Investigations on the Physiological Basis for Fertility Awareness

Professor Emeritus Erik Odeblad

At the National Conference organized by the Ovulation Method Research and Reference Centre of Australia in Melbourne in October 2001, the following paper was contributed by Erik Odeblad, Emeritus Professor of Medical BioPhysics in the University of Umeå, Sweden. The paper was read at the Conference by Drs John and Lyn Billings in combination.

Professor Odeblad has won international distinction for his superlative investigations into the activity of the woman's cervix during the menstrual cycle, particularly the secretion of different types of mucus, the pattern of their secretion being regulated by the hormonal control of the cycle and therefore defining the various phases of infertility and fertility.

The title of this presentation contains two words which we must discuss a little, namely Fertility and Awareness. In the present context the word Fertility means the capacity to produce offspring. This capacity is, as we know, different for women and men. In a broad sense women have a cyclically varying fertility from puberty to menopause, while men are fertile from puberty onwards for the rest of their lives. The Fertility which is the subject for this presentation is mainly the normal cyclically varying capacity to conceive in women. To be fertile a woman has to have a mature egg cell available for conception and implantation, and hopefully carry the offspring until delivery. The other word, Awareness, means that a woman can know when she possesses this capacity. Human beings have existed for a long time, and we may speculate that perhaps people have once had some natural feelings about the fertile periods in women. We know nothing about this in prehistoric and early historical time, and when people started to write in early times, this was probably restricted to men only. Who has heard about a woman Hippocrata or a woman Aristotela?

Even as late as in the sixteenth century there hardly existed any female authors, and it might have been forbidden to write about these things if a woman had tried. So, compared with all other knowledge we have, this subject cannot rely on any traditions, like many other fields such as chemistry, housekeeping, warfare, geometry etc. So, we have to develop this field, the history of which is less than a century.

Parameters we can study

There are several parameters we can do research on, e.g., body temperature, vaginal discharge, urinary samples, blood samples, subjective feelings, ultrasound etc. This presentation will be limited to some work on the vaginal discharge of the cervical mucus and the sensations women experience when observing and keeping records of her observations, that is, practising the Ovulation Method (Billings Ovulation Method®). Many of these women became very interested in understanding what is going on in their bodies, they want to see the cervical mucus under the microscope, they want to convey their knowledge and interest to other women and help to develop scientific knowledge in this area and understand the relationships between the objective measurements and their subjective sensations of mucus and other manifestations of the cycle. Even after many years of my own work, actually more than 50 years, there are more problems to solve than are already solved.

I will subdivide this presentation into three parts;

1) the stimulation of mucus secretion,

2) the properties of mucus and

3) how the perception and awareness of the fertile period occurs.

