

Is the Menstrual Cycle Affecting the Skin Prick Test Reactivity?

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Allergen skin prick tests (SPT) are of value in the diagnosis of immunoglobulin E (IgE)-mediated diseases. They are used to evaluate rhinitis, asthma, and anaphylaxis including reactions to food, certain drugs, and venom. These tests are usually preferred to other methods, because they are simple, inexpensive, and give immediate results. Several methods other than SPT are used to detect allergen specific IgE, such as radioallergosorbent test (RAST). Allergen skin prick tests correlate well with *in vitro* tests such as RAST, modified RAST, and the Pharmacia CAP system. However, in general, SPT are more sensitive and specific in detection of allergy when compared to these.^{1,2} Allergen skin prick tests have been used since the 19th century for the diagnosis of allergic diseases. Skin testing was first introduced in 1873 by Blackley³ who observed a wheal-and-flare reaction after rubbing grass pollen on skin. Later, Lewis defined the triple response of skin induced by histamine. This phenomenon consists of a central wheal, circumferential erythema and local erythema where histamine is applied.⁴

SUMMARY Allergen skin prick tests (SPT) are very sensitive and specific tests to detect allergic sensitization in atopic patients. Certain factors like antihistamines, antidepressant therapies or circadian rhythms can alter the results of SPT. In women, the changes in endogenous hormone levels throughout the menstrual cycle may affect the allergic responses and natural course of allergic diseases. The aim of this study was to investigate the probable influence of the phases of the menstrual cycle on SPT reactivity to allergen extracts and histamine. Forty-two female patients with seasonal allergic rhinoconjunctivitis were enrolled in the study. Skin prick test reactivities to allergens and histamine were measured at the beginning of the menstrual cycle (3rd or 4th day), mid-cycle (14th or 15th day) and end-cycle (27th or 28th day) consecutively. Serum estradiol, progesterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) levels were determined simultaneously. We observed the most significant reactions to allergens when SPT is performed at mid-cycle. However, SPT reactivity to histamine did not vary throughout the menstrual cycle. Serum estradiol and LH levels showed positive correlation with SPT reactivity to allergens at mid-cycle. Our results suggest that SPT give the best results when they are performed at mid-cycle. Additionally, allergens seem to cause mast cell degranulation to a greater extent in subjects in which endogenous hormones like estradiol and LH are elevated.

Factors that can alter the SPT reactivity to histamine or allergens include age,⁵ sex,⁶ racial group,⁷ pharmacologic treatment, particularly antihistamines and tricyclic antidepressants,⁵ site of SPT administration,⁸ circadian rhythm,⁹ and seasons of the year.⁵ Women exhibit the weakest histamine whealing capacity during the first day of the menstrual cycle and a second weak response around the twentieth day.¹⁰ Undulations of endogenous cortisol can affect the late

phase of SPT reactivity.¹¹ Exogenous sex hormones like estrogen and progesterone individually or in combination as in oral contracep-

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tives attenuate SPT reactivity to histamine.¹² Additionally, appropriate doses of progesterone induce conversion of T helper cells from a Th0 to a Th2 phenotype.¹³ Finally, Kalogeromitros *et al.*¹⁴ have reported that the menstrual cycle influences SPT reactivity to histamine, morphine and allergens.

It is well documented that endogenous sex hormone levels of estradiol, progesterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) are undulating throughout the menstrual cycle.¹⁵ We aimed to detect the possible change in SPT reactivity to histamine and selected allergens during different phases of the menstrual cycle in atopic women. Moreover, we intended to determine the appropriate day(s) to perform SPT.

MATERIALS AND METHODS

Subjects

Forty-two atopic female patients without menstrual cycle irregularity were enrolled in the study. The mean age was 28.52 ± 6.59 (age range between 19 and 42) years. All patients were diagnosed with seasonal allergic rhinoconjunctivitis based on history, physical examination and laboratory findings. Allergic sensitizations of all patients were demonstrated by SPT. All patients had positive SPT reactivity to a grass/cereal allergen mixture. Additional inclusion criteria that all selected patients met were 1) an orderly menstrual cycle lasting 28-3 days; 2) menarche at age 11-14 years; 3) absence of pregnancy or labor for at least 1 year prior to the study and 4) not being on oral contraceptives. All of the patients were required to avoid

oral and/or nasal antihistamines, corticosteroids or other treatment that might suppress SPT reactivity such as H2 receptor blockers, decongestants, antidepressants for at least two weeks prior to SPT. Moreover, underlying diseases such as diabetes mellitus, obesity, thyroid disease, autoimmune disease and chronic renal failure were absent in the patients included in the study.

