MICROSCOPY ATLAS

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PHASE CONTRAST MICROSCOPY OF CERVICOVAGINAL DISCHARGE

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1

INTRODUCTION

Antoni van Leeuwenhoek (1632–1723), a cloth trader, developed and perfected the first simple microscope, with a magnification up to x200, in Delft (the Netherlands). August Köhler (1866–1948) revolutionized the light illumination of microscopes in 1893, as a member of Carl Zeiss AG in Jena (Germany), by focusing the light on the front aperture of the condenser, which allowed it to focus on the object. This technique is still used today. Frits Zernike (1888–1966) developed the phase contrast technique in 1930 (Groningen, the Netherlands) and was awarded a Nobel prize for the invention in 1953.[1] He recognized that the diffraction pattern in the rear focal plane of the objective exhibits characteristic differences in non-absorbing objects that affects the phase of the transmitted light compared to absorbing objects. The light is made visible by shifting and weakening the phase of the non-diffracted "direct" light in contrast to the diffracted light by means of a "phase plate".[2, 3] The practical implementation of phase contrast microscopy took place in 1941 at the Carl Zeiss company, in Jena. This technique is currently also used, for instance, in fluorescence and confocal microscopy for optical contrasting. The differential interference contrast (DIC) or Nomarski-Contrast method (introduced by inventor Georges Nomarski, Paris)[14] is rarely used in practical use.

In gynecological practice, different microscopy techniques are applied, including: bright- and dark-field microscopy and phase-contrast. Cytological Papanicolaou smears, as well as Gram-stained microbiological preparations are read using conventional bright-field microscopy, the latter requiring x1.000 magnification and oil immersion.

Native specimens of vaginal discharge are sometimes observed using methylene blue 0.5% staining, for better visualization of bacteria and yeast cells in the poorly contrasted background. It must be kept in mind that methylene blue is toxic for trichomonads, thus making their identification harder. Most authors, nevertheless, recommend the use of phase-contrast microscopy, without staining, for assessment of, preferably, fresh vaginal samples.[4]

Using the latter technique, skin cells and hairs can also be observed (in addition to cervicovaginal discharge). The evaluation is simple and quick, providing significantly more information compared to simple bright-field microscopy. *(Figure 1)*

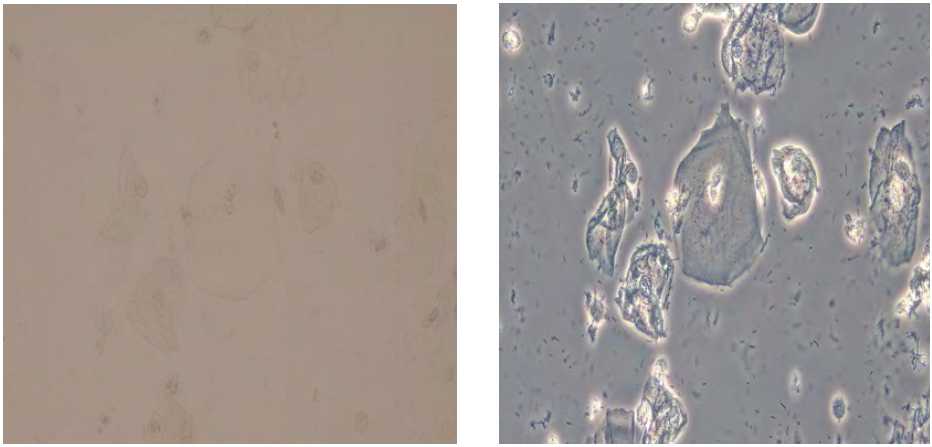


FIGURE 1a and 1b Comparison of the same field of a microscopic slide of vaginal discharge using bright light (a) and phase contrast (b) microscopy.

Phase-contrast microscopy can be performed immediately after the gynecological examination and should be considered a fundamental component of it. However, deferred observation of the slide is also possibile.[4]

Wet mount microscopy (WMM) is recommended as the first step for the diagnosis of bacterial vaginosis (BV), vulvovaginal *Candida spp*. infections, and trichomoniasis in various guidelines, including the *International Society for the Study of Vulvovaginal Disease Recommendations for the Diagnosis and Treatment of Vaginitis* (download it at https://www.issvd.org/guidelines).[4-8]

Nevertheless, some critical points must be stressed: 1) the fact that microscopes are not always available, 2) academic teaching centers often fail to adequately teach trainees to use this diagnostic tool, and 3) the diagnosis depends on the training and expertise of the clinician.

While nucleic acid amplification tests (NAATs) have already been established as the gold standard for diagnosing trichomoniasis, the place of the molecular tests that effectively diagnose BV and candidiasis (or candidosis*) remains to be validated. Despite their good clinical performance, NAATs are expensive and may fail to evaluate the full picture. Other recent solutions include tools that automatically read wet mount slides (GYNI™, GynTools, Israel). [9, 10] Nevertheless, the use of WMM is still far from being considered anachronistic, given its simplicity, low cost, good clinical performance, and allowing the evaluation of other factors such as hormonal status and inflammation, for instance.[5]

Phase-contrast microscopy of fresh discharge allows functional and hormonal evaluation through the characteristics of the desquamated vaginal epithelial cells, and the differentiation of eubiosis (normal microbiota) *vs.* dysbiosis (abnormal microbiota), BV, mixed vaginitis, trichomoniasis, candidiasis, inflammation and the identification of "toxic"/ activated leucocytes, sperm, or vaginally applied medication.

* Some authors prefer to use the term "candidosis", as "-osis" is the suffix usually used for fungal infections

Phase-contrast microscopy is not suitable for the diagnosis of sexually transmitted infections caused by gonococci, *Mycoplasma genitalium, Chlamydia trachomatis, Treponema pallidum* or herpes simplex viruses. It also does not allow the differentiation between Gram-negative and Gram-positive bacteria, nor to diagnose cervical or vaginal dysplasia.

Halo effects can occur due to superimposition of structures in thicker samples. Therefore, for phase-contrast microscopy, thin specimens are mandatory, as this will minimize overlap.

It is recommended to use x400 magnification for gynecological WMM (objective x40 combined with an ocular lens x10).

The aperture diaphragm (if present in the condenser) must remain fully open.

2

PHASE-CONTRAST MICROSCOPY

2.1 Advantages and disadvantages

TABLE 1
Advantages and disadvantages of phase-contrast microscopy for evaluation of vaginal discharge

Advantages	Disadvantages
• Immediate • Staining not required • Quick • Low cost • Easy to perform • Relatively fast learning curve • Good performance from the clinical point of view (except for *Trichomonas vaginalis*) • Allows the diagnosis of "mixed infections" • Good sensitivity and specificity for cytolytic vaginosis and desquamative inflammatory vaginitis/aerobic vaginitis • Evaluation of the hormonal status • Evaluation of inflammation	• Preparations can be destroyed • Experienced/trained reader necessary • Poor sensitivity for trichomoniasis

2.2 Physics, tuning, and set-up of the phase contrast microscope

The phase-contrast objectives are typically labeled as follows:

- Objective x20: Ph 1
- Objective x40: Ph 2 *(Figure 2)*

The communicating aperture in the condenser/capacitator is marked with the same number (1 or 2) and must match the number in the objective *(Figures 3 a, b)*.

It is essential to assure that the annular aperture in the condenser and the phase ring in the objective are coordinated and perfectly aligned. To check it, the investigator can remove the ocular lens and look into the microscope through the tube: if the condenser and the objective are aligned, one shall see only one bright ring; if not, he will observe a bright and a dark ring, which do not exactly cover each other.

FIGURE 2 40 fold phase contrast objective with a blue ring and green letters "Ph 2".

FIGURE 3a Adjusted phase ring in the condensor.

FIGURE 3b Phase ring in the condensor pulled out.

The light waves are first directed through a converging lens onto the ring diaphragm of the condenser, which creates a cone of light. Those waves then pass through the object onto the lens, where they meet the phase ring. When the waves pass through the object, a part of them is deflected by diffraction. The diffracted light beam passes the object without a phase shift and does not hit the phase ring in the lens. The non-diffracted light rays experience a phase shift of ¼ wavelength through the transparent slide preparation and hit the phase ring, causing the light ray to be delayed again by ¼ wavelength. There is now a phase difference of half a wavelength. In the intermediate image plane, wave crests and troughs meet each other because of the phase differences. The consequence is cancellation by interference, and the result of this are light and dark contrasts, which make the transparent object visible for the eye. The structural details of the object appear dark on a lighter background or vice versa, depending on the different phase rings, which allows a plastic picture.

2.2.1 The Köhler illumination principle

If the illumination of the specimen is not perfectly set, the microscope will perform below the expected capability. Guidelines for optimal light guidance were first published by August Köhler in 1883, and remain a basic principle of light microscopy.

The general principles of Köhler are:

1. The condenser is turned up close under the specimen stage and a stained specimen is focused on the bright field.
2. The light field diaphragm at the base of the microscope should now be nearly completely closed, so that the aperture can be seen as a point. If this point is not in the center of the field, it must be moved to this position by adjusting the centering screws of the condenser or by moving the collector.
3. The height of the condenser is adjusted so that the edge of the field diaphragm is in focus.
4. Finally, the field diaphragm is opened again until the center field is illuminated.

Specific procedures of phase-contrast:

1. The stained specimen must be switched to an unstained one (i.e. vaginal discharge). The light field diaphragm must be completely open and brightness set to the maximum.
2. The phase aperture must be set to match the phase contrast objective (Ph 1 for x20 or Ph 2 for x40); the way it is done varies between microscopes (usually using a revolver or, in many modern microscopes, a slider
3. After removing the ocular/eyepiece and looking into the microscope, a bright ring (image of the condenser diaphragm) and a grey ring (image of the phase ring in the objective) are seen and they must be in the center and completely covering the bright ring.

2.2.2 Cleaning of the microscope

The front lenses of the objectives and the oculars, but also all other lenses, can be contaminated by dust, grease (from the skin or eyelashes), salt crystals from dried saline solution, KOH, and dried discharge.

Alcohol or disinfecting agents are not recommended, because they can damage the lens cementation.

A soft microfiber cleaning cloth with water or a special cleaning agent recommended by the manufacturing company is the preferred option.

The microscope must be covered with a hood, when not in use.

3

PREPARATION OF A VAGINAL WET MOUNT SLIDE

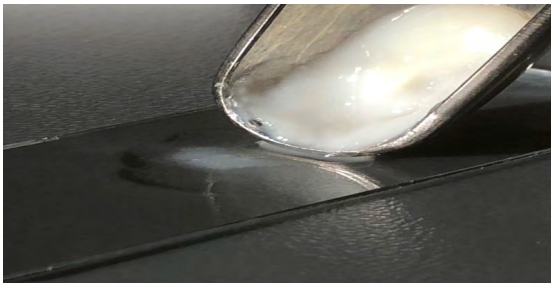


FIGURE 4 Preparing a sample for wet mount microscopy, by adding vaginal discharge from a speculum blade to a drop of saline.

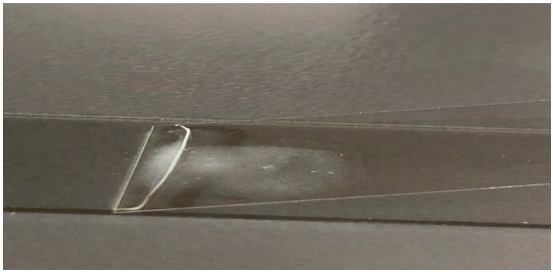


FIGURE 5 A cover slip must be applied before observation. Some pressure should be added to avoid the formation of air bubbles. The excess of saline/discharge should be cleaned with absorbent paper.

FIGURE 6 View of the slide with cover slip, ready for reading.

