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# The biology of the cervix: biophysical and biochemical aspects
# A general review
## Biologia szyjki macicy. Aspekty biofizyczne i biochemiczne
## Przegląd ogólny

**Abstract:**
Cervical mucus is a viscous fluid, produced by the cervical glands. It plays a fundamental role in the selection, transportation and capacitation of spermatozoids. Mucus from the lumen of the cervix appears to be a morphologically heterogeneous entity. The studies show the presence of four different morphological mucus types, namely L, S, P and G, studied by scanning electron microscopy, 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. What is surprising in this investigation is to observe the care that Nature has bestowed upon the selection and filtering of the sperm, being extraordinarily generous with the number of spermatozoids secreted in each ejaculation (40-50 million). Later, it puts them through a large number of tests and difficulties in advancing. At the end, only one of them, the best, is responsible, together with the ovule, for the generation of a new human life.
**Key words:** cervical mucus, cervix, ferning, scanning electron microscopy, sperm selection.

**Abstrakt:**
Śluz szyjkowy to lepki płyn wytwarzany przez gruczoły (krypty) szyjki macicy, który odgrywa zasadniczą rolę w selekcji, transporcie i kapacytacji plemników. Istota śluzu szyjkowego jest morfologicznie heterogeniczna. Na podstawie badań spod mikroskopu elektronowego wyróżniono cztery różne typy śluzu szyjkowego L, S, P i G a jego ilość i proporcje zmieniają się w zależności od faz cyklu miesiączkowego kobiety. Każdy z 4 typów śluzu szyjkowego jest wytwarzany na różnych poziomach krypt rozsianych w świetle kanału szyjki i charakteryzuje się odmiennym typem krystalizacji oraz unikalną ultrastrukturą, prawdopodobnie związaną z układem sieci glikoproteinowej. Zaskakującą obserwacją wynikającą z badań nad śluzem szyjkowym jest ta, jak dużą troską otacza Natura proces selekcji i filtracji plemników podczas gdy ich koncentracja może sięgać ok. 40-50 milionów w każdym wytrysku . W drodze do wyłonienia „lidera" wśród tak wielu plemników podlegają one licznym testom i sprawdzianom, których uwieńczeniem jest poczęcie nowego życia.
**Słowa kluczowe:** śluz szyjkowy, test liścia paproci mikroskop elektronowy, selekcja plemników

## 1. The biology of the cervix

Reviewing the functions of feminine genital mucus, we see that they are very diverse: on one hand, they protect against infection and on the other, they receive and conduct sperm through the genital tract. In order to carry out their role successfully, there are important anatomic changes from the vagina to where the gametes are found in fertilisation. One of these changes is located in a well differentiated area, which narrows like a barrier. We refer to the transition between the vagina and the uterus, called the cervix. Here, the path of spermatozoids is filtered, principally by the action of the mucus found on the epithelium in this area (cervical mucus).

Cervical mucus is a viscous fluid produced by what we commonly call cervical glands or cervix. It plays a fundamental role in the transportation and capacitation of spermatozoids through the feminine genital apparatus. Together with this, it is well known that this mucus suffers modifications which produce different biophysical characteristics throughout the cycle (Elstein, 1978). It therefore becomes an important element in the identification of ovulation, both in clinical practice as well as through self-evaluation of the woman about mucus in the introitus of the vagina, in Methods of Natural Family Planning, especially Billings (Billings and Westmore, 1980; Parrilla and Delgado, 1997).

Today we know that human cervical mucus is a heterogeneous entity, formed by different mucosal units which vary in proportion and in presence throughout the feminine cycle. (Ryder and Campbell, 1995).

Cervical mucus is an intricate system of crypts, previously referred to as "cervical glands" (Odeblad, 1972). The term "crypts" is used to describe the folds of the columnar epithelium of the cervical mucus. They may be oblique, transversal, or longitudinal and they may fork or extend in a downward direction. This area is covered with an epithelium composed of only one layer of cylindrical cells, with a basal nucleus and cytoplasm rich in mucus. Ninety-five percent of this epithelium is composed of secretory cells and five percent of ciliated cells. The cilia flap towards the vagina and one of their functions is to create a muco-ciliary current which expels cells and particles towards the vagina. Cervical epithelium contains receptors for estradiol and progesterone, which is why we know that the cervix is a "target organ" for these hormones. In accordance with this concept, cervical secretion varies in quality and quantity in response to hormonal changes occurring during the menstrual cycle (Pérez y cols, 1995).