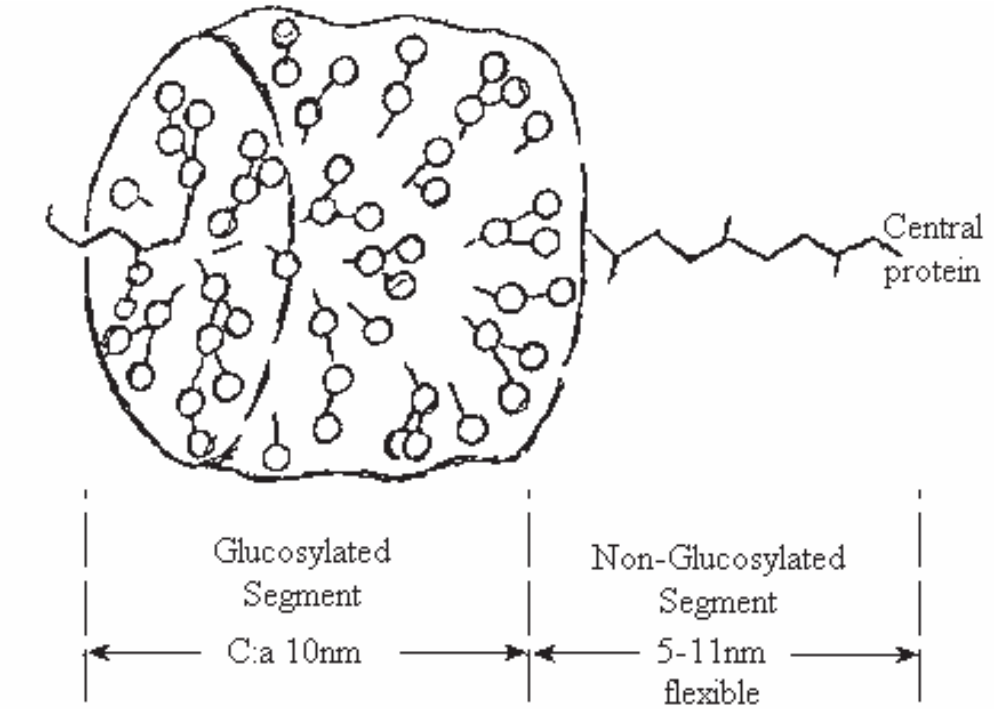

Figure 1A. *Sketch of a mucin molecule showing the glucosylated and non-glucosylated segments and the approximate size.*

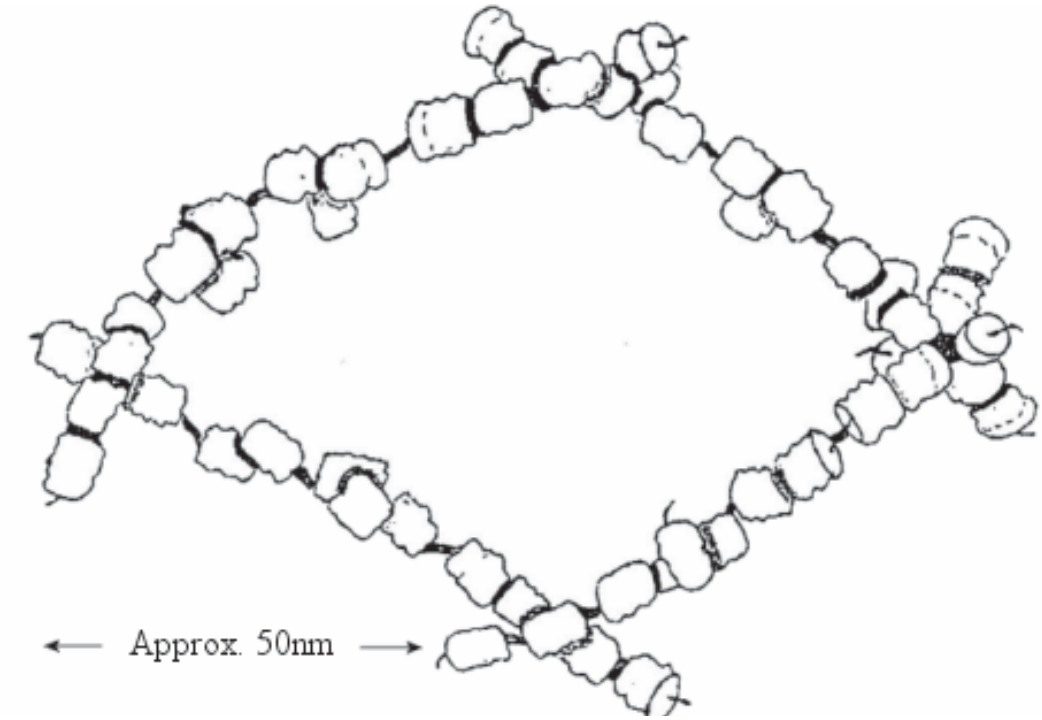

Figure 1B. *indicating how mucin molecules bind with various types of intermolecular bonds forming a mesh in G mucus.*

Stimulation of mucus secretion

Mucus consists essentially of two parts, the mucin and the water with its dissolved components. Mucin is built up of mucin molecules which interact, forming a network. The aqueous solution is present in the three dimensional meshes of this network.

