SCANNER ELECTRON MICROSCOPY OF THE CERVICAL MUCUS

F. BONILLA-MUSOLES

Department of Obstetrics and Gynecology
School of Medicine, Valencia (Spain)

SUMMARY

The cyclical changes that appear in the fibrillar structure of the cervical mucus are described using scanner electron microscopy (SEM).

During ovulation the fibrillar mesh is formed of microfibers of 500 to 5000 Å that form a large open network, that is not very dense noted by ample spaces (up to 40 μ) between the fibers.

During the rest of the cycle the mesh is very dense and the fibers are very thick and less elastic.

Clin. Exp. Obst. Gyn. - ISSN: 0390-6663
X, n. 4, 1983

INTRODUCTION

The ultrastructure of the cervical mucus, although known for almost two decades (see revision in [1]), has been little investigated due to:

– the difficulties in the technics of the preparation of the specimens;

– the works in which this is described ([3-5, 15, 28]) are inconclusive;

– the impossibility of the study of the central part of the samples;

– the distinctive reologic properties of the mucus.

The works of transmission electron microscopy (see in [1]) have been very inconclusive. Only scanner electron microscopy (SEM) has been able to confirm the fibrillar structure suggested by Odeblad ([23, 24]).

Nevertheless, the images that have been furnished differed greatly, some having been described to have a pure fibrillar aspect, and of a very clear image ([3-17]), some not so clear ([26-28]); others having the aspect of a "honeycomb" ([19, 20]), or those, recently, which negated the fibrillar aspect ([22]).

All the investigations in this field, have been intended to prove the possible existence of cyclical changes in the fibrillar network that could justify the action against the sperm transport during the cycle ([13]), except during ovulation ([3-21, 26-28]), and under steroid administration ([25]).

MATERIAL AND METHODS

15 specimens of cervical mucus coming from patients with normal cycles corresponding to days 8, 14 and 22, all of which have a grade of purity of the vaginal mucus II of AF Huerlin were obtained by means of a pipette and prepared for SEM study.

The exact hormonal data were obtained by means of vaginal cytology, determinations with RIA of FSH, LH, 17-β-estradiol and plasma progesterone.

The specimens after dehydration by liofilizing in cold ([15]) were fixed in a 2% solution of glutaraldehid in cacodilat 0.1 M (pH = 7.4) for 1 hour. The "critical point technique" was then employed using Freon-11. The samples were covered in a vacuum chamber with an evaporator at 5 times 10^{-5} torr, with carbon and gold. The samples were examined with a model JSM-50A SEM with an acceleration voltage of 10 kW.

RESULTS

Indisputably the structure of the cervical mucus is fibrillar, even though the thickness and size of these fibers vary extensively during the cycle.

Under the estrogen and progesterone effect the fibrillar network is very compact, formed of coarse filaments of variable thickness and strongly ramified (figs. 1 and 2). The thickest fibrillar fascicules (figs. 1 and 2) reach the point of having 5 μ and, in contrast to other descriptions of the literature, we have found that their thickness varies in the same fibrilar segment. Thus one can't speak of a uniform thickness. From the thickest fibers emerge ramifications of first and second order whose thickness vary between 1 and 3 μ, and 0.5 and 2 μ respectively. The network that they form (fig. 1) is very dense, leaving spaces between fibers having a width of 1 to 5 μ.

In this way a very impenetrable network, that is able to show the practically absolute impossibility of the ascent of the sperm. On the other hand, the network is made up of thicker fibers than those that are observed during ovulation (fig. 3) which leads one to suspect not only that the fibrillar resistence is greater, but also that the elasticity of the same is much less, which supposes the slightest distension of the network.

During ovulation the microscopic aspect changes radically, the thickest fibers have a thickness less than in the rest of the cycle (some 3 μ), and even though the ramification that appears is larger, it is formed of much thinner secondary and tertiary fibers (0.3 to 1.5 μ) which are also much longer. Because of this the spaces that remain between the fibers are much wider (between 10 and 40 μ), which easily permits the passage of the sperm. This greater thinness of the fibers that make up the mesh permits its displacement which can be seen with the blood elements in which they are stationed.

