NCPTS
National Cervical Pathology Training Service

NORMAL CERVICAL CYTOLOGY
Cytomorphology for Year 1-2 Registrars

Overview

- includes cells sampled from surface epithelium, exfoliated from genital tract epithelium and material trapped in cervical mucus
- types and proportions of cells depends on clinical factors such as age and hormonal status, as well as proficiency and method/s of sample taking

The normal cervical cytology sample may include:

- squamous epithelial cells
- endocervical glandular epithelial cells
- endometrial glandular cells +/- stromal cells
- leucocytes (neutrophils, histiocytes)
- normal vaginal flora e.g. Döderlein bacilli (lactobacilli)
- spermatozoa
- miscellaneous contaminates e.g. lubricating jelly

Squamous epithelial cells

Non-keratinising stratified squamous epithelium covers the ectocervix. From the surface layer downward to the basement membrane, there are four layers:

- Superficial cells
- Intermediate cells
- Parabasal cells
- Basal cells

Superficial squamous cells

Superficial squamous cells have matured fully under the influence of oestrogen.

- are large, polygonal-shaped flat cells with dense cytoplasm.
- nucleus is small, pyknotic and opaque (cannot see any internal structure)
- cytoplasm stains pink (eosinophilic). May have kerato-hyaline granules (keratin precursors) seen as blue-black granules in the cytoplasm.

These mature cells eventually undergo cell death and exfoliate from the surface. are a Common degenerative features include soft perinuclear halos, nuclear degeneration and occasionally, anucleate ghost cells.

Intermediate squamous cells

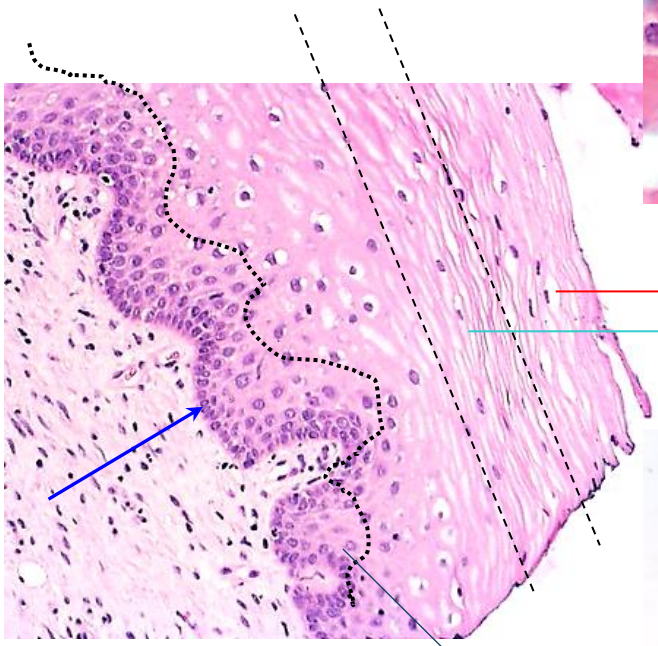
- are about the same size as superficial squamous cells
- cytoplasm is usually cyanophilic
- nucleus is round to oval with a clearly defined nuclear membrane and well-preserved chromatin. Sometimes called an “open nucleus” because the internal structure of the nucleus can be seen. This is the feature that defines an intermediate squamous cell compared with a superficial squamous cell, where the nucleus is opaque.

Parabasal squamous cells

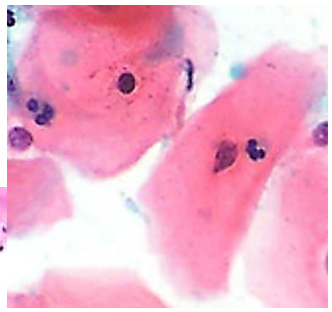
- uncommon in normal samples because come from the lower layers of the squamous epithelium.
- presence increases over 35 years of age, post-partum and in post-menopausal age group
- nuclei are slightly larger and N:C ratios are increased compared with intermediate squamous cells
- The cytoplasm stains cyanophilic.

Basal squamous cells

- are rarely seen in cervical cytology samples
- look like parabasal cells morphologically but are much smaller with a large nucleus, giving a high N:C ratio.