Figure 1A shows a simplified drawing of a mucin molecule with its two parts, the glucosylated segment and the naked segment. A central protein is surrounded by attached sugar molecules in the glucosylated segment, and the central protein continues in one direction as the naked part. Several different types of sugar molecules are present in the glucosylated segment, and between them are many water molecules bound to the sugars with weak bonds (“hydrated water”).

The sugar composition is most probably different between the mucus types G, L, S, P2 and P6. This different composition contributes to the different ways in which the mucin molecules aggregate to form the different mucus types (Figure 1B).

Mucus G is stimulated by progesterone, mucus L, S, P2 and P6 are stimulated by estrogens, but (probably) at different blood levels. P6 mucus may also be stimulated by noradrenaline, which peaks around ovulation.

The way in which estrogen executes its action has been quite carefully investigated in recent years, and a simplified compilation of these results is shown in Figure 2. Briefly, estrogen molecules are bound to an estrogen receptor, which then becomes activated and finds a predestined location on DNA, which transcribes

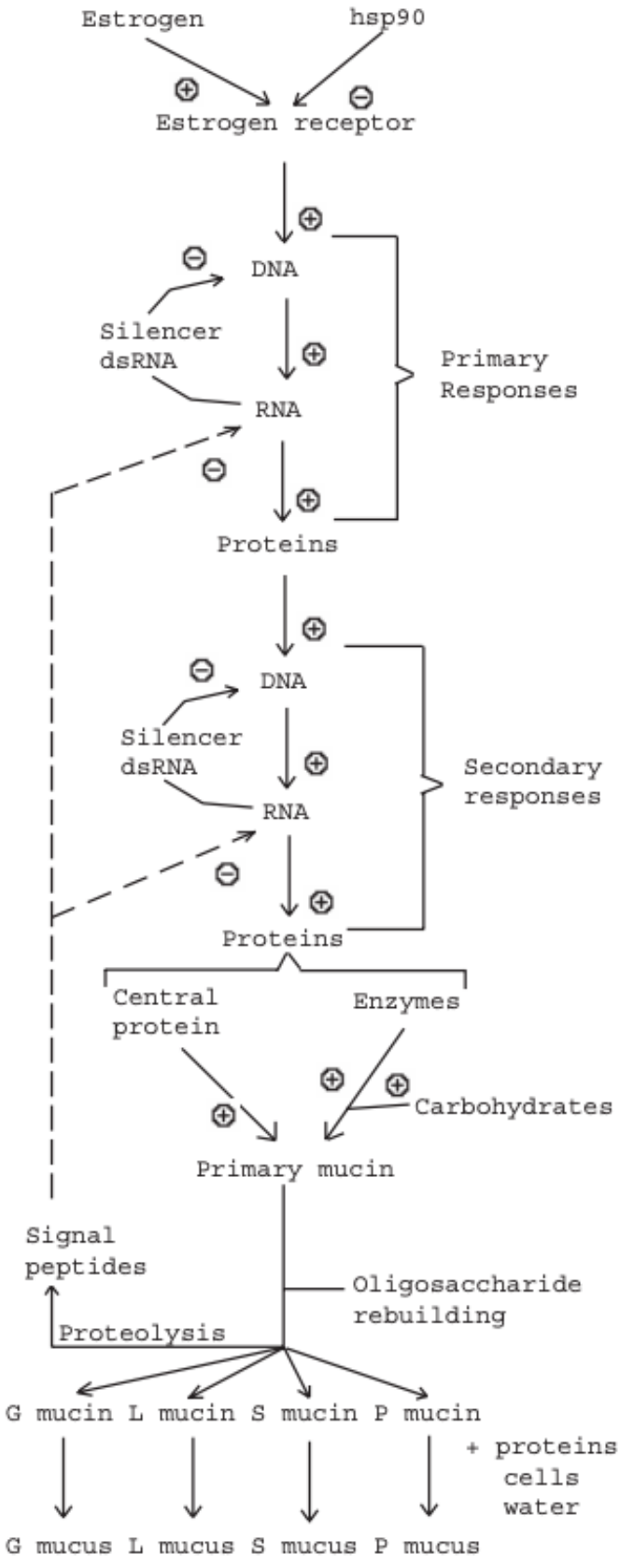

***Figure 2**. A flow diagram, showing the essentials of cervical mucin biosynthesis. Only a few steps are explained in the text.*

to RNA which in turn translates the message to proteins. Some of these proteins again go to predestined places on DNA and elicit similar reactions with protein biosynthesis.