It seems then that in this way the elasticity of the cervical mucus (of its semisolid component) increases greatly. In this way two factors favourable to the ascent of the sperm are combined: a larger size of the spaces between the mesh and greater elasticity of the same.

DISCUSSION

The fibrillar structure of the cervical mucus is due to the semisolid components of which it is made up (glycoproteins), and to these are due the physical properties which are characteristic (viscosity, cohesion).

The fibrillar system is made up of a basic unit, identical in all of them: fine fibers of glycoproteins. Each one of these possesses a central polypeptidic axis, formed of hydroxilized amino acids, to which are united lateral chains of carbohydrates.

The first suspicion of a fibrillar make-up was obtained from the biochemical studies of its macromolecular structure, assuming the formation of complex meshes. The use of the nuclear magnetic resonance ([23]) allowed us to get, without modifying the tissue structure ([2]), a perfect idea of the fibrilar organization of the mucus.

According to this, the dimensions of the fibers, and of the spaces between fibers, varied depending on the hormonal period. In ovulation the fibers measured around 0.5 μ in diameter, and were separated by spaces which varied between 1 and 10 μ. The fiber chains that have a parallel orientation, present small ramifications between them that unite them adapting a "tricot-like" form. During the secretory phase the mesh is much more dense and closed. During the estrogen phase the fibers follow a parallel direc-

