In vivo imaging of estrogen receptor concentration in the endometrium and myometrium using FES PET--influence of menstrual cycle and endogenous estrogen level.

In Vivo Imaging of Estrogen Receptor Concentration in the Endometrium and Myometrium using ^{18}F-FES PET – Influence of Menstrual Cycle and Endogenous Estrogen Level-

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Abbreviated title: Change of ER measured with FES-PET

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ABSTRACT

Purpose: The goal of this study was to measure estrogen receptor (ER) concentration in the endometrium and myometrium using 16α-[^{18}F]fluoro-17β-estradiol (FES) PET and to investigate the relationship between changes in these parameters with the menstrual cycle and endogenous estrogen levels. **Methods:** Sixteen female healthy volunteers were included in this study. After blood sampling to measure endogenous estrogen level, FES-PET image was acquired 60 min postinjection of FES. After whole-body imaging of FES-PET, averaged standardized uptake value (SUV) in the endometrium and myometrium were measured, and the relationship between FES uptake and menstrual cycle or endogenous estrogen level was evaluated. **Results:** Endometrial SUV was significantly higher in the proliferative phase than in the secretory phase (6.03 ±1.05 vs. 3.97 ± 1.29, p = 0.022). In contrast, there was no significant difference in myometrial SUV when comparing the proliferative and secretory phases (p = 0.23). Further, there was no correlation between SUV and endogenous estrogen level in the proliferative phase. **Conclusions:** The change of ER concentration relative to menstrual cycle as characterized by FES-PET was consistent with those from previous report using immunohistochemical technique. These data suggest FES-PET is a feasible noninvasive method of characterizing changes in ER concentration.

INTRODUCTION

16α-[^{18}F]fluoro-17β-estradiol (FES) is a radiopharmaceutical that binds to the estrogen receptor (ER) and expresses the existence of ER [1]. FES can help characterize the diagnosis and efficacy of hormonal therapy in patients with ER-positive breast cancer [2-6]. Indeed, Mintun et al. [2] reported that in vivo uptake of FES in primary breast carcinoma correlates with in vitro measurements of ER concentration and thereby represents a noninvasive quantitative measurement of ER in breast carcinoma. Although several studies reported the expression of ER in many organs other than breast using immunohistochemistry [7], such findings has not known yet in human study even in normal ER-rich tissue like endometrium or myometrium. Because FES is expected to be a good tracer to reveal the change of ER concentration in normal tissue as well as in disease, normal control data should be required. In immunohistochemical studies, the relationships between the level of ER and menstrual cycle in endometrium and myometrium are reported as follows [8-17]; In endometrium, the level of ER increase from early- to late proliferative phase, then fell down in secretory phase. In myometrium, the cyclical change of ER varies in different layers. The inner part of the myometirum (stratum subvasculare) adjacent to endometrium shows the same behavior as the endometrium does. On the other hand, the outer part

of the myometirum (stratum vasculare and supuravasculare) shows the constant ER expression through the whole menstrual cycle. It is well known that the endogenous estrogen level alters during menstrual cycle, which has two peaks in late proliferative and mid secretory phase and thus, the effect of estrogen level on FES uptake should also be studied before clinical application. Therefore, the goal of this study was to measure ER concentration in the endometrium and myometrium using FES-PET and to investigate the relationship between changes in these parameters with the menstrual cycle and the endogenous estrogen level in healthy volunteers.

MATERIALS AND METHODS

Subjects

Sixteen female normal volunteers were included in this study. Participant age range was 21 to 28 years old (23.6 ± 2.2 yrs; mean ± SD). Medical interviews, encompassing previous malignancy and gynecological surgery, menstrual state and cycle, and the last menstrual period, were conducted in all subjects. Written informed consent was obtained from all subjects participating in this study, which was approved by the institutional review board of University of Fukui Hospital.

Synthesis of FES

FES synthesis was performed according to the method of Kiesewetter et al. [1] with modifications as reported by Mori et al. [18] in detail. A cassette-type automatic [^{18}F]fluorodeoxyglucose (FDG) synthesizer (TRACERlab MX$_{FDG}$, GE Medical Systems, Milwaukee, WI) that was modified for the synthesis of FES was utilized and fluorination, two-step hydrolysis, and neutralization were performed under the appropriate condition to synthesize FES. After the final purification, the radiochemical purity was greater than 99% and the yield was 42.4 ± 3.2 % (EOB). The specific activity calculated by the analytical HPLC system was more than 111

GBq/μmol.