Accordingly, there is one primary and one secondary response. The secondary response gives rise to the central protein of the mucin molecule and also to several enzymes which help to attach sugar molecules to the protein, so that a mucin molecule is formed.

There is one important consideration about this. To make a perfect mucin molecule, an enzyme must be at the correct place just at the precise time, a requirement which cannot always be fulfilled, especially as the number of enzyme molecules in every cell can vary and is very small. Therefore, there is an inherent variation of the properties of mucus. As mucus gives rise to the mucus symptom, this also becomes an inherent variable from cycle to cycle, something that every woman has experienced. It is a naturally occurring phenomenon. If the variations become very excessive, the cells have a control system which eliminates the deviating product, the so-named endosome-lysosome pathway.

There are four different cell populations in the cervix involved in the mucus symptom, L-cells, S-cells, P2-cells and P6-cells. These work independently and differently between different women, who often detect very large differences when discussing each other's mucus charts.

The Properties of Mucus

Mucus can be characterised in different ways, such as viscosity, transparency, stretchiness (strings), sperm conducting capacity etc. Because the mucus leaving the cervix is a mixture of the types G, L, S, P2 and P6, this composite mixture varies from day to day. Figure 3 shows a typical average curve of this variation.

The functions of the various mucus types and their contributions to the mucus symptom and fertility awareness can briefly be described as follows:
In the beginning of a normal menstrual cycle the G mucus will, shortly after menstruation, fill up the cervical canal and act as a barrier between the vagina and uterine corpus. G mucus is then a high-viscosity elastic stopper. It contains immunoglobulins and other antimicrobial agents, protecting the upper genital organs. Very small amounts of G mucus normally become detached from the plug and reach the vulva, but this varies from one woman to another.

When the estrogens begin to rise, the L-cells also begin to produce mucus. It has lower viscosity and begins to descend through the vagina and reach the vulva. Often this downflow is facilitated by mucolysis due to a mucolytic enzyme coming from the isthmus glands, located between cervix and corpus and carried by mucus P2 which exhibits a maximum in the beginning of the fertile phase. When the L mucus exceeds 50% of the total mucus, the woman perceives the beginning of the fertile period as a change in the character of the vaginal outflow, usually to a wet sticky sensation. The G mucus decreases steadily, and with increasing estrogens, the S mucus now begins to appear. When S mucus reaches the same amount as the L mucus, the woman notices a change in the character of mucus perception to a wet-slippery sensation. After still one or two days, the estrogen reaches a maximum, as also does the S mucus.

Now the P6 mucus begins to appear in rising quantity. The S mucus seldom reaches more than 35% and the P6 mucus seldom reaches more than 10%, but still changes the quality of sensation to an extremely slippery and lubricative sensation. A contributing factor to this sensation is probably an increase of the mucolytic activity again. The mucus aggregates are broken into smaller pieces which makes the mucus much more lubricative. Mucolysis may in some cases be excessive. This results in a loss of continuity of mucus, the vaginal outflow becomes watery, and loss of slipperiness and lubrication occurs.