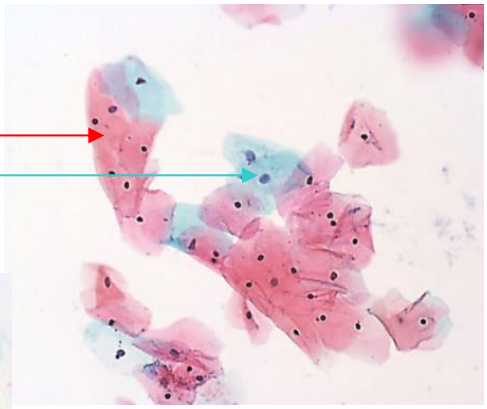
Histology of mature squamous epithelium with uppermost superficial cells (S) and intermediate cells (I) directly beneath. The basement membrane is located between the basal epithelium and the underlying stroma and is shown by the blue arrow.

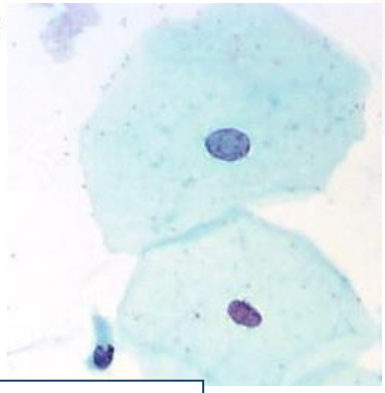


Superficial cell with a pyknotic nucleus and eosinophilic cytoplasm.

Superficial cells

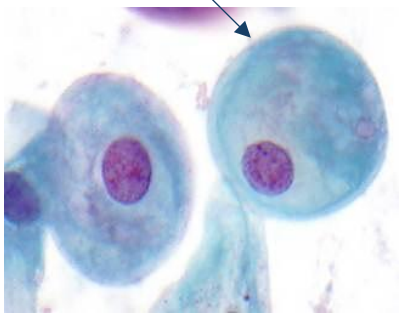
Intermediate cells





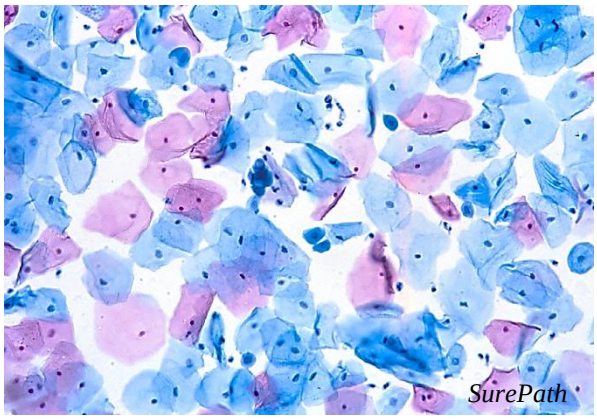
Intermediate cell with a vesicular nucleus and basophilic cytoplasm. The lower of the two cells is close to being a superficial cell as the nucleus is starting to condense.

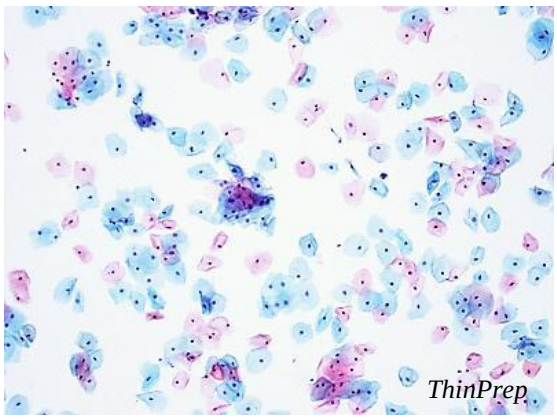
Parabasal cells



Parabasal cells with larger open nuclei. The N:C ratio varies depending on the degree of transition between a parabasal and an intermediate cell. The cytoplasm is round to oval with a smooth dense cytoplasmic border.

Squamous cells are the predominant cell type in most cervical cytology samples, shown here in SurePath and ThinPrep preparations





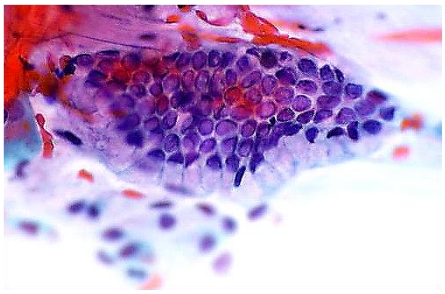
Endocervical glandular cells

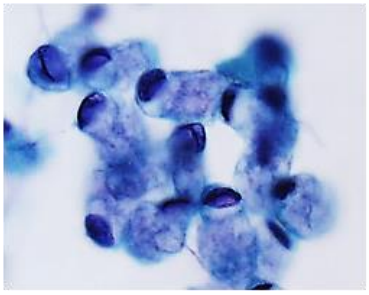
Endocervical cells are present in most cervical cytology samples. At least ten well-preserved endocervical or squamous metaplastic cells are required before reporting that a transformation zone component is present in the sample. In some normal clinical situations, the squamocolumnar junction (SCJ) is situated up in the endocervical canal (e.g. post-menopausal women) so endocervical/metaplastic squamous cells may not be sampled.