PET Imaging

All subjects fasted more than 4 hours prior to FES-PET examination to eliminate the excretion of FES from the hepatobiliary system and the gastro-intestinal tract, which would otherwise interfere with image interpretation in the pelvic space. Three ml of blood was obtained just before FES injection to measure the endogenous estrogen level (estradiol, E2). FES-PET data acquisition started 60 min after the injection of 185 MBq of FES. Emission scans were performed for 3 min in the pelvic portion (2 bed positions) and 2 min in remaining positions (5 bed positions) to cover the area from the head to the inguinal region. Postinjection transmission scans for 2 min at pelvis and 1 min in other parts were performed after the emission scans for attenuation correction. The acquired data were reconstructed using an iterative reconstruction method with 14 subsets and 2 iterations. The reconstructed image was converted to standardized uptake value (SUV) image according to the subject's body weight and net injected dose for the data analysis.

MRI Imaging

All subjects underwent MRI examination on the same day of FES-PET or on one day before FES-PET to obtain positional information regarding the endometrium and myometrium. T1- and T2-weighed images (WI) in the transaxial plane and T2-WI in the sagittal plane were acquired with 1.5-Tesla superconducting MRI system (Signa, GE Medical Systems, Milwaukee, WI). The imaging sequence of T1-WI and T2-WI was 533/8 and 4700/90 (TR/TE, unit;msec), respectively.

Data Analysis

On mid-sagittal image of FES-PET, circular regions of interest (ROIs: 8 mm in diameter) were placed using guidance by T2-WI of MRI in the sagittal plane. By comparing FES-PET and MRI visually, endometrium which usually has high uptake of FES compared with myometrium was identified. Myometrium was defined as the faint FES uptake area surrounding the endometrium. Three ROIs were placed on the endometrium and 9 were placed on the myometrium shown in Figure 1. The averaged SUV in endometrium and myometrium were plotted against the days from the onset of menses and the endogenous estrogen level.

In this study, the menstrual cycle was divided into 2 groups because of the limited number of subjects. Those participations who were 6 to 14 days after the onset of

menses were defined as being in the proliferative phase and those who were 15 to 28 days from the onset of menses were defined as being in the secretory phase. SUV between these 2 phases were compared in the endometrium and myometrium. Nonparametric Mann-Whitney U-test was used for statistical comparisons. Spearman nonparametric correlation analysis was performed to analyze the relationship between SUV in endometrium and SUV in myometrium and E2. In each statistical analysis, $p < 0.05$ was considered to represent statistical significance.

RESULTS

Characteristics of the subjects and summary of the results are shown in Table 1.

One subject had irregular menstrual cycle and was excluded from the analysis of the relationship between FES uptake and menstrual cycle. From the definition of menstrual cycle stated in the section of data analysis, 6 subjects were classified as being in the proliferative phase and 7 were classified as being in the secretory phase. Two subjects were in the menstrual phase and were also excluded from this analysis. In the analysis of the relationship between FES uptake and E2 level, no subjects were excluded.

Figure 2A shows the relationship between endometrial SUV and the menstrual cycle. SUV was significantly higher in the proliferative phase than in the secretory phase (6.03 ± 1.05 vs. 3.97 ± 1.29, $p = 0.022$).

The relationship between myometrial SUV and the menstrual cycle is illustrated in Figure 2B. No significant difference in SUV was noted when comparing the proliferative and secretory phase (2.75 ± 0.22 vs. 2.53 ± 0.37, $p = 0.23$).

Further, there was no relationship between FES uptake and E2 level in the endometrium (Figure 3A) or myometrium (Figure 3B) in the proliferative phase.