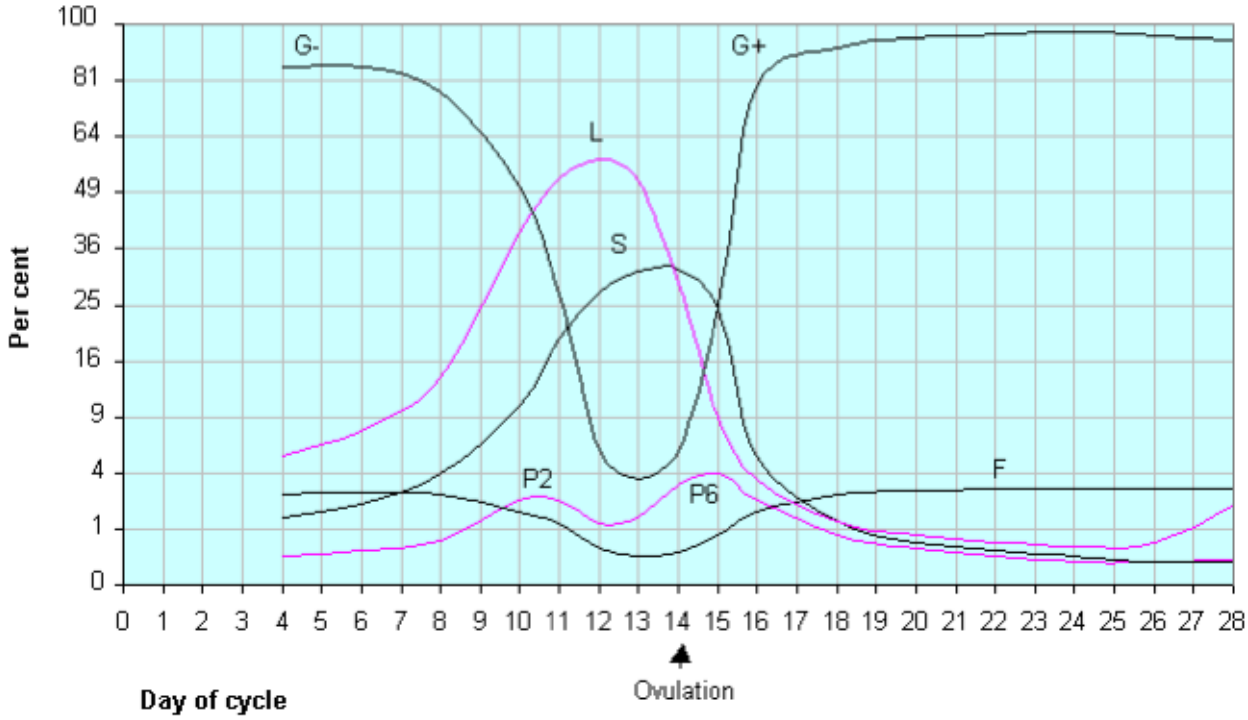

Figure 3. *Percentages of different mucus types. Average from 32 normal cycles, the same material as underlying figure 4 and described in the text.*

A group of 14 healthy women, aged 18-25 years recorded carefully their sensations during 32 cycles. Figure 4 shows the result of their record. They were all virgins and there was accordingly no problem with postcoital discharge of sperm, or sexual excitation which could confuse their records. Some important findings were made: Both the amount of mucus and the strings showed a maximum two days before ovulation and the slipperiness and wetness both culminated a few hours before ovulation (determined to within 8 hours by ovarian palpation, in a few cycles within 3 hours).

Shortly after ovulation the G mucus rises rapidly to nearly 100%, again giving effective mechanical and microbiological protection.

In cervical mucus there is always a small quantity of secretion which does not belong to any of the five types of mucus mentioned so far. This “mucus” may have a complex origin. It can be endometrial or even tubal secretion, or it may come from the surface glycoproteins which are present in all cells of the body. All these together form this mucus F.

Exfoliation from the vagina is always present at the vulva. This secretion seems to affect sensation only to a small extent, but in cases of vaginal infection it can become dominant and strongly interfere with the effort to make an accurate charting.