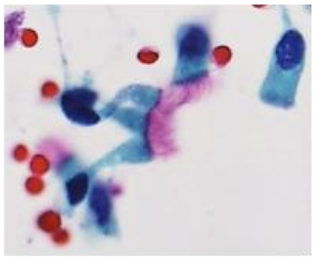
- are tall columnar cells with basal nuclei and apical cytoplasm that is usually finely vacuolated and cyanophilic
- usually appear in tightly cohesive flat sheets and can be seen either forming a palisade or “picket-fence” (side on view) or a honeycomb pattern (vertical view from above).

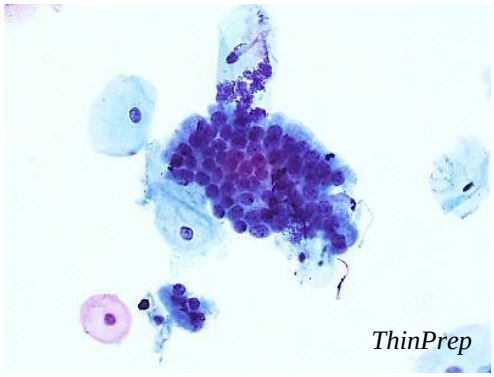
- nuclei have finely granular grey-blue chromatin. One to two small nucleoli may be present. A small dense dark nipple-like protrusion may be observed on endocervical nuclei, particularly at maximum oestrogen activity during mid-cycle.

- a small number of cells have cilia supported by a terminal bar
- endocervical cell cytoplasm is fragile and fragments may be seen interspersed with bare free nuclei.

