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# Influence of the menstrual cycle on skin-prick test reactions to histamine, morphine and allergen

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**Summary**

The purpose of this study was to examine the possible influence of the phases of the menstrual cycle on dermal reactivity to skin-prick testing. We studied 15 atopic, menstruating women with seasonal rhinoconjunctivitis and/or asthma, with known sensitivity to olive and parietaria (mean age 25·2 years) and 15 non-atopic, healthy, female controls (mean age 24·7 years). Skin-prick tests with histamine, morphine, and in the atopic group with parietaria/and/or olive, were repeated three times during the same menstrual cycle, corresponding to bleeding (day 1–4), midcycle (day 12–16) and the late progesterone phase (day 24–28). None of the patients had received oral antihistamines or exogenous hormones for at least 1 month prior to testing. Results indicate a significant increase in weal-and-flare size to histamine, morphine, and parietaria on days 12–16 of the cycle, corresponding to ovulation and peak oestrogen levels. This was observed in both atopic and non-atopic women. Differences in skin reactivity to histamine and morphine between the groups were not significant. Therefore, in women, the phase of the menstrual cycle is another factor that may influence skin-test results.

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## Introduction

Skin-prick testing is a very sensitive method of evaluating for the presence of specific IgE antibodies. Skin testing has been used to diagnose allergic diseases since the 19th century. In 1865, Blackley first introduced scratch testing on the forearm, scratching then placing cereal pollens at the site, thereby inducing a weal-and-flare reaction [1]. Later, in 1924, Lewis described the classical histamine induced 'triple response' of the skin [2], consisting of local erythema, circumferential erythema and a central weal.

Numerous factors can influence the reactivity of the skin to skin testing with various reagents such as histamine, opiates or allergens. These include the site of the body [3,4] age [5] sex [6,7], race [8], ingestion of drugs, most notably of anti-histamines, and circadian rhythms [9,10].

The effect of the circadian rhythms on skin-test responses to histamine was first reported by Cromia (1952), with a peak in the late evening and a decrease in the morning [11]. A number of more recent studies have reporting circadian variations in skin reactivity to histamine and allergen, as well as bronchial reactivity and T-lymphocyte function [9,12–16].

Fluctuations in endogenous hormones influence skin-test reactivity [9]. In addition, exogenous sex hormones, in the form of combination oral contraceptives (oestrogen and progesterone), have been reported to decrease skin reactivity to histamine [16]. Prolonged doses of oral corticosteroids can also decrease dermal responses to histamine and codeine [17].

During the menstrual cycle endogenous variations in sex hormone levels occur. In this study, we examined skin-test reactivity in the different phases of the

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**Table 1.** Characteristics of the groups

| Parameter | Atopic | Non-atopic |
|---|---|---|
| $n =$ | 15 | 15 |
| Sex | F | F |
| Age | | |
| Range (years) | 20–30 | 20–30 |
| Mean± SEM | 25·4 ± 0·8 | 25·3 ± 0·8 |
| Onset of menses | | |
| Range (years) | 12–14 | 12–14 |
| Mean± SEM | 12·87 ± 0·1 | 12·73 ± 0·2 |
| Cycle | | |
| Range (days) | 28–29 | 28 |
| Mean± SEM | 28·2 ± 0·1 | 28 |
| IgE | | |
| Range (IU/ml) | 25–320 | 20–120 |
| Mean± SEM | 102·9 ± 18* | 52·3 ± 7* |

* None of the differences were statistically significant except IgE levels ($P < 0·005$). SEM = standard error of the mean.

cycle and found that weal-and-flare reactions were significantly increased in mid-cycle (days 12–16) corresponding to the time of follicular rupture and peak oestrogen levels.

## Materials and methods

### *Patient selection*

We studied 15 women with seasonal rhinoconjunctivitis and/or asthma who had documented reactivity to olive and/or parietaria on skin-prick tests. These are the two most prevalent allergens in our region. Selected patients met the following inclusion criteria:

(1) a regular menstrual cycle lasting 28–30 days;
(2) no pregnancy or delivery for at least 1 year prior to testing;
(3) onset of menses at age 12–14 years;
(4) oral antihistamines, oral corticosteroids or other agents that suppress skin-test reactivity were not used for at least 1 month prior to testing;
(5) denied use of oral contraceptives.

