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Effect of systemic hormonal cyclicity on skin

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Synopsis

Fluctuations in estrogen and progesterone during the menstrual cycle can cause changes in body systems other than the reproductive system. We conducted several studies to determine a possible correlation between phases of the menstrual cycle and specific skin properties. Healthy Caucasian women (ages 21–48), who had a typical 26–29 day menstrual cycle, participated in the studies. Measurements of skin barrier strength, dryness, response to lactic acid stinging, skin surface lipids, and microflora were obtained every week for two to three months. Ultraviolet B susceptibility in terms of minimal erythemal dose was also studied. The skin barrier was the weakest between days 22 and 26 of the cycle. Elevated neuronal response (lactic acid sting) was not observed to vary much with the cycle. Skin was driest between day 1 and day 6, while skin surface lipid secretion appeared to be highest on days 16–20 of the hormonal cycle. The highest microbial count was around days 16–22, and there was a high UV-B susceptibility between days 20 and 28 of the menstrual cycle.

INTRODUCTION

Menstrual cyclicity is a major biological process for women during their reproductive years and is associated with significant changes in hormonal status and behavior. Androgen excess from the ovaries or adrenal overproduction from the cutaneous metabolism of precursor hormones may be responsible for acne in some women (1–3). In addition to acne, other skin changes like skin thickness and echodensity (4), changes in transepidermal water loss (TEWL) and blood flow (5–6), neuronal responses like pain (7), and irritant stimulus (8) have been implicated as consequences of hormonal changes.

Clinical studies were designed to study changes in skin during the course of systemic fluctuations in estrogen and progesterone in a monthly menstrual cycle. In the first study, blood levels of progesterone and estradiol were measured once a week for a month. Additionally, barrier functions, neuronal responses, skin moisturization, and skin surface lipids and their correlation with hormonal changes in blood were studied. In

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another study, the quantity of skin microflora was correlated with hormonal changes, and in a third study UV sensitivity was observed.

MATERIALS AND METHODS

SUBJECTS

Females of ages 21–48, with no evidence of acute or chronic disease, including dermatological or ophthalmologic problems, were enrolled in the study. In order to qualify, the women had to be in a spontaneous ovulatory menstrual cycle. The subjects were not on any hormonal therapy or infertility treatment and had not taken any oral contraceptives for six months prior to the study. The facial skin had to be free of warts, nevi, moles, sunburn, suntan, scars, and active dermal lesions.

On the day of the study, the subjects were instructed to refrain from using any lotions, creams, or other products on the face. Subjects were allowed to equilibrate for at least 30 minutes prior to testing in a controlled environment of 68°–70°F and 25% relative humidity.

HORMONAL CYCLICITY

Twenty-six female participants, ages 21–48, participated in the study. Blood was drawn from the subjects once a week at the same hour of the day by a trained technician following the standard operating procedures for phlebotomy at the clinical testing laboratory. The blood was centrifuged (100 rpm for 15 min) to pellet the blood cells and collect the serum. The serum was kept frozen until analyzed.

Progesterone and estradiol were assayed from serum, employing a competitive immunoassay using direct chemiluminescence technology. Progesterone in the patient sample binds to an acridium ester-labeled mouse monoclonal anti-progesterone antibody. Unbound antibody binds to a progesterone derivative, covalently coupled to paramagnetic particles in the solid phase. Relative light units (RLU) detected by the system determine the quantity of progesterone.

Estradiol in the patient sample competes with acridium ester-labeled estradiole in the reagent for a limited amount of rabbit anti-estradiol antibody in the antibody reagent. Rabbit anti-estradiole is captured by mouse anti-rabbit IgG, which is coupled to paramagnetic particles in the solid phase. RLU detected by the system determine the quantity of estradiol (9).

EFFECTS OF SYSTEMIC HORMONAL FLUCTUATION ON SKIN

The same participants as in the previous study used a simple TEA stearate-based lotion two weeks prior to commencement of the study and for the first month of the study. The subjects applied the assigned test materials on the full face twice a day and were instructed not to change their daily cleansing regimen and makeup for the course of the study.

