

Ocular ferning during the menstrual cycle in healthy women

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PURPOSE. *The aim of the study is to investigate whether tear ferning patterns change during different phases of the menstrual cycle.*

METHODS. *The tear ferning test was performed on twelve normal women of childbearing age at three day intervals throughout one complete menstrual cycle. Serum hormone levels (progesterone, estrogen, testosterone) were measured.*

RESULTS. *Eight women showed type I ferning, and the other four had type II ferning initially. These patterns did not change during the menstrual cycle. Serum hormone levels were all in the normal range. Since no change in ferning pattern was detected during the menstrual cycle, the ferning test can be done at any time in women.*

CONCLUSIONS. *This study showed no effect of different menstrual cycle phases on tear ferning patterns. (Eur J Ophthalmol 2001; 11: 15-18)*

KEY WORDS. *Tear ferning test, Menstrual cycle, Androgens*

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INTRODUCTION

When a drop of mucus is placed on a microscope slide, and is left to dry at room temperature, it crystallizes in the form of arborizations or ferns. This ferning was first described by Papanicolau in gynecology and was used to identify the ovulation time on the basis of the different crystallization forms (1). The same phenomenon, i.e., detecting characteristic crystallization patterns of tear mucus in the form of ferns, has been proposed by Tabbara and Okumoto as a test for mucus in diseases with decreased goblet cell density in conjunctiva (2). It is believed that the ferning results from the interaction of electrolytes with the mucins and that different concentrations of electrolytes are the cause of differences in ferning patterns (3).

The purpose of this study was to investigate whether the tear ferning patterns change during different phases of the menstrual cycle in normal women of childbearing age.

MATERIALS AND METHODS

Twelve normal women between the ages of 24 and 36 years, with regular menstrual cycles, served as volunteers for this study. Each woman had a complete physical examination. All were found to be in excellent general health with no gynecological disease. None of the women were using any hormonal contraceptive methods. The presence of ovulation was confirmed by a serum progesterone radioimmunoassay in the third week of the menstrual cycle (21st day progesterone). By convention, days of cycle are identified by number, starting with the first day of menstruation. Blood was drawn by venipuncture and estradiol (E₂) and testosterone levels were also measured.

All patients underwent a complete ophthalmological examination including Schirmer test I and tear breakup time. The tear ferning test was repeated throughout the menstrual cycle at three day intervals starting from the first day of menstruation. The average length of cycle in our subjects was 28 days, so the test was

done ten times during each complete menstrual cycle (days 1, 4, 7, 10, 13, 16, 19, 22, 25, 28).

Tear was collected from the middle portion of the lower fornix of the right eye, using a spatula. To prevent contamination or dilution of the tear sample, topical anesthesia was avoided. For the same reason, tear samples were collected before any other test was done. The ferning test was always done at the same time of day, to minimize any possible effect of diurnal variations. The tear sample was dropped on a light microscopy slide and allowed to dry by evaporation at room temperature. Mucus crystallization was observed within 5-7 minutes from collection, using a light microscope at 40x magnification.

The ferning patterns were classified according to the system of Rolando (4). Thus, the type I pattern showed numerous acute angle branchings without spaces between the ferns. Type II was similar to type I but with many more spaces and a lower branching frequency. Type III showed thicker and smaller ferns, often resembling a short-armed right-angled cross, with very little branching and very large spaces. Type IV showed an amorphous pattern with no ferns. Types I and II are seen in normal tears and types III and IV in abnormal tears. Days 1, 4, 7, 10 and 13 reflect the follicular phase, days 16, 19, 22, 25 and 28 the luteal phase of cycle, accepting that ovulation generally occurs on the 14th day of cycle. The results of ferning tests in the two phases were compared for each subject.

RESULTS

At the initial ferning test (day 1), none of our subjects showed type III or type IV patterns. Eight had type I, and the other four type II patterns. Throughout the menstrual cycle, ferning type remained constant for each case; i.e., subjects with type I pattern remained type I and those with type II remained type II. The only exception was a case with type I pattern which showed type II ferning on her second test (day 4). The remainder of her ferning tests were all type I. This was most probably due to an artifact arising during tear sampling rather than a true change in ferning pattern.

