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RECENT RESEARCH IN CERVICAL SECRETION: SOME BIOPHYSICAL ASPECTS

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Summary

Mucus from the lumen of the cervix appears to be a morphologically heterogeneous entity. It contains different types of secretions, the proportion of which vary throughout the menstrual cycle. The different mucosal types show different types of crystallization, different patterns of ultrastructure (probably related to the arrangement of the glycoprotein network) and are produced in different secretory zones of the crypts in the cervix.

One of these mucus types is P mucus. Recent studies about it shows the relation between mucus symptom and P mucus levels in the samples from the cervix, although this is the least abundant of the different mucus types, about 5-7 percent in the peak day. It has been also studied P cells, secreting P mucus (Odeblad, 10).

There are also in the female genital tract some binary cell-secretion systems. A single type of cells or a single type of secretion often shows well-defined function. However, we want to draw attention to systems in which two types of secretions or cells seem to form a symbiotic unit with a meaningful function of importance in the female tract (Odeblad, 9). For example, the L and S mucus together, are necessary for the sperm selection. Also P and S mucus interaction contribute to sperm selection. Some cells are also involved, for example, in the peak symptom, which can be experience in the vestibular part of the vulva.

INTRODUCTION

The previous studies by Odeblad (7,8) indicate that human cervical mucus, contains four different types of secretion, called G (gestagenic), and E (estrogenic) mucus types. The estrogenic mucus types are called L, S and P. The four mucus types, G, L (loaf), S (string) and P (peak) presents different viscosities (NMR) and a different morphology in the crystals of air-dried mucus samples. Main characteristics of the mucus types:

L MUCUS: it is secreted under estrogenic situation, starting 6-7 days before ovulation. It has the typical "ferning" morphology. We observed that it had a structure with a straight or curved axis and branches protruding from it at 90ŗ angle. These branches could also act as an axis for new branches, again at a 90ŗ angle (Fig 1).

In the fixed samples from the cervical canal, a structure similar to a marine sponge was founded in the samples with a high percentage of L mucus. The pores had diameters of 0.4-1.3μm.

S MUCUS: it is secreted under estrogenic situation, starting 2-3 days before ovulation.

In the air dried samples, S mucus consists of a parallel arrangement of crystals, sometimes with little branches (Fig 2).

In the fixed mucus samples, S mucus presents a networking of parallel or crossing fibres, with pores of a greater diameter (1.5-7μm) than the L ones.

P MUCUS: it is secreted under estrogenic situation, specially during the peak day, which means the day of ovulation and the following day.

We found different subtypes for P mucus:

P6B: presented a star-like morphology with six well-defined axes. There were branches protruding from the axes at a 60ŗ angle (Fig 3).

Pt: consisted of triangular crystals. Both, axis and branches seemed to be formed by crystals, which were not always joined.

In the fixed mucus samples, from the cervical canal, P mucus appears in plain surfaces with parallel folds.

G MUCUS: it is secreted under gestagenic situation, during the infertile fases of the cycle. Consisted of free crystals with a different morphology and some times a large number of cells (Fig 4).

In the G mucus fixed samples, it presented large plain surfaces and sometimes red blood cells or other cells. With a high magnification we were able to see the pores (0.1-0.5μm).

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These four mucus types are produced in different and specific crypts in the different areas inside the cervix (Fig 5).

In a non pregnant woman, aged 25-30 and not having used contraception, the cervix is 22 mm long (average) and the average diameter is 6mm at ovulation time. The crypt distribution starting from below and going upwards is as follows: the G crypts dominate in the lowest 4 to 5mm. Then, there is a zone of L crypts, occupying the next 9-10mm, followed by the S zone, for 5-6mm. The highest three to four mm contains the P crypts. The borderlines between the zones are not clearly defined; there is some overlapping (Odeblad, 8).