DISCUSSION

Physiological FES uptake in the endometrium is affected by the menstrual cycle secondary to changes in endogenous estrogen level in healthy women. However, the present study demonstrated that endometrial SUV was not directly correlated with plasma E2 level or FES uptake in the myometrium. Yoo et al. [19] reported that FES preferentially binds the ERα subtype with 6.3-fold higher affinity than that for ERβ. Further, the uterus is one of the target organs of E2 and expresses both ERα and ERβ. ERα predominates in the uterus, breast, kidney, liver, and heart, whereas tissues that have high ERβ levels include prostate, testes, ovaries, gastrointestinal tract, lung, bladder, hematopoietic and central nervous systems. Many tissues contain both ERα and ERβ, such as breast, epididymis, thyroid, adrenal, bone. Wang et al. used immunohistochemistry to demonstrate that the myometrium and leiomyomas have ERα-dominant expression [20]. These results suggested that uterine ER expression is a good target for study with FES-PET. However, physiological FES uptake in the uterus of healthy women remains unclear, and may vary with the menstrual cycle observed in FDG-PET [21].

Several investigators have used immunohistochemistry to characterize the relationship between ER expression and menstrual cycle measured in the endometrium

[8-17] and myometrium [9-16]. In these reports, endometrial ER concentration in the proliferative phase was higher than that in the secretory phase, which is consistent with results from the present study. Further, several studies have reported a significant difference between myometrial ER concentration in the proliferative phase and the secretory phase. By contrast, the present study showed no significant difference in myometrial FES uptake when comparing the proliferative phase and secretory phase. This finding is supported by some papers [14 – 16]. Noe and Vienonen reported [15, 16] that in the myometirum ERα was regulated in the layer adjacent to the endometrium in an endometrium-like pattern during the menstrual cycle, whereas expression pattern in the outer part of the myometrium were more stable. In the studies which showed a significant difference between myometrial ER concentration in the proliferative phase and the secretory phase, only the myometrium immediately underlying the endometrium was analyzed as the representative of the whole uterine muscular wall [9-11]. In the present study, placed ROI on the myometrium which surrounded endometrial ROI rather evaluated the outer part of the myometirum because of the limited spatial resolution.

In this study, there was no significant relationship between FES uptake and E2 even in the proliferative phase. A previous study reported that a large concentration of

endometrial ER in the late proliferative phase correlates with the plasma estradiol surge [22]. Further, Levy et al. reported that a significant correlation was observed between ER concentration and E2 in the proliferative phase [8]. In the present study, there was a trend toward a relationship between E2 and SUV in the endometrium in the proliferative phase ($p = 0.09$), but the difference did not reach the level of statistical significance. Repetition of the present study with a large population size may result in data consistent with those reported by Levy et al.

The relationship between ER concentration in the myometrium and endogenous estrogen level demonstrated no statistical significance. FES uptake in the myometrium was almost constant through the entire menstrual cycle and may be independent from the change of endogenous estrogen level in healthy volunteers.

Although FES represents the level of unoccupied ER and ER measured with immunohistochemistry represents total ER, our results and the previous reports showed the same behavior. Katzenellenbogen et al. reported [23] that the FES uptake in the uterus was suppressed by the coinjection of high dose of estradiol or the tamoxifen pretreatment in the rat. But in proliferative phase, although total ER and occupied ER by endogenous estrogen in the endometrium will increase, the proportion of unoccupied ER measured with FES will not change. In secretory phase, the opposite phenomenon

will occur and the proportion of unoccupied ER will not also change. Therefore, it is speculated the behavior of total ER and unoccupied ER will be similar. In myometrium, total and unoccupied ER in proliferative phase did not change when the endogenous estrogen increased. The proportion of occupied ER against total ER may be enough small although further examination will be required.

FES has been used for the evaluation of breast tumors [2-6] and may also have clinical application in patients with uterine endometrium-related gynecological diseases [24, 25]. Indeed, Okazawa et al. reported [25] that the combination of FDG- and FES-PET improved the diagnostic accuracy in various uterine endometrium-related gynecological diseases including uterine endometrial cancer, adenomyosis, and endometrial hyperplasia. For the assessment of FES uptake, SUV or lesion to normal ratio (L/N) will be feasible. The present study demonstrated that FES uptake in the endometrium varied with the menstrual cycle, whereas myometrial FES uptake was stable throughout the entire menstrual cycle. These data suggest that the myometirum may serve as a good internal control during FES studies of other organ systems and the menstrual cycle should be taken account for when the endometrium was considered for an internal control.

CONCLUSION

The change of ER concentration relative to menstrual cycle as characterized by FES was consistent with those from previous report using immunohistochemical technique. Further, these data suggest that FES-PET is a feasible noninvasive method of characterizing changes in ER concentration.