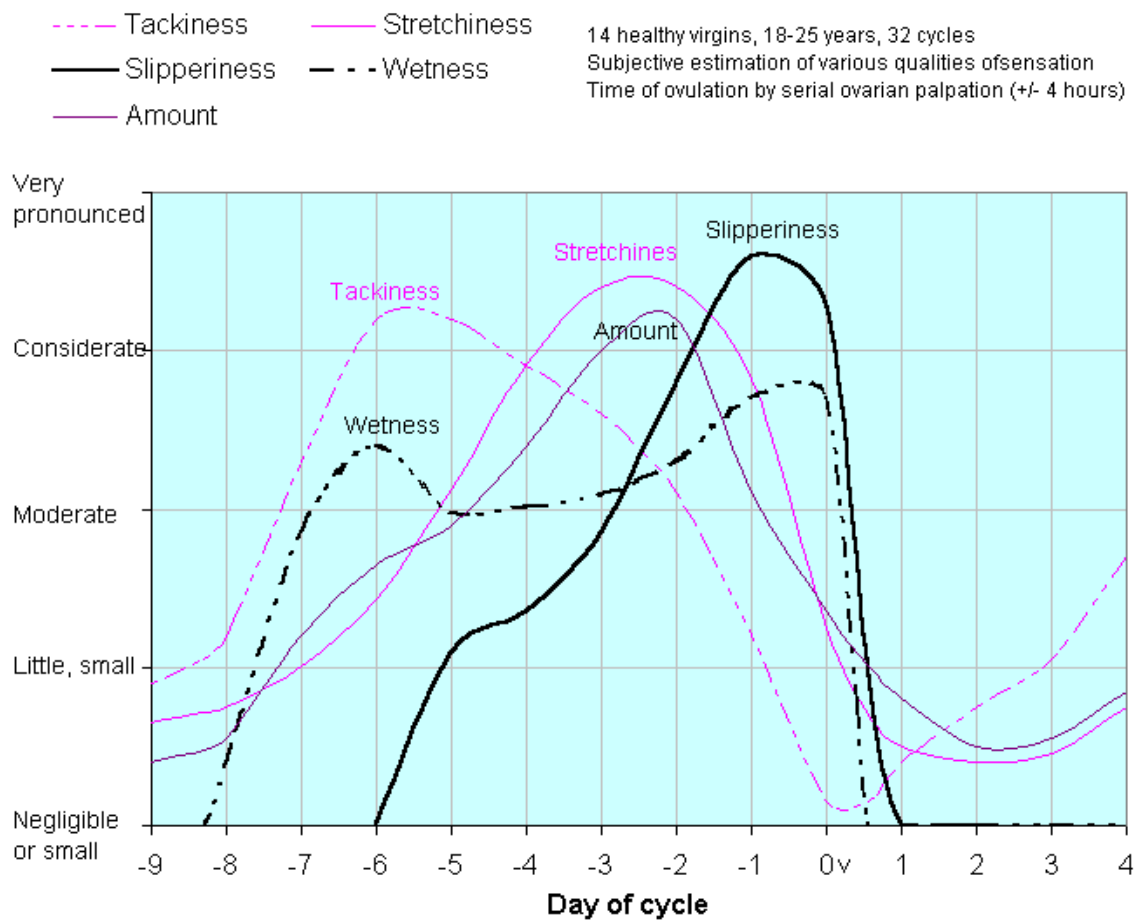

Figure 4. *Average of estimated strength of five parameters experienced by 14 healthy women around the fertile phase. Material described in the text.*

The variation of the length and quality of the mucus symptom

All women charting their cycles know that the mucus symptom can differ considerably from one cycle to another. There are several factors behind this variability. One factor may be changes in the estrogen level pattern in different cycles. However, our own investigations indicate that also in cycles with the same estrogen curve (within 1ó of variation), the mucus symptom for one particular woman varies considerably. To try to understand this we have to consider in some detail the *biosynthetic* properties of mucus.

First, cells may be differently supplied with oxygen, glucose, amino-acids and estrogen from the circulating blood. Colpo-microscopic observations on L-mucus- secreting mucosa on the portio show that capillaries are located some 12-30 mm apart and that the diffusion length to individual cells may vary between 4 and 15 mm. Because diffusion is inversely related to the square of the distance, the supply to different cells may vary with a factor of ten.

The age of secreting cells may also play a role. We know from cell-counting studies that these cells are continuously ageing and are replaced on a time scale of a few months to a year. It is very possible that “new” and “old” cells (at least in a pre-apoptotic state) differ in metabolic and secretory properties.