There is no need to use a sterile swab for sampling. Cotton swabs can absorb the discharge and contaminate the sample with fibers. Plastic spatulas or endocervical brushes can be great tools for the purpose of sampling discharge. Alternatively, it is possible to remove a small amount of discharge from the lateral middle vaginal wall with the speculum blade and touch with it into the saline (or 10-15% KOH) drop, which is already prepared on a glass slide *(Figure 4)*. It is also possible to spread the sample evenly in the glass slide and then add the drop of saline or KOH. The sample is then covered with a coverslip and pressed to avoid the formation of air bubbles. *(Figure 5 and 6)*

3.1 Cells in the wet mount preparation of (cervico-) vaginal discharge

3.1.1 Vaginal epithelial cells

The proliferation of the vaginal epithelial cells is influenced by the estrogens levels, which fluctuate during the menstrual cycle, are low during lactation, in prepubertal children, and postmenopausal women not using menopause hormone therapy.

Basal cells are the smallest, round cells of the basal layer of the vaginal wall with a size of 12–20 µm, with a rather large round nucleus and a sparse cytoplasm around it, often with granulations. Their presence is a sign of severe vaginal atrophy or erosions.

Parabasal cells are round to oval immature cells, measuring between 15-30 µm and with a central round nucleus measuring 9–10 µm. These cells are the predominating ones in vaginal atrophy and are also commonly found in the estrogenized vagina of young women with aerobic vaginitis (AV)/desquamative inflammatory vaginitis (DIV) or with other inflammatory diseases, such as trichomoniasis or erosive vaginal lichen planus.

Intermediate cells, along with superficial cells, predominate in the well estrogenized vagina of adult women. There are small and large intermediate cells, depending on the grade of their proliferation. Small intermediate cells occur during the perimenopausal time or under a strong influence of progesterone (i.e. during pregnancy, progestin or androgen therapy) and are called navicular cells, because they look like a small boat. The nucleus of the intermediate cells is round, vesiculous, measuring 7–10 µm. The larger intermediate cells measure 40–60 µm.

The large superficial cells have a diameter of 50 – 60 µm, are polygonal, transparent and with a small, dense pycnotic nucleus of 5 – 7 µm. They dominate the superficial layer of the estrogenized vaginal epithelium.

3.1.2 Endometrial epithelial cells

Endometrial cells can be encountered in the vagina in samples collected during the menses or breakthrough bleeding. More rarely, glandular cells can be seen in vaginal samples, in the context of vaginal endometriosis or vaginal adenosis.

3.1.3 Non-epithelial cells

The leukocytes are divided into granulocytes, lymphocytes, and monocytes. They have a round to oval shape and exhibit distinct cell characteristics with a mean diameter between 7 µm for lymphocytes and 20 µm for monocytes. The segmented nucleus of granulocytes, which is not activated in the healthy genital tract, is characteristic and easily observed in phase-contrast microscopy.

The so-called "toxic" leukocytes are activated granulocytes/neutrophils resulting from infection or inflammation. In such cases, the originally segmented nucleus dissolves into a granulated mass in the center of the now spherical cell.

There is an agreement, that the presence of more than 25 toxic leukocytes per field at x400 magnification or a ratio of leukocytes to epithelial cells higher than 1:1 indicates an infectious or inflammatory process in the lower genital tract, warranting further investigation, for instance for cervicitis. In some cases, however, it may just happen in the context of a large ectopy, or if the sample is collected close to the cervical ostium (with long chains of polymorphonuclear neutrophils.

Erythrocytes are identified by their round shape, the absence of a nucleus, and featuring a central concavity, which appears as a dark spot in phase-contrast. This characteristic should not be misinterpreted as a nucleus. Degraded erythrocytes may resemble dark, sometimes damaged round "shades of grey".

4

DIAGNOSTIC CRITERIA

Criteria to diagnose vaginal dysbiosis or infection:

- Symptoms
- Clinical signs
- pH
- WMM
- Gram stain and Nugent score
- Identification of sexually transmitted organisms by non-cultural methods
- In some special cases a vaginal/cervical culture can be useful (e.g. gonococci, group A streptococci)

TABLE 2
Clinical and microscopic signs of vaginal dysbiosis and infections

	Vagina	Discharge	pH	Phase contrast microscopy
Healthy vagina	normal	normal	3.8-4.7	dominance by lactobacilli, no severe inflammation and no parabasal cells
Bacterial vaginosis	normal	grey, creamy	>4.5	dysbiosis, clue cells
Candidiasis	red or normal	White or yellow, "cottage cheese"	Any	blastospores, pseudomycelia
Trichomoniasis	red or normal often vulvitis	can be yellow, fluid, with air bubbles or appear normal	>4.5	trichomonads (or not) often vulvitis, dysbiosis, toxic leukocytes
AV/DIV	inflammation, parabasal desquamation	more or less yellow	5-7	toxic leukocytes, cells, no clue cells, dysbiosis, but less bacteria than in BV
Cytolytic vaginosis	normal	similar to what is seen in candididis	<4.1	abundand lactobacilli, bare nuclei, cytoplasm fragments, no inflammation

4.1 How to diagnose bacterial vaginosis?[6, 11]

- Amsel criteria – at least 3 out of 4 criteria present (pH>4.5, fishy odor, creamy discharge, presence of clue cells)
- WMM
- Nugent Score (Gram-stain, microscope x1,000)
- BV Blue Test (Gryphus Diagnostics USA, Sekisui Diagnostics (United Kingdom) (evaluation of sialidase activity)
- Affirm VP III (BD Diagnostics, Sparks, USA) and others (DNA probe test)
- GYNI (Gyn Tools, Israel) (automatic reading of wet mount and pH)
- NAATs/PCR (Allplex Vaginitis Screening Assay [Seegene Inc. South Korea], BD Max [Becton, Dickinson, Franklin Lakes, NJ, USA] , Aptima Vaginal Panel (Hologic, Malborough/ Massachusetts, USA), Xpert Xpress MVP [Cepheid, Sunnyvale, CA, USA])

4.2 How to diagnose AV/DIV?[11, 12]

- Yellow purulent discharge for weeks or months
- Markedly elevated pH
- Vaginal inflammation (in DIV also desquamation), which into redness, leukocytosis ("toxic" leukocytes), but no evidence of infection
- Patients may have associated autoimmune conditions or be using rituximab[12]
- Phase-contrast microscopy: toxic leukocytes, parabasal cells, no clue cells, strongly disturbed, but sparse microbiota
- Gram stain: findings similar to those seen using WMM (except toxic leukocytes)
- Cultures not helpful
- GYNI (Gyn-tools, Israel)

4.3 Future promising diagnostic tool

Fluorescence *in situ* hybridization (FISH) enables simultaneous taxonomically unambiguous identification of microorganisms, assessment of their spatial arrangement, and morphological features in the material under investigation. By employing a panel with various fluorescence-labeled probes covering relevant pathogen and pathogen groups, complex mixed cultures as well as polymicrobial biofilms can be visualized in their original composition.

FISH can be applied to examine smears in a liquid transport medium (swabs), as well as biopsy specimens e.g. from vagina or abortion material, urine sediment, ejaculate and other samples.[12]

Figures 7-9 and their legends are kindly provided by Prof. Alexander Swidsinski, Berlin.

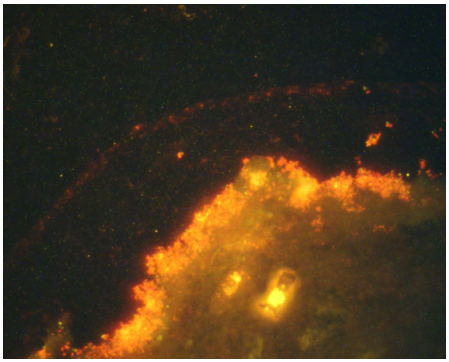


FIGURE 7 Multicolor single-cell fluorescence in *situ hybridization* (sc-FisH) of vaginal biopsies from a woman with bacterial vaginosis demonstrating the polymicrobial nature of adherent biofilms. *Gardnerella spp.* is red (marked by Gard 664-Cy5), *Fannyhessea spp.* is yellow (marked by Arto 124 Cy 3). *Gardnerella spp.* creates the structure of the biofilm, while *Fannyhessea spp.* dissolves within its properties (for particulars see above Swidsinski et al. 2019)[14].

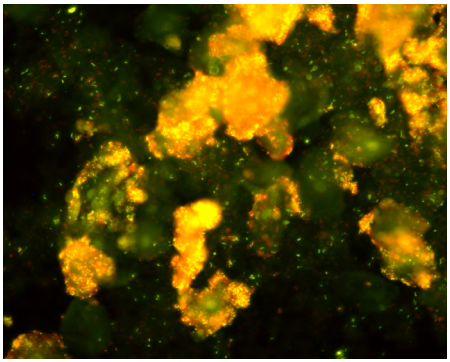


FIGURE 8 Multicolor single sc-FisH of a vaginal smear from a woman with bacterial vaginosis. *Gardnerella spp.* is red (Gard 664-Cy5), *Lactobacillus (L.) iners* is yellow (Liners Cy5). One can clearly see, that the cells are not overlaid by overgrowing vaginal bacteria, but primarily building a biofilm on the surface of desquamated epithelial cells. The concentrations in vaginal secretions are quickly falling with the distance to the surface of epithelial cells. Clearly, clue cells are not overlaid by bacterial overgrowth within vaginal secretions; rather the primarily forming biofilm rapidly decreases from the surface of epithelial cells. *L. iners* merges with *Gardnerella spp.* with no signs of antagonism between the two species.

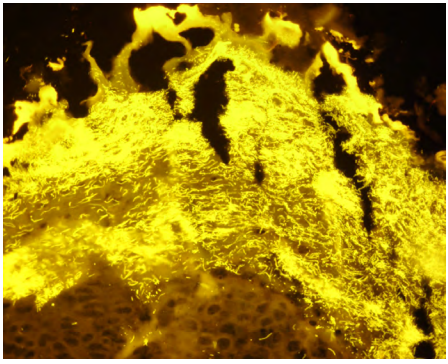


FIGURE 9 Sc-FisH of a biopsy from a woman with recurrent vulvovaginal candidiasis, yellow flourescence (Caal Cy3). The invasive nature of *Candida spp.* is evident. No biofilm can be found on the same biopsy surface. A *Candida spp.* invasion is always accompanied by coinvasion with microbial strains, mainly lactobacilli. Less often, it is accompanied by colonization with *Gardnerella spp.* or Enterobacteriaceae.

5

CLINICAL AND MICROSCOPIC EXAMPLES

All microphotographs shown were taken with x400 magnification, using phase contrast microscopy. Unless otherwise mentioned, the slides were prepared with saline (in some cases 10% KOH solution was used).

5.1 Normal findings

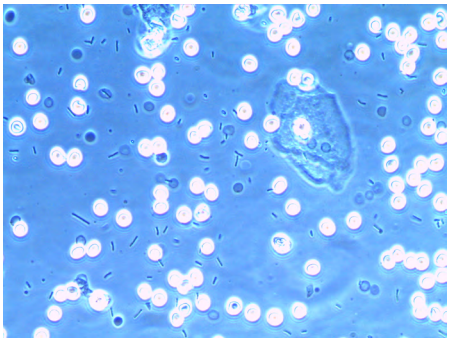


FIGURE 10 Menstruation. Erythrocytes (some partially broken), lactobacilli.

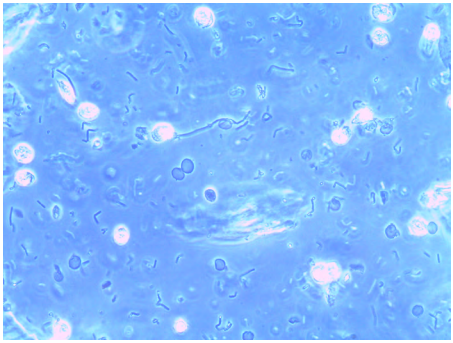


FIGURE 11 Menstruation. Lactobacilli, erythrocytes (dark), leukocytes.

