Cervical Mucus, key to fertility - originality and wonder of Erik Odeblad's Discoveries

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The Biology of the Cervix

The cervix is an intricate system of crypts, previously referred to as "cervical glands" (Odeblad, 1972)[3]. The anatomical structures of the cervix, which indicate the place where each kind of mucus is located, were studied in detail by Odeblad.[4]

Thus, Odeblad (1997)[5] 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. Producers 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.

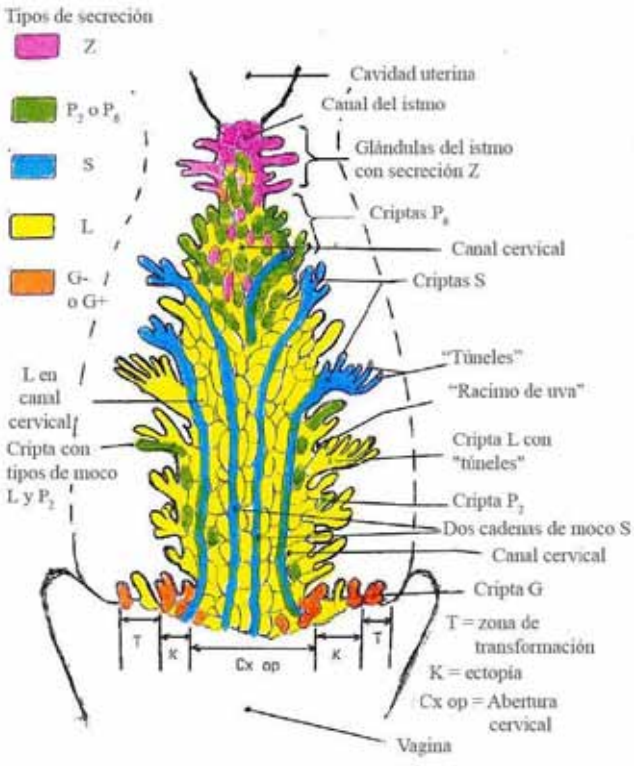

Fig. 1. Odeblad, E. *Bulletin of the Natural Family Planning Council of Victoria, Vol 21, Nº 3, September, 1994.*

Cervical epithelium contains receptors for estradiol and progesterone, which is why we know that the cervix is a "target organ" for these hormones.

At the moment of secretion, the cellular membrane breaks, producing the liberation of secretory granules (Odeblad,1973)[6]. 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.
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)[7].

The Biophysics of Cervical Mucus

At the beginning of his studies, Odeblad thought that all the crypts produced secretions at the same time.

In 1966, Odeblad discovered that some of these crypts were responsible for the fine mucus secreted on days close to ovulation and produced crystals when the mucus was air dried on a slide. Other crypts producing thick mucus did not crystallize and were obtained in the infertile phases of the cycle.

The first type of mucus discovered by Odeblad in 1966[8] was named E (estrogenic) and the second, G (gestagenic).

In 1977[9], Odeblad proved that Type E had 2 components:

1. S (sperm conveying)

2. 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 an angle of 90º.

In 1977 Dr. Kevin Hume learned that Professor Odeblad was to make a visit to Sydney, Australia, in response to an invitation coming from a group of veterinary scientists.

Dr. Hume was able to attend Professor Odeblad's presentation and afterwards informed him of the development of the Ovulation Method, providing him with copies of the Ovulation Method teaching materials which he took away for further study.

About two years later he reported that he had gone back over the records of his own research into the activity of the cervix of the uterus during the ovarian cycle, in his capacity as Professor of Medical Bio-Physics in the University of Umeå. He said that he had been surprised and delighted to find that the work that had been carried out in Melbourne precisely coincided with his own studies in Umeå and that the guidelines that had been devised in Melbourne for the use of the Ovulation Method were certainly correct. Professor James Brown added that the Ovulation Method has a rule to provide for every situation the woman may encounter during the reproductive era of her life.

"In 1983 I had the privilege of working with Drs. John and Evelyn Billings in Melbourne and also with Professor James Brown and other research workers of the Ovulation Method", Odeblad said.