A control group of 15 sex-matched healthy, non-atopic women who met the same criteria, were chosen from the hospital setting.

### *Skin testing*

Skin-prick tests were performed at the out-patient Allergy and Dermatological Clinic of the A. Sygros Hospital, Athens, Greece by an allergist (D.K.). Briefly, tests were performed using standard techniques [9] and prick lancets (Hollister-Stier/disposable), on the upper back, on both sides of the spine. The following solutions were used: histamine 1% (1/20) glycerinated for skin tests (Dome/Hollister-Stier, Sens, France); morphine O,O15 mg/ml (National Pharmaceutical, Athens, Greece) in buffered sterile albumin diluent (Dome/Hollister-Stier, Sens, France); olive extract 1/20 glycerinated for skin tests, (Dome Hollister-Stier, Sens France) and parietaria extract (as for olive).

All 30 subjects and controls were tested with histamine and morphine. The allergens olive and parietaria were only applied in the atopic group. The non-atopic group was skin tested to a panel of common inhalant allergens, including olive and parietaria, and found to be non-reactive. All patients were tested using the same batch of solutions.

Skin tests were done in the morning between 09.00 h and 11.00 h in all subjects. They were performed on days 1–4 of the menstrual cycle, repeated between days 12–16 and again between days 24–28 of the same cycle.

The weal-and-flare responses of the skin were measured at 15 min by D.K. The outline of each reaction was first marked on the skin, and then transferred to transparent adhesive tape and filed as a permanent record. Interpretation was not blinded, but results were not compared until the study was completed. Each area was later measured with a electronic planimeter, HAFF E318, with scale of 1 : 1. The mean of two measurements was used and expressed in $cm^2$.

### *Total IgE*

Serum total IgE levels (in IU/ml) was measured in atopic and non-atopic women with a Kallested microplate assay kit (Pasteur diagnostics) based on the two site immuno-radioactive technique (IRMA) [18].

### *Statistical comparisons*

For each substance, differences in weal-and-flare size between the three phases of the menstrual cycle and between the atopic and non-atopic groups were analysed using multivariate analysis of variance (MANOVA) (SPSS program/IBM) and specific comparisons between days 12–16, days 1–4 and days 24–28 using non-parametric sign-test with continuity correction. IgE levels were compared using log transformed $t$-test. A $P < 0·05$ is considered significant.