The subjects reported to the clinical testing facility once a week at the same hour of the

day, for a month. Data was organized according to the subject's time of the menstrual cycle and labeled as the day of the month relative to the onset of menstruation, which was day 1. The following measurements were obtained every week for four weeks.

Barrier functions and repair. Transepidermal water loss was used as the parameter for barrier integrity (10–12). The test sites were the right and left facial cheek areas. A sticky tape (Tesa Tuck, Tape Systems, Charlotte, NC) was used to cover the test area and, after a firm stroke in both directions, the tape was peeled off (12); three strippings were obtained. Transepidermal water loss (TEWL) measurements were recorded using an EP-1 evaporimeter (Servomed, Sweden) at three sites within the stripped area, pre- and post-stripping. The skin was stripped in increments of three, followed by TEWL measurements, until a TEWL of 18 $g/m^2/hr$ was reached. Damage to the skin barrier is described in terms of the increase in the rate of water loss. The exact number of strippings required to damage the skin barrier (TEWL = 18 $g/m^2/hr$ or more) was calculated by plotting TEWL vs the number of strippings and interpolations.

Lactic acid sting. As per the protocol of Frosch and Kligman (13), a 10% solution of lactic acid (98% purity, Sigma, San Diego, CA), prepared in phosphate buffer saline, was used for this study. Lactic acid was applied on the nasolabial fold of one side of the face, and saline was applied to the other side. Any adverse reaction (itching, burning, stinging) reported by the subjects was recorded after 2.5 minutes and 5 minutes. The stinging intensity was graded by the subjects as mild, moderate, or severe (scored as 1, 2 or 3, respectively). The sums of the scores at both time points were calculated as the cumulative intensity of the stinging effect.

Moisturization. Skin moisturization was measured via the Nova Meter DPM 9003 (NOVA Technology Corporation, Portsmouth, NH) following the protocols of Tagami (14) and Barel and Clarys (15). Surface electrical capacitance provides a representation of skin surface hydration that is inversely proportional to electrical impedance. The Nova measures an output proportional to the skin's electrical capacitance in the Mhz frequency range. Data acquisition is software-controlled. The higher the skin water content, the higher the output (in arbitary units), and hence, the more moisturized is the skin.

Skin surface lipids. Skin surface lipids were evaluated using a sebumeter SM810 (Courage and Khazaka, Cologne, Germany) as described by Cunliffe *et al.* (16). Three-hour accumulation of skin surface lipids was measured. Briefly, three hours after washing the forehead with a mild liquid soap, a translucent plastic strip 0.1-mm thick and with an area of 64 mm^2 is applied on the skin and held at constant pressure for a defined time interval, during which sebum absorbs to the strip. The sebumeter measures the increase in the transparency of the strip when it becomes soaked with sebum. The strip is backed by a mirror, which presses it against the skin with a force of 10N by means of a spring. The instrument contains a timing device, which allows for a 30-second measurement. The transparency of the strip is evaluated by means of a microprocessor and is read off a digital instrument directly as gram of sebum per square centimeter.

Skin microflora (17–18). Twenty-eight female participants, ages 21–48, were selected for the study based on the criteria described above. The mornings of each visit the subjects washed their face thoroughly with a given mild (anionic, sodium laureth sulfate base) liquid soap and warm water. The subjects were instructed not to wash or even touch their faces for the next three hours. Three-hour bacterial growth was obtained in a saline wash collected from the forehead using a cup-scrub method. A glass cup was held

against the forehead and 1 ml of saline (Dulbecco's phosphate-buffered saline) was poured into the cup. The skin was scrubbed with a rubber policeman (ten strokes) and washed, and then the saline was aspirated and collected in 9 ml of PBS.

The sample was analyzed for aerobic bacterial count: One milliliter of sample was diluted into 10-ml tubes of Difco's TAT broth base in order to obtain a 1:10 and 1:100 dilution. Also, 1 ml of sample was plated directly in Trypticase soy agar (TSA) (Fisher Scientific, Pittsburgh, PA) so that the samples containing lower counts of bacteria could be detected. All TSA plates were then incubated for 48 hours at 37° C and the colonies counted. The results were expressed as microorganisms per square centimeter of skin. To characterize the bacteria, gram staining was conducted. The grown populations were described by their genus if they appeared to be a certain majority. Plates having more variability in microflora required the use of BBL Crystal Mind software and identification kits (Becton Dickenson Microbiology Systems, Becton Dickenson and Co., Cockeysville, MD) to further characterize some organisms (19).