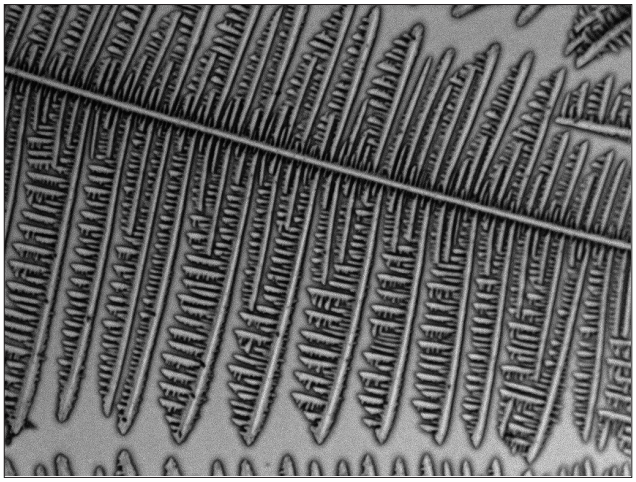

Fig. 1: L mucus in dried sample. We can clearly observe the structure of the central axis, and the branches protruding at a 90º angle LM x10.

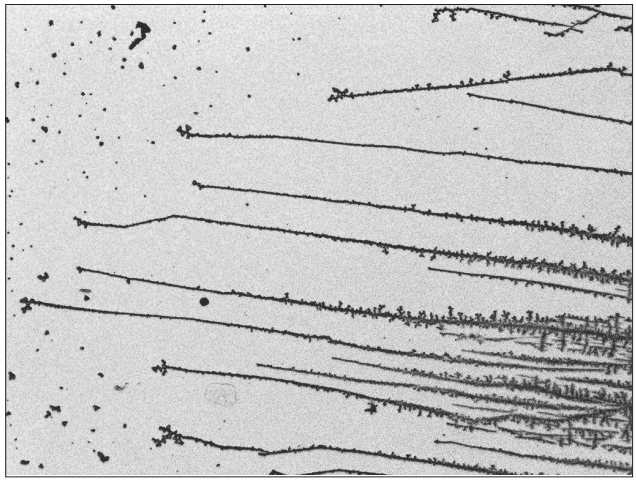

Fig. 2: S mucus in dried samples. Subtype S2 consists of a parallel arrangement of crystals which are joined, but without branches. Subtype S3 consists of a parallel arrangement of crystals but with short protruding branches LM x 4.

Background. Cervical mucus is an hydrogel produced by the cérvix glands. It is involved in sperm migration and maturation through the female genital tract, and provides a barrier to prevent the pathogens entering the endometrium. This mucus changes with the menstrual cycle, which means it has different biophysical and biochemical characteristics (Menarguez, 5; Menarguez, 6) throughout the cycle. Mucus is therefore an indirect but important element for identifying ovulation, not only for clinicians but also for women using natural family planning methods (Hilgers, 3). Nowadays, we know that cervical mucus is not a homogeneous entity.

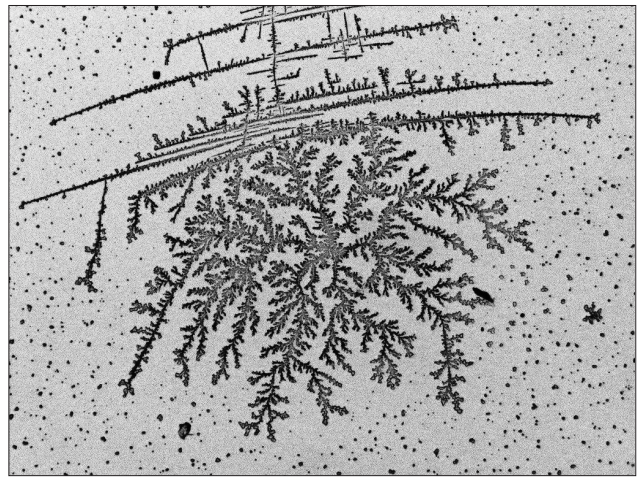

Fig. 3: Pa (active) is the most frequent subtype, the axes of which are not well defined. S mucus at the top of the Figure, and Pa in the center, which axes are not well defined LM x 4.