## Results

The two patient groups had similar age and menstrual

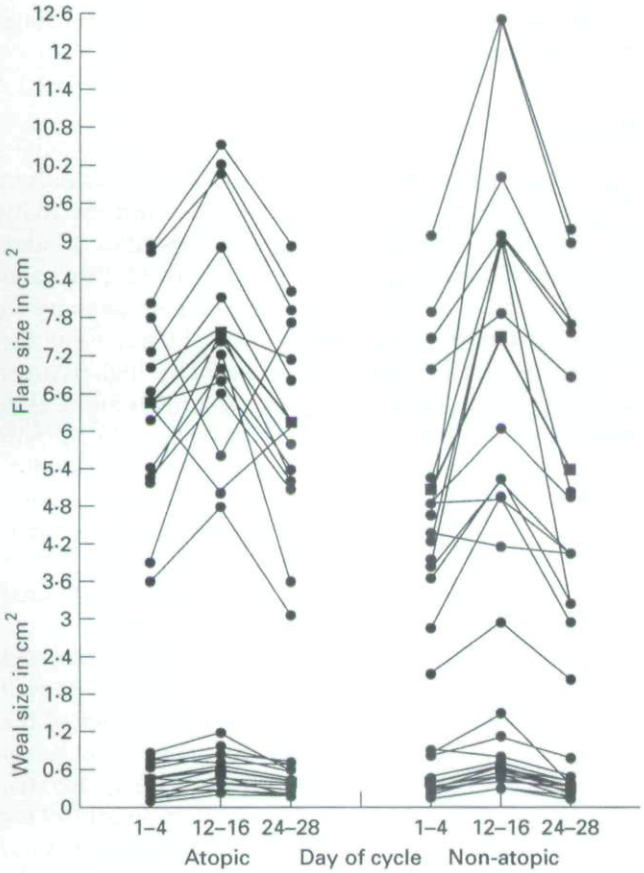


**Fig. 1.** Mean weal-and-flare response to histamine prick test ($cm^2$) in 15 atopic and 15 matched non-atopic females on different days of the menstrual cycle. Differences between weal-and-flare sizes on days 12–16 vs days 1–4 and 24–28 were significant in both atopic and non-atopic groups. See text for mean values and *P* values.

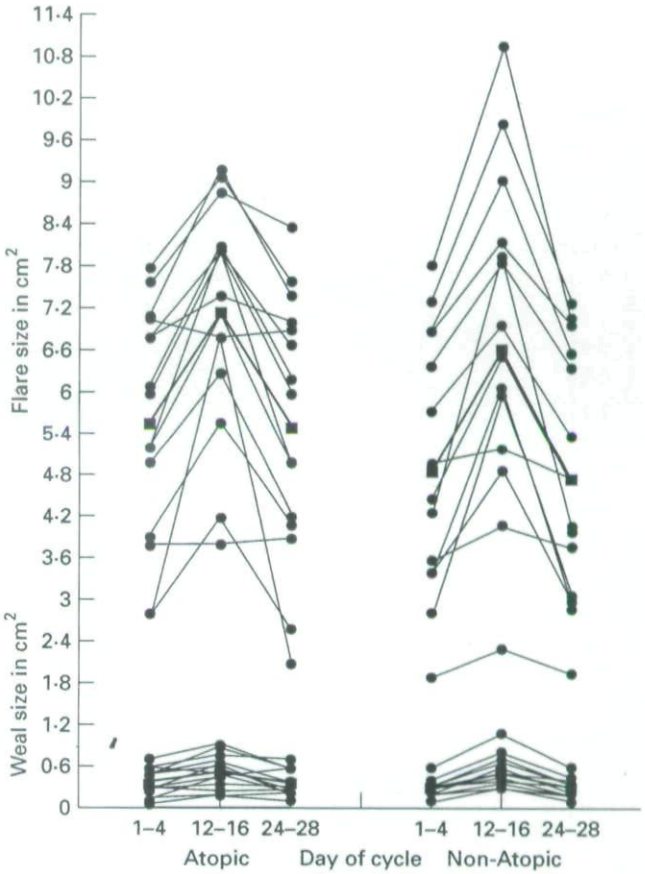


**Fig. 2.** Weal-and-flare area to morphine prick testing in atopic ($n = 15$) and non-atopic females ($n = 15$) as $cm^2$, on different days of the menstrual cycle. Differences between weal-and-flare sizes on days 12–16 vs days 1–4 and 24–28 were significant in atopic and non-atopic groups. See text for mean values and *P* values.

**Table 2.** Mean weal-and-flare size ($cm^2$) to histamine and morphine prick tests in 15 atopic (A) and 15 non-atopic (NA) menstruating-females

| | Histamine | | | | Morphine | | | |
|---|---|---|---|---|---|---|---|---|
| | Weal | | Flare | | Weal | | Flare | |
| Day of cycle | A | NA | A | NA | A | NA | a | NA |
| 1–4 | 0·45 | 0·38 | 6·45 | 5 | 0·38 | 0·28 | 5·6 | 4·9 |
| 12–16 | 0·61 | 0·63 | 7·56 | 7·43 | 0·54 | 0·52 | 7·17 | 6·7 |
| 24–28 | 0·44 | 0·31 | 6·14 | 5·38 | 0·36 | 0·29 | 5·54 | 4·8 |

See text for *P* values.

characteristics (Table 1). IgE levels were significantly higher in the atopic group ($P < 0{\cdot}005$).