UV effects (20–21). Twenty female participants, ages 21–48, were selected for the study based on the inclusion and exclusion criteria listed above. The source of radiation was a xenon-arc Berger solar simulator (Solar Light Co, Philadelphia, PA) for UV-B irradiation, using an interference filter with a range of 280 nm to 320 nm and a peak of 300 nm in addition to WG 320 and UG-11 filters. The test site was the backs of the subjects. The minimal erythemal dose (MED) of the subjects was obtained every week for eight weeks. Seven sites (~ 2 cm in diameter) were exposed to UV-B in 25% increments, and erythema was visually graded after 24 hours. The minimal energy level (mJ/cm^2) to induce a slightly pink erythema after 24 hours is the MED. These MED measurements were obtained every week for two months. During the course of the study, the subjects were instructed to refrain from applying any topical agents to their backs and to totally avoid exposure to the sun.

DATA ANALYSIS

Data were plotted versus the day in the cycle, considering as day 1 the day of the onset of menstruation. For each person, the day of the cycle was documented at each visit. For every physiological property measured in this study, frequency histograms were plotted, displaying the number of subjects showing the highest (or lowest) score at a particular day of the cycle *versus* the day of the cycle.

RESULTS

HORMONAL CHANGES

Progesterone and estradiol levels from the blood work were correlated to the time of the month for each panelist. There was a large variability in the levels of estradiol (10–430 RLU, 30–400 picograms/ml) and progesterone (0–700 RLU, 0–2,800 ng/dl), and so the four measurements for each person (per month) were scrutinized and the day of the highest hormone level was recorded and referred to their day of the month. The histogram of the number of subjects having the highest hormone level at a particular day of the cycle versus the days of the cycle is reported in Figure 1. Figure 1 also displays the

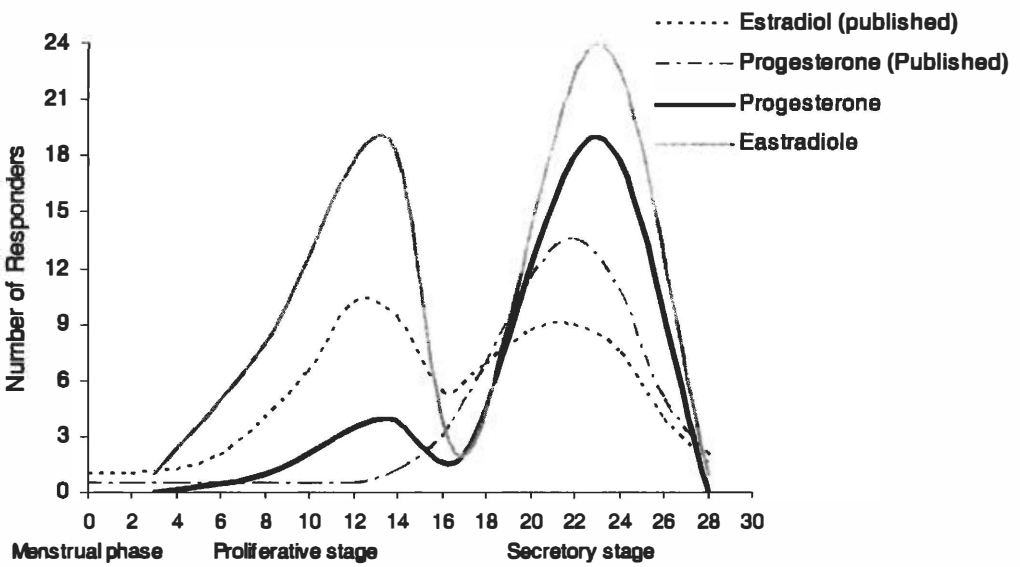

Figure 1. Hormonal changes in blood over the course of a month. Published levels are the dotted lines with arbitrary units. It is clear that the increase and reduction of hormones over the course of a month match published data when calculated based on the number of subjects exhibiting the highest levels over the course of the month.