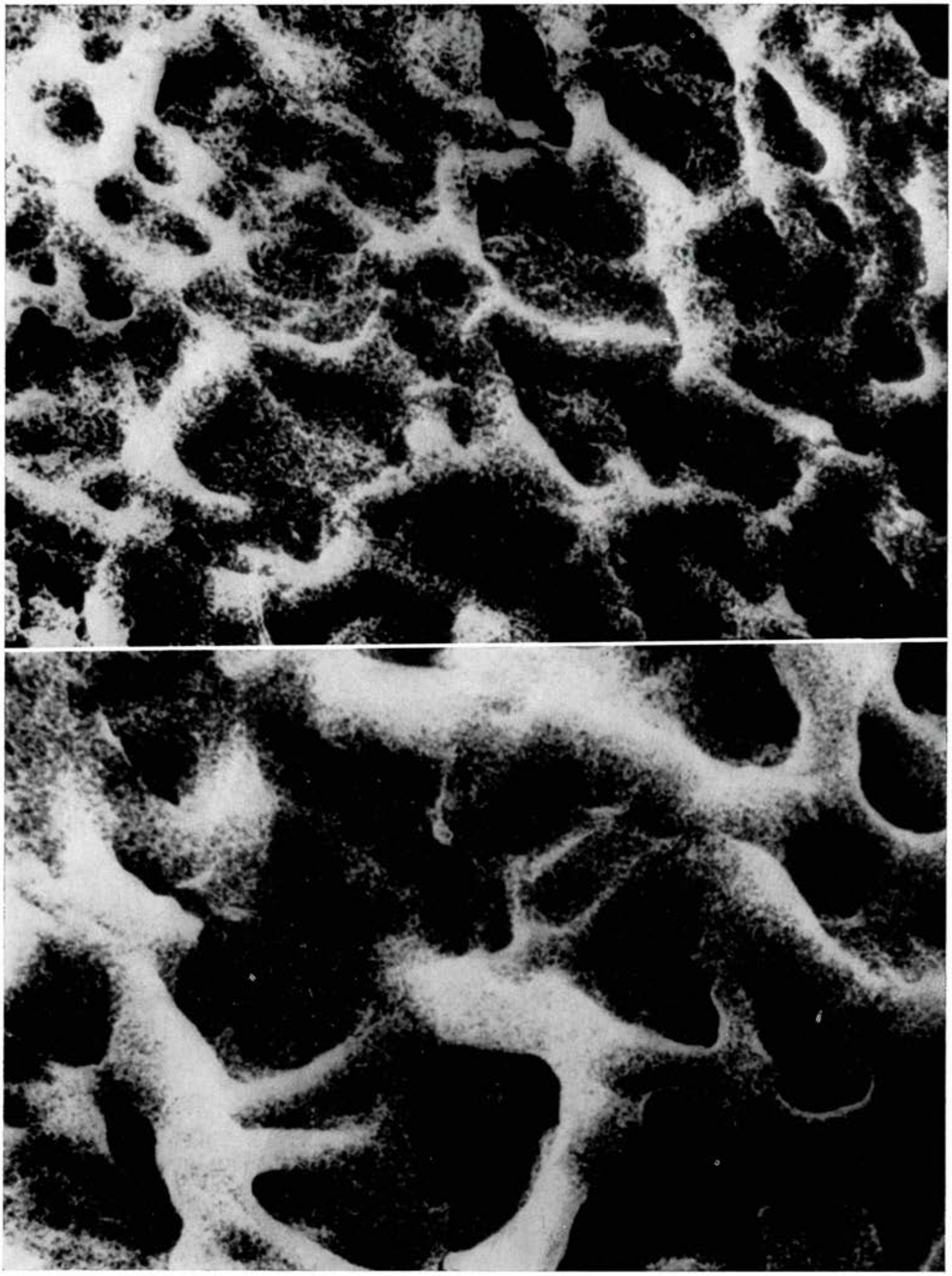

Fig. 1. — SEM of the cervical mucus with low magnification, and during the initial estrogen phase. The mucoprotein filaments are very thick, probably little distensible, and the mesh that is formed leaves very small spaces. All of that makes the penetration by sperms very difficult (1000-1500 ×).