Informed consent for the described investigations was obtained from all patients. Approval for the study was given by the ethics committee of our hospital.

Study design

The starting day of the menorrhagia was accepted as day 0 of the menstrual cycle. The first SPT was performed on the 3rd or 4th day of the menstrual cycle (beginning of the menstrual cycle) on all patients. A second and third SPT was performed on the 14th or 15th day (middle) and 27th or 28th day (end) of the menstrual cycle, respectively. A blood sample was obtained from each patient to determine serum hormone levels at the time of SPT.

Allergen skin prick tests

Allergen skin prick tests were performed in the morning between 09.30 a.m. and 11.00 a.m. to avoid the effect of the circadian rhythm. A standardized positive control (histamine 1.7 mg/ml), negative control (9 mg NaCl, 4 mg phenol, 563 mg glycerol/ml) and grass/cereal mixture (*Hulcus lanatus*, *Lolium perenne*, *Festuca pratensis*, *Phleum pratense*, *Poa pratensis*, *Dactylis glomerata*, *Hordeum vulgare*, *Avena sativa*, *Secale cereale*, *Triticum sativum*) allergen extracts (Al-

lergopharma Ltd, Reinbek, Germany) were used for SPT. These allergens are the most prevalent allergens in our region. Allergen skin prick tests were performed according to the EAACI guidelines.¹⁶ Results were evaluated as described in literature.¹⁷ The largest diameter and perpendicular diameter of both wheal and erythema were measured by this technique. The sum of the diameters divided by two was accepted as the mean diameter (millimeters). The wheals' mean diameters were used for statistical analysis.

Hormonal determination

Blood samples were obtained from all patients to determine serum levels of estradiol, progesterone, LH and FSH just before performing SPT. All blood samples were taken at about the same time to avoid the circadian rhythm of the hormones. Patients' sera were collected from the samples. Serum hormone levels were measured by using automated chemiluminescent immunoassay technique (DPC-Immulite-2000, Los Angeles, USA).

Statistical analysis

The mean diameter of the SPT reactivity to the allergen mixture and histamine in three phases of the menstrual cycle were compared by two-way analysis of variance (ANOVA) for significance. Also the mean diameters of the SPT reactivity to grass/cereal allergen mixture and histamine were compared to each other determined at the beginning of the menstrual cycle vs. the middle of the menstrual cycle; beginning of the menstrual cycle vs. the end of the menstrual cycle; middle of the men-

strual cycle vs. the end of the menstrual cycle using standard statistical tests (paired *t* tests) as post-hoc test. Whole serum hormone levels were compared with the same technique, as well. Correlations between the mean diameters of SPT reactivity to the allergen mixture and histamine and serum hormone levels were evaluated by Spearman rank correlation test. *P* values less than 0.05 were accepted as statistically significant.

RESULTS

The mean diameters of SPT reactivity to histamine and grass/cereal allergen mixture and whole hormone levels throughout the menstrual cycle are shown in Table 1. All patients showed positive SPT reactivity to the grass/cereal allergen mixture. The mean diameter of SPT reactivity to histamine was the same in all measures ($p > 0.05$) (Fig. 1). Maximum mean diameters of SPT reactivity to a grass/cereal

allergen mixture were determined at the middle of the menstrual cycle ($p < 0.001$). There was no significant difference between SPT reactivities to the grass/cereal allergen mixture determined at the beginning and the end of the menstrual cycle ($p > 0.05$) (Fig. 1).

Maximum serum estradiol levels were determined at the middle of the menstrual cycle. There was a significant difference between serum estradiol levels determined at the middle and at the beginning of the menstrual cycle ($p < 0.001$). But there was no significant difference between serum estradiol levels determined at the middle and at the end of the menstrual cycle ($p > 0.05$). Serum progesterone levels determined at the end of the menstrual cycle were higher when compared to those determined at the beginning and at the middle of the menstrual cycle ($p < 0.001$ and $p < 0.01$, respectively). Serum FSH levels were high at the beginning

and at the middle of the menstrual cycle and decreased by the end of the menstrual cycle (for beginning vs. middle of the menstrual cycle $p < 0.05$; for beginning vs. end of the menstrual cycle $p < 0.001$; for middle vs. end of the menstrual cycle $p < 0.001$). Serum LH levels showed a peak at the middle of the menstrual cycle ($p < 0.001$ for each period compared). All hormone levels determined were in the range of the physiological hormonal changes of the menstrual cycle as described in the literature.¹⁵