Anterior segment examination with slit-lamp microscope revealed no abnormalities. Schirmer test I results were

all more than 15 mm and tear breakup time was longer than 10 seconds.

In all these volunteers, serum progesterone on day 21 of the cycle was more than 5 ng/ml, and this is consistent with ovulation having occurred (5). Serum levels of the other hormones, estradiol and testosterone, were also within normal limits for adult women (the normal range for luteal phase estradiol is 50-150 pg/ml and the normal level of testosterone is 20-80 ng/dl). Serum hormone levels in women with types I and II patterns were similar.

DISCUSSION

Unlike the reproductive system of men, the reproductive system of adult women shows regular cyclic changes in periodic preparation for fertilization and pregnancy. This is called the menstrual cycle. The first part of the cycle, the follicular phase, involves the development of a mature follicle in the ovary and secretion of a large amount of estrogen, mainly estradiol. Then on day 14-16 of the cycle, ovulation takes place. The follicle that ruptures during ovulation changes into the corpus luteum and secretes large amounts of progesterone. This is called the luteal phase (6).

In line with these cyclic changes, regular alterations occur in the mucus produced by the uterine cervix. Mucus from the cervical canal in the preovulatory (follicular) phase normally responds to a high-estrogen environment and dries in a crystalline pattern (ferning). After ovulation (luteal phase), under the influence of progesterone, the mucus no longer forms ferning pattern. Papanicolaou was the first to show this (1). It has since been proposed as a test to evaluate the presence of mucus in diseases with decreased goblet cell number in the conjunctiva (2) and called that tear mucus ferning test (TMFT). However, the name "tear ferning test" is probably preferable because mucus is not the only factor responsible. The exact mechanisms and the biochemical agents involved in ferning are still not known, but more recent studies indicate that tear ferning is largely dependent on the electrolyte concentration, especially the ratio of monovalent to divalent ions (7). The presence of a macromolecule is necessary, but this need not be mucus: it may well be proteins originating from the lacrimal gland. Thus, the ferning test does not depend solely

on the mucus from conjunctival goblet cells, and the aqueous part of the tear film containing electrolytes and proteins originating from the lacrimal gland plays a major role. A quantitative tear ferning method has also been proposed (8). The tear ferning test shows good sensitivity and specificity for diagnosis of the dry eye syndrome (9).

Like cervical mucus, there is a direct correlation between salivary ferning and fertile period (10). But, unlike in cervical and salivary ferning, we could not identify any change in the tear ferning pattern during the different phases of the menstrual cycle. This lack of influence on the ferning test of different menstrual cycle periods, meaning the cyclic changes in estrogen and progesterone, is consistent with findings regarding lacrimal gland function. Estrogen does not appear to alter either the structure or function of the lacrimal gland (11-13), and estrogen receptors cannot be detected in lacrimal tissue (14). This lack of estrogen action helps to explain the lack of effect of the menstrual cycle on tear production in humans (15).

Androgens may play a major role in supporting lacrimal gland cell number and secretory function. Experiments have shown that ovariectomy causes functional and biochemical atrophy of the lacrimal gland in rabbits (16). Ovariectomy reduces the level of testosterone by 88.5% in these animals and dihydrotestosterone partially or totally prevents the functional and bio-

chemical changes of this surgery. The acinar area in lacrimal tissue of males of several species, including rats, rabbits and humans, is significantly larger than in females (17). These observations suggest that lacrimal gland secretory function depends on the circulating levels of androgens. In fact, a correlation has been found between dry eye and decreased testosterone levels in women (18). Unlike estrogen and progesterone the testosterone level in women is not cyclic and was in fact quite constant throughout the menstrual cycle (15). Thus, the constant pattern of mucus ferning during a complete menstrual cycle seems to be another indirect indication that the lacrimal gland is androgen-dependent.

In summary, the present study found no alterations in tear ferning test patterns during different phases of the menstrual cycle, thus indirectly supporting the theory that lacrimal gland integrity and secretory function depend largely on the androgens. Therapeutic implications of androgens may be a practical outcome of this finding in the future.

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