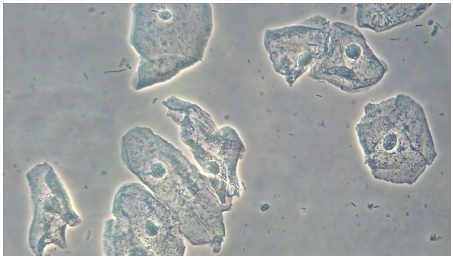


FIGURE 12 Sample collected almost after menstruation – lactobacilli very scarce.

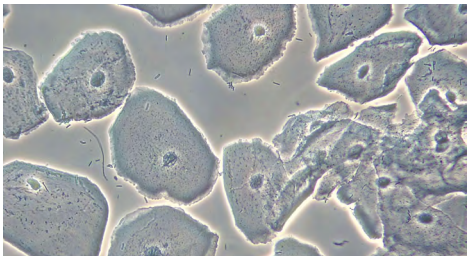


FIGURE 13 Sample collected after antibiotic; lactobacilli almost absent.

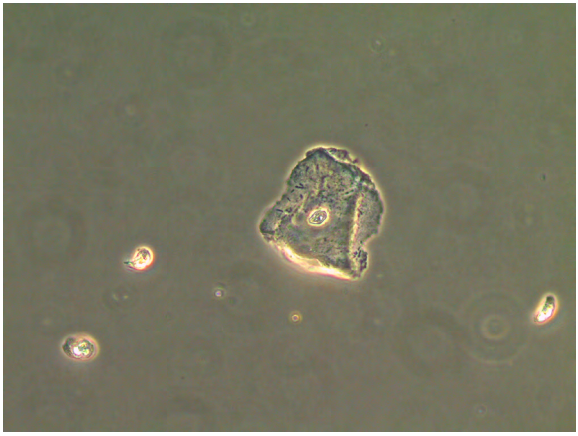


FIGURE 14 Premenopausal woman treated with ampicillin: lactobacilli pratically absent.

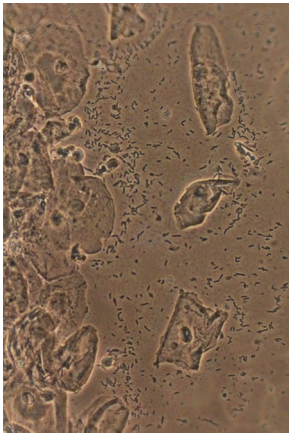


FIGURE 15 Normal sample: Lactobacilli, superficial cells.

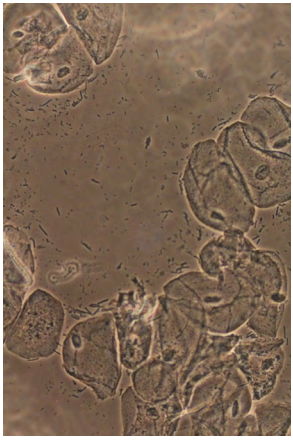


FIGURE 16 Different lactobacilli, some other bacteria.

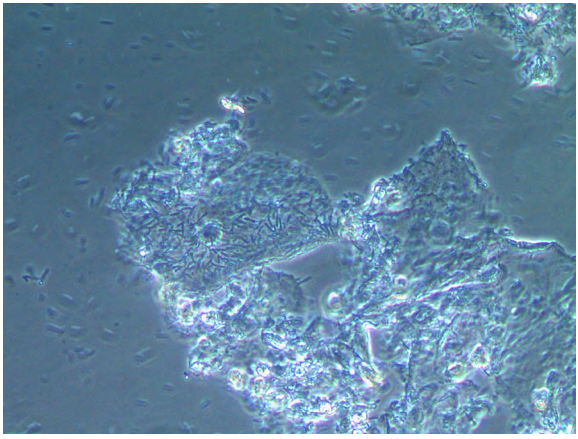


FIGURE 17 Premenstrual lactobacilli adherence on a vaginal superficial cell, some granulocytes.

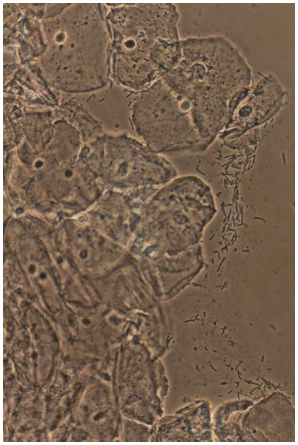


FIGURE 18 Normal lactobacilli.

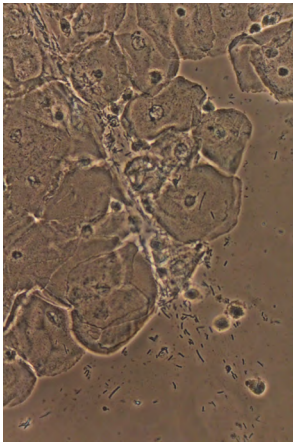


FIGURE 19 Normal lactobacilli.

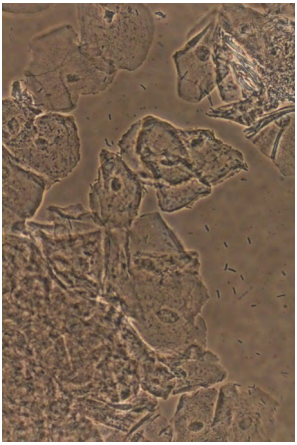


FIGURE 20 Normal lactobacilli.

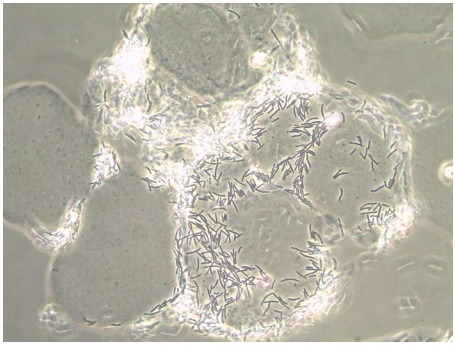


FIGURE 21 KOH – solution, lactobacilli, epithelial cells beginning to dissolve.

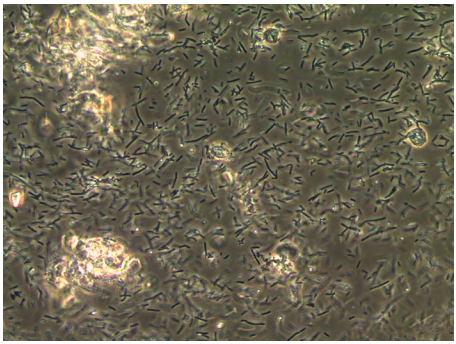


FIGURE 22 Premenstrual progesterone-induced cytolysis. Naked nuclei of lysed epithelial cells and abundant lactobacilli.

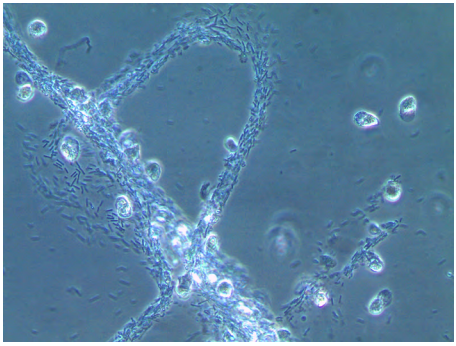


FIGURE 23 Lactobacilli, non-toxic leukocytes.

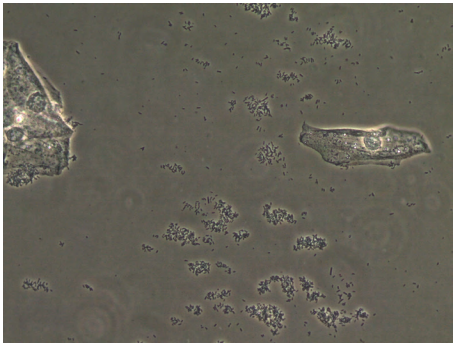


FIGURE 24 Short lactobacilli, possibly *Lactobacillus iners*.

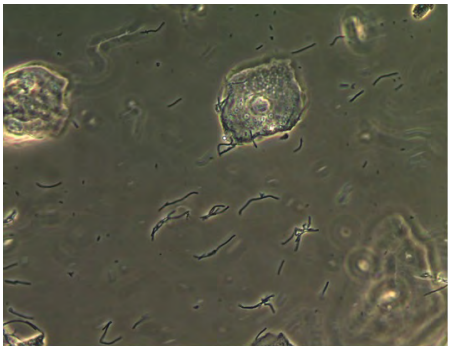


FIGURE 25 Lactobacilli of a vaginal tablet.

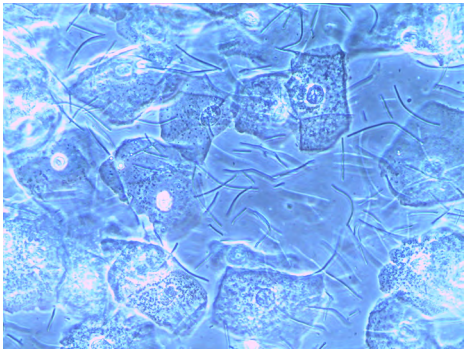


FIGURE 26 Rather long lactobacilli ("leptothrix").

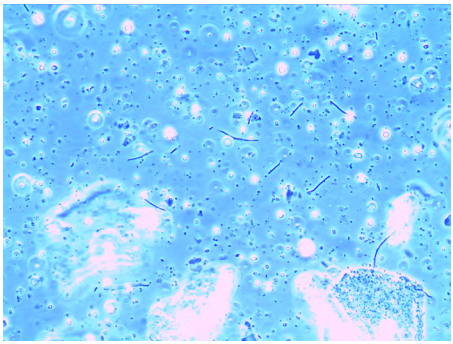


FIGURE 27 Long lactobacilli, in the background dysbiosis and many droplets of a vaginal cream.

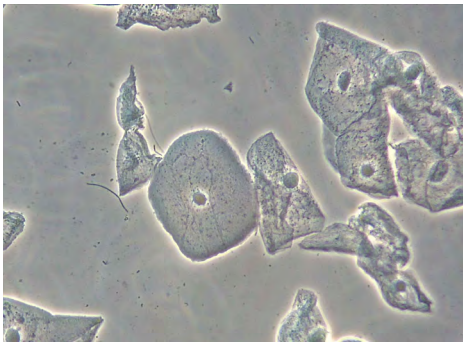


FIGURE 28 Rather long lactobacilli ("leptothrix") after antibiotics.

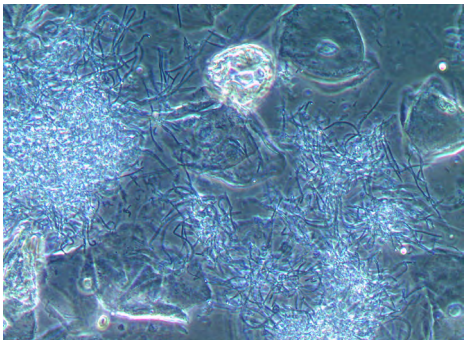


FIGURE 29 Long lactobacilli ("leptothrix").

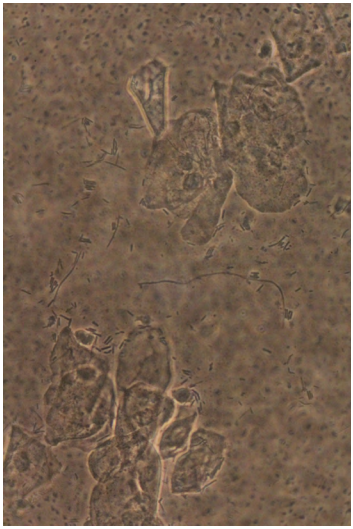


FIGURE 30 “Leptothrix”.

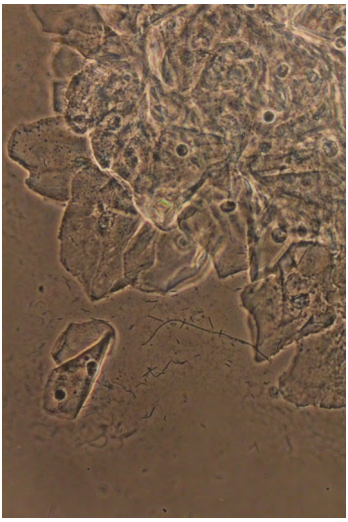


FIGURE 31 “Leptothrix”.