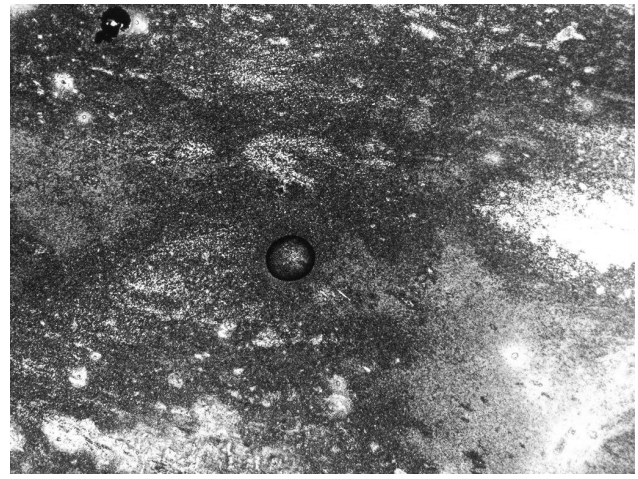

Fig. 4: G mucus from the cervical canal in dried samples. It has free-crystals content with no predetermined form LM x 4.

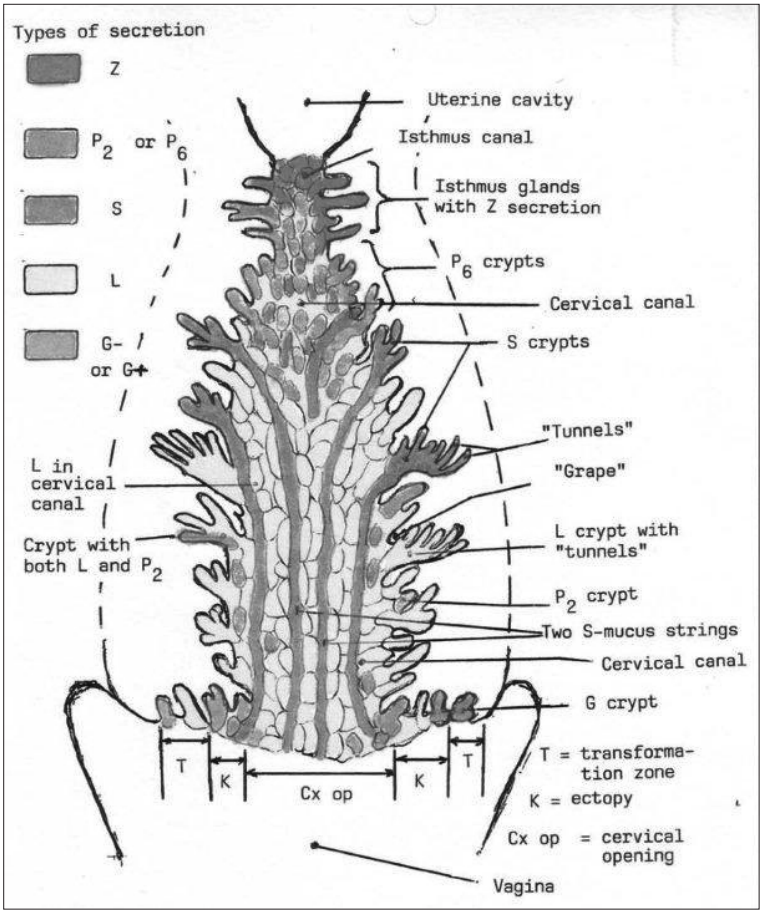

Fig. 5: Odeblad´s diagram showing that the four mucus types are produced in specific crypts in different areas of the cervix.