There was a negative correlation between the mean diameters of SPT reactivity to the allergen mixture and serum LH levels at the beginning of the menstrual cycle ($r = -0.405$, $p < 0.01$). However, during the same period serum progesterone levels correlated weakly with SPT reactivity to the allergen mixture ($r = 0.384$, $p < 0.05$) (Fig. 2a). At the middle of the menstrual cycle, the mean diameter of SPT reac-

Table 1 Mean diameters of skin test reactivity to histamine and allergen mixture and serum hormone levels during the three phases of the menstrual cycle. See text for *p* values.

	Beginning of menstruation	Mid-cycle of menstruation	End-cycle of menstruation
Skin test reactivity to histamine (mean \pm SD) (mm)	6.89 \pm 0.84	6.91 \pm 0.65	6.81 \pm 0.63
Skin test reactivity to allergen mixture (mean \pm SD) (mm)	7.64 \pm 1.5	8.64 \pm 1.05	7.63 \pm 1.03
Estradiol (pg/ml)	39.38 \pm 14.12	150.80 \pm 70.99	95.79 \pm 69.04
Progesterone (ng/ml)	1.18 \pm 0.9	2.09 \pm 1.88	6.47 \pm 3.92
FSH (IU/l)	5.53 \pm 2.52	6.35 \pm 3.52	2.72 \pm 1.25
LH (IU/l)	3.28 \pm 1.46	14.05 \pm 10.94	1.71 \pm 1.01

tivity to the allergen mixture correlated significantly with serum LH and estradiol levels (respectively $r = 0.41$, $p < 0.01$; $r = 0.437$, $p < 0.005$) (Fig. 2b, Fig. 2c). At the end of the menstrual cycle, serum FSH levels and mean diameter of SPT reactivity to the allergen mixture displayed a weak negative correlation ($r = -0.321$, $p < 0.05$). Mean SPT reactivity to histamine correlated significantly with serum estradiol levels during the middle of the menstrual cycle ($r = 0.442$, $p < 0.01$). Additionally, a significant correlation between the mean diameter of SPT reactivity to histamine and all serum hormone levels was observed only at the middle of the menstrual cycle.

DISCUSSION

At present, SPT reactivity to allergens is the most reliable and safest test used routinely for the diagnosis of IgE mediated allergic diseases. Unfortunately, many factors like age, sex, drug ingestion and daily, monthly or seasonal variation of our biological rhythm may affect the sensitivity of this test to allergens and positive controls (histamine, etc.).⁵⁻⁹ It has been documented in the literature that female sex hormones, pregnancy and hormonal changes throughout the menstrual cycle may alter mast cell degranulation by affecting the secretion of mediators like IL-4, IL-13 and IgE that have major roles in allergic sensitization.¹⁸ Therefore, for women, the hormonal fluctuations during the different phases of the menstrual cycle may be the most important factor that must be considered while evaluating the results of this test. Unfortunately, few studies that have been conducted about this issue revealed conflicting results.