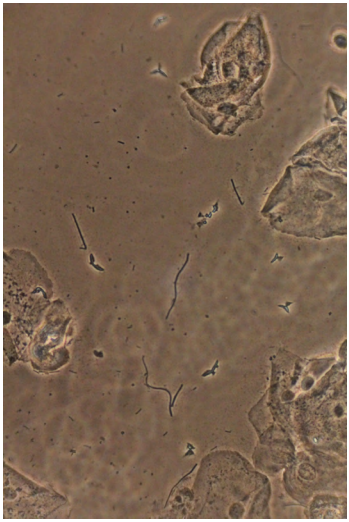


FIGURE 32 “Leptothrix”.

5.2 Cytolytic vaginosis

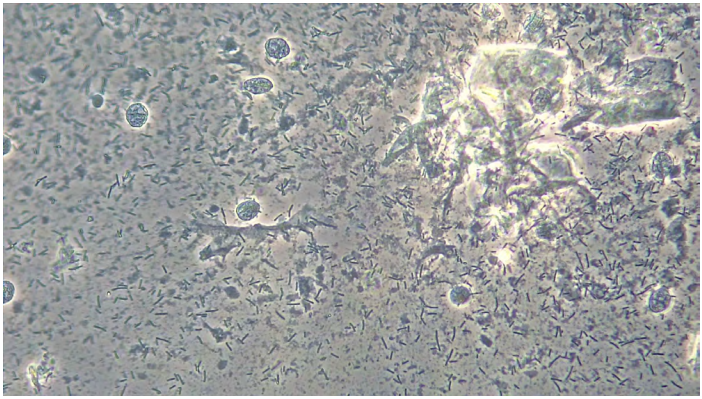


FIGURE 33 Cytolysis, naked nuclei, lactobacilli.

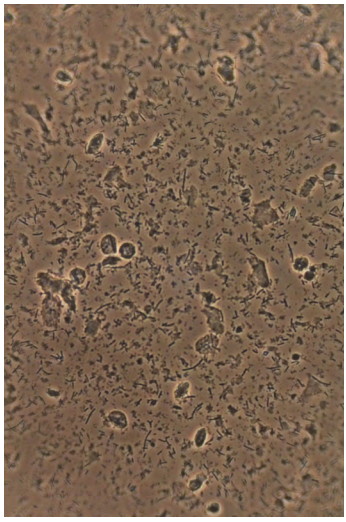


FIGURE 34 Cytolysis, naked nuclei, lactobacilli.

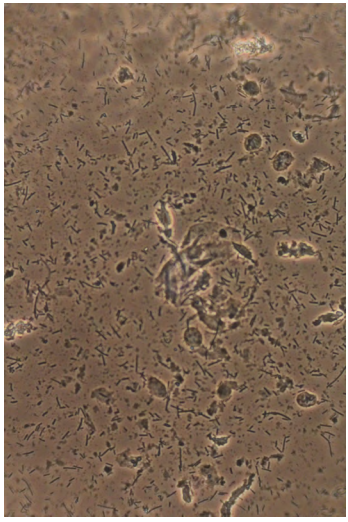


FIGURE 35 Cytolysis, naked nuclei, lactobacilli.

5.3 Atrophy

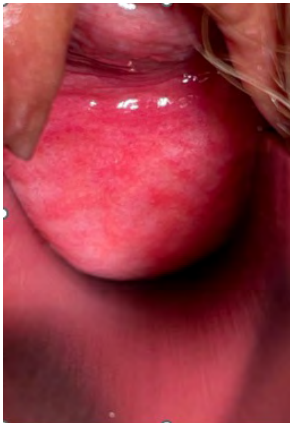


FIGURE 36 65 years old, pH value 6, vaginal atrophy..

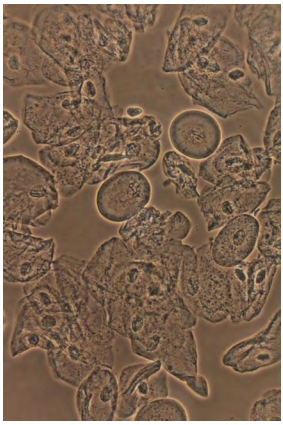


FIGURE 37 Predominating intermediate cells and 3 parabasal cells during lactation, no lactobacilli.

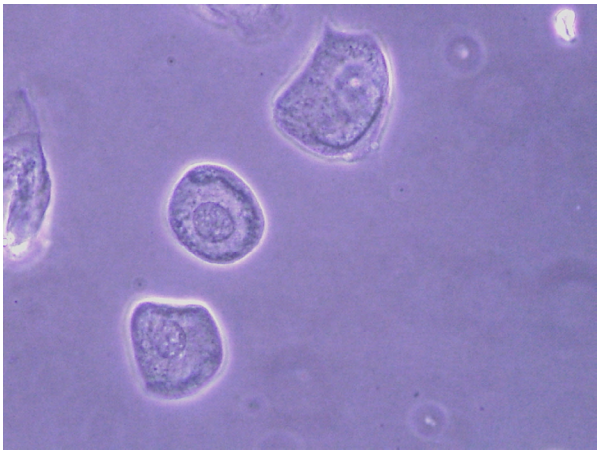


FIGURE 38 32-year-old lactating individual presents with atrophy: parabasal and small intermediate cells, with absence of lactobacilli.

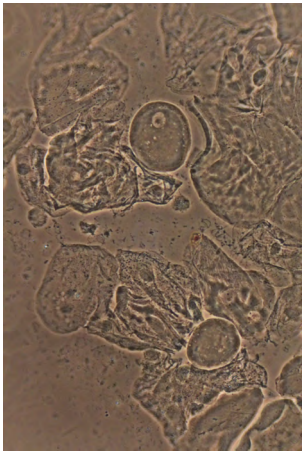


FIGURE 39 Vaginal atrophy caused by low estrogen dosage in contraceptive pills.

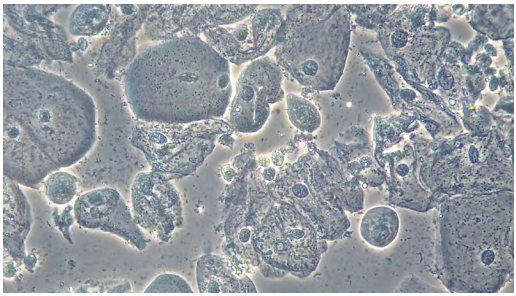


FIGURE 40 Superficial, intermediate and parabasal cells, toxic leukocytes, dysbiosis of a peri-/postmenopausal woman with atrophy.

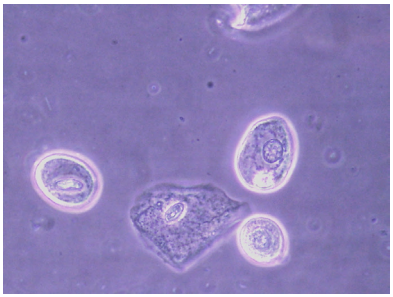


FIGURE 41 75-year-old, no hormone therapy, atrophy. Parabasal cells.

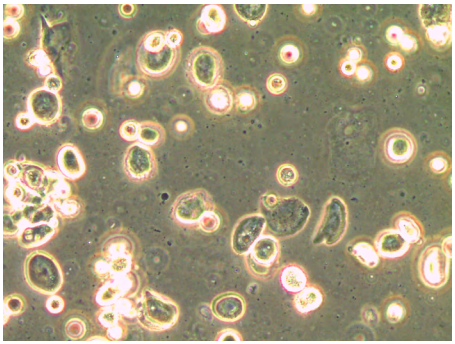


FIGURE 42 80-year-old, postmenopausal atrophy. Parabasal and basal cells, toxic leukocytes, dysbiosis.

5.4 Bacterial vaginosis/dysbiosis

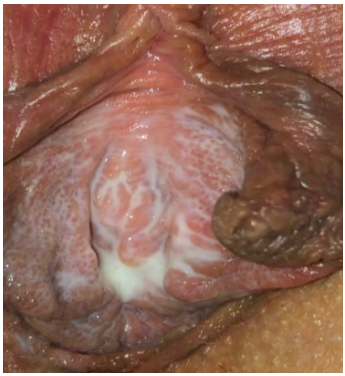


FIGURE 43 Bacterial vaginosis: grey creamy and foamy discharge. The gas bubbles originate from bacterial gas/amine production.

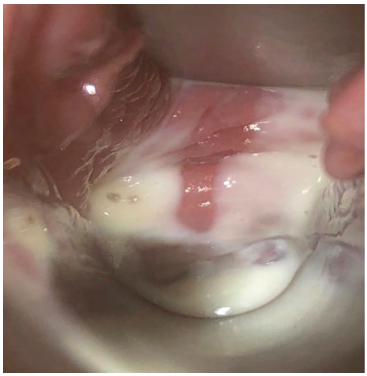


FIGURE 44 Bacterial vaginosis: intravaginal grey creamy and foamy discharge.

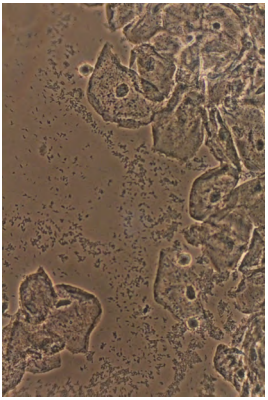


FIGURE 45 Severe dysbiosis.

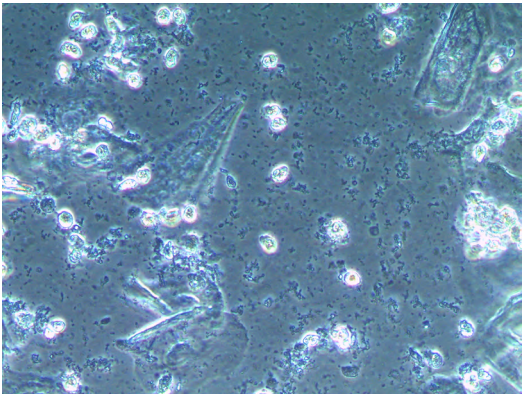


FIGURE 46 Severe dysbiosis, no clue cells. Clinically bacterial vaginosis with an inflammatory component.

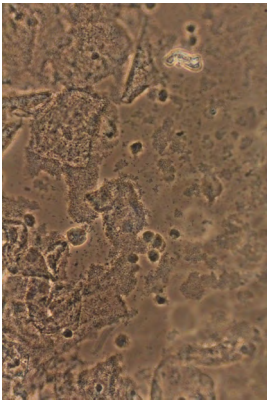


FIGURE 47 Severe dysbiosis, clue cells, pseudo – clue cells (bacterial vaginosis).

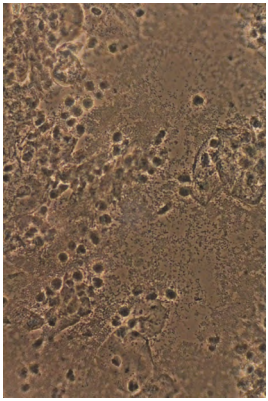


FIGURE 48 Dysbiosis, no clue cells, toxic leukocytes.

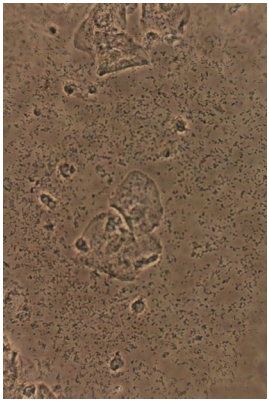


FIGURE 49 Dysbiosis, no clue cells (bacterial vaginosis).