rise and fall of the hormones during the menstrual cycle, obtained by Toot *et al.* (22). In the normal menstrual cycle, progesterone is produced after ovulation (days 15–28), with peaks of 250–2,800 ng/dl of progesterone in serum (22). The levels of estradiol generally reach a maximum one day before the lutinizing hormone (LH) peak, after which there is a marked reduction followed by a rise again during the luteal phase, 5–7 days after ovulation (22) (Figure 1).

EFFECT OF SYSTEMIC HORMONAL FLUCTUATIONS ON SKIN

Barrier. The effect of the menstrual cycle on skin barrier functions is reported in Figure 2, which displays the histogram of the number of subjects exhibiting the weakest barrier (i.e., the number of subjects who required the lowest number of strippings to disrupt skin barrier at a certain day in the cycle versus the days in the cycle). In Figure 2, the histogram is superimposed onto the published data for progesterone and estradiol levels in serum. It is clear from Figure 2 that most respondents had the worse barrier between days 22 and 26. It must be borne in mind that the deterioration of the barrier was extremely slight, but consistent for the time of the month.

Sting. Since all subjects were not stingers, no significant difference in the stinging response over the course of the month could be observed. From Figure 3 it can only be concluded that a subgroup of the panelists (the stingers) seems to have a slightly higher peak in sensitivity between days 2 and 12, thus exhibiting a possibility of higher neuronal response in the proliferative phase.

Dry skin. Figure 4 shows the number of subjects exhibiting the driest skin at the different time points during the course of a month. All the subjects had the driest skin during the proliferative phase, with most subjects exhibiting the driest skin during the

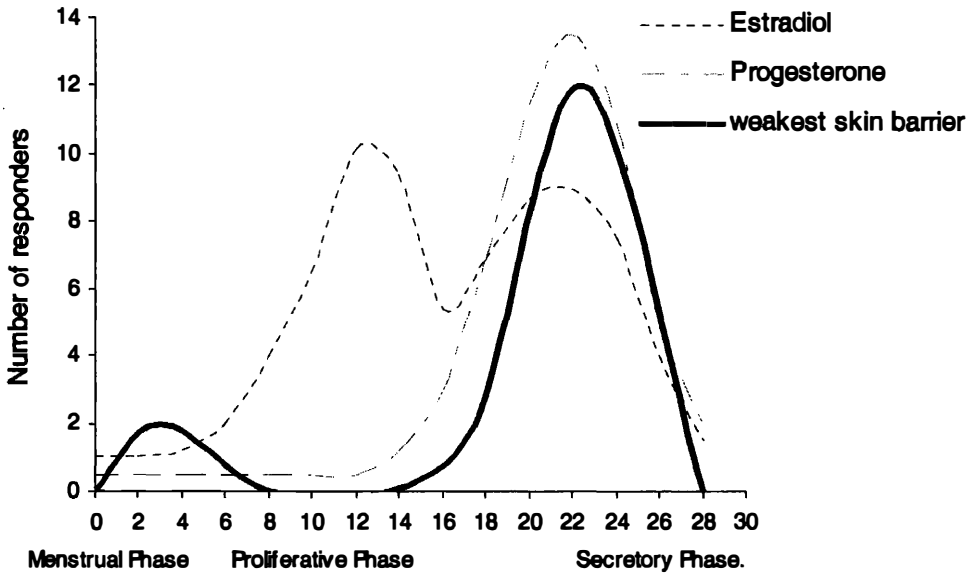

Figure 2. Skin barrier strength in relation to the menstrual phase. The highest number of subjects exhibiting the worse barrier was between days 20 and 27 of the cycle.