Non-ciliated cells, which are secretory, are covered by microvilli and contain a massive number of cytoplasmic granules which may displace the nucleus towards the base of the membrane. The secretory activity of these cells decreases after menopause. However, there is less epithelial atrophy in the cervix than in the endrometrium and the vagina, especially protected and regenerated during pregnancy. At the moment of secretion, the cellular membrane breaks, producing the liberation of secretory granules in (Odeblad, 1973). The quantity of secretion liberated depends on: (a) the number of secretory units in the cervical canal; (b) the percentage of cellular mucus secretion per unit, and (c) the response of the secretory cells to the circulating hormones. In a normal woman of child-bearing age, there are about 400 secretory units of mucus in the cervical canal. The daily production of mucus varies from 600 mg at midcycle to 20-60 mg during other periods (Odeblad, 1977).

The peak day is the "golden standard" of the cycle (Billings 2008). In the middle of 1970s we only knew about the existence of L, S and G mucus types (Odeblad, 1977) and it appeared that no combination of these types could explain the peak sensation. Odeblad started to "hunt" a "peak mucus". I could be presented for fist time in San Antonio (USA), and published in 1992.

**2. The biophysics of cervical mucus**

When studies of the cervix began, it was thought that all the crypts produced secretions at the same time. In 1966, Odeblad showed that some of these crypts were responsible for the fine mucus secreted on days close to ovulation and produced crystals. Other crypts producing thick mucus did not crystallise and were obtained in the infertile phases of the cycle.

It is interesting to note that taking a sample of cervical mucus, spreading it out, and drying it on a slide, gives us very precise information about the day of the cycle that the sample corresponds to.

However, it is important to remember that "in vivo", crystals do not exist, that is, the "spread out" technique developed by Prof. Odeblat, which permits the observation of the crystals, reflects what is occurring in the inside of the cervix, produced by evaporation of the water. Therefore, what we observe are basically CINa crystals or, at times, CIK. These ions "in vivo" are dissolved in the water that composes the aqueous phase of cervical mucus. Nevertheless, the technique developed by Odeblad allows us to know, through crystallisation (which varies according to the moment of the cycle) the potential fertility of a sample.

The first type of mucus discovered in 1966 was named E (estrogenic), and the second, G (gestagenic). Afterwards, quite advanced in his investigations, Odeblad (1977) proved that Type E had 2 components: S (sperm conveying) and L (locking in low-quality spermatozoa). He observed that Type S mucus crystallised into thin, parallel, needle-like structures, yet Type L showed a fern-like morphology, with crystals forming a central axis from which long branches fanned out at a 90º angle.

They continued to study the three types discovered until then ( G, L and S). In 1992, Odeblad described an additional type of mucus, present in lesser quantity, called P (peak), as it has its maximum secretion on the Peak (ovulation) day.

It took so long time, about 15 years to find and prove the existence of the P mucus. The most important difficulties had to be overcome were:

1) The crypts producing P mucus are localized high up in the cervix and often surrounded by the enzyme-secreting glands. This made it difficult to get crypt samples.
2) P mucus is present in small amounts; often much less than 5% of the total mucus quantity.
3) The quantity and quality of the P mucus varies with age (more in young, less in elder women) and parity (more in multiparous women).
4) P mucus has the same NMR properties as S mucus.
5) There are several subtypes of P mucus both in crystal patterns and in functions.
6) And independent confirmation or support of a scientific result is necessary, and this came in 1990 (Temprano).

Later, Menárguez showed in her thesis (1998), by scanning electron microscope that S, L, G and P types had all different and specific macromolecular network patterns. Minor alteration in the macromolecules may give rise to various subtypes.