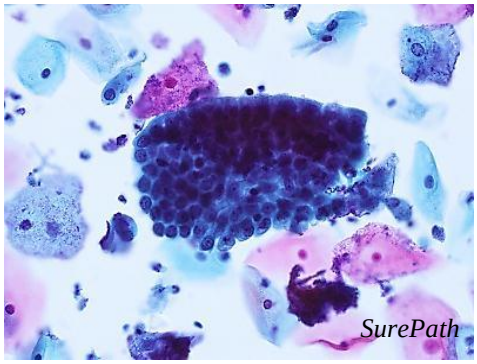








Endocervical cells in ThinPrep



Endocervical cells in SurePath

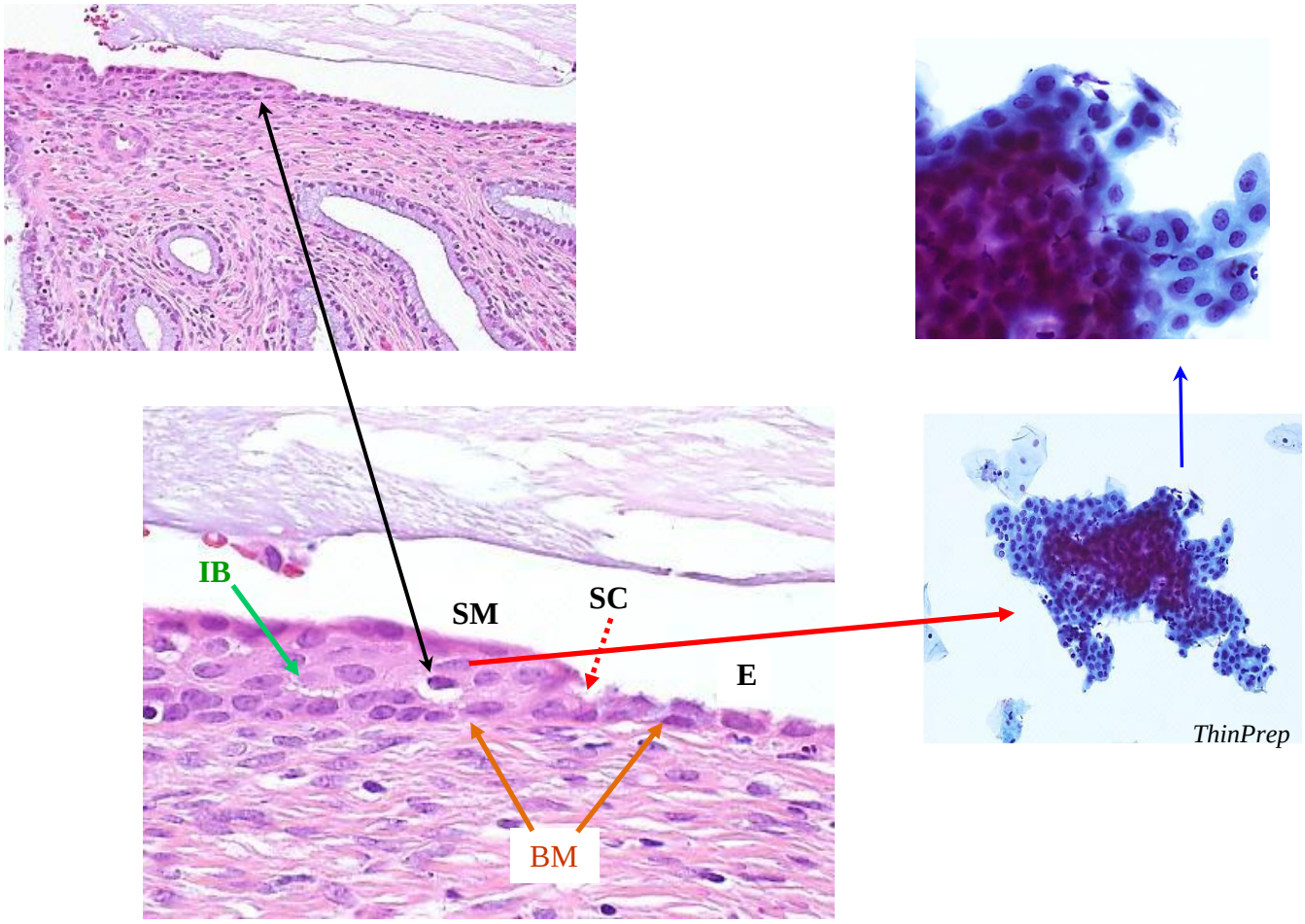
Squamous metaplasia

- a normal physiological process by which original endocervical epithelium is replaced with squamous epithelium.
- the area between the edge of the original squamous epithelium (i.e. the original squamocolumnar junction) and the current endocervical epithelium (i.e. the new squamocolumnar junction) is the Transformation Zone (TZ). The squamocolumnar junction (SCJ) is the point where the squamous metaplastic epithelium and endocervical epithelium meet.
    - the metaplastic process is triggered at puberty by the effect of the lower pH of vaginal secretions on columnar epithelium, which becomes exposed to the vagina because of growth.
    - begins with differentiation of endocervical reserve cells into immature metaplastic squamous cells which gradually become fully mature non-metaplastic squamous cells.

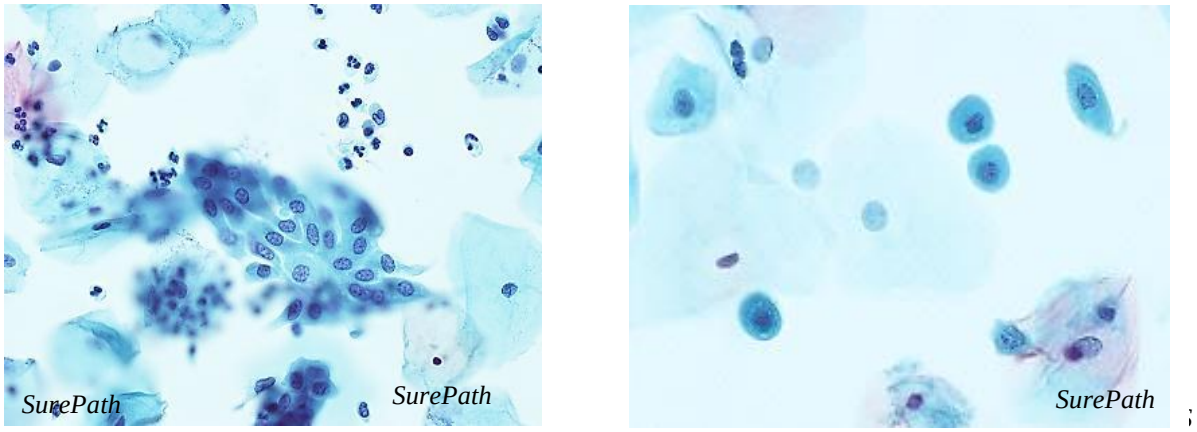
Cytologic appearance of squamous metaplasia varies depending on cell maturity.