"The hormonal response of G, L, and S mucus was studied. We found that L mucus was stimulated by medium and increasing levels, and S mucus by high levels, of oestrogen. Later I showed that S mucus was also stimulated by noradrenaline. G mucus was stimulated by progesterone. In the first infertile phase of the cycle the progesterone level is low, but sufficient to stimulate G crypts feebly (G- mucus). After ovulation, progesterone levels are high and stimulate G crypts strongly. This G mucus is very dense (G+ mucus)" (Odeblad).

In 1992[10] 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.

Several studies indicate that P6 mucus most probably is responsible for the peak sensation of slipperiness (2011)[11] .

On the other hand, we have to consider Odeblad´s studies with Nuclear Magnetic Resonance (NMR). These studies started in 1953, when he was awarded the Rockefeller Foundation Fellow at the University of California, Berkeley and Standford.

He was the first researcher to bring NMR to Europe through Sweden.

In 2003, Paul C. Lauterbur received the Nobel Prize in Physiology of Medicine. In his Nobel lecture, he said: "Actual medical measurements were started when Erik Odeblad, a Swedish M. D., constructed apparatus and devised methods to study very small quantities of human secretions for medical purposes".

Also in 2003 I had the privilege of publishing a paper with Prof. Odeblad in Human Reproduction[12]. It was also coverfront and Prof.Odeblad was delighted by that. It referred to the work of my doctoral thesis, which was done with his priceless help.

On 25 May 2012, Prof. Erik Odeblad received the European Magnetic Resonance Award 2012 in a special ceremony in Umea, Sweden.

The two prizes-one in Basic Science, the other in Medical Sciences- were combined into a single one. Odeblad was not the only one with a happy face in the room; members of the Swedish scientific community attending the presentation were clearly gratified that their colleague was finally being recognized. So also his family. Better late than never.

Samples from the Obstetrics and Gynecology Service of the A Coruña University Hospital, collected by Dr. Temprano, are presented. Endocervical secretion is obtained by aspiration with an insulin syringe. It is spread on a slide, then making a radial extension, with a needle, parallel to the glass. This Odeblad technique allows us to differentiate the most fluid types of secretion from the densest ones. The sample is allowed to dry at room temperature.

To determine the percentages of the types of cervical secretion, 10 fields are evaluated by optical microscopy (OM). Using Odeblad´s diagram about percentages of the different mucus types, we can know if this sample corresponds to a fertile or infertile secretion and to date the day of the cycle in relation to ovulation.

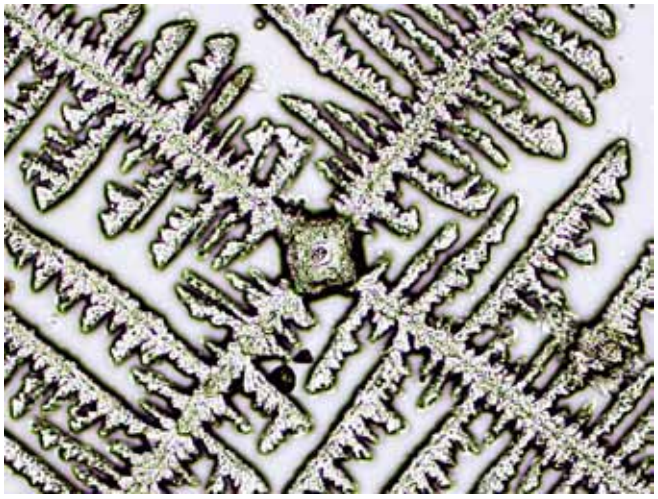

Fig. 2. L mucus. Showed a fern-like morphology with crystals forming a central axis from with long branches fanned out at a 90º. 55 years. Perimenopausal. Mucogram +++. Day 30º (-2). L78% S22% G1%. Big ferning. Cristallyization +++. 10x. m103, f 1771.[13]