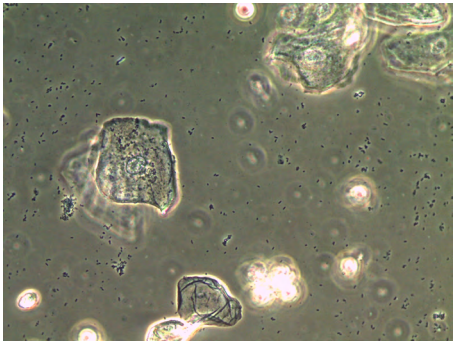


FIGURE 50 Dysbiosis, some short lactobacilli, possibly *L. iners*, no bacterial vaginosis.

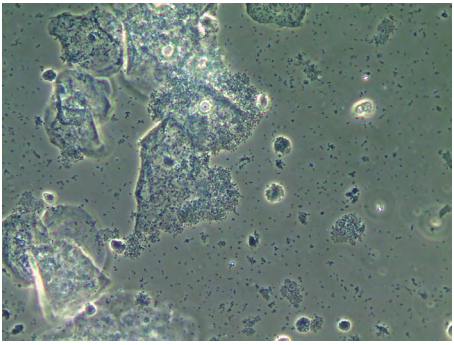


FIGURE 51 Bacterial vaginosis. Clue cells and pseudo-clue cells, few non toxic leukocytes.

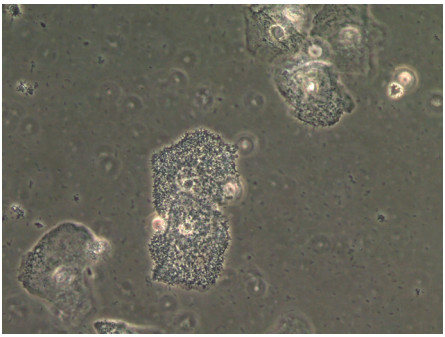


FIGURE 52 Clue cells.

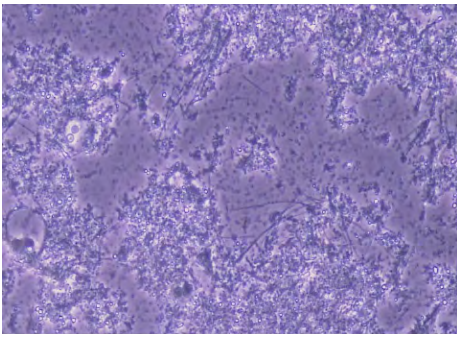


FIGURE 53 Bacterial vaginosis: "clouds" of bacteria/clue cells, and long bacteria.

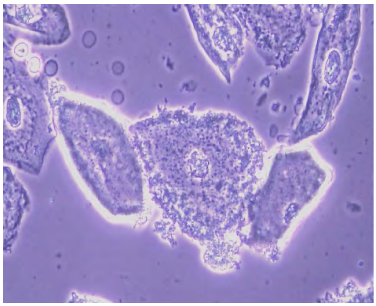


FIGURE 54 Bacterial vaginosis: clue cell, during menstruation.

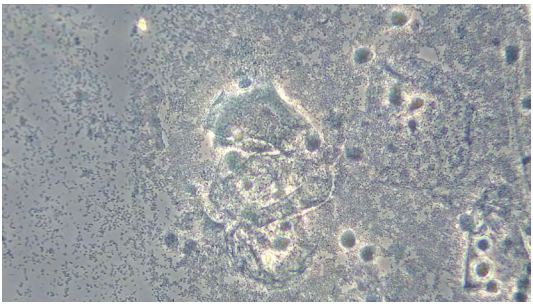


FIGURE 55 Bacterial vaginosis and inflammation: masses of bacteria, no clue cells, toxic leukocytes.

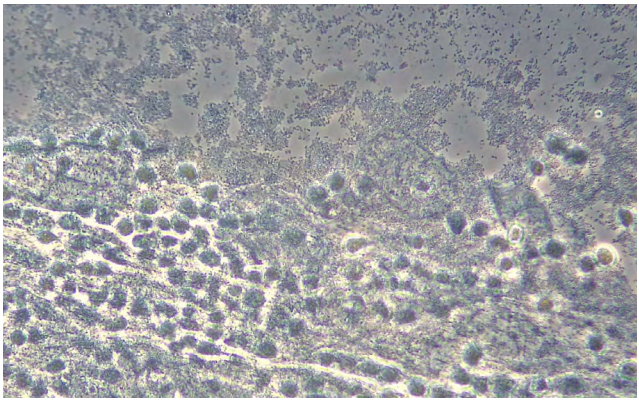


FIGURE 56 Bacterial vaginosis and cervicitis. Clue cells, pseudo-clue cells, toxic leukocytes in clusters with cervical mucus.

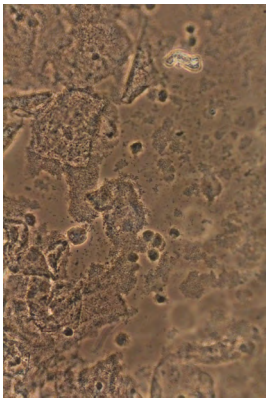


FIGURE 57 Bacterial vaginosis. Presence of clue cells

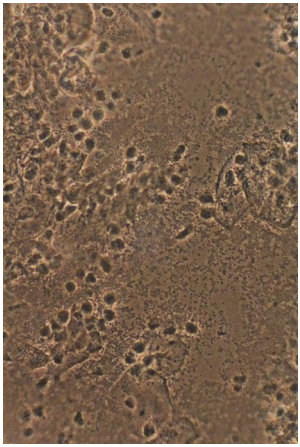


FIGURE 58 Bacterial vaginosis, in this case with inflammation associated.

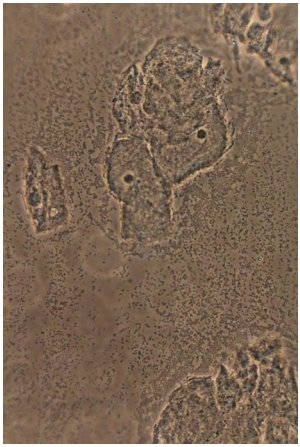


FIGURE 59 Severe dysbiosis.

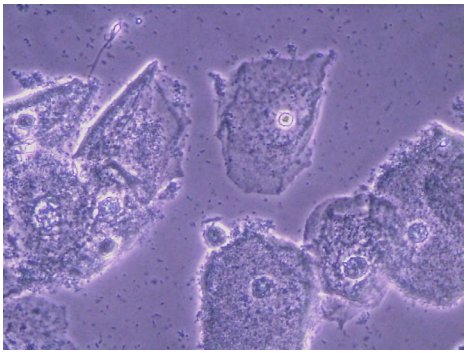
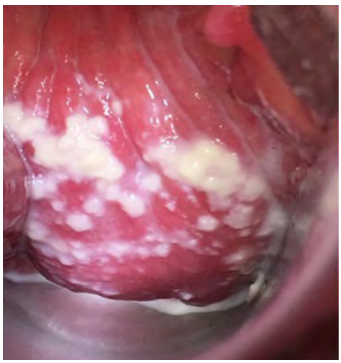


FIGURE 60 Clue cells, dysbiosis, bacterial vaginosis, and sperm.

5.5 Candida vaginitis



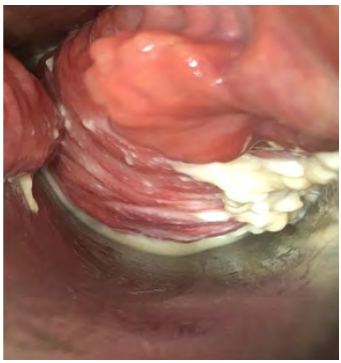
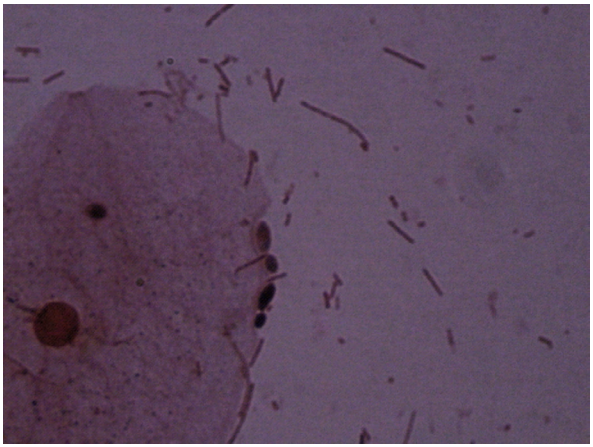


FIGURE 61 a+b *Candida albicans (vulvo-)vaginitis.*



FIGURES 62 Gram stain, 1,000x (oil immersion). Adherence of Candida cells to the vaginal epithelial cells in the beginning of a Candida vaginitis. Blastospores are the colonizing forms of Candida spp. In case of infection they begin to adhere on the vaginal epithelium, form germ tubes and pseudohyphae, many pseudohyphae are called pseudomycelium (true hyphae are formed by dermatophytes and by moulds, but *C. albicans* is also able to form true mycelia in some cases and also chlamydospores).

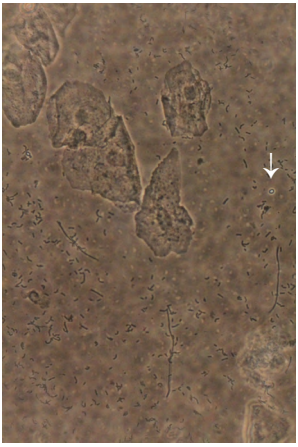


FIGURE 63 Normal and long lactobacilli, one small blastopspore (arrow), probably *C. (Nakaseomyces) glabrata*. Currently this species is named *Nakaseomyces (N.) glabrata* (some name it *N. glabratus*). We use for better understanding the name *C. glabrata.*

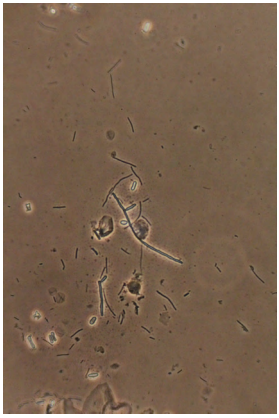


FIGURE 64 Candida vaginitis. Pseudomycelia, blastospores, lactobacilli.

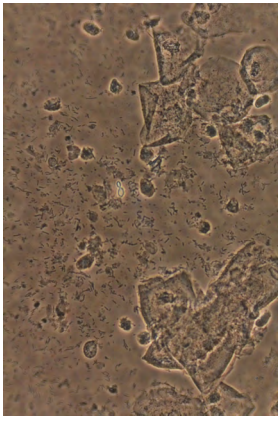


FIGURE 65 Premenstrual cytolysis, blastospores.

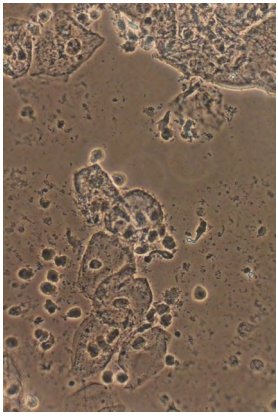


FIGURE 66 Premenstrual cytolysis, two small blastospores, probably *C. glabrata.*

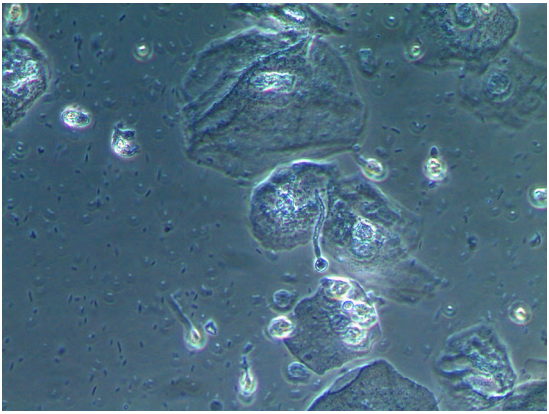


FIGURE 67 Short lactobacilli, blastospore with germ tube/ pseudomycelium, non-toxic leukocytes.