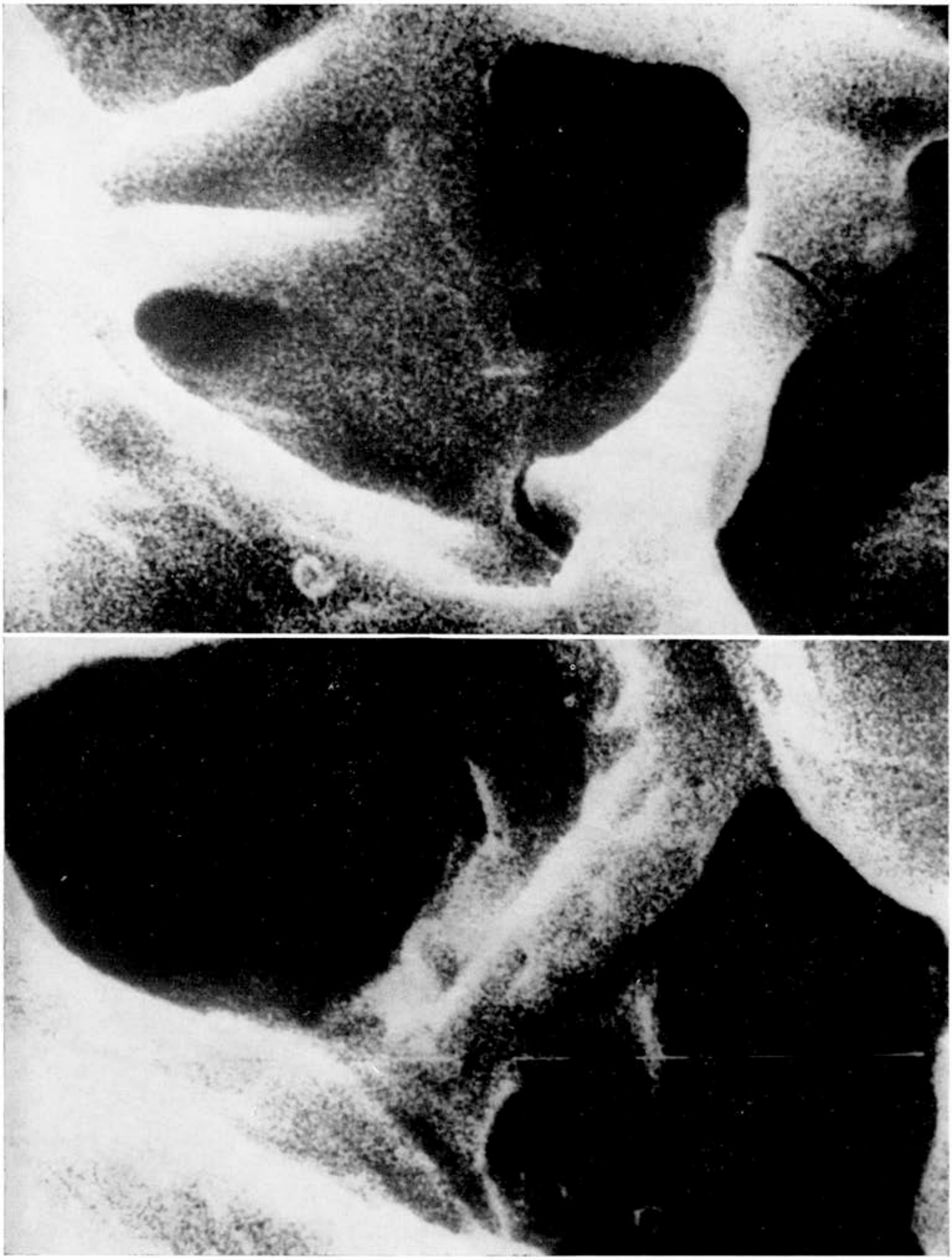

Fig. 2. — Cervical mucus in the estrogenic phase. This picture is similar in every way to that which is seen in the advanced progesterone phase. Thick ramificated mucoprotein filaments, leave minimum spaces (5000-7000 ×).