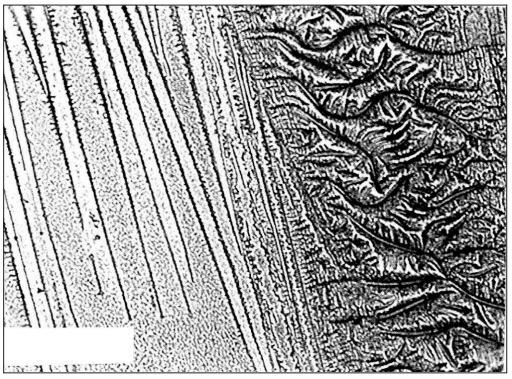

Fig. 7: Boarderline between S mucus and L mucus. LM x 180.

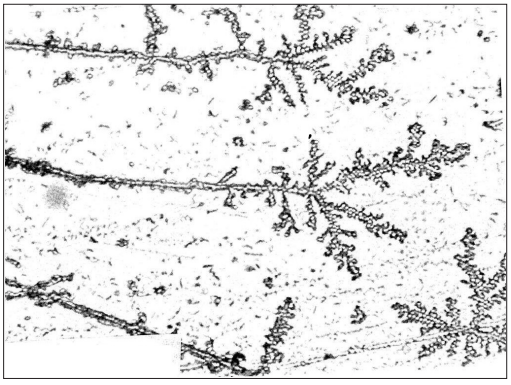

Fig. 8: Brooms, P6 mucus on S filament. LM x 750.

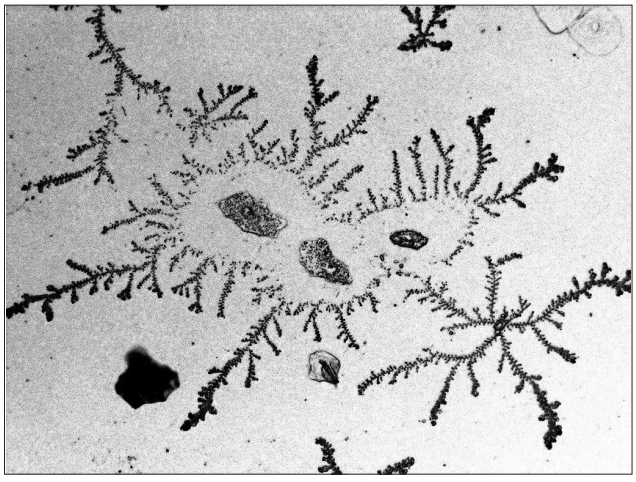

Fig. 6: P6 cells with secreted material arranged in triangular structures in several directions (Pt) LM x 10.

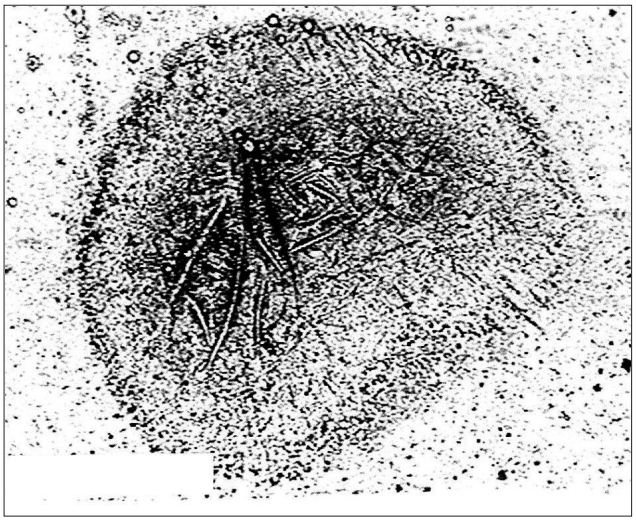

Fig. 9: Grain drop. LM x 45.

different types of secretion, which percentages that vary throughout the cycle.

OBJECTIVES AND METHODS

The aim of the study was the morphological characterization of the different mucus types, with samples taken from the lumen of the cervix and the study of the cells-secretion from the cervix. We also wanted to draw attention to systems in which two types of secretions or cells seem to form a symbiotic unit with a meaningful function of importance in the female genital tract.