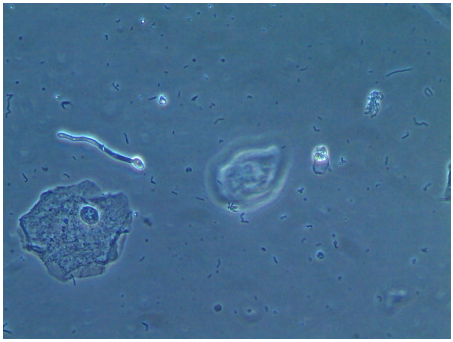


FIGURE 68 Blastospore and blastospore with germ tube/pseudomycelium.

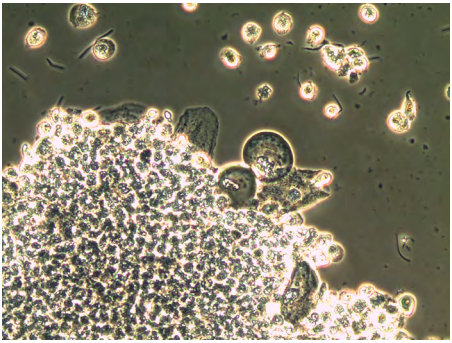


FIGURE 69 Very dense layers of non-toxic leukocytes, lactobacilli and blastospores.

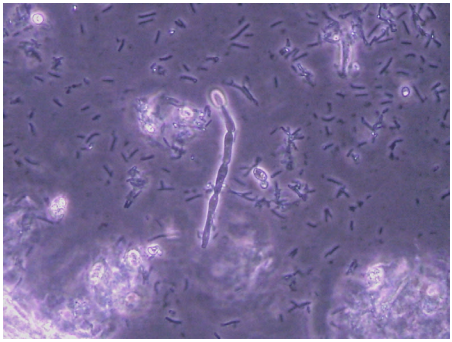


FIGURE 70 Candida vaginitis by *C. albicans* (confirmed by culture): lactobacilli, blastospores, pseudomycelia. In the right upper part a head of a sperm can be seen.

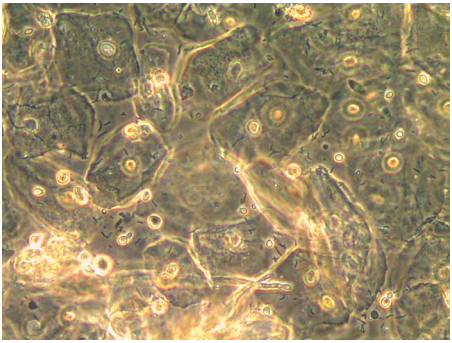


FIGURE 71 Too dense layer of a wet mount. But still blastospores and pseudomycelia can be identified!

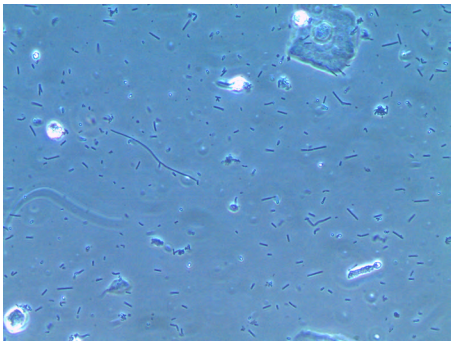


FIGURE 72 Short and long lactobacilli *(L. vaginalis)* and pseudohyphae/blastospore of *C. albicans* (confirmed by culture).

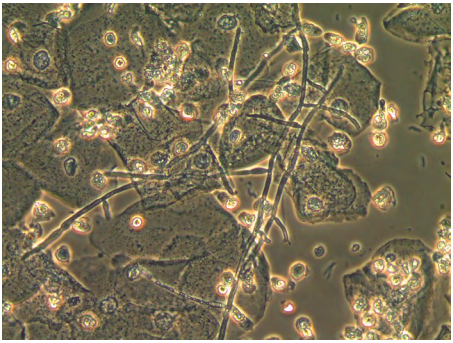


FIGURE 73 Vulvovaginal candidiasis, pseudomycelia.

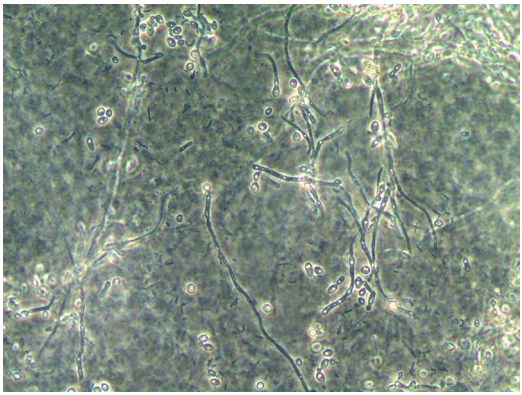


FIGURE 74 KOH 15%, dissolved vaginal epithelial cells, masses of *Candida spp*. blastospores and pseudomycelia.

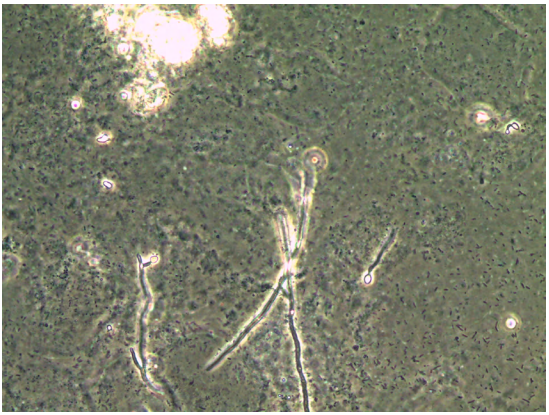


FIGURE 75 Wet mount prepared with KOH – the solution dissolved epithelial cells. Blastospores and pseudomycelia.

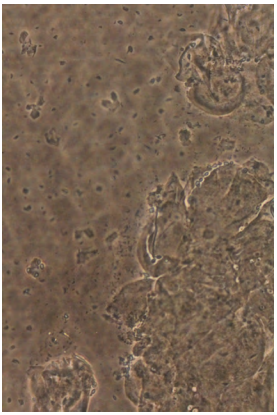


FIGURE 76 Pseudomycelium and blastospores.

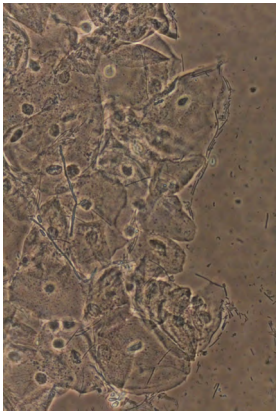


FIGURE 77 Long lactobacilli ("leptothrix"), pseudomycelia.

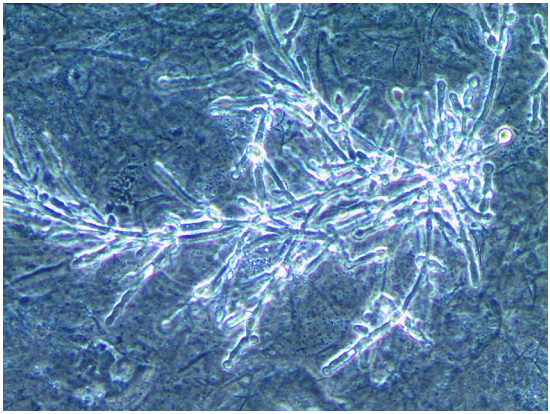


FIGURE 78 Pseudomycelia.

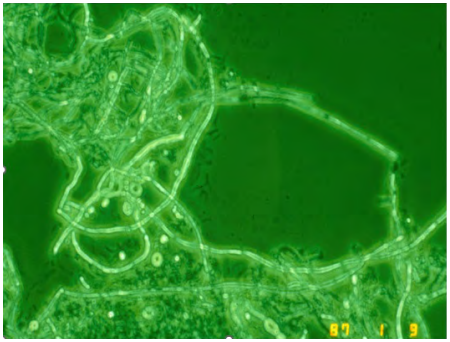


FIGURE 79 Tampon fibres, no yeasts! Green background. Color by green filter wich protects the eye in long microscope sessions.

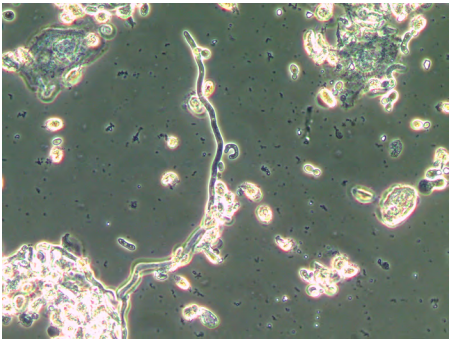


FIGURE 80 Dysbiosis, pseudomycelia, blastospores, non toxic leukocytes, cytolysis.

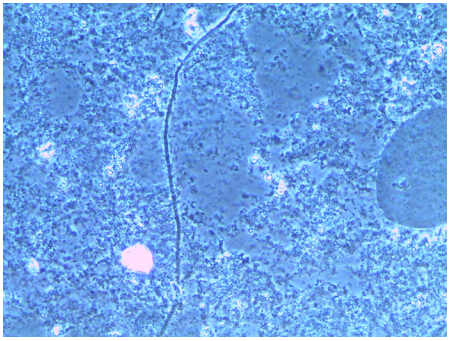


FIGURE 81 Severe dysbiosis (BV), long pseudomycelium, some blastospores.

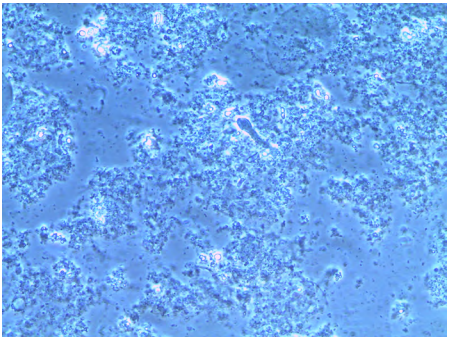


FIGURE 82 Severe dysbiosis/BV, blastospore and big pseudohyphe.

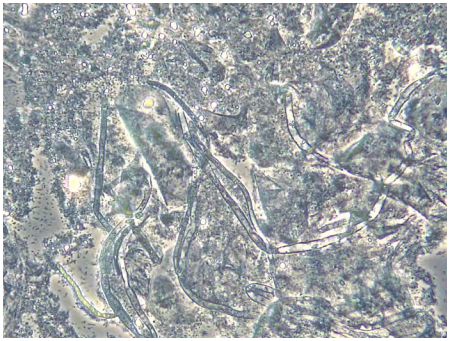


FIGURE 83 Dysbiosis and pseudomycelia.

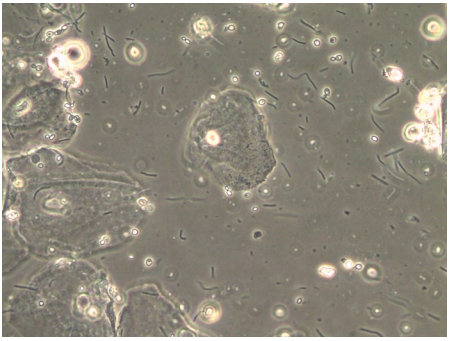


FIGURE 84 Lactobacilli and many small blastospores of (confirmed by culture) *C. glabrata*, which have a mean size of 3-6 mü, while *C.- albicans* have a mean size of 7-10 mü and would appear larger in the microscopic field

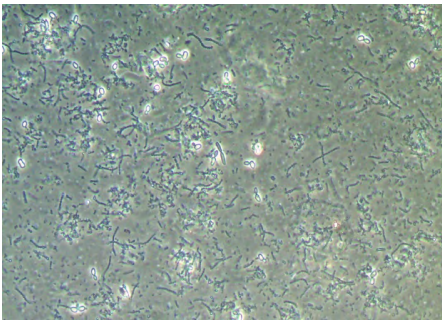


FIGURE 85 KOH 15% solution. Masses of small blastospores (confirmed by culture *C. glabrata*), and masses of streptococci, probably *Streptococcus agalactiae*. The patient had no symptoms and was not treated.