It is generally believed that a steroid-sensitive cell contains about 10,000 steroid receptor molecules. We *still do not know what stimulates receptor synthesis and degradation*. Because of the competition with hsp90, the available number of receptor molecules for estrogen may vary considerably; a qualified guess

may be by around 5,000. This number has to be shared between 7-12 primary response predestined sites on DNA. Furthermore, these sites have to be available during the correct time by DNA decoiling from the respective histone complexes by acetylation. We still do not know how this process is regulated in a steroid-sensitive cell.

In Figure 2 several negative-feed-back processes are indicated. Also, several others exist, e.g., one of the primary response products, a special protein, suppresses the action of the activated estrogen receptor. All these considerations are probably valid for all estrogen- stimulated mucus types, L, S, P2 and P6, and, in principle also for the progesterone-stimulated types G- and G+.

If we can draw any conclusions at all from this knowledge, or lack of knowledge, it might be that there is room for considerable variation of quantity and quality of cervical mucus, and consequently for being aware of fertility. But also, that this variability is part of human physiology. And still, it is not possible to foresee any better technological or non-technological method giving better information on fertility awareness than the Ovulation Method (Billings Ovulation Method®).

Perception and awareness of the fertile period

Mucus perception occurs at the surface of the minor labia of the vulva. The mucus membrane there is covered with stratified epithelium similar to that of the gingiva. It differs from the vagina which has a thicker epithelium which reacts strongly on estrogen stimulation. On the labia minora the proliferative response to estrogens is much weaker. The underlying connective tissue behaves in an opposite manner. The vaginal submucosal connective tissue does not show so pronounced a cyclic variation as it does in the minor labia, which can be markedly swollen around ovulatory time.

The total area of the sensitive part of the labia minora shows pronounced changes with age and parity. This is indicated in Figure 5. Even if there are very small cyclical variations of the epithelium, they show long-term sensitivity for estrogenic hormones and grow slowly from early adolescence to mature age, and also during pregnancy. Also, the regression after pregnancy is a slow process. In the menopause there can occur pronounced atrophy sometimes leading to Kraurosis vulvae with dyspareunia and painful micturition. This condition can even start before menopause. Also, all vaginal infections make the vulva sensitive and tender.

The sensation of the mucus symptom is possibly due to the presence of Merkel and Pacini tactile receptors located under the epithelia. They differ considerably in their properties. The Merkel receptors have very long adaptation time, which means that they can sense long-time mechanical stimulation but do not respond to rapidly changing pressure. The Pacini receptors respond quickly to stimuli varying on the time scale of seconds, while the time scale for Merkel receptors is in the order of many minutes. The spinal segment innervating the minor labia is probably S4 but may in part also be S3. The nerve cells of the dorsal root have been shown to contain estrogen receptors, a remarkable finding. This means that estrogens may more or less regulate the response of these neurons. It is still not known if this may be relevant to mucus symptom perception.