Samples from the lumen of the cervix were spread out on slides and air dried (Odeblad, 7,8; Menarguez, 4). The phenomenon of "ferning" was observed and assessed in these samples using both light microscopy (LM) and scanning electron microscopy (SEM) (Ceric,2, Vigil, 11). Further samples from the lumen of the cervix were spread out on cover slips and fixed with glutaraldehyde (2,5%) to be studied by SEM. Also smears have been removed from the vestibular area and examined using LM.

Some binary cell-secretion systems in the female genital tract. A single type of cells or a single type of secretion often shows well-defined function (Fig 6).

However, we want to draw attention to systems in which two types of secretions or cells seem to form a symbiotic unit with a meaningful function of importance in the female genital tract.

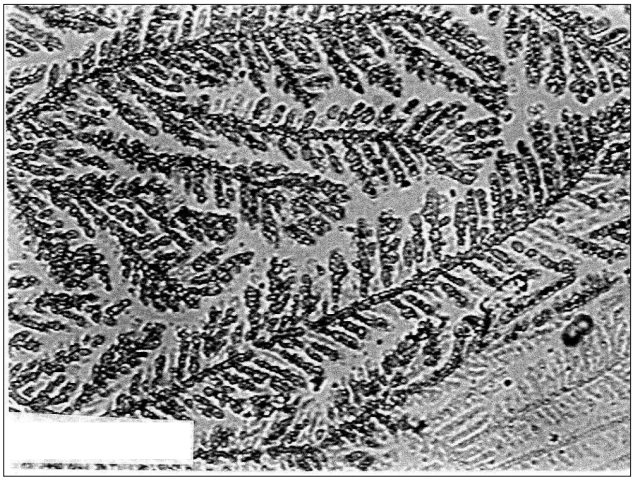

Fig. 10: P2 mucus, 60º degrees-branching, some grains are visible between the branches. LM x 250.

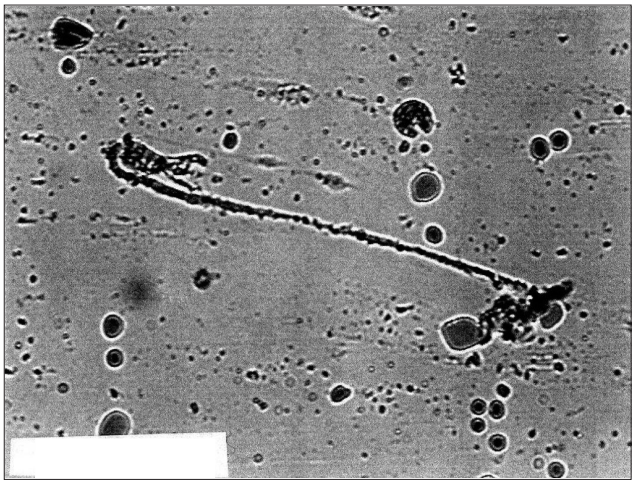

Fig. 11: A rope-like objet between two vestibular cells. LM x 280.

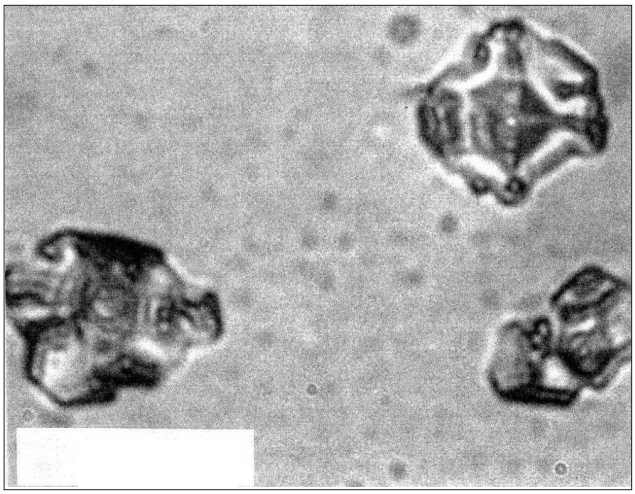

Fig. 12: G mucus in the postovulatory phase, with inactive P cells. Note the presence of small atypical crystals on the cells indicating a very low secretory activity.