- Usually appear singly, or may be in flat sheets or loose groups if less mature

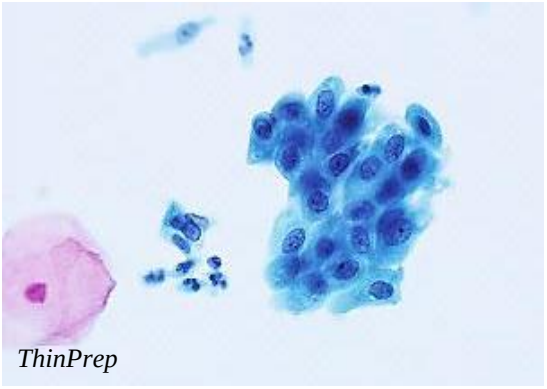
- distinction from parabasal cells can be difficult.
    - The clinical situation often helps – e.g. are likely to be parabasal if post-partum.
    - is unusual to see large numbers of all three different squamous cell types of maturation (i.e. superficial, intermediate and parabasal). If numerous superficial and intermediate cells are observed with numerous less mature rounded cells it is more likely that these cells are metaplastic rather than parabasal cells.
    - Identifying the cells as normal is more important than classify them definitively as parabasal or metaplastic.

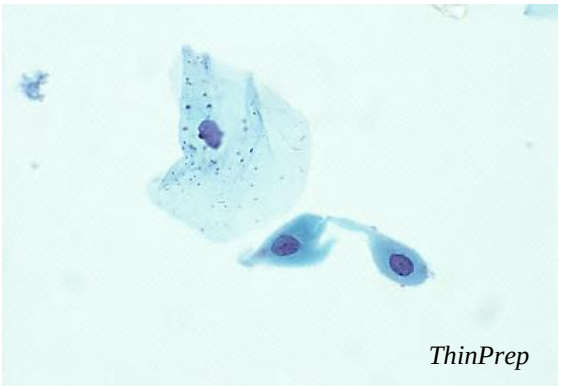


Histology: **Maturing squamous metaplasia** (SM) alongside endocervical cells (E) at the squamocolumnar junction (SC). Fine cytoplasmic extensions/intracellular bridges (IB) are seen throughout the metaplastic epithelium, particularly in the lower layers close to the basement membrane (BM).



Metaplastic squamous cells in SurePath. Note the dense cytoplasm.





Metaplastic squamous cells in ThinPrep. The cells often lie singly and can show cytoplasmic processes or round up in the vial fluid.

Atrophic squamous cells and Reserve cells

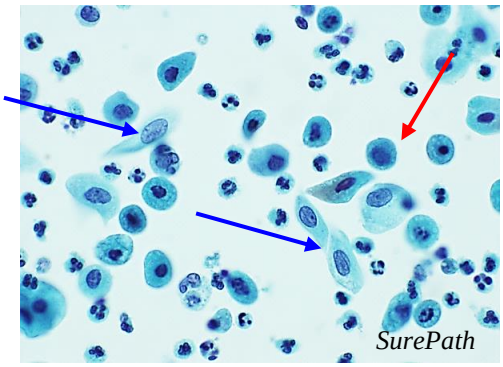
Atrophy occurs because of low levels of oestrogen, or a relative decrease in oestrogen compared with progesterone levels. e.g. the postmenopausal period, postpartum and where women are taking progestagens for contraception (such as Depo Provera).

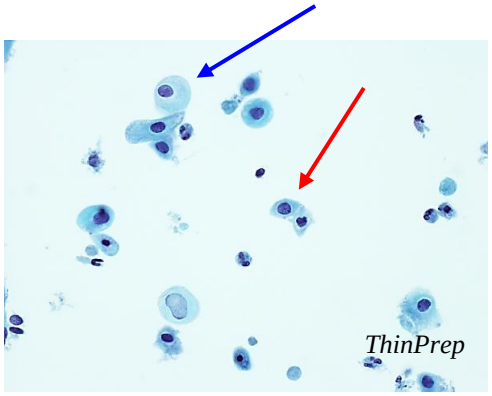
Cytologic appearances of atrophy

- may see single dissociated parabasal cells, sheets and/or loose clusters of atrophic cells.
- cells contain increased glycogen and may show cellular degeneration.
- granular background of degenerative cellular debris is common, but this may have been removed by LBC processing.

