. did not find significant changes in ER^+ frequency and concentrations,[18] whereas Weimer et al. found the highest ER values in the late luteal phase.[19] In line with our observations of increased PgR levels during the ovulatory phase, an increase in PgR level in the early luteal phases has also been reported.[17,18] Our results, therefore, are in agreement with the overall trends of these previous studies, showing an increased ER level in the follicular phase and an increased PgR level in midcycle.

There was an increased level of ER and a de-

creased level of PgR in menopause, as reported previously.[24,25] A possible explanation for the lower percentage of ER^+ in premenopausal patients could be a blockade of ER by endogenous estrogens. Thorpe et al. have shown that some PgR^+ breast carcinoma biopsies are ER^+ when immunoenzymatic assays (IEA) are used, but are ER^- when ligand binding assay (LBA) is used, suggesting that occupied ER in the nuclear fraction of premenopausal women may not be measured by LBA.[26] A greater discordance of ER status between LBA and immunohistochemistry (IHC) methods has also been noted in premenopausal patients, particularly when the receptor concentration was close to the LBA cutoff of 10 fmol/mg protein.[27] However, other studies comparing methods for measuring both unoccupied and occupied ER and only occupied ER, such as enzyme immunoassays (EIA) and isoelectrofocusing[28] or EIA and ligand binding assays,[24] have not confirmed this hypothesis. Therefore, increased ER levels in menopause may reflect a real change in steroid receptor contents. In breast cancer cell lines with ERs (i.e., MCF7), E2 down-regulates the ER content.[29,30] Down-regulation of ER synthesis in premenopausal patients has also been shown at both the protein level[17] and the mRNA level,[31] whereas Pg decreased ER content.[32] It can be speculated that the increased ER content during menopause might be due to the decreased E2 level and the fact that cyclical Pg secretion in premenopausal women limits estrogen stimulation of ER synthesis. Consistent with these observations, we found a weak negative association of ER with E2 in premenopausal women and in the overall population.

In this study, a high percentage of ER^-/PgR^+ tumors was noted in premenopausal patients (22%) compared with menopausal patients (9%). Other large studies using the same ER cutoff (10 fmol/mg protein) pointed out an increased proportion of ER^-/PgR^+ tumors in premenopause, as determined by LBA.[33,34] In a population of more than 4000 breast carcinoma patients, Thorpe et al. found that the percentage of ER^-/PgR^+ tumors decreased steadily from 16% at the age of 30 years to 3% at the age of 80 years.[33] Fernö et al.[34] also found a higher ER^-/PgR^+ percentage in women between 35 and 50 years of age than in women over 50 years of age (10% and 4%, respectively). Conversely, we observed a higher percentage of ER^+PgR^- tumors in menopause compared with women in cycle. This could be due to decreased PgR induction by estradiol in ER^+ menopausal women.

It can be hypothetized that differences in ER and PgR status between pre- and postmenopause might be due to distinct tumor biology and, thus, may truly reflect differences in hormone dependency. However, it is unlikely that the differences in ER status observed during the menstrual cycle reflect changes in the intrinsic characteristics of the tumor; rather, it is likely that these differences are related to changes in the hormonal milieu. This study used a cutoff level of 10 fmol/mg protein to define receptor status. This is the most commonly used threshold in Europe,[35] but the choice of ER and PgR cutoff levels (usually ranging from 3 fmol/mg to 10 fmol/mg protein) has been controversial[36] and remains arbitrary. Moreover, the cutoff levels often are set by the technical limit of the lower value. Under our routine LBA assay conditions, the sensitivity and reliability of ER determination are limited for low ER values, particularly in tumors with a low level of cytosolic protein. Cutoff levels based on detection of a minimal amount of ER expression, as determined by sensitive LBA, EIA, or IHC, thus, may reflect more accurately the hormonal phenotype of some tumors with low ER content. This might be particularly helpful for cycling women in which down-regulation of ER may explain the low ER expression level of tumors that may be hormone-dependent.

If it is speculated that the changing ER levels throughout the cycle reflect more a physiologic variation than a change in hormone sensitivity, then the difference between menstrual cycle phases raises the question of whether it is appropriate in clinical practice to use the same cutoff points when separating positive and negative receptors. For example, to obtain the same percentage of PgR positivity in all phases of the menstrual cycle, the cutoff level should be set at 32 fmol/mg in the ovulatory phase and at 19 fmol/mg in the follicular phase, for a cutoff level in the luteal phase of 10 fmol/mg.

Over the last few years, ER determination by IHC has been used extensively, because it is a sensitive method that requires a minimum amount of tissue, and the proportion of carcinoma cells can be checked (thus, avoiding false negative results). Moreover, IHC is not affected by the level of endogenous steroids and provides information on the heterogeneity of ER expression in tissue. Studies comparing ER expression between IHC and DCC generally show good correlations.[37,38] IHC studies on normal breast cells found a higher ER expression level in the first phase of the menstrual cycle.[12–15] Our results of an increased ER level in the follicular phase during the menstrual cycle in breast carcinoma are consistent with these data.

Our study shows that the menstrual cycle phase can influence ER and PgR status of breast carcinoma, leading to a possibly different interpretation of hormone sensitivity in a given tumor, depending on the hormonal status at the time of surgery. Although there was a weak negative association between E2 and ER in

premenopause, the variations in steroid receptor levels could not be related directly to the levels of circulating E2 and Pg or of gonadotrophin. Further studies analyzing different cutoff levels and taking into account the hormonal status at the time of surgery are needed to define which threshold is more reliable for predicting hormonosensitivity and prognosis.

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