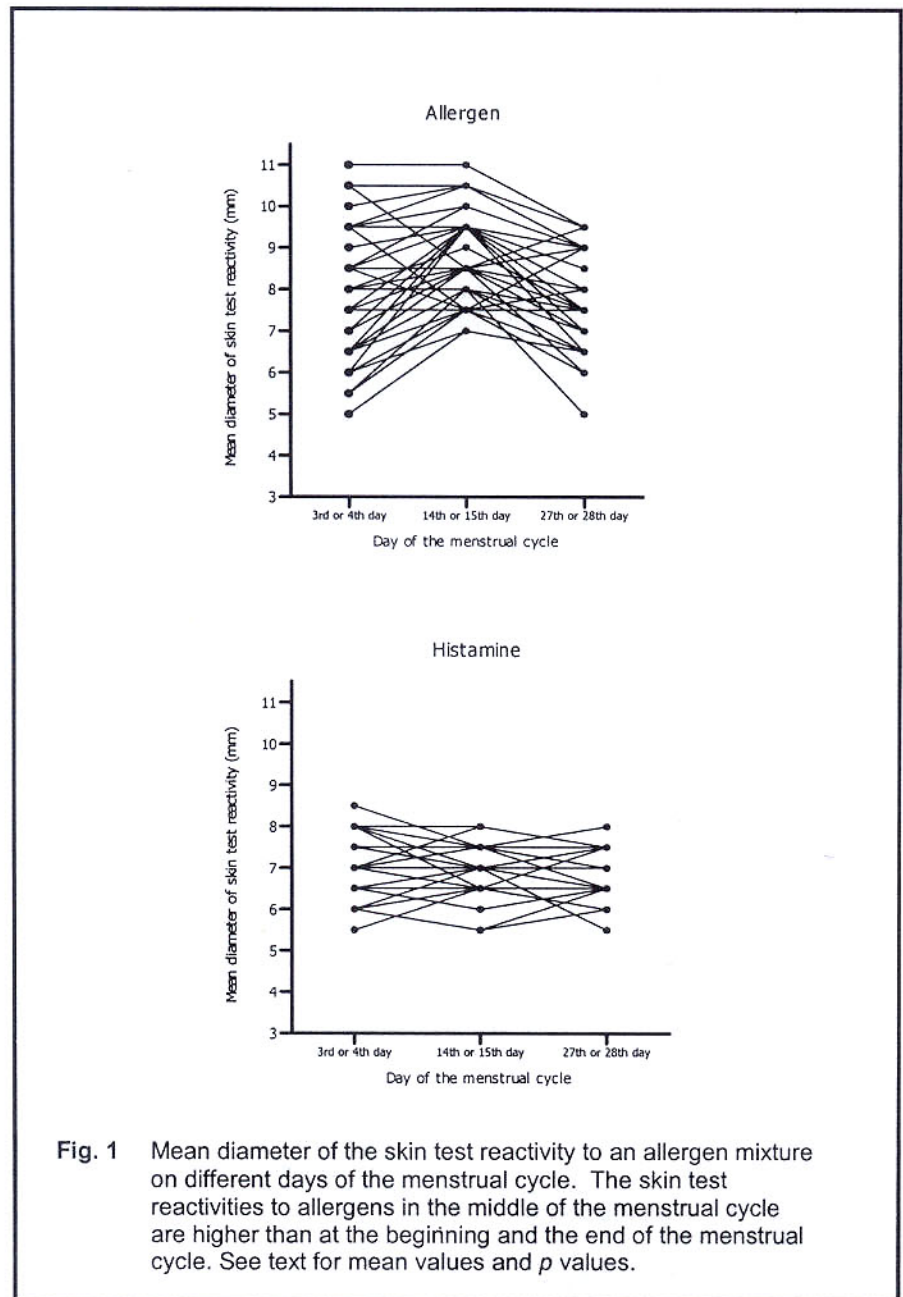


Fig. 1 Mean diameter of the skin test reactivity to an allergen mixture on different days of the menstrual cycle. The skin test reactivities to allergens in the middle of the menstrual cycle are higher than at the beginning and the end of the menstrual cycle. See text for mean values and p values.

For example Hansen-Pruss and Raymond determined the highest reactivity to allergens on the 3rd day of the cycle while Ozkaragoz and Cakin found the greatest reactivity between days 1 and 4 of the cycle.^{19,20} Recently, Kalogeromitros *et al.*¹⁴ reported a significant increase in wheal size to histamine, morphine and allergens (only for the atopic group) between days 12 and

16 of the cycle in atopic and non-atopic women. However, they did not investigate the possible relationship between these hormones and SPT reactivity by measuring synchronous levels of serum gonadotropins and sex hormones which modulate the menstrual cycle. Thus, we have studied SPT reactivity to histamine and sensitized allergens simultaneously with the serum lev-

els of the gonadotropins FSH and LH and the sex hormones estradiol and progesterone in our study. Finally, we have investigated the possible relationship between SPT reactivity and the serum levels of the hormones mentioned above.