To investigate if the subjectively estimated slipperiness on the peak day correlated with the P subtypes, 37 cycles in 34 women were investigated. The studies were performed from 1981 to 2005. Preliminary only the subtype P6 shows a correlation, so that problem was studied in more detail. The women, all experienced in BOM, came for mucus sampling in the evening of the peak day and mucus was extracted and smeared out in thin layers on several slides, so that all mucus could be carefully investigated and the areas covered by all known types could be evaluated. The women had estimated her slipperiness in four levels, very week, week, strong and very strong. Very week and week were not significantly different and therefore treated as one group: W. Strong and very strong were similarly treated as one group,

S. When the groups W and S were compared there was a significant difference: the P6 amounts were:

From W: 0,57%; From S: 1,80%.

This indicates that P6 mucus most probably is responsible for the peak sensation of slipperiness.

During these studies, we also observed that P6 mucus is present in two morphological varieties. P6r and P6s. Sometimes we needed to take a new sample after an hour. In these resamplings we nearly always got only the P6r variety, so that component is supposed to form more rapidly than P6s.

In this notations r means rapidly and s means slow. Apparently they are both about equally effective for the sensation of slipperiness.

When left to dry on a slide, this type presents a crystalline morphology composed of a central axis from which spring branches that form 60º angles, in contrast with the 90º angles formed by type L[1].

The anatomical structures of the cervix, which indicate the place where each kind of mucus is located, have also been studied in detail. Thus, Odeblad (1997) describes that the crypts producing type G mucus are found at the beginning of the cervix, near to its union with the vagina, a logical place to produce this protective stopper, characteristic of the phases of infertility. Productors of types L and S are found in the intermediate area of the cervix, and those of type P mucus preferably at the end, quite near the uterine body, which facilitates its function[2].

[1] The functions of these types of mucus have been described in detail. Although there are still many aspects to discover, today we can affirm that mucus G, present in the infertile phases, forms a stopper in the cervix. This stopper (or plug) closes it, makes it impenetrable to spermatozoids and defends the woman from infections, as it is especially rich in immunoglobulin and enzymes, such as lysosyme, which intervene in the general immunity of the body.
Mucus L, secreted several days before and up to ovulation, filters the spermatozoids, producing a very precise natural selection, as the diameter of the pore makes sperm advancement difficult. It does not impede it completely, but only the best sperm may pass.
Type S mucus, secreted at the moment of ovulation, is the great highway along which the spermatozoids can swim, once they have been duly filtered by type L.
Finally, type P mucus, of intermediate viscosity and a diameter of intermediate pore between type S and L, secreted at the end, would work as a filter and final carrier of sugars, which provide the glucoproteins of the mucus.
Cervical mucus in involved in sperm migration and maturation through the female genital tract, and provides a barrier to prevent the pathogens entering the endometrium (Menarguez, 2011). Rapid swimmers with normal morphology advanced within the S mucus string. But slow swimmers with abnormal shape deviate laterally into the L mucus. So the intimate contact of the two mucus types, S and L, is necessary for the sperm selection. G mucus is very rich in immunoglobulins against infections (ibidem).

[2] What is surprising in this investigation is to observe the care that Nature has bestowed upon the selection and filtering of the sperm, being extraordinarily generous with the number of spermatozoids

**3. The biochemistry of cervical mucus**

After considering the biophysical aspects, some ideas about the chemical composition of cervical secretion will lead to better understanding.

Cervical mucus, from a biochemical point of view, is a polymer of glucoproteins (mucin) with a high molecular weight. It forms a matrix in a gel phase, inside of which is included the aqueous phase of low molecular weight, called cervical plasma. Both phases form the mucus "per se" (Daunter, 1984).

The mucus is composed basically of water (90-98%), where diverse electrolytes, principally Na+ and Cl-, are dissolved. These electrolytes crystallise on taking a sample and allowing it to air-dry. They deposit upon the organic subtract, which remains unevaporated, and give us an idea of the molecular structure below. Further, in the aqueous phase, there are soluble proteins, chiefly albumin and globulins. Mucin is the essential substance that confers its characteristic properties to the mucus and, at the same time, is responsible for the differences among the four types of cervical mucus. It constitutes 1-2% of the total.