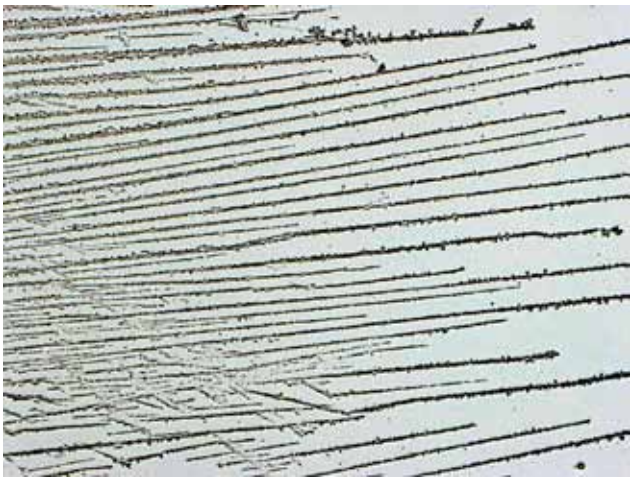

Fig. 3. S mucus. Crystallized into thin, parallel, needle-like structures. long micels. Fig. 381. Day 14º (0). L71% S29%. 10x. m118, f 2168.[14]

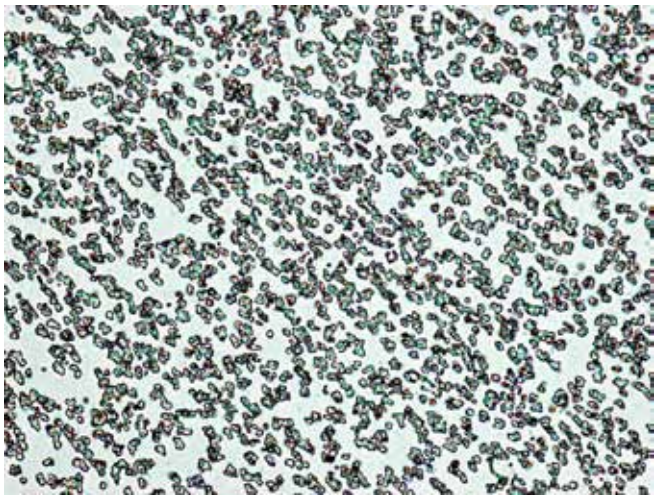

Fig 4. G mucus. Contained free crystals with a different morphology and sometimes a large number of cells. Day 13º (-4). L64% S7% G21%. G –. Criystals grouped, not organized. 40x. m131, f 2529.[15].

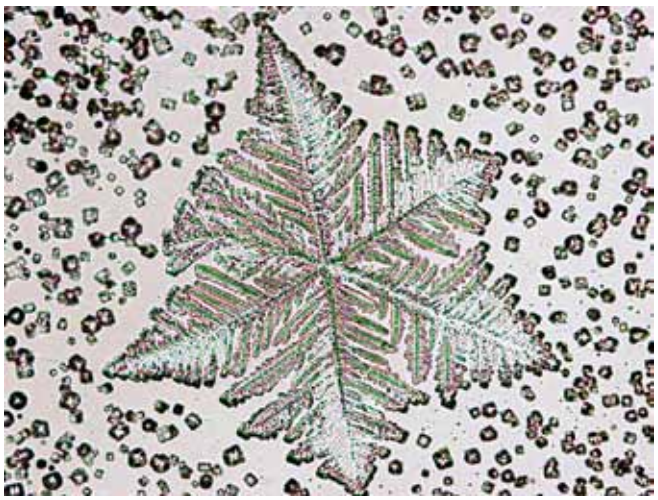

Fig. 5. P mucus. Presents a crystalline morphology composed of a central axis from with spring branches that form 60º angle in contrast with the 90º angle formed by type L. Cubic crystals indicates high estrogenic level. Day 18º (+1). L5%9 S15% G26%. Star P6. 20x. m130, f 2466 [16,17] .

The Biochemistry of Cervical Mucus

Cervical mucus, from a biochemical 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)[18].

The mucus is composed basically of water (90-98%), where diverse electrolytes, principally Na+ and Cl-, are dissolved. These electrolytes crystallize on taking a sample and allowing it to air-dry. They deposit upon the

organic subtract, which remains un-evaporated, and give us an idea of the molecular structure below.

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.

The glycoproteins network of the different types of cervical mucus is as follows:

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

This was measured by scanning with an electron microscope[19]

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

Samples from Umea University, collected by Prof. Odeblad and Dra. Menarguez, are presented. Endocervical secretion is obtained by aspiration with an insulin syringe. It is spread on a slide, then making a radial extension, with a needle, parallel to the glass. This Odeblad technique allows us to differentiate the most fluid types of secretion from the densest ones. The sample is allowed to dry at room temperature.