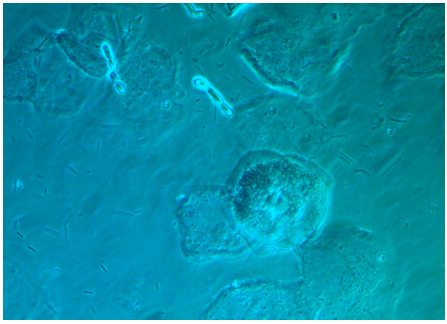


FIGURE 86 Blastospores of (confirmed by culture) *Pichia kudriavzevii* (former *C. krusei*). This yeast (like *N. glabrata*) is closer related to the genus *Saccharomyces* and less pathogenic in the vagina of immunocompetent women. For better understanding we still use *C. krusei.*

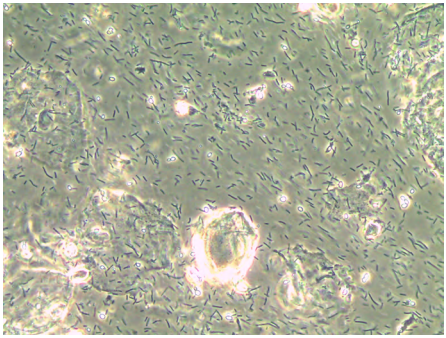


FIGURE 87 Many small blastospores of *C. glabrata* (confirmed by culture) , lactobacilli. No symptoms (colonization), no treatment!

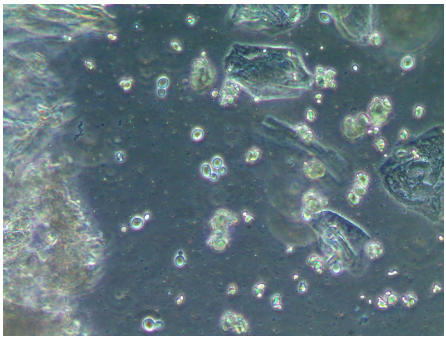


FIGURE 88 Big blastospores of *Saccharomyces cerevisiae* (confirmed by culture), lactobacilli, some leukocytes (not sharp).

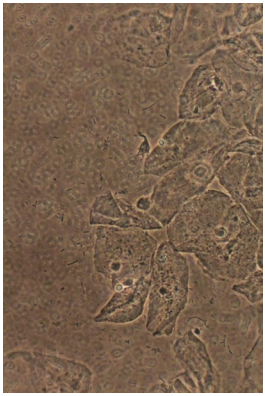


FIGURE 89 Blastospores of *S. cerevisiae* (confirmed by culture).

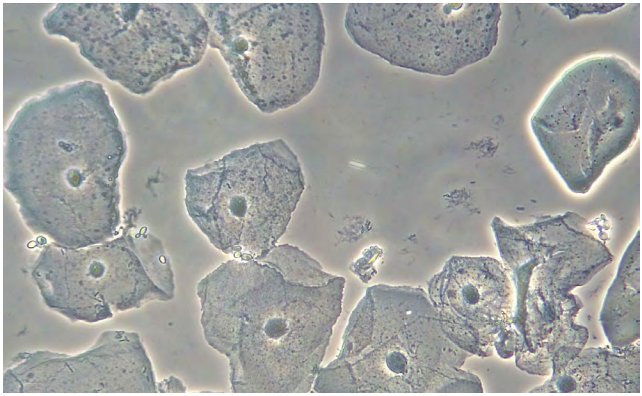


FIGURE 90 Blastospores of *C. lusitaniae* (confirmed by culture). This species is asymptomatic in not immunosuppressed women.

5.6 Trichomoniasis

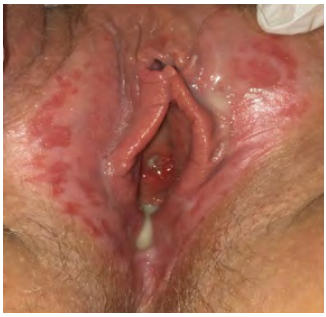


FIGURE 91 Severe vulvitis in a patient with trichomoniasis.

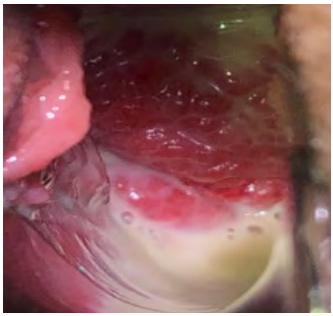


FIGURE 92 Trichomoniasis.

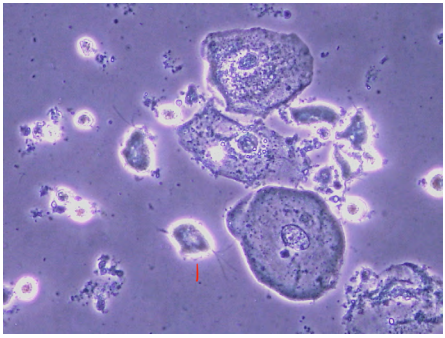


FIGURE 93 Trichomoniasis. Trichomonas vaginalis with flagellates, typical pike at the other pole, undulating membrane near the pole of the flagellates (red sign). Severely disturbed vaginal microbiota, but no clue cells, no BV, toxic leukocytes.

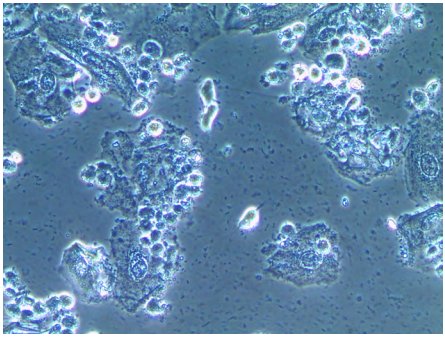


FIGURE 94 Trichomoniasis and severely disturbed microbiota, no typical clue cells, presence of toxic leukocytes.

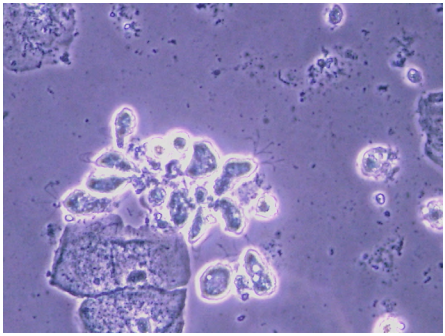


FIGURE 95 Trichomoniasis.

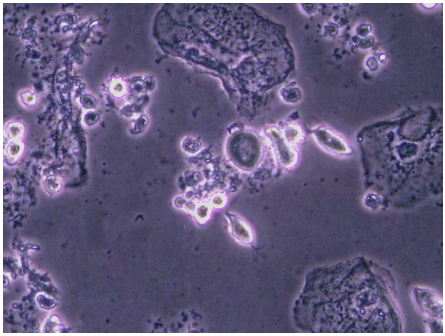


FIGURE 96 Trichomoniasis.

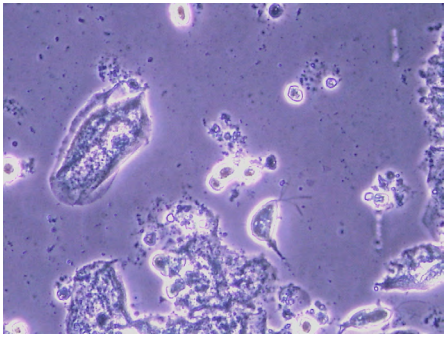


FIGURE 97 Trichomoniasis. The pike and the flagellates.

5.7 Aerobic vaginitis (AV)/desquamative inflammatory vaginitis (DIV)

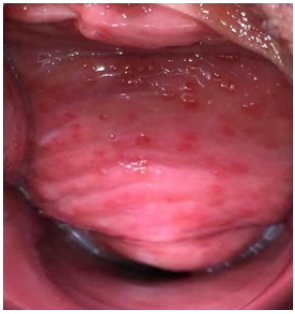


FIGURE 98 AV: vaginal inflammation with pH value 5.5 and yellow discharge (not to be seen here).

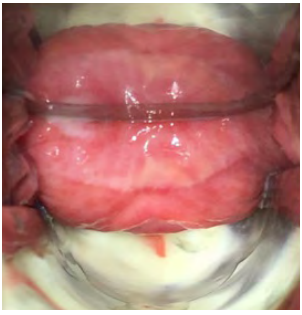


FIGURE 99 DIV: severe vaginal inflammation, pH value 6 and signs of desquamation with severe yellow discharge.

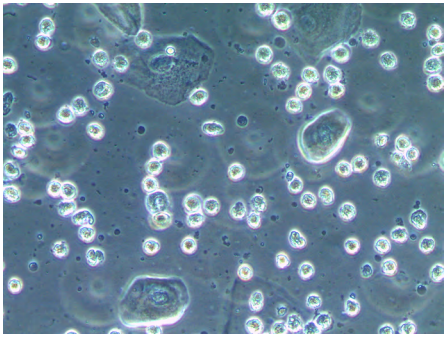


FIGURE 100 AV/DIV. Toxic leukocytes, sparse coccoid microbiota, no clue cells, small intermediate cells/large parabasal cells, one intermediate cell. The pH - value of the discharge in figures 100 – 104 is markedly elevated. S. agalactiae or S. anginosis are often identified on culture.

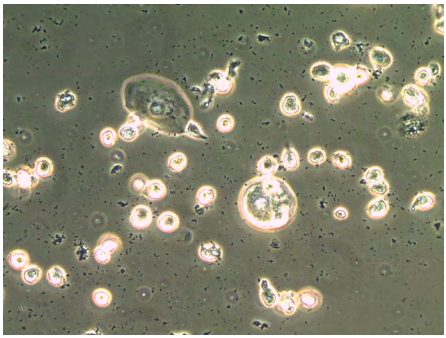


FIGURE 101 Severe AV/DIV.

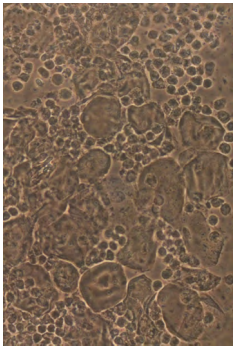


FIGURE 102 Severe AV/DIV. many toxic leukocytes, parabasal cells, intermediate cells, sparse microbiota.

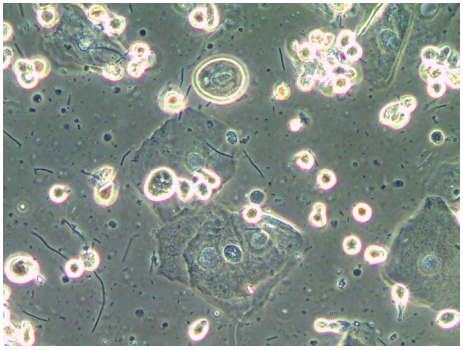


FIGURE 103 Light AV: intermediate cells, three parabasal cells, coccoid microbiota, but still many lactobacilli.

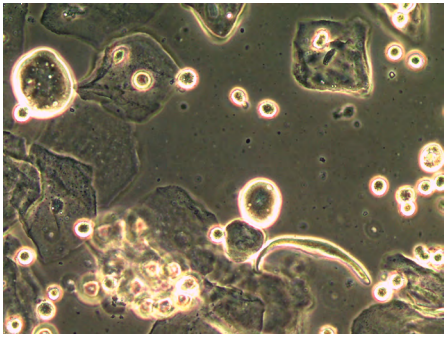


FIGURE 104 DIV: superficial and intermediate cells, parabasal cells, toxic leukocytes, sparse coccoid microbiota.

5.8 Diverse

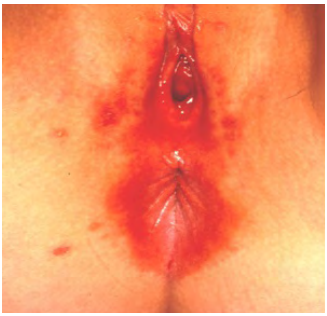


FIGURE 105 *Streptococcus. pyogenes* /Group A S. vulvovaginitis – 6-year-old.