It is reasonable to think that the nature and composition of the mucin molecules of the four types of cervical mucus is similar but different, as the configuration of the networks that form the meshes, seen through a scanning electronic microscope, is also different. We are able to affirm, therefore, that different molecules of mucin originate different network structures, producing the four types of cervical mucus.

As we have indicated before, the mucin molecule consists of two parts:

A) glycosylated segment with carbohydrates,

B) naked or non-glycosylated peptide.

This double-segmented structure makes possible the existence of a certain variation in the disposition of the molecules, which form a glucoproteinic frame.

The networks of the different types of cervical mucus: Mucus G: 0.1-0.5 μm; mucus L: 0.4-3 μm; mucus S: 1.5-7 μm; mucus P: 0.4-2 μm (Menárguez, Pastor, Odeblad, Hum. Reprod. 2003) can be compared with a scanning electronic microscope.

The different sizes of the pores of the network are very important in understanding the functions of the mucus in spermatic migration, as we have seen before, since the size of the head of the spermatozoid is 5μm.

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secreted in each ejaculation (40-50 million). Later, it puts them through a large number of tests and difficulties in advancing. At the end, only one of them, the best, is responsible, together with the ovule, for the generation of a new human life.

The variability in the molecular architecture of the mucins is responsible for the different aggregations of the ions Na+ y Cl- in dried mucus, which lead to different patterns of crystallisation. It is important to understand that the crystals that we "see" in the optical microscope are only a "reflection" of a molecular disposition lying below.

What is observed in the images of the air-dried mucus are basically ClNa crystals. The different patterns of crystallisation corresponding to the different types of cervical mucus are due to complicated molecular interactions between mucin (and other organic material) and the Cl- and Na+ ions. The aqueous phase contains, in addition, other soluble components, such as traces of metals, seric proteins, enzymes and locally originated immunoglobins.

At midcycle, the concentration of soluble components decreases, due to an increase in the quantity of water (Daunter, 1984). Likewise, the concentration of sugars and proteins decreases in the ovulatory phase.

For several years, the objective of our investigation was to find a pre-ovulatory biochemical parameter which would indicate ovulation with sufficient anticipation, making the window of combined fertility more precise, bearing in mind spermatic survival.

Odeblad has suggested that the answer to this question may be in our immune system, the very same that saves us from sure death by infection. Any newborn with a deficient immunological system will soon die unless measures are taken to isolate it from an army of infectious agents (Alberts et al., 1989).

Immunology was born from the habitual observation that people who recover from certain infections are "immune" to the illness from that moment on. Many of the responses of the immune system initiate the destruction and elimination of the invading organisms and the toxic molecules they produce. Therefore, reacting against foreign molecules in the host organism and not against its own molecules is exactly what the immune system does. This capacity of distinguishing between foreign and innate molecules is another fundamental trait of the immune system.

There are women in Africa who have had an HIV-positive diagnosis for 12 or more years, who have had no access to antiretrovirals, and yet, have not developed the disease (Menarguez and Odeblad, Fermasa, 2003). When their immune systems were studied, a surprisingly elevated amount of lysosyme or muramidase, an agent belonging to the general immunity of the body, was found in tears.

This enzyme, found in high concentrations in the women who had not developed the disease in Africa, and present in cervical mucus as a defence against genital tract infections, may be considered, together with many others, one of the biochemical pre-ovulatory parameters.

The antebacterial properties of the cervical mucus have been described since the 60´0 (Rozansky, 1962).

Lysosyme or muramidase, together with other enzymes such as alpha-amylase, DNase, acid and alkaline phosphatase, are found among the soluble proteins dissolved in the liquid phase of cervical mucus (Schill and Schumacher, 1972).

The specific activity of these enzymes, expressed in u/mg of cervical mucus, shows a decline at midcycle, which coincides with an increase in their production, as well as an increase in the water content (Tsibris, 1982).

Most of the enzymes described in cervical mucus show a cyclic pattern (Moghissi, 1986), which implies a descent from 3-5 days prior to ovulation.