To determine the percentages of the types of cervical secretion, 10 fields are evaluated by optical microscopy (OM). Using Odeblad´s diagram about percentages of the different mucus types, we can know if this sample corresponds to a fertile or infertile secretion and to date the day of the cycle in relation to ovulation.

Part of the sample was fixed with glutaraldehide and metallized for its observation under scanning electron microscope. The results of the fixed samples were as follows:

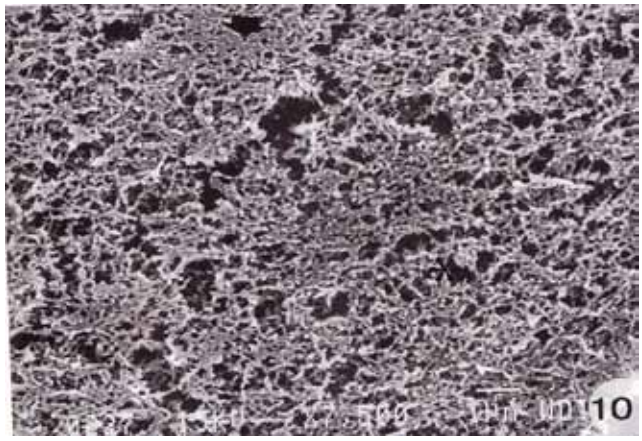

Fig. 6: FUNCTIONS OF THE L MUCUS: L crypt. MEB x 13500. Sizes of the pores of the glycoprotein network: 0.4-3 µm. L mucus, secreted several days before and up to ovulation, filters the spermatozoids (5 µm), producing a very precise natural selection, as the diameter of the pore makes sperm advancement difficult. It does not impede it completely, but only the best sperm may pass. f 101[20]

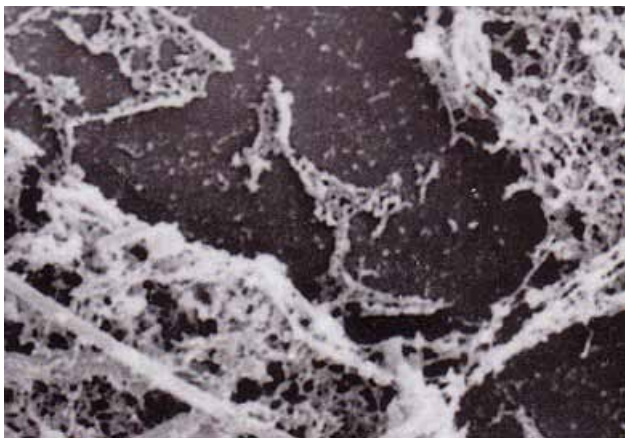

Fig 7: FUNCTIONS OF THE S MUCUS: Type III mucus. Sample 10 A. 55% L, 30% S, 5% P. MEB x 7000. Sizes of the pores of the glycoprotein network 5-7 µm. S mucus, secreted around ovulation, is the great highway along which the spermatozoids (5 µm) can swim, once they have been duly filtered by type L. f. 132[21]

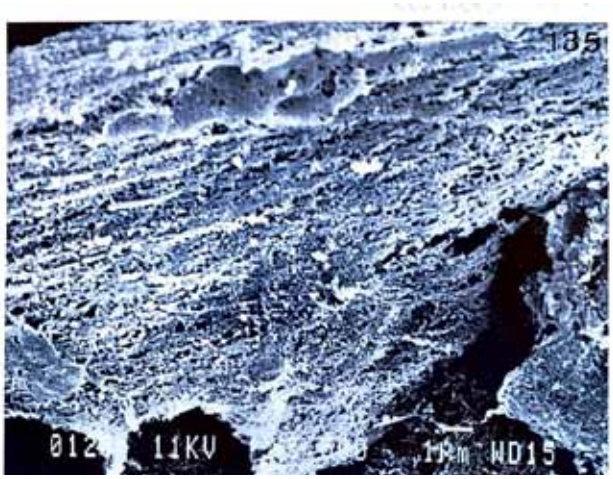

Fig 8: FUNCTIONS OF THE P MUCUS: Type IV mucus. Sample 12 A. 25%S, 50% G, 5% P. MEB x 9900. Sizes of the pores of the glycoprotein network: 0.4-2 µm. P mucus would work as a filter of spermatozoids (5 µm)and final carrier of sugars, which provide the glucoproteins of the mucus. F 135[22]