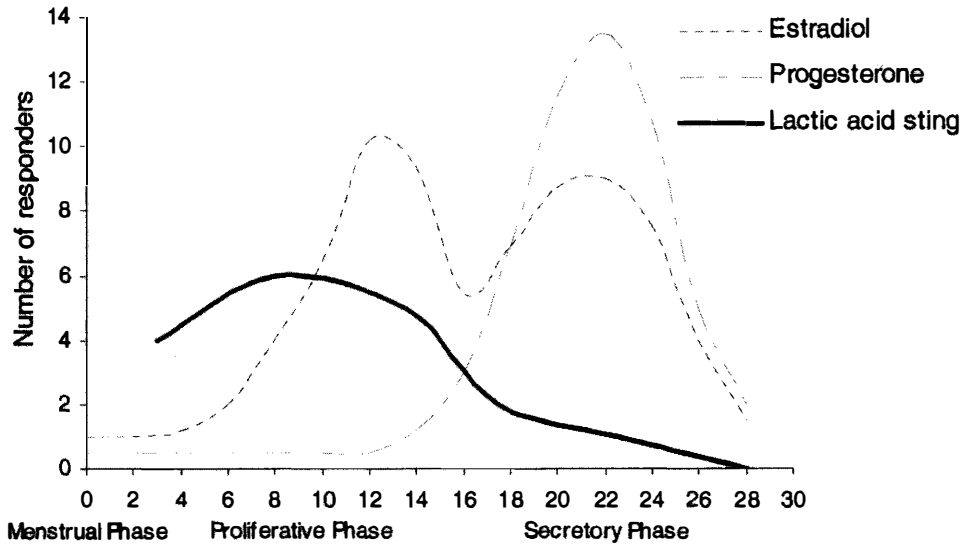

Figure 3. Lactic-acid sting sensitivity in relation to the menstrual cycle. Most subjects exhibited the highest sting around days 4–14 of the cycle. Since all the subjects were not stingers, this effect does not appear as clearly correlated to the time of the month.

first few days of the menstrual cycle, when estrogen levels are lowest. This is expected because the absence of estrogen does not favor the retention of water.

Skin surface lipids. The histogram of the number of high sebumeter scores versus the day of the cycle is reported in Figure 5. The subjects exhibit the oiliest skin from day 9 to day 28. Lipid or sebum production appeared to coincide with the secretion of the luteinizing hormone.

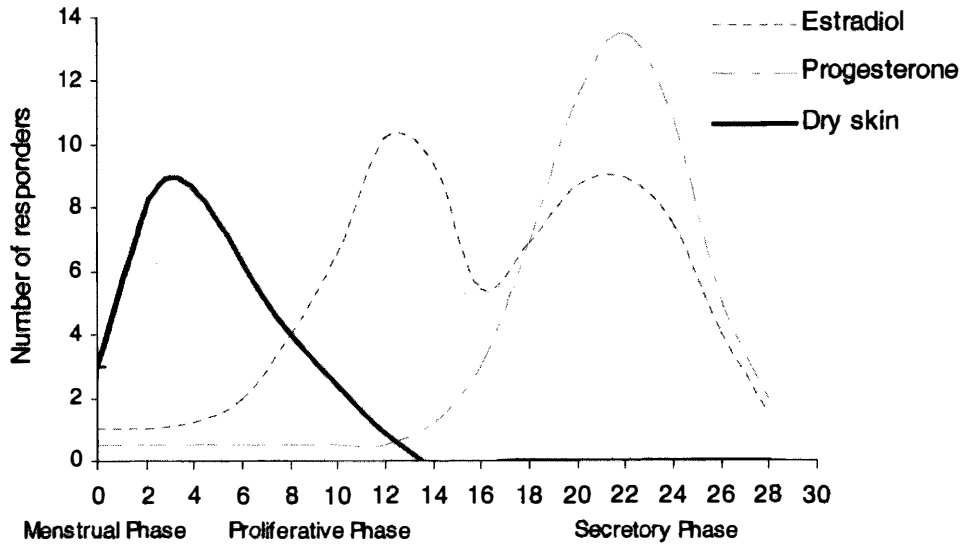

Figure 4. Skin moisturization in relation to the menstrual cycle. Most subjects exhibited the driest skin in the first week of the cycle.