Weal-and-flare responses in $cm^2$ to histamine for the 15 atopic and 15 healthy non-atopics were maximal at days 12–16 of the cycle (Fig. 1). Mean areas are shown in Table 2. Differences were significant when comparing days 12–16 to days 1–4 (for weal $P < 0{\cdot}02$ in atopics and $P < 0{\cdot}002$ in non-atopics; for flare $P < 0{\cdot}008$ in atopics and $P < 0{\cdot}002$ in non-atopics) and days 24–28 (for weal $P < 0{\cdot}0002$ in both atopic and non-atopic groups; for flare $P < 0{\cdot}008$ and $P < 0{\cdot}0002$ in atopic and non-atopics, respectively).

Weal-and-flare responses to morphine in the atopic and non-atopic women are shown in Fig. 2. Mean areas, outlined in Table 2, were significantly greater on days 12–16, as compared with days 1–4 (for weal $P < 0{\cdot}008$ in atopics and $P < 0{\cdot}0002$ in non-atopics; for flare $P < 0{\cdot}002$ in atopics and $P < 0{\cdot}0002$ in

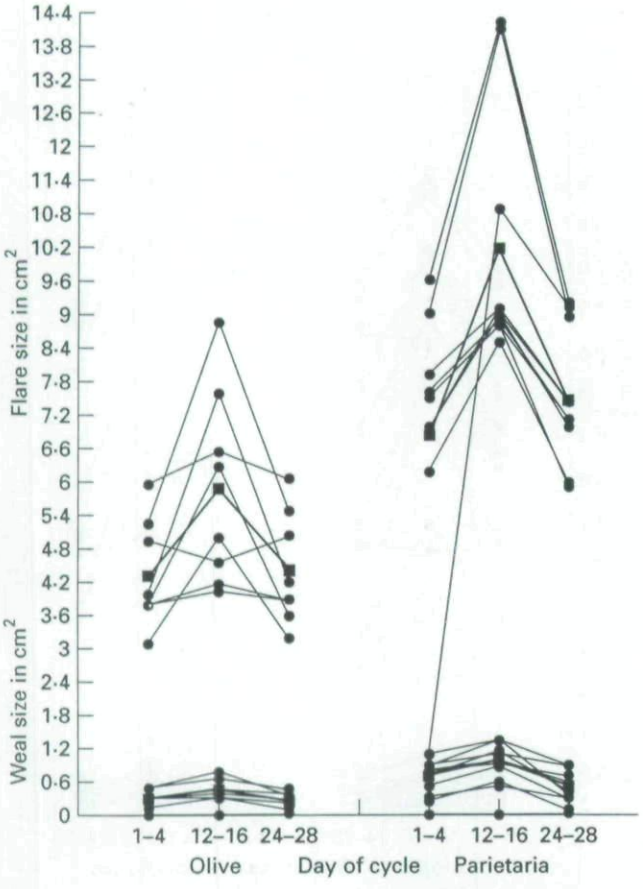


**Fig. 3.** Weal-and-flare response of atopic menstruating females to olive and parietaria in relation to days of cycle. See text for mean values and *P* values. Differences between days 12–16 vs days 1–4 and 24–28 were significant for weal size to olive and parietaria and flare size to parietaria.

non-atopics), and as compared with days 24–28 (for weal $P < 0{\cdot}004$ in atopics and $P < 0{\cdot}0002$ in non-atopics; for flare $P < 0{\cdot}008$ in atopics and $P < 0{\cdot}0002$ in non-atopics).

Differences between the atopic and non-atopic groups were not statistically significant for either histamine or morphine. Weal-and-flare responses to olive and parietaria in the 15 atopic patients are shown in Fig. 3. The mean weal sizes for olive in the skin-test positive patients were $0{\cdot}35\,cm^2$ on days 1–4 vs $0{\cdot}52\,cm^2$ on days 12–16 and $0{\cdot}3\,cm^2$ on days 24–28. Differences between days 12–16 and days 1–4 ($P < 0{\cdot}02$) and days 24–28 ($P < 0{\cdot}008$) were significant. Differences in mean flare values for olive were not significant.