Muramidase or lysosyme, more precisely, shows a pre-ovulatory descent followed by a post-ovulatory ascent (Shill, Schumacher, 1972).

Diverse studies on alkaline phosphatase, amino peptidase, esterase lactate dehydrogenase, and guaiacol peroxidase have demonstrated that the concentration of all these enzymes is high during the follicular phase and decreases rapidly three or four days before the LH peak.

The lowest level of enzymes is normally reached on the day of the LH rise. The following day, there is a significant, brusque increase in their concentration, which is maintained during the luteal phase.

Different authors have corroborated the antimicrobial effect of the cervical mucus, with special attention paid to lysosyme.

Has no ever asked why the immune system does not react destroying the spermatozoids, being, as they are, foreign cells to the feminine organism?.

Odeblad, in his studies on cervical mucus, has described that the largest content of immunoglobins and defences against infections, in general, is found in the G mucus, present in a practically absolute percentage in the infertile phases of the cycle.

Human lysosyme is a basic protein with a polypeptide chain of 120 amino acids, structured with 4 disulphuric bonds, with a Pm of 15000, and acts by hydrolysis of glucosidic bonds. Its mechanism of action, part of the non-specific system of antimicrobial defence in different body fluids, consists of bacteriolysis by attack of the muramic acid, found in the cellular walls of bacteria.

The basic character of lysosyme and the strong interactions that it generates make its determination difficult, especially in complex biological matrixes, such as cervical mucus, of which only small quantities of samples are available.

Capillary electrophoresis, which works with volumes of sample on the order of nanolitres, is an especially useful instrument for this. It has demonstrated its capacity of separation and identification of lysosyme in egg white. Therefore, it is an ideal candidate to amplify the work, studying the variation in the concentrations found in cervical mucus as a possible indicator of fertility.

All the assessed studies evidenced that the observation of the CM allows the identification of the days with the highest probability of pregnancy (Fernandez-Hermida, 2018). Cervical mucus proteome changes throughout the menstrual cycle have been revealed. Few proteomic studies on the constitutive protein composition of CM of fertile women have been conducted to date so, cervical mucus is a reliable predictor of fertility.

Further characterization of CM proteins would contribute to a better understanding of the key role they have on fertility, reproduction and biological regulation. CM may represent moreover a source of biomarkers for gynecological diseases (Fernandez-Hermida, 2018).

## 4. Cervical mucus and its clinical applications

Over the last few years, fertility problems have also been an object of our study and open a very interesting new field of investigation. An example of this is the thesis presented by Dña. Isabel Valdés (2012). The study was carried out, in Madrid along fifteen years. Couples who wished to have a child and had difficulties or for those who wished to avoid a pregnancy at that moment, were attended in the public health system. They were attended 224 couples, that were looking for a pregnancy during at least one year without success. The success rate was 41,5%, 93 pregnancies, only with the parameters of cervical mucus and basal body temperature.

On commenting these results with those responsible for assisted reproduction in different centres in Spain, they agreed with us that before submitting the woman and her husband to so many tests that leave them physically and emotionally exhausted and are so expensive for the public health system, it would be well worth-while to obtain a diagnosis of fertility and infertility; show them the mucus and the temperature, and in that way see how many pregnancies are achieved before beginning with more invasive testing.

Our colleagues also agreed that there were many difficulties, as they do not have trained personnel, and many still do not know of the technical advances in the diagnosis and recognition of the fertile and infertile phases of the cycle.

The practical applications of the investigation of the cervix are directly related to the Billings method and there is a continual transfer of ideas between investigators and the work carried out in clinical practice.

The deviations in the normal cyclical curves of different types of mucus, the studies of cellular biology, and the teaching of the greatest number of investigators are possible fields of investigation in the future.

Finally, we would like to emphasise that the cervix is an organ of great biological complexity and very precise functions. It is sensitive to infections and external factors, such as the effects that hormonal treatments, often carried out without adequate medical control, produce in it.

The maintenance of reproductive health in the female should also consider these questions. Women have a right to a healthy cervix as part of their reproductive health and our investigation pretends, as far as possible, to help them achieve it.

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