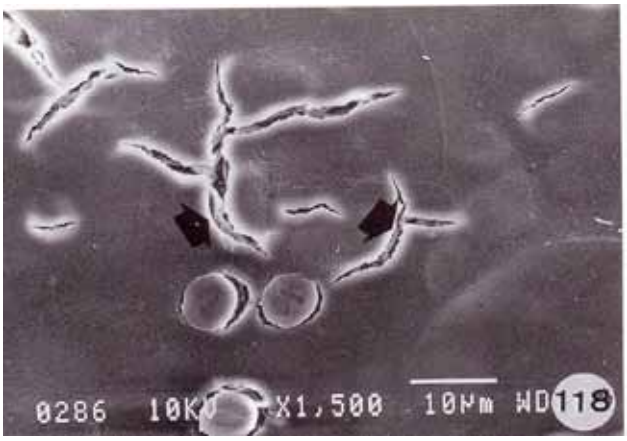

Fig 9: FUNCTIONS OF THE G MUCUS: G crypt. MEB x 2625. Sizes of the pores of the glycoprotein network 0.1-0.5 µm forms a stopper in the cérvix which closes it, makes it impenetrable to spermatozoids (5 µm) and defends the woman from infections, as it is especially rich in immunoglobulin and enzymes, such as lysosyme, which intervene in the general immunity of the body. F 118[23]

In his studies on cervical mucus, Odeblad has described that the largest content of inmunoglobulins 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.

What is observed in the images of the air dried mucus are basically ClNa crystals. The different patterns of crystallization corresponding to the different types of cervical mucus are due to complicated molecular interactions between mucins (and other organic material) and the Cl- and Na+ ions.

The Cervical Mucus and Clinical Applications

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.

a) The Collection from the lumen of the cervix.

The cervix was viewed with a speculum. The cervical orifice was cleaned with dried gauze and then an insulin syringe was inserted, without a needle, into the cervical orifice to obtain samples by aspiration. The mucus was stretched as far as possible with the syringe as it was being deposited on the slide, before being spread out.

b) Observation of the samples.

In order to observe air-dried cervical mucus samples with light microscopy, the samples were spread out on a slide in all directions using a needle (spread-out technique) (Odeblad, 1995)[24]. Afterwards, the samples

were air-dried at room temperature for at least 15 min before the study.

In the spread-out samples from the cervical canal the mucus was studied with light microscopy. To do so, 10 fields, chosen at random from the sample, were analyzed using a 10 x 10 grid in the microscope eyepiece, to note which cervical mucus type was present in each square. The number of squares in the 10 fields for each mucus type was added up and divided by the total number of squares to obtain the percentages of each mucus type.

With this semiquantitative study, the percentages of the different mucus types in each sample were calculated to determine the day of the cycle to which the sample belonged, using the Odeblad model (Odeblad, 2018)[25].

This technique proved to be:

a) quick
b) easy to perform at the different times of the cycle
c) easy to evaluate by counting the percentages of the different mucus types.

This can be very useful for improving the efficiency of the ferning test.

Our observations confirmed that there are cyclic changes in the ferning of the mucus, depending on the day of the cycle on which the samples are taken, which can be expressed as percentages. This quality would allow it to be used clinically, not only for problems of infertility but also for natural family planning.

Conclusions

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 for any difficulties in advancing, and at the end, only one of them, the best, is responsible, together with the ovule, for the generation of a new human life.

"We would like to emphasize 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 on 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 attempts, as far as possible, to help them to achieve it" (Prof. Odeblad)[26]

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17. "The study of crystallization in endocervical secretion samples was one of the objectives of Dr. Helvia Temprano in her Doctoral Thesis. Since its publication in 1990, it has continued to be the priority line of research. The Atlas publishes 672 figures extracted from the more than 3.000 photos taken of 131 cervical mucus samples obtained between 2008 at 2009 in the Gynecological Endocrinology and Fertility Recognition Consultations of the Maternal and Child Hospital of La Coruña. Also 22 samples obtained in a stay at 2011 at the University Medical Center of Piura (Peru). With all her experience, this Atlas that is now coming to light is probably the best source of knowledge published worldwide on the importance of the study of human cervical secretion"

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