Mean weal values for the patients skin-test positive to parietaria were $0{\cdot}69\,cm^2$ on days 1–4 vs $1{\cdot}0\,cm^2$ on days 12–16 and $0{\cdot}5\,cm^2$ on days 24–28 ($P < 0{\cdot}003$ for days 12–16 compared with days 1–4, and 24–28). Mean flare values were $6{\cdot}9\,cm^2$ on days 1–4 vs $10{\cdot}2\,cm^2$ on days 12–16, and $7{\cdot}5\,cm^2$ on days 24–28 ($P < 0{\cdot}003$ for days 12–16 vs days 1–4 and 24–28).

## Discussion

The potential influence of hormonal fluctuations during the phases of the menstrual cycle on skin-test reactivity has not been thoroughly investigated. Studies to date report conflicting data. The first studies by H. Pruss and Raymond in the 1940s, reported maximal reactivity to allergens on day 3 and 18 of the cycle [19] and using the P–K passive transfer test, demonstrated the highest titres of reagins on day 3. In the 1970s, Ozkarogor and Cakin reported that 70 of 246 skin-test reactions (23%) with allergens were significantly greater during menses (i.e. corresponding to days 1–4) [20]. On the contrary, Bosse and Ladebeck found that skin reactivity to histamine is lowest on day 1 and 20 of the cycle [21]. Another report supports greatest reactivity on day 1 and 29 and minimal reactivity on day 15 [16].

In our study, dermal reactivity to histamine and morphine, a non-specific mast cell degranulator, as well as allergens was found to vary during the phases of the cycle and was significantly greater around ovulation (days 12–16) than during menses (days 1–4) and the last days of the cycle (days 24–28). In order to control for changes in dermal reactivity related to circadian rhythm, skin tests were performed within a 3 h period in the morning, in all patients. In addition, none of the patients we studied were using exogenous corticosteroids or sex hormones. Our results are most in accordance to those of Bosse and Ladeek [21] and demonstrate the need for further studies to clarify the issue. Differences in dermal reactivity to histamine and morphine between atopic and non-atopic women were not significant.

The normal function of the genital cycle in the female is dependent on interactions between the hypothalamus, the hypophysis and the ovaries. Circulating levels of central stimulating factors and endogenous hormones rises and fall in a predictable pattern [22].

During days 1–4 of the cycle, corresponding to bleeding, serum levels of follicle stimulating hormone (FSH), leutinizing hormone (LH), 17 p-oestradiol (< 50 pg/ml) and progesterone (< 1 ng/ml) are all low. On days 12–16, around the time follicular rupture or ovulation occurs, progesterone remains low while levels of FSH, LH and 17 p-oestradiol (up to 200 pg/ml) peak. Days 24–28 correspond to the end of the corpus luteum, when FSH and LH are low, while progesterone as well as oestrogen are mildly to moderately elevated. Thus, maximal skin-test reactivity in our patients corresponded to elevated oestrogens (and FSH, LH) and low progesterone levels.

The differences in skin-test reactivity observed during the phases of the menstrual cycle may theoretically be due to changes in vascular permeability and vasodilation mediated directly or indirectly by changes in oestrogen and progesterone levels. But, female sex hormones can also affect immune function in general [23], influencing T cell populations, the production of specific antibodies, clotting factors, pro-inflammatory mediators and mast cell degranulation.

Oestrogens induce antibody production and increase serum levels of IgG and IgA in rodents (24–26). As suggested by Paavonen *et al.* these effects may be related to their ability to decrease T suppressor cells [27]. Rodents exposed to antigen expand mature B cell populations in response to oestrogens. In addition, daily administration of oestrogens prevents the physiological fall in levels of specific antibodies after the last dose of antigen [28,29]. Oestradiol also acts on plasminogen, in the coagulation pathway, to form plasmin [22]. The latter can activate complement leading to production of anaphylatoxins, that can induce mast cell degranulation. Thus, oestrogens can be considered to enhance the humoral immune response.