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FIGURE LEGENDS

Figure 1 Sagittal PET image of [^{18}F]-FES distribution in a healthy woman (left) and representative regions of interest (ROIs) placed on the endometrium (white circles) and myometrium (dotted circles) (right). Image was acquired 60 min postinjection of 185 MBq of FES.

Figure 2 Changes in FES uptake in the uterus during the menstrual cycle. Endometrial FES uptake (A) was significantly higher in the proliferative phase (closed circle) than in the secretory phase (open circle). By contrast, myometrial FES uptake (B) was similar when comparing the two phases.

Figure 3 Relationship between FES uptake and endogenous estrogen level. Linear regression analysis (solid line) was performed only in the proliferative phase (closed circle). No significant linear correlation was observed in the endometrium (A) ($y = 0.05\ x + 2.95$, $r^2 = 0.56$, $p = 0.09$) or the myometirum (B) ($y = 0.006\ x + 2.37$, $r^2 = 0.20$, $p = 0.38$). Open circle represents SUV in the proliferative phase and open square represents SUV in the menstrual phase.

Figure 1

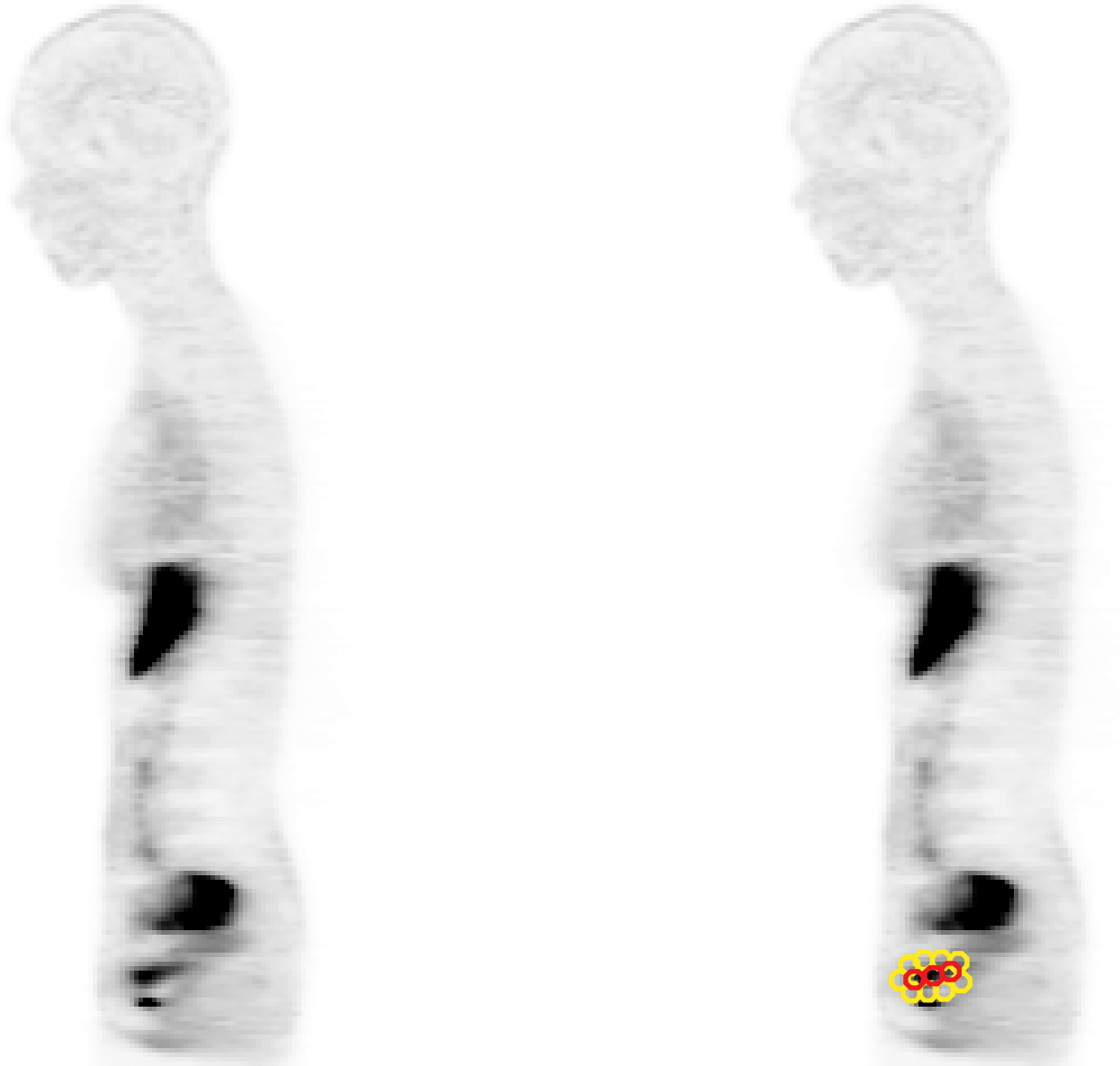


Figure 2A

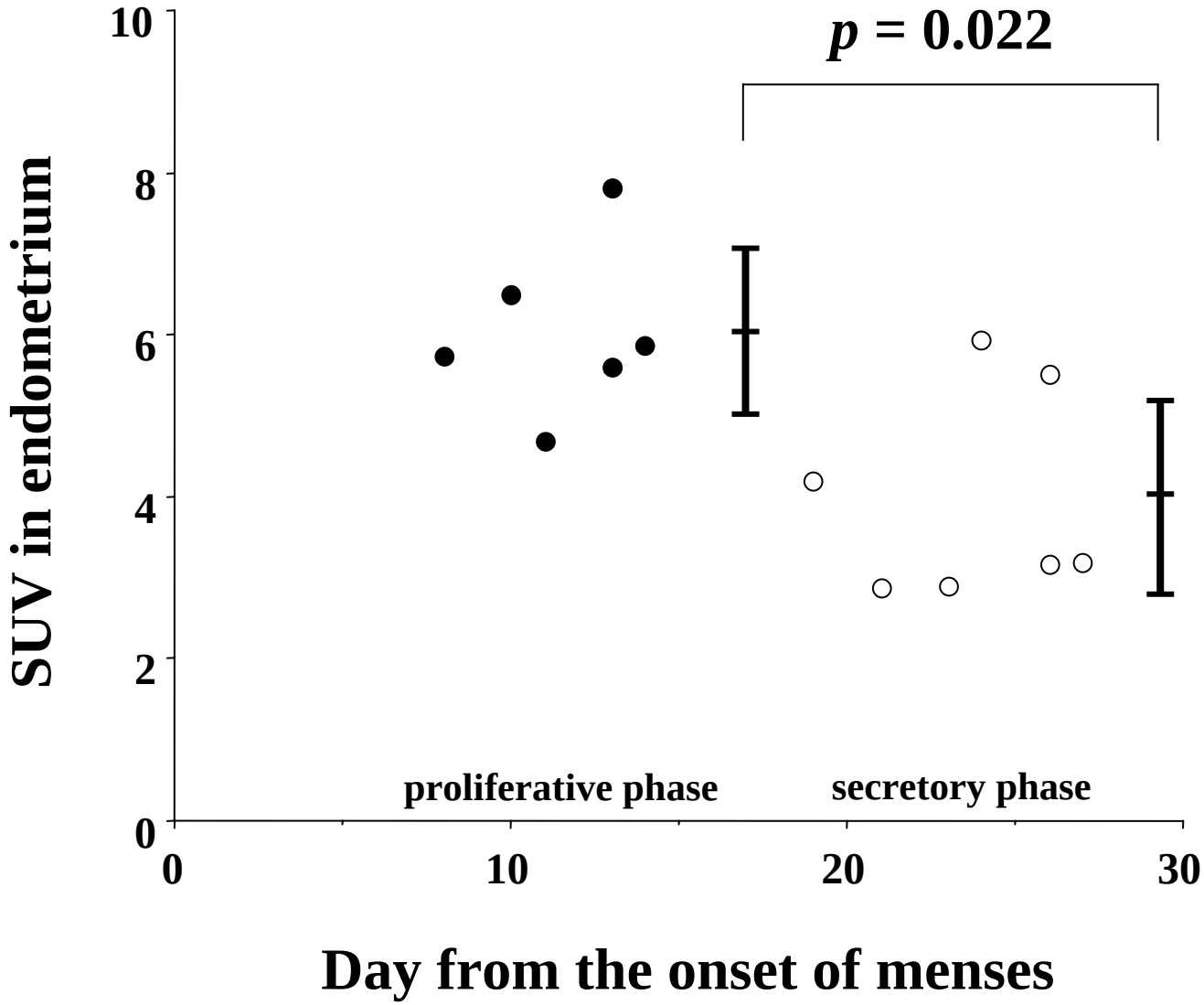
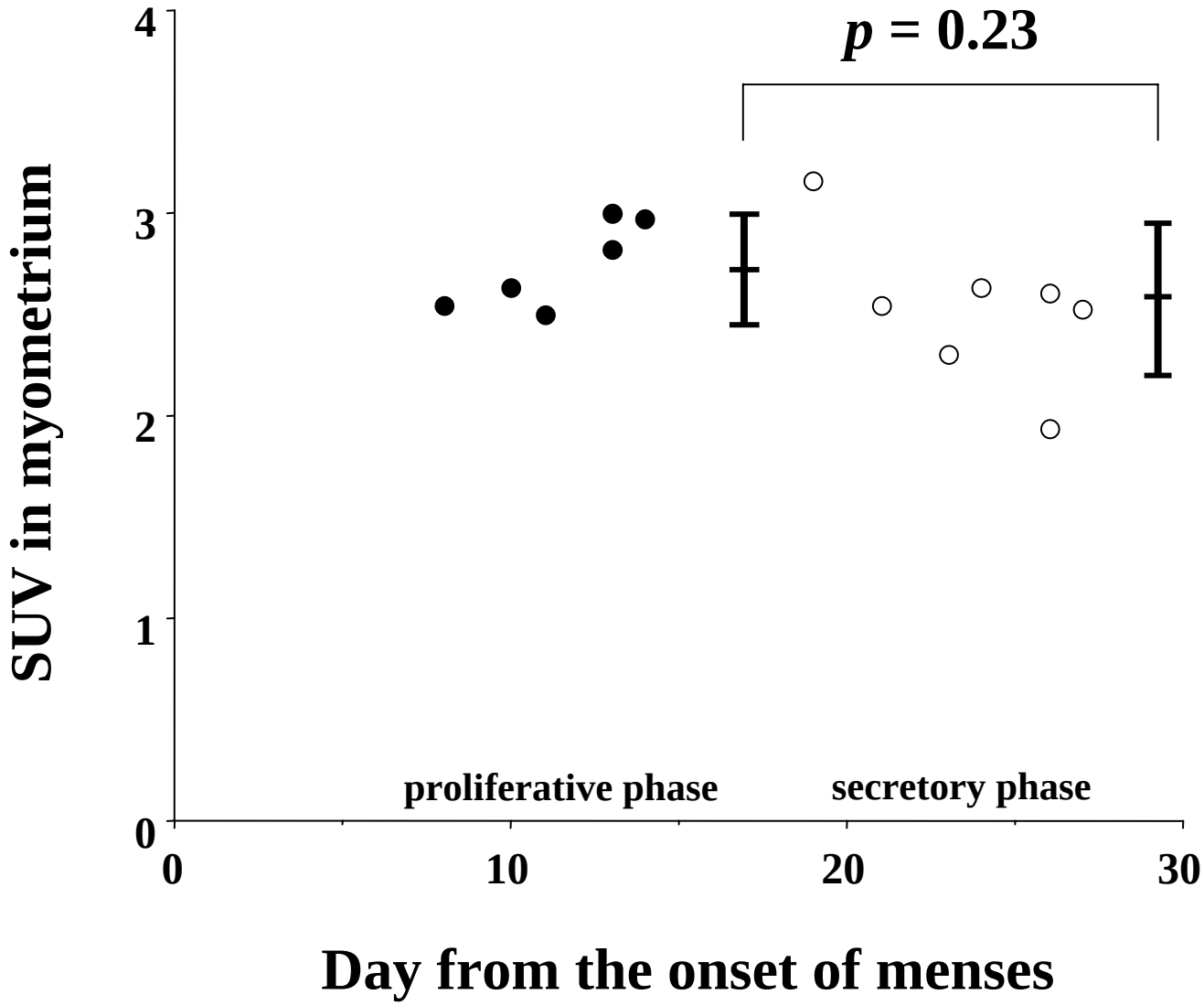

p = 0.022
SUV in endometrium
10
8
6
4
2
0
proliferative phase
secretory phase
0
10
20
30
Day from the onset of menses