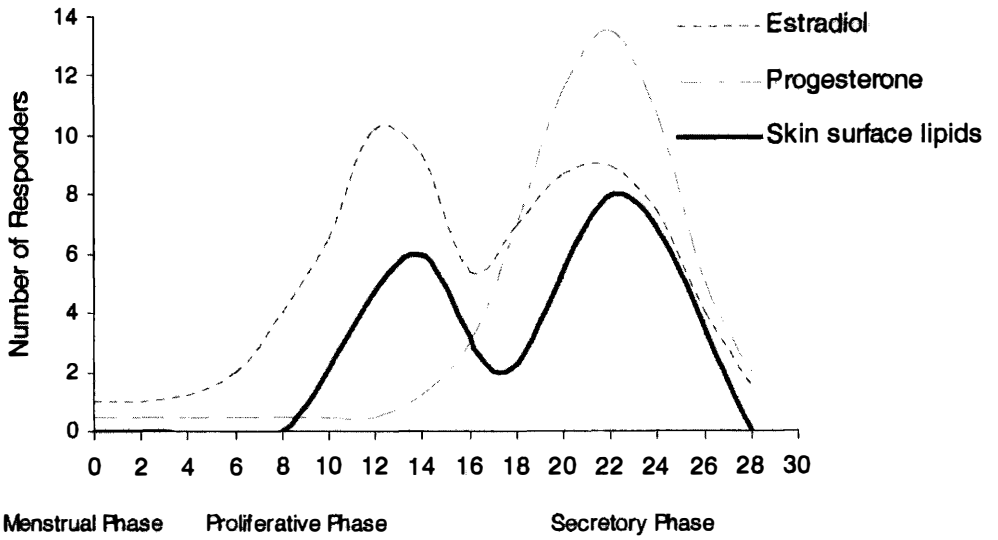

Figure 5. Skin surface lipids in relation to the menstrual cycle. There appear to be two peaks: days 12–15 and days 20–25 of the cycle. These peaks appear to coincide with the appearance of estradiol levels in the blood.

Skin microflora. As observed in Figure 6, the highest microbiological count was around days 20–25. *Staphylococcus epidermidus* was the most prevalent organism, and no consistent shift in bacterial population was observed over the course of the month.

UV sensitivity. The histogram of the number of panelists exhibiting the least MED at a day in the cycle versus the days in the cycle is displayed in Figure 7. The least MED is indicative of high UV susceptibility. As observed in the graph, there appears to be a higher susceptibility between days 20 and 28 of the menstrual cycle. Studies conducted

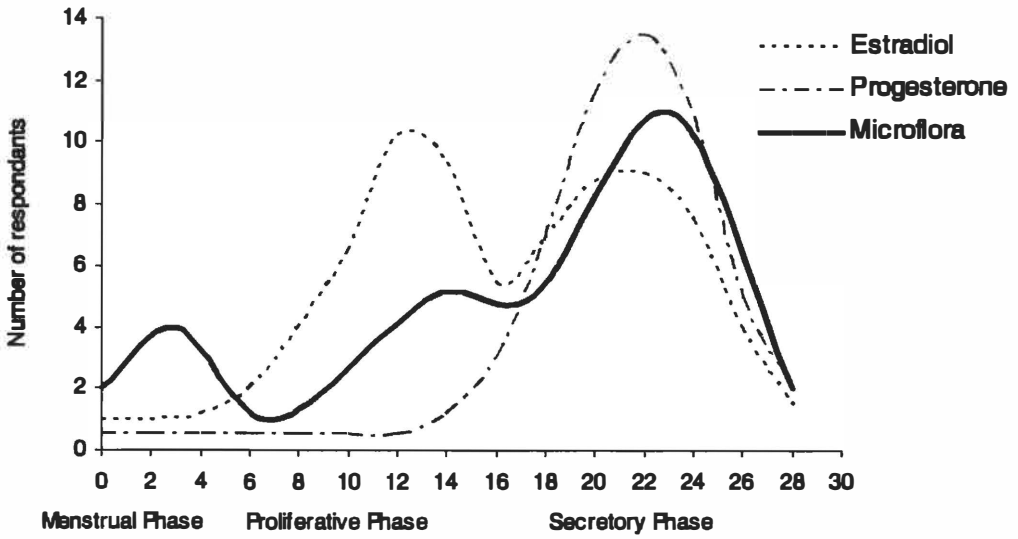

Figure 6. Skin surface microflora in relation to the menstrual cycle. The highest microbial count was around days 16–22 of the monthly hormonal cycle. This study implies a correlation between the bacterial population and sebum production on the skin surface.