Oestrogen receptors have recently been found on rat peritoneal mast cells and oestradiol reportedly augments non-specific mast cell degranulation *in vivo* [30]. Although concentrations of oestradiol were very high (non-physiological) and responses of human skin mast cells have not been looked at, this raises the intriguing concept that oestrogen can potentially effect mast cell degranulation in response to certain stimuli.

In the presence of progesterone, T-suppressor cell populations are increased [31] and clonal lymphocyte proliferation is depressed [23]. Progesterone also suppresses, in a dose-dependent fashion, IgE mediated histamine release from basophils, as shown in patients with cyclic urticaria by Mittman *et al.* [32].

In the fluid of the ovarian follicle, where sex hormones receptors are abundant, high oestrogen levels in combination with low progesterone levels increase local production of PGE and F [33]. In the skin, prostaglandins have potent vasoactive and pro-inflammatory potential. But, the role, if any, of sex hormones on prostaglandin production and release in the skin has not been investigated.

Thus, elevation in concentrations of oestrogens or progesterone, or both, could potentially induce changes in responsiveness of vasculature, and of the local immune milieu. Such changes in the skin could be translated into changes in dermal reactivity to histamine and IgE mediated (i.e. allergen induced) or non-IgE mediated (i.e. morphine induced) mast cell degranulation. This is only speculative, however.

In conclusion, results of this and other studies, strongly suggest that the phase of the menstrual cycle is an additional endogenous factor that should be considered when evaluating skin-test reactivity in menstruating women. This needs to be considered when comparing changes in skin reactivity, as for example pre- and post-allergen immunotherapy. Further studies are needed to clarify the role of sex hormones on immediate skin-test reactions and on the dermal immune response, in general.

## References


1 Blackley CH. Hay fever: its causes, treatment and effective prevention; experimental researches. 2nd edn. London: Baillière, Tindal's Cox, 1880.

2 Lewis T, Grand RT. Vascular reactions of the skin to injury Part II. The liberation of a histamine-like substance in injured skin the underlying cause of factitious urticaria and of weals produced by burning; and observations upon the nervous control of certain skin reactions. Heart 1924; 11:209–13.

3 Bowman KL. Pertinent factors influencing comparative skin tests on the arm. J Allergy 1935; 7:39–43.

4 Clarke CW, Mitchell J, Nunn AJ, Pepys J. Reproducibility of skin prick tests to five common allergens. Clin Allergy 1982; 12:21–5.

5 Skassa WJ, Bousquet J, Menardo JL, Michel FB. Skin test reactivity to histamine from infancy to elderly. J Allergy Clin Immunol 1987; 80:711–16.

6 Haahtela T, Bjorksten F, Heisakala M, Suoneimi I. Skin prick test reactivity to common allergens in Finnish adolescents. Allergy 1980; 35:425–8.

7 Hendrick DJ, Davies RJ, D'Sousa MF, Pepys J. Analysis of skin prick test reactions in 656 asthmatic patients. Thorax 1975; 30:2–8.

8 Van Niekerk CH, Prinloo AEM. Effect of skin pigmentation on the response to intradermal histamine. Int Arch Allergy Appl Immunol. 1985; 76:73–5.

9 J Bousquet. In vitro methods for study of allergy: Skin test techniques and interpretation. In Allergy Principles and Practice. Eds. A. Middleton, Jr., C. Reed, N. F. Adkinson, J. Yunginger. CV Mosby Co., Missouri, 1988; 420–30.

10 Lee RE, Smolensky MH, Leach CS, M Govern JP. Circadian rhythms in the cutaneous reactivity to histamine and selected antigens, including phase relationship to uninary cortisol excretion. Ann Allergy 1977; 38:231–5.

11 Cromia FE. Experimental histamine pruritus. I. Influence of physical and psychological factors on threshold reactivity. J Invest Dermatol 1952; 19:21–4.

12 Reinberg A. Hours of changes in responsiveness and susceptibility. Perspect Biol Med 1967; 1:11–3.

13 Reinberg A, Sidi E, Ghata J. Circadian reactivity rhythms of human skin to histamine or allergen and the adrenal cycle. J Allergy 1965; 36:273–8.