As noted above, Kalogeromitros *et al.*¹⁴ indicated that skin reactivity to histamine was significantly higher in mid-cycle. Contrarily, we demonstrated that SPT reactivity to histamine, which was the positive control of the test, did not vary during the menstrual cycle. On the other hand, we have concluded that SPT reactivity to allergens was significantly higher in the middle compared to the beginning and the end of the menstrual cycle similar to the results of Kalogeromitros *et al.*¹⁴ Therefore, our results correlated with the results of Kalogeromitros *et al.*¹⁴ to some extent; the major difference is that we observed no change in SPT reactivity to histamine through the phases of the menstrual cycle. Histamine and mast cell degranulators (such as morphine, compound 48/80, etc.) are used as a positive control for allergen SPT.²¹

Histamine is the most useful positive control agent as it is the most important and functional vasoactive mediator of allergic response and mast cell degranulation.²² Previous *in vitro* studies demonstrated that increased estrogens at middle of the menstrual cycle might lead to enhanced histamine receptor expression on the nasal epithelia and endothelial cells.²³ Vliagoftis *et al.*²⁴ also showed an augmentation of mast cell degranulation by estradiol in peritoneal mast cells.²⁴ Therefore, it has been hypothesized that female sex hor-

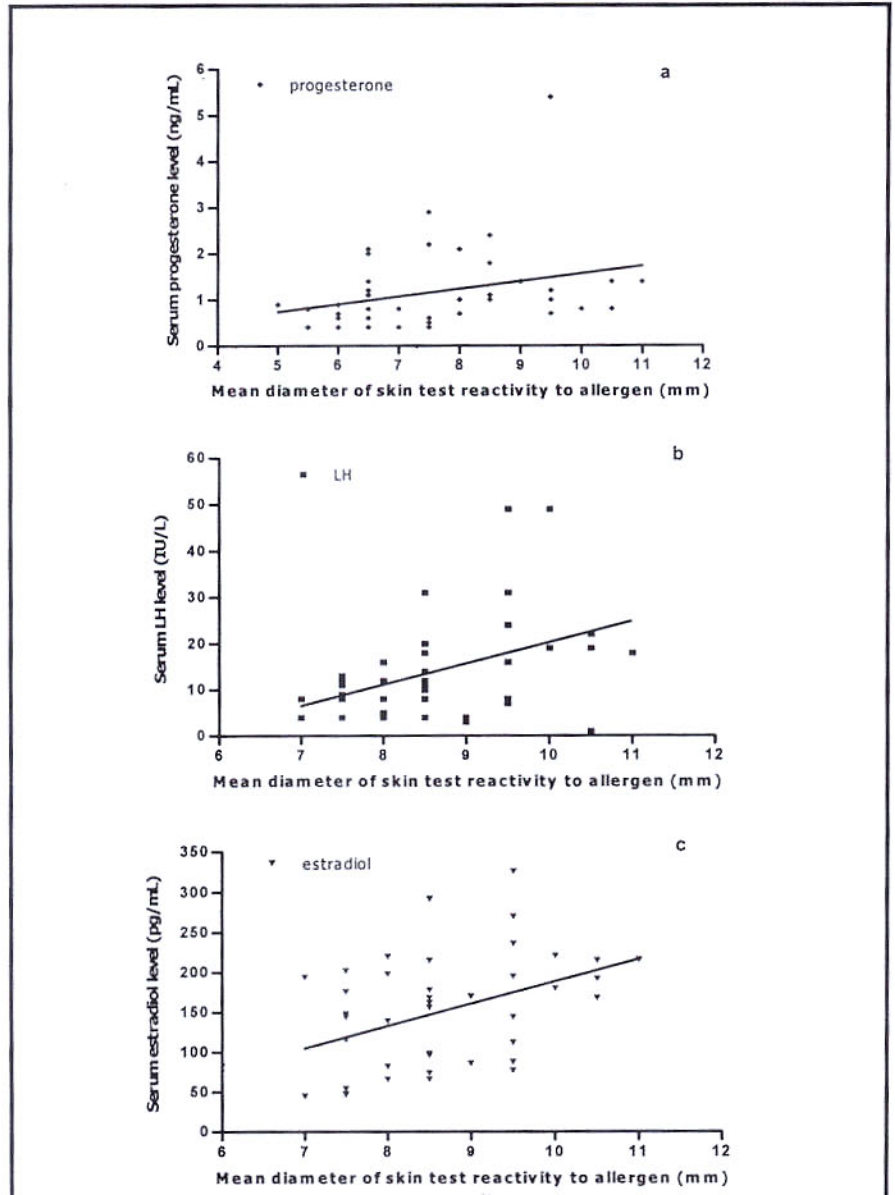


Fig. 2 a) Weak correlation between the mean diameter of skin test reactivity to the allergen mixture and serum progesterone levels at the beginning of the menstrual cycle ($r = 0.384, p < 0.05$); b) in the middle of the menstrual cycle, a significant correlation between the mean diameter of skin test reactivity to the allergen mixture and serum LH levels ($r = 0.41, p < 0.01$); c) significant correlation between the mean diameter of skin test reactivity to the allergen mixture and estradiol levels, in the middle of the menstrual cycle ($r = 0.437, p < 0.005$).