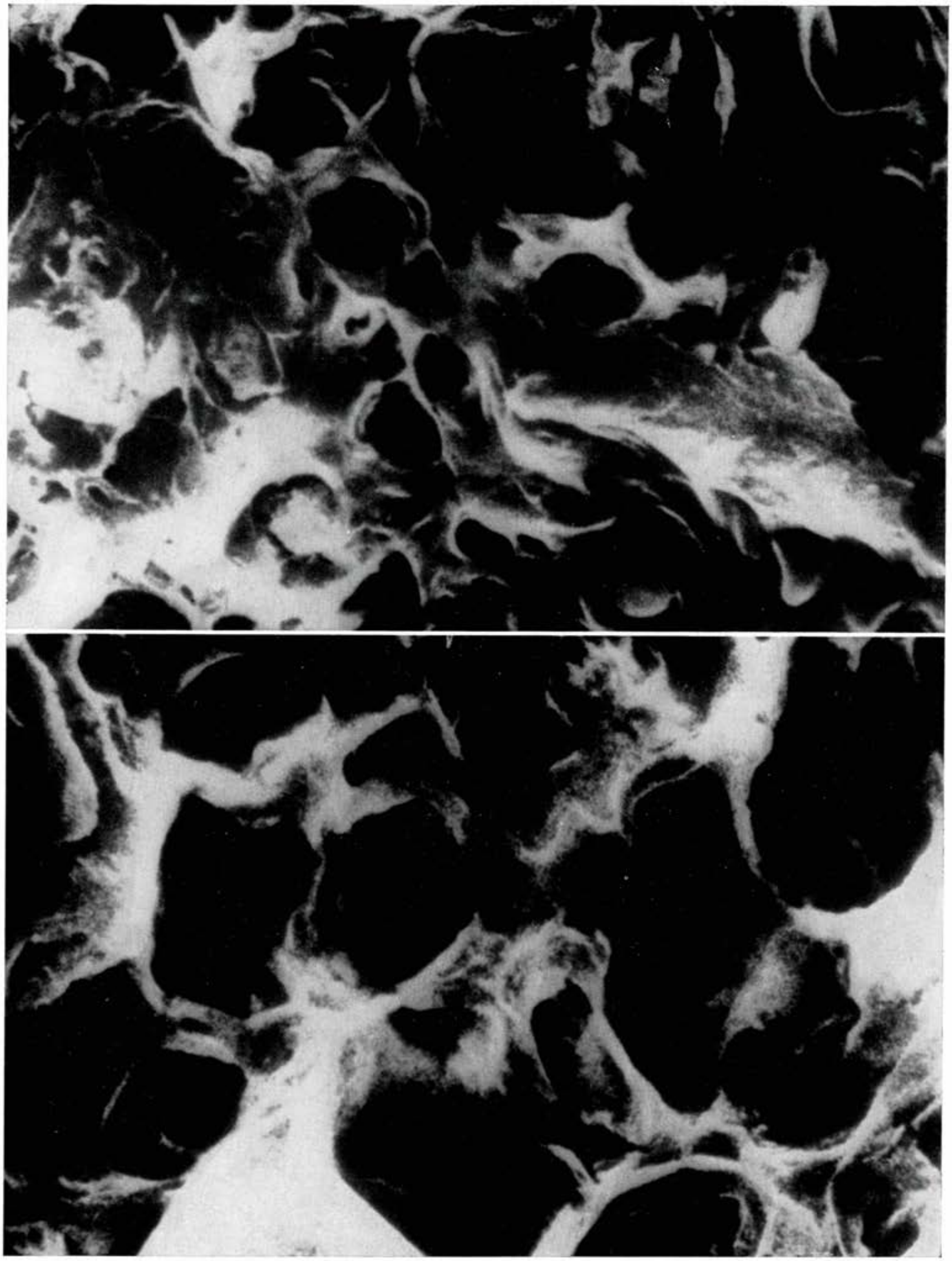

Fig. 3. — Cervical mucus during ovulation. The picture is radically different from that which is seen during estrogen and progesterone phase. The filaments are much longer, finer, more elastic, and the mesh that is formed is much more lax, showing much wider spaces between them (4000-5000 ×).

tion which disappears under the progesterone.

The transmission electron microscopy ([25]) was able to corroborate the organization in the form of a mesh. Each fiber was made up of microfibers of 200 to 500 Å, which formed long fibers of 500 to 2500 Å organized in parallel filaments of a thickness of 5000 Å separated by spaces of 5-15 μ; Elstein on the other and ([19, 20]) found the mucus to have a structure made up of globular particles of 1000 to 1500 Å in diameter.

Chretien ([3]), first to describe its organization with SEM, returned to show clearly the reticular structure formed of alpha filamens (3000-6000 Å), beta filaments (1000-1500 Å) and gamma filaments (500-700 Å). The first ones form the support, from which the beta and gamma filaments arise through ramification.

Later studies ([26, 28]) have confirmed all of this and have observed the cyclical changes ([6, 8, 12, 17, 18, 21, 26]), consisting of increases of interfibrillar space, the diminishing of the fibrillar mesh, specially the finer fibers, and the longer length of the same. Our findings confirm earlier results, recently not admitted ([22]), eventhough the fibers possessed, according to our results, variable thicknesses, and upon these depend the "Spinnbarkeit" and distension of the mucus in ovulation.