Figure 2B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SUV in myometrium
4
3
2
1
0
proliferative phase
secretory phase
0
10
20
30
Day from the onset of menses

Figure 3A

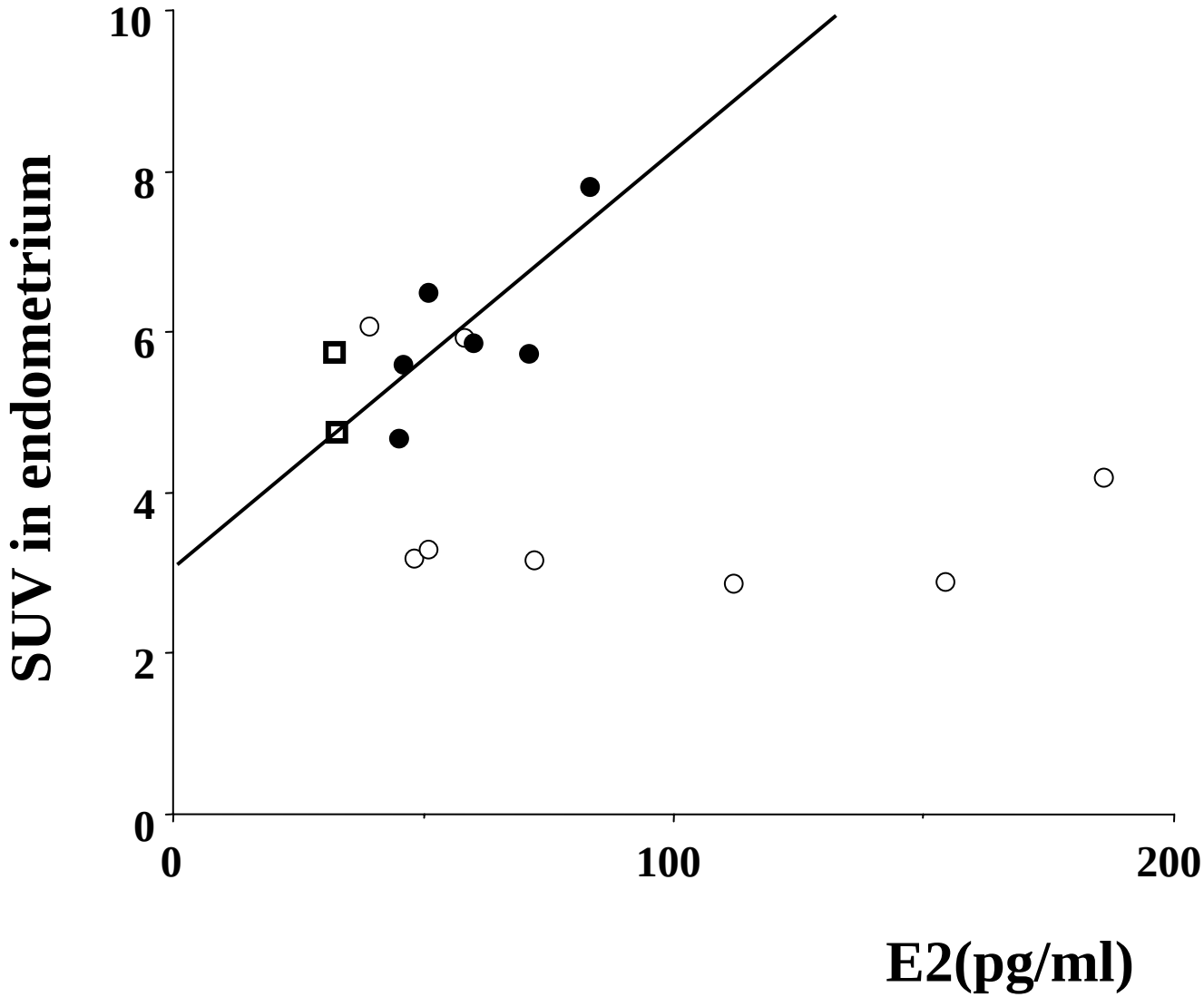

Figure 3B