14 Ascoff J, Wever R. Human circadian rhythms: a multi-oscillatory system. Fed Proc 1976; 35:2326–32.
15 Fernades G, Halberg F, Good RA. Circadian rhythms in T, B, and natural killer cells. In Smolensky MH, et al. eds. Recent advances in the chronobiology of allergy and immunology. Pergamon Press Inc., 289–99. New York, 1980.
16 Fitzerland GA, Barnes P, Brown MJ, Dollery CT. The circadian variability of circulating adrenaline and bronchomotor reactivity in asthma. In Smolensky MH, et al, eds. Recent advances in the chronobiology of allergy and immunology. Pergamon Press Inc, 89–94. New York, 1980.
17 Olson R, Karprink M, H Shelansky S et al. Skin reactivity to codeine and histamine during prolonged corticosteroid therapy. J Allergy Clin Immunol 1990; 86:153–9.
18 Woodhead H, Addison G, Hales C. The immunoradiometric assay and related techniques. British Med Bulletin 1974; 30:44–9.
19 Hansen-Pruss OC, Raymond R. Skin reactivity during the menstrual cycle. J Clin Endocrinol Metabol 1942; 2:161–5.
20 Ozkaragoz K, Cakin F. The effect of menstruation on immediate skin reactions in patients with respiratory allergy. J Asthma Res 1970; 7:171–6.
21 Bosse E, Ladebeck HE. Die kutane Histaminzeaktion in Kozzelation zum Cycles der Frau. Z. Haut Geschlechtisky 1972; 47(8):365–7.
22 Fransis J, Greenspan S, Peter H, Forshan. Basic and clinical endocrinology LANGE medical publication/Los Altos California Chapter 14. Alan Goldfien M.D. and Scott E. Monroe M.D. The ovaries 1986; 385–429.
23 Grossman JC. Regulation of the immune system by sex steroids. Endocrine Reviews 1984; 5:435–55.
24 Stern K, Davidson I. Effect of oestrogen and cortisone on immune hemoantibodies in mice of inbred strains. J Immunol 1955; 74:479–82.
25 Von Haam E, Rosenfeld I. The effect of oestrone on antibody production. J Immunology 1942; 43:109–13.
26 Wira CR, Sandoe VP. Hormonal regulation of immunoglobulins: Influence of oestradiol on immunoglobulins A and G in the rat uterus. Endocrinology 1980; 106:1020–6.
27 Paavonen T, Anderson LC, Adlercreuts H. Sex hormone regulation of in vivo immune response. Oestradiol enhances human B cell maturation via inhibition of suppressor T cell in pokeweed mitogen–stimulated cultures. J Exp Med 1981; 154:1935–45.
28 Feigen GA, Fraser RC, Peterson NS, Dandlikes WB. Sex hormones and the immune response. I Host factors in the production of penicillin specific antibodies in the female guinea pig. Int Arch Allergy Appl Immunol 1978; 57: 385–98.
29 Feigen GA, Fraser RC, Peterson NS. Sex hormones and the immune response. II Pertubation of antibody production by oestradiol 17$\beta$. Int Arch Allergy Appl Immunol 1978; 57: 488–97.
30 Viagothis H, Dimitriadou V, Boucher W et al. Oestradiol augments while tamoxifen inhibits rat mast cell secretion. Int Arch Allergy Immunol 1992; 98:398–409.
31 Holdstock G, Chastenay BF, Crawitt EL. Effects of testosterone, oestradiol and progestone on immune regulation. Clin Exp Immunol 1982; 47:449–52.
32 Mittman RJ, Bernstein DI, Steinberg DR, Enrione M, Bernstein IL. Progesterone responsive urticaria and eosinophilia. J Allergy Clin Immunol 1989; 84:304–10.
33 Speroff L, Glass HR, Kase GN. Endocrinologia gynecologica clinica e infertilita. Ermes-SRL-Milano, 1986, p. 295 (edizione italiana).