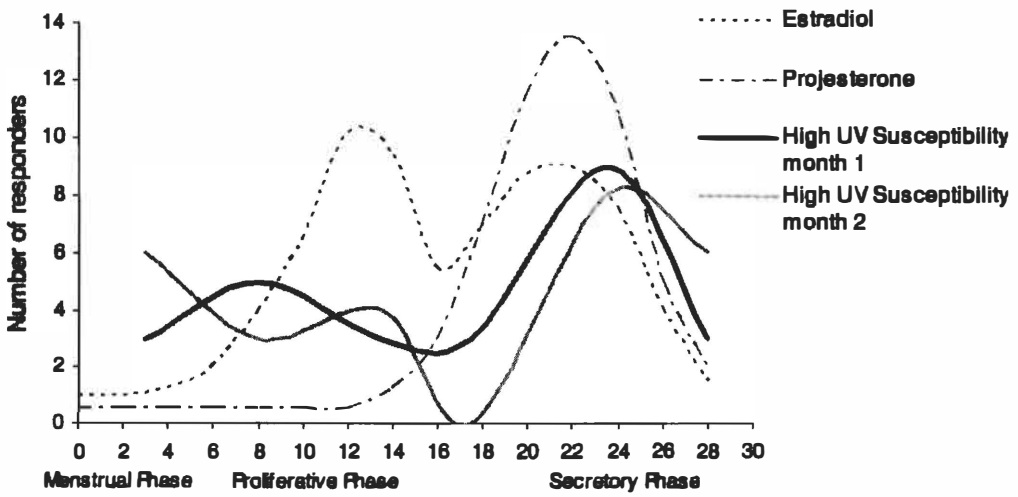

Figure 7. UV susceptibility in relation to the menstrual cycle. Several subjects exhibited a lower MED and thereby a higher UV susceptibility between days 20 and 28 of the menstrual cycle. In this study UV susceptibility appears to be concurrent with an impaired barrier. It is possible that this variation in MED is due to the combined effect of several factors, including hormonal levels and stratum corneum integrity.

by Jemec and Heidenheim (23) indicate an increased UV-induced inflammation following topical application of estrogen, but they observe no significant change in UV response in correlation with the blood levels of estrogen.

DISCUSSION

The menstrual cycle starts with the menstrual phase on days 1 to 6 when the thickened

lining of the uterus (endometrium) is shed, causing menstrual bleeding. Days 7 to 14 are the follicular or proliferative phase, and days 15 to 28 are the luteal or secretory phase when the egg is released (ovulation). In the normal menstrual cycle, progesterone is produced during ovulation. Generally, during days 1–6 of the cycle there is less than 100 ng/dl of progesterone in the blood. During days 7–14 the level rises to 20–150 ng/dl, and on days 15–28 there is a peak of 250–2,800 ng/dl of progesterone in the blood (22). A similar trend was observed in this study, as observed in Figure 1. It is clear that the subjects used in these studies were within the normal range of monthly hormonal fluctuation.

During early follicular development, circulating estradiol levels are relatively low. About one week before ovulation, levels begin to increase, at first slowly, then rapidly. The levels generally reach a maximum one day before the luteinizing hormone (LH) peak. After this peak and before ovulation, there is a marked and precipitous fall. During the luteal phase, estradiol rises to a maximum 5–7 days after ovulation and returns to baseline shortly before menstruation (22).