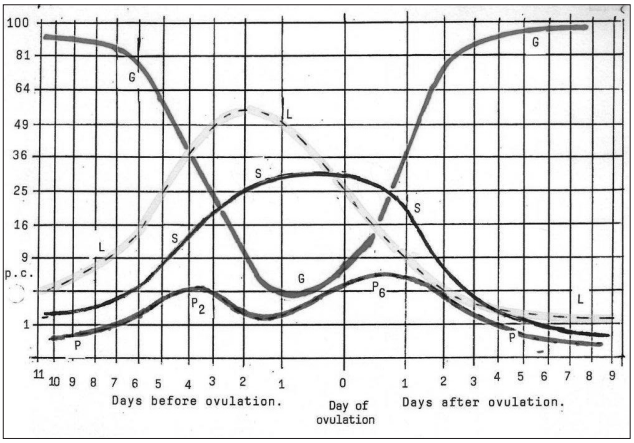

Fig. 13: Odeblad´s diagram which shows cyclic changes in the quantity of the differents mucus types, along the cycle. They are expressed in percentages.

The first time this became evident was when the sperm propagation in gently prepared cervical mucus was studied. Rapid swimers with normal morphology advanced within the S mucus string. But slow swimers with abnormal shape deviated laterally into the L mucus surrounding the string. So the first conclusion is that the intimate contact of the two mucus types, S and L, is necessary for the sperm selection (Fig 7).

Interactions between S and P6 mucus and between S and Pt (the inmediate precusor of P6 mucus type) are very common. They form broom-like structures in the spread-out dried smears of mid-cycle cervical mucus. The function of these brooms in vivo are not yet understood, but second conclusion could be that they may also contribute to sperm selection (Fig 8).

Another common structure in mucus smears are the so-named grain drops, GD. In a smear of an area of a few cm2, they may be found in 4 to 9 numbers. They look-like a collection of thousands of grains of micrometer size, the whole drop may be 0.3 – 1mm in diameter. A more careful observation reveals a crystalline center, the crystals resembling L mucus, but may be some other mucus not yet identified (Fig 9). Becouse de GDs are so common, they might have some function, perhaps mucolitic function, between GDs and cervical mucus, which we are now studying.

Grains together with crystals are also founded in the P2 mucus. It has been possible to follow P2 mucus from upper to lower cervix. In the lower part of the cervix, near the cervical opening, the P2 mucus units are surrounded by watery contents suggesting mucolytic activity (Fig 5) . Forth conclusion should be that this mucolytic process occurs early in the fertile period and may help to "clean" the cervical canal from G mucus and be ready to hold the L and S secretions (Fig 10).

The peak symtom depends largely on the secretion of, first, the L, and then, both, L and S, and finally S and P6 mucus types. The peak symptom is not experiensed in the cervix and not in the vagina, but in the vestibular part of the vulva. Therefore smears have been removed from the vestibular area and examined in the microscope. Small quantities of L and S mucus have been found, but P6 dominates.

Also another type of structures were seen, the "rope-like objects", R L O-s.

These could be finally be explained as the result of normal apoptosis (programmed cell dead) of the surface cells of the vestibulum. In Fig 11, we can observe how this apoptosis gives rise to elongated cell residues (ropes), apparently with great affinity for P6 mucus which enhances the slippery peak sensation. Both together, P6 and cell residues, enhace the slippery peak sensation and helps women to identify ovulation (Bigelow,1).

After the peak, in the postovulatory phase, the G mucus dominates and fills the cervical canal. This has been consider to be a trivial part of the cycle, but recent findings has made it more interesting. P cells with a very low secretory activity, have been found in the G mucus. Series of studies indicate that these cells persist until and after the next menstruation, when they start to be active and later, develop to Pt, P2, P4 y P6 cells.