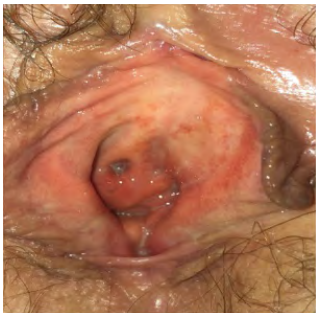


FIGURE 106 Group A streptococcus vulvovaginitis – 32-year-old. Lactating. Atrophy.

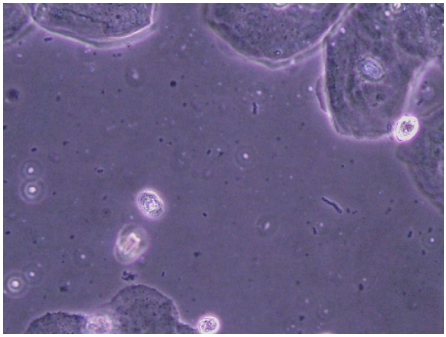


FIGURE 107 Chain of streptococci in a patient with *S. pyogenes* vulvovaginitis.

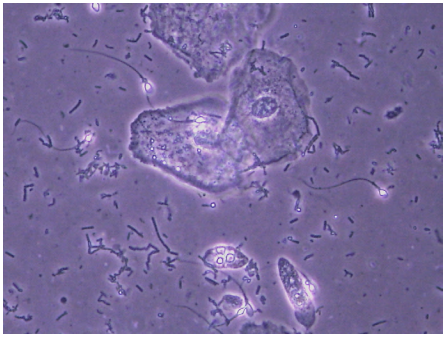


FIGURE 108 Sperms, lactobacilli, some not toxic leukocytes.

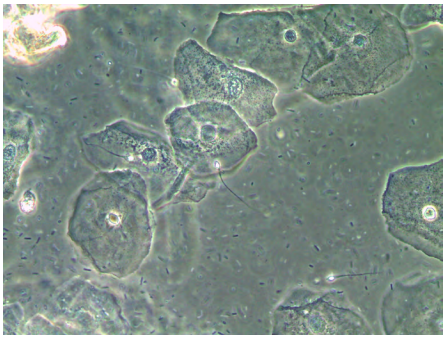


FIGURE 109 Immobile sperms, short lactobacilli.

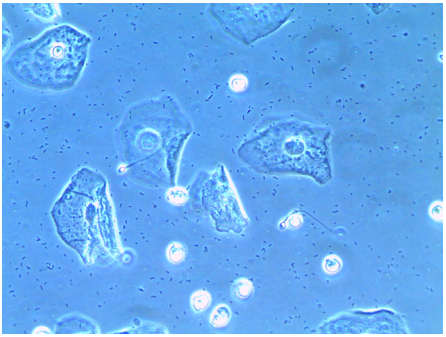


FIGURE 110 Immobile sperms, short lactobacilli *(L. iners?)*, some others, between normal to abnormal microbiota, some non toxic leukocytes.

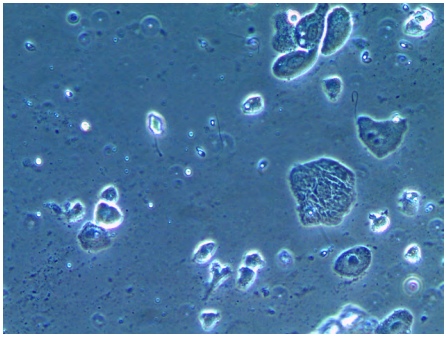


FIGURE 111 Immobile sperms, heads and tails of sperms, disturbed microbiota without lactobacilli, parabasal cells (postmenopausal).

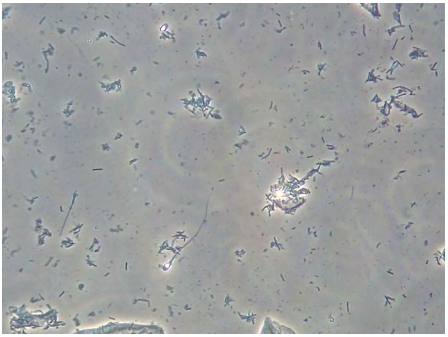


FIGURE 112 Heads and tails of sperms, lactobacilli, cytolysis.

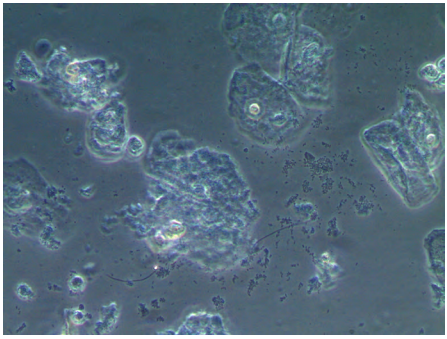


FIGURE 113 Dysbiosis, tails of sperms.

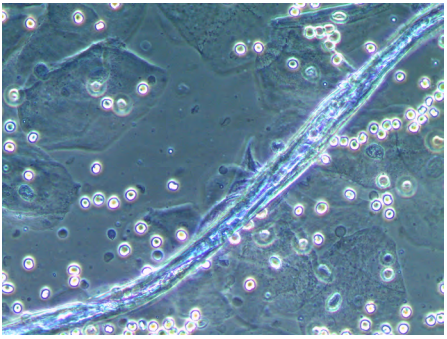


FIGURE 114 Menstruation, erythrocytes, fiber of a tampon.

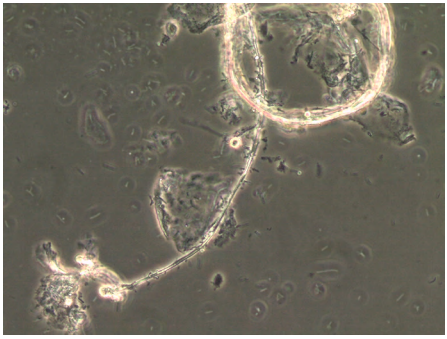


FIGURE 115 Fiber.

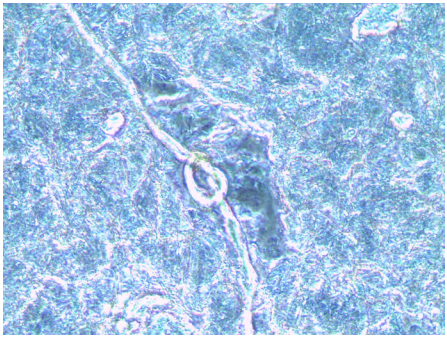


FIGURE 116 Knot of a fiber within thick layers of epithelial cells.

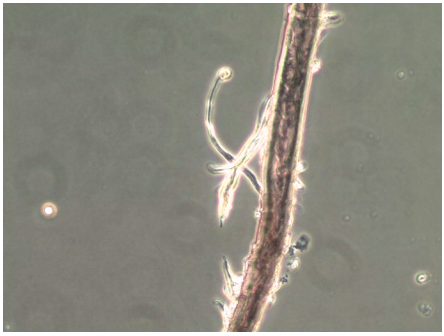


FIGURE 117 Split hair.

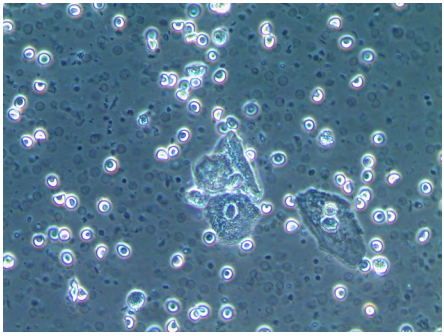


FIGURE 118 Erythrocytes.

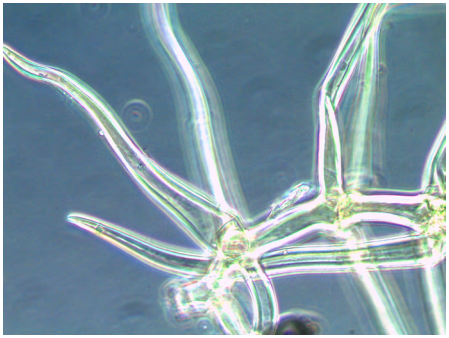


FIGURE 119 Unknown object.

FIGURE 120 No yeast, probably a fiber.

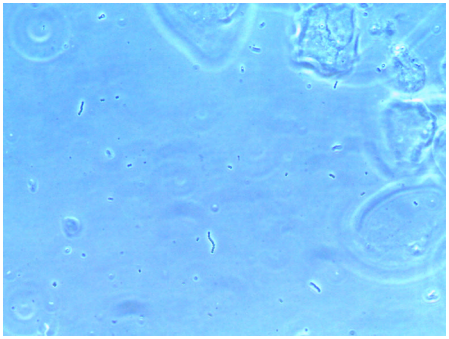


FIGURE 121 Streptococci, well to distinguish from lactobacilli by a perl chain-like appearance.

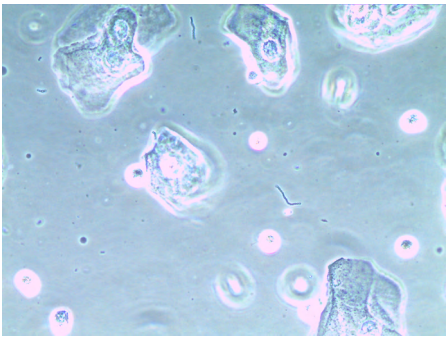


FIGURE 122 Streptococci, no lactobacilli, intermediate cells. See also comment to Figure 121!

FIGURE 123 Mucus, fern phenomenon.

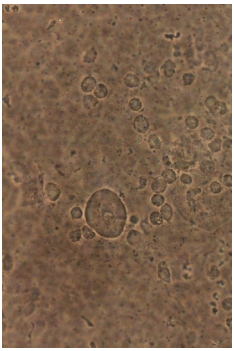


FIGURE 124 41 years old, vaginal erosive lichen planus: parabasal cell, toxic leukocytes.

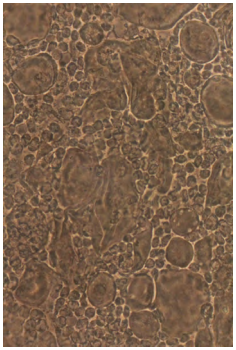


FIGURE 125 Erosive vaginal lichen planus: parabasal cells, toxic leukocytes.

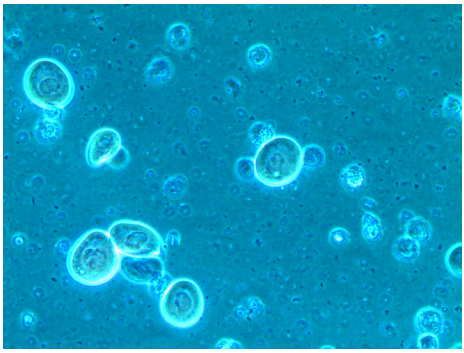


FIGURE 126 Erosive vaginal lichen planus in a premenopausal woman: predominating parabasal cells, toxic leukocytes, severely disturbed microbiota, pH 7.

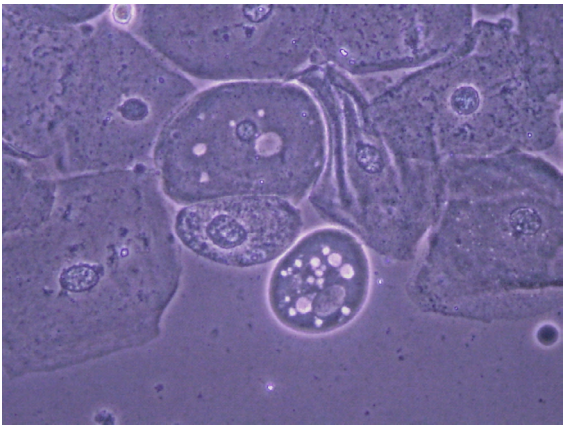


FIGURE 127 Abnormal microbiota without lactobacilli, intermediate cells and severely abnormal epithelial cells with vacuoles of a HIV-positive patient.

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