mones, particularly estrogens in the middle of the cycle may cause increased nasal or bronchial hyper-reactivity and congestion.^{25,26} More-

over, Kalogeromitros *et al.*¹⁴ reported enhanced SPT reactivity to histamine in the middle of the menstrual cycle and attributed this phenomenon to

enhanced skin reactivity due to increased estradiol levels. This differed from our results. This increased response to histamine was postulated to be due to the vasodilatory effect of histamine leading to increased vasopermeability. However, the vasomotor tonus depends not only on histamine but also on the autonomic skin response.²² The autonomic skin response is established by sympathetic and parasympathetic activation in the skin and does not change with time.^{22,27} Therefore, SPT reactivity to histamine should not change with time, circadian rhythm, or menstrual cycle. Our results support this latter assertion as SPT reactivity to histamine did not change during the menstrual cycle in our study. On the other hand, we observed that serum levels of estradiol correlated significantly with SPT reactivity to histamine only in the middle of the menstrual cycle. We thought that this could be a coincidence because the highest levels of estradiol were determined during this period as a result of normal physiology.

Unlike previous studies, we measured serum levels of gonadotropins and sex hormones along with SPT, and have investigated the presence of a possible relationship between SPT results and the serum levels of these hormones. Our results also indicated that SPT reactivity to allergens was significantly higher during the middle of the menstrual cycle, that is around ovulation, when compared to other phases which may be attributed to the elevated serum levels of gonadotropins or sex hormones. Our results supported that the levels of gonadotropins and sex hormones altered SPT results because serum levels of estradiol were significantly

increased in the middle of the menstrual cycle and had a significant positive correlation with the mean wheal size showing SPT reactivity to allergens. The serum levels of FSH, LH, estradiol and progesterone show fluctuations through the menstrual period.¹⁵

Different SPT reactivity to allergens during the phases of the menstrual cycle may be due to changes in mast cell degranulation or the secretion rate and vascular permeability caused by this hormonal fluctuation, particularly by estrogens.^{14,24} Additionally, it has been demonstrated in human and animal models that female sex hormones may alter the immune modulation like T lymphocyte differentiation, specific antibody production, pro-inflammatory mediator synthesis, cytokine synthesis, and receptor expression.^{28,29} That the significant increase in SPT reactivity to allergens at mid-cycle was observed when estrogen levels were at the highest point support the view that high estrogen levels may alter SPT reactivity to allergens. Since estrogens can enhance mast cell degranulation and mediator secretion, we postulate that the changes in the mast cell degranulation rate due to the elevated levels of estrogens during mid-cycle may play an important role for the significantly increased SPT reactivity to allergens.

This effect is supported by other mechanisms. First, estrogens, such as estradiol, enhance the mast cell degranulation and secretion rate directly and this effect can be prevented by estrogen receptor antagonists such as tamoxifene.²⁴ Secondly, estradiol also accelerates the activation of plasminogen in the coagulation pathway to form plasmin.^{30,31}

The latter mediator may activate the complement cascade leading to anaphylatoxins that can induce mast cell degranulation. Thirdly, high estrogen levels may lead to an increased local production of prostaglandin-E which is a potent vasoactive mediator.³² In addition to the increased mast cell degranulation effect, higher levels of estrogens may lead to increased vascular permeability and vasodilatation by an increased histamine receptor expression and a decreased vasomotor tone.²⁴ The clinical reflections of these effects may be observed as increased swelling and hyperactivity of the nasal mucosa and bronchial hyperactivity during the middle of the menstrual cycle related to higher estrogen levels.^{25,26,33}

In conclusion, the results of our study and other previous studies demonstrate that the menstrual cycle is an important factor altering SPT reactivity to allergens in allergen specific SPT. Additionally, we also concluded that serum estradiol levels demonstrated a significant correlation with SPT reactivity to allergens. Thus, these results should be considered when evaluating allergen reactivity in menstruating women. In the light of our study supported by previous reports, we suggest that SPT gives the best results when performed during mid-cycle in menstruating women.

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