Vulvar sensing mucosa

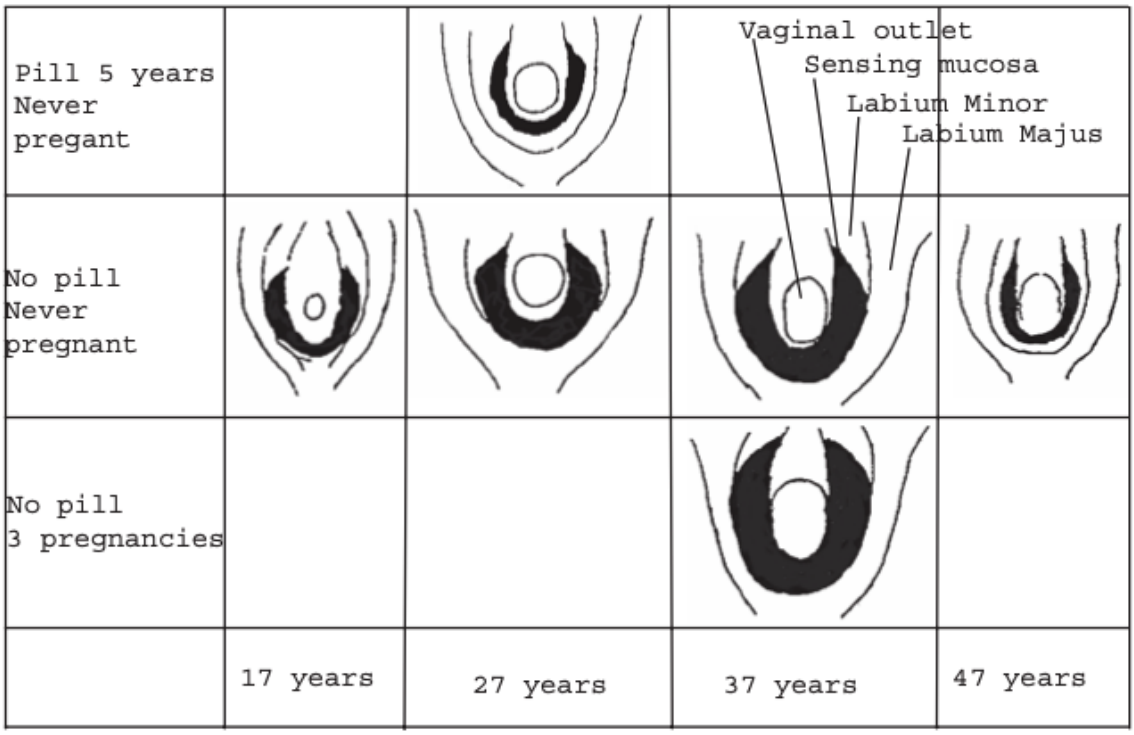

Figure 5. *Average size of the sensitive area of the vulva. Averages on 56 healthy women. The ages are average in each group.*

The response to the mucus symptom is, as experience shows, strongly dependent on body movements, like walking around, walking on stairs, biking, sports, etc. Activism in the pelvic muscles during micturition etc. also increases sensitivity as also more passive pelvic movements such as coughing and sneezing. Rest-activity and day-night periods lead to intravaginal mucus accumulation and release of “stored” mucus.

It is not quite clear where in the brain the sensed impulses are projected. Available information suggests the inside of Sensory Area 1.

We know that the estrogen molecules compete with the protein hsp90 when finding the estrogen receptor. hsp90 increases in amount at elevated body temperature (fever) and sometimes in stress. This circumstance may contribute to the variability of the mucus symptom.

Changes of the rhythm of the cycle, such as amenorrhoea, post-pill situation, stress induced amenorrhoea, etc. may also alter the character of the mucus symptom. Normal lactational amenorrhoea is, however, usually no problem; the first ovulation is usually easily recognizable.

The ovulatory noradrenaline peak may also give other symptoms which some women can learn to recognize, e.g., changes in pupillary size and heartbeat frequency.

Also, the vulvar ovulatory swelling may often be accompanied by swelling in the groin and/or a swollen lymph node on the ovulating side.

All these are signs of ovulation which do not require any extra technology.

Understanding the physiological basis for fertility awareness is important for all teachers of NFP, especially the Ovulation Method (Billings Ovulation Method®), in order to increase the safety, acceptability and continuity of the method and for the autonomy of the couples practising the method.

References

Estrogen receptor. *Rhodes, D and Klug, A*.: Zinc fingers. Sci. Am. Febr. 1993: 32-39.

Steroid receptors in general. *Chawla, A. et al*.: Nuclear receptors and Lipid Physiology: Opening the X files. Science 294, (No 5548), 2000: 1866-1870.

RNA inhibition of transcription. *Sharp, Ph. A. and Zamore Ph. D*.: RNA interference. Science 287, (No. 5452): 2000: 2431 and 2433.

Estrogen receptor and hsp90. *Geogropouloa, C. and Welch, W. J.:* Role of major heat shock proteins as molecular chaperones. Ann. Rev. Cell Biol. 9, 1993: 601-634.

Primary and secondary response to steroid hormones. *Yamamoto,K.R.:* Steroid receptor regulated transcription of regulated genes and gene networks. Ann. Rev. Genetics 19, 1995: 209-252.