In these studies, the skin barrier was the weakest between days 22 and 26. A weak barrier is defined as having fewer layers and/or weaker cohesivity of the layers of the stratum corneum. Skin thickness and echodensity has been reported to change during the spontaneous menstrual cycle (4). Studies conducted by Eisenbeiss *et al.* (4) report a statistically significant increase in the skin thickness from phase A (2–4 days) to phase B (12–14 days), but not from phase B to phase C (21–23 days). In studies conducted by Harvell *et al.* (5), TEWL was higher on the day of minimal estrogen/progesterone secretion as compared to the day of maximal estrogen secretion on both back ($p = 0.037$) and forearm ($p = 0.021$) sites, suggesting that the skin barrier function is less complete on the days just prior to the onset of the menses as compared to the days just prior to ovulation. Significant differences in baseline blood flow also existed for the day of maximal estrogen secretion as compared to the day of maximal progesterone secretion, with higher baseline blood flow recorded on the day of maximal progesterone secretion on both the back ($p = 0.021$) and forearm ($p = 0.009$) sites (2).

Since all subjects in this study were not "stingers," there was only a slight trend toward elevated neuronal response between days 2 and 12 of the cycle. Neuronal responses, like pain symptoms of many disorders, are reported to vary with menstrual stage. Studies conducted by Giamberardino *et al.* (7) indicate that menstrual phase dysmenorrhea status can have interacting effects on pain thresholds. Skin response to a challenge with sodium lauryl sulfate has been found to be significantly stronger at day 1 than at days 9 through 11 in the menstrual cycle (8). The influence of the menstrual cycle on skin-prick test reactions to histamine, morphine, and allergen indicate a significant increase in weal-and-flare size to histamine, morphine, and parietaria on days 12 to 16 of the cycle, corresponding to ovulation and peak estrogen levels (24). In this study, sting response appeared to correspond more to skin dryness, since skin was also driest between day 1 and day 6 of the cycle.

Estrogens have an important function in many components of human skin, including the epidermis, dermis, vasculature, hair follicle, and the sebaceous, eccrine, and apocrine glands (25). Estrogens have significant roles in skin aging, pigmentation, hair growth, sebum production, and skin cancer (25). Estrogen improves the physical properties of skin by improving water retention and the quality of vascularization. In addition,

estrogens have been reported to improve the extracellular matrix responsible for the tone and appearance of the skin (26). The lowest production of progresterone and estrogen was on the first few days of the cycle, when skin appeared to be the most dry. In addition, skin surface lipids appeared to be highest on days 16–20 of the hormonal cycle. The pattern of skin oiliness appears to follow the estrogen release pattern, indicating a possible effect of this hormone on skin surface lipids.

The highest microbial count was around days 16 to 22 of the monthly hormonal cycle. The microflora resident on human skin show great inter-individual and intra-individual differences. It is essentially composed of micrococci, staphylococci, and aerobic and anaerobic coryneforms, as well as pityrosporum species, which, in accordance with the different environment in the different regions of the body, are in a steady state. With increasing age, human skin microflora undergo qualitative changes: the streptococci, which are found in infants, disappear and coryneform bacteria occur, which are mainly responsible for odor production (27). Human "native" intracellular sebum, before secretion, is composed of squalene, waxes, and triglycerides. Once secreted, the sebum is colonized by various xenobiots whose development is controlled by several defensive humoral mechanisms and by contact with ambient oxygen. Oxygen and microorganisms transform "native" sebum, the lysis of triglycerides to fatty acids (28). A correlation between bacterial population and sebum production has been implied (29) and is clearly visible in this study.

Several subjects exhibited a lower MED and thereby a higher UV susceptibility between days 20 and 28 of the menstrual cycle. Studies conducted by Jemec and Heidenheim (23) indicate an increased UV-induced inflammation following topical application of estrogen, but they observe no significant change in UV response in correlation with the blood levels of estrogen. In this study, a weak barrier follows the maximum systemic production of estradiol and progesterone.

In this study, UV susceptibility appears to be concurrent with barrier condition. Although epidermal stratification and the formation of a stratum corneum has been hypothesized to provide protection against UV-B radiation (30–31) the differences in UV-B transmission in both stratum corneum and epidermis are too small to account completely for variation in MED at the different times of the cycle. It is possible that this variation in MED is due to the combined effect of several factors, including hormonal levels and stratum corneum integrity.

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