As long as they are dormant, they are inactive (they do not receive enough of oxigen, ATP, etc..within the G mucus. This is an ilustrative example of "team work" between a secretion (G mucus), and cells (P cells) (Fig 12).

Probably in the future more of these binary systems may be found helping to understand more of the human reproductive physiology.

Human cervical mucus located inside the cervical canal is a morphological heterogeneous entity with different types of secretion, the percentages of which vary during the cycle (Fig 13). They show a different ferning and ultrastructure, related to the arrangement of the glycoprotein network, and are produced in different zones of the crypts in the cervix

What is surprising in this investigation is to observe the care that Nature has bestowed upon the selection and filtering of sperm, being extraordinarily generous with the number of spermatozoids secreted in each ejaculation (400-500 million).

Later, it puts them through a large number of test and difficulties in advancing, and at the end, only one of them, the best is responsible, thogether with the ovule, for the generation of a new human life.

CONCLUSIONS

1. The zones of the cervical cryps are very specific areas of mucus synthesis where different mucus types are produced, which will then combine to constitute what we know as cervical mucus.
2. Human cervical mucus, located inside the cervical canal is a morphological heterogeneous entity which different type of secretion, the proportion of which vary during the cycle.
3. The different mucus types show a different ferning and ultrastructure, related to the arrangement of the glycoprotein network.
4. There are also in the female genital tract some binary cell-secretion systems in which two types of secretions or cells seem to form a symbiotic unit with a

meaningful function of importance in the female tract. L and S mucus together, are necessary for the sperm selection and also P and S mucus interaction contribute to same proposal. Some cells are also involved, for example, in the peak symptom, which can be experience in the vestibular part of the vulva, and can be useful for identifying ovulation.

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NAUJAUSI GIMDOS KAKLELIO SEKRECIJOS TYRIMAI: BIOFIZIKINIAI ASPEKTAI

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Santrauka

Raktažodžiai: gimdos kaklelio gleivės, vaisingumas, morfologija, mikroskopija, ląstelių sekrecija.

Gimdos kaklelio gleivės yra morfologiškai įvairialypės. Gleivėse randama skirtingų komponenų, kurių proporcija keičiasi priklausomai nuo menstruacijų ciklo. Skirtingoms gleivėms, kurios yra išskiriamos atskirų gimdos kaklelio sekretorinių zonų nišų, būdingi skirtingi kristalizacijos tipai ir ultrastruktūros modeliai (tikriausiai susiję su glikoproteinų tinklo išsidėstymu). Vienas iš gleivių tipų yra P gleivės. Paskutinieji klinikiniai tyrimai atskleidė sąsają tarp gleivių pobūdžio ir P gleivių kiekio iš gimdos kaklelio mėginių. Nors P gleivių išsiskiria mažiausiai, tačiau piko dieną jos sudaro 5-7 proc. visų gleivių. Darbo metu išnagrinėtos gimdos kaklelio P ląstelės, išskiriančios P gleives. Moters lytinių takų gleivinėje rasta kelios dvilypės ląstelių, produkuojančių gleives, sistemos. Vienos rūšies ląstelės arba ląstelės, produkuojančios vienos rūšies gleives, paprastai pasižymi gera funkcija. Tačiau mes norėtume atkreipti dėmesį į ląstelių sistemas, kurios gali gaminti dviejų rūšių gleives ar ląsteles, kurios gali suformuoti simbiotinį vienetą, turintį reikšmingą funkciją moters lytiniuose takuose. Pavyzdžiui, L ir S gleivės kartu yra būtinos spermatozoidų selekcijai. Tam taip pat svarbi P ir S gleivių sąveika. Kai kurios ląstelės yra įtrauktos, pavyzdžiui, į piko simptomus, kurie gali būti jaučiami makšties prieangyje.

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