BIBLIOGRAPHY

1) Bonilla-Musoles F.: *El moco cervical.* In: *El cuello uterino y sus enfermedades*, F. Bonilla-Musoles (ed.), Jims, Barcelona, 1978.
2) Cazabat A. M., Volochine B., Kunstmann J. M., Chretien F. C.: *Acta Obst. Gyn. Scand.*, *61*, 385, 1982.
3) Chretien F. C.: *Contracept. Fert. Sexual.*, *1*, 9, 1973.
4) Chretien F. C., Gernigon G., David G., Psychoyos A.: *Fertil. Steril.*, *24*, 746, 1973.
5) Chretien F. C., Gernigon C., David G., Psychoyos A.: *J. Gyn. Obst. Biol. Repr.*, *2*, 341, 1973.
6) Chretien F. C., Gernigon G., David G., Psychoyos A.: *J. Gyn. Obst. Biol. Repr.*, *5*, 313, 1976.
7) Chretien F. C.: *J. Gyn. Obst. Biol. Repr.*, *3*, 711, 1974.
8) Chretien F. C., Cohen J., Psychoyos A.: *J. Repr. Fertil.*, *40*, 447, 1974.
9) Chretien F. C.: *J. Gyn. Obst. Biol. Repr.*, *3*, 115, 1974.
10) Chretien F. C.: *Acta Obst. Gyn. Scand.*, *40* (Suppl.), 15, 1975.
11) Chretien F.C., Psychoyos A.: *Cervical mucus (man).* In: Hafez E. S. E. (ed.), *Scanning electron microscopic atlas of mammalian reproduction.* Springer, New York, 234, 1975.
12) Chretien F. C., Cohen J., Psychoyos A.: *J. Reprod. Med.*, *14*, 192, 1975.
13) Chretien F. C., Psychoyos A.: *Temporary obstructive effect of human cervical mucus on spermatozoa throughout reproductive life: A scanning electron microscopy study.* Proc. Polish Gyn. Soc. Bialowizea, 1975.
14) Chretien F. C.: *J. Microsc. Biol. Cell.*, *24*, 23, 1975.
15) Chretien F. C., Cohen J., Psychoyos A.: *J. Gyn. Obst. Biol. Repr.*, *5*, 313, 1976.
16) Chretien F. C.: *J. Gyn. Obst. Biol. Repr.*, *6*, 451, 1977.
17) Chretien F. C., David G.: *Eur. J. Obst. Gyn. Repr. Biol.*, *816*, 307, 1978.
18) Daunter B., Chantler E., Elstein M.: *Brit. J. Obst. Gyn.*, *83*, 738, 1976.
19) Elstein M., Daunter B.: *The structure of cervical mucus.* In: *The Cervix*, Singer A. and Jordan J. (eds.), London, Saunders, 137, 1976.
20) Elstein M., Mitchell M., Syrett J. T.: *J. Obst. Gyn. Brit. Cwlth.*, *78*, 180, 1971.
21) Gould K. G., Martin D. E., Graham C. E.: *Scanning electron microscopy of human cervical mucus.* Proc. Workshop on SEM in Reprod. Biol. ITT Research Institute, Chicago, 1976.
22) Lee W., Blandau R. J., Verdugo P.: *Workshop conference on the uterine cervix in reproduction.* Rotach-Egern Insler V., and Bettendorf G. (eds.), Thieme, Stuttgart, 68, 1977.
23) Odeblad E.: *Acta Obst. Gyn. Scand.*, Suppl. 1, *59*, 79, 1968.
24) Odeblad E.: *Int. J. Fertil.*, *7*, 313, 1962.
25) Singer A., Reid B. L.: *J. Repr. Fertil.*, *23*, 249, 1970.
26) Zaneveld L. J. D., Tauber P. F., Port C., Propping D., Schumacher G. F. B.: *Am. J. Obst. Gyn.*, *122*, 650, 1975.
27) Zaneveld L. J. D., Tauber P. F., Port C., Propping D.: *Obst. Gyn.*, *46*, 419, 1975.
28) Zaneveld L. J. D., Tauber P. F., Propping D., Hafez E. S. E.: *Cervical mucus.* In: *Scanning electron microscopy of human reproduction.* Hafez E. S. E. (ed.), Ann. Arbor Science Publ. Inc. Ann. Arbor, 159, 1978.