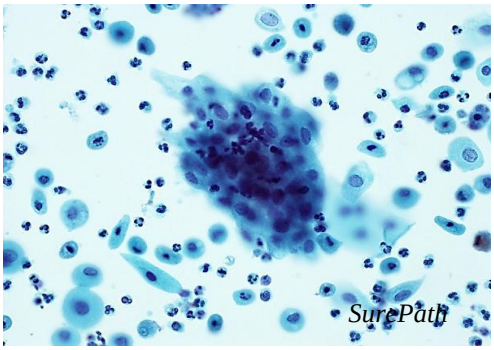
The degree of atrophy varies considerably among individuals of the same chronological age. Atrophy can be severe where parabasal cells predominate, or mild to moderate where intermediate squamous cells predominate with a reduction or lack of superficial squamous cells.

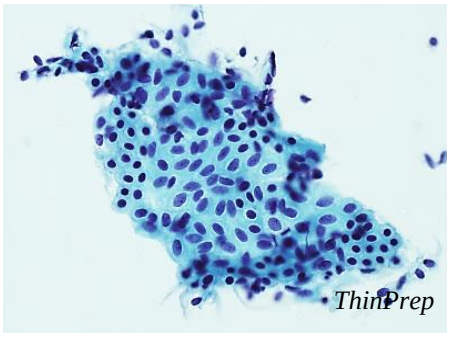
Because atrophic squamous cells have high N:C ratios inherently, the presence of a high grade squamous intraepithelial lesion (HSIL) or endometrial cells (either normal or atypical) can be misinterpreted in atrophic samples. Experience in examining a large number of normal samples showing atrophy is of considerable assistance in learning how to recognise atrophic samples where a significant lesion is also present.





Atrophic squamous cells. The upper images show single atrophic squamous cells, mostly parabasal cells (red arrows) with some larger intermediate squamous cells (blue arrows). The lower images show flat parabasal cell sheets demonstrating cohesion and retention of cell polarity.





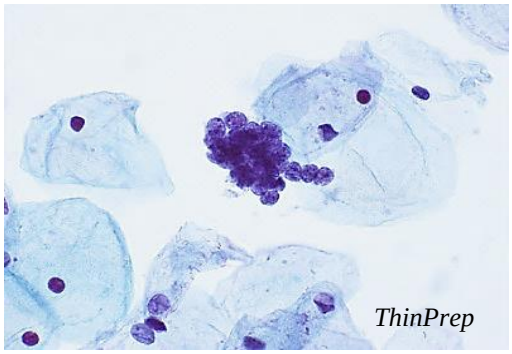
Reserve cells are located directly above the basement membrane and are precursor cells which differentiate into functional mature epithelium.

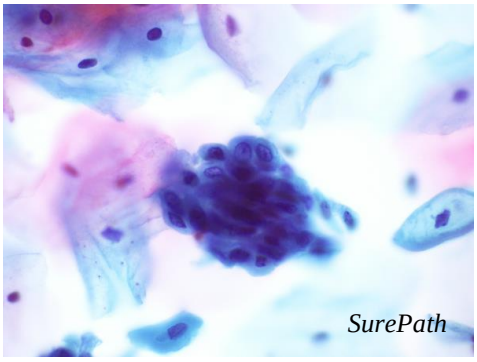
- Endocervical reserve cells transform into either endocervical cells or metaplastic squamous cells.
- Reserve cells are seen very infrequently in cervical cytology samples but may be seen in situations such as marked atrophy when the epithelium is thin, immature and fragile.

Reserve cells have:

- cells are more oval than round, and are often present in grape-like clusters
- have small, pale-appearing nuclei and extremely high N:C ratios with a bland chromatin pattern

Distinguishing true reserve cells from bare nuclei which have come from fragile atrophic squamous epithelium can be very difficult. When reserve cells are seen in clusters, their monotony and predictability distinguish them from the abnormal bare sticky nuclei seen with high-grade squamous lesions. Lymphocytes have a distinct rim of cytoplasm and a more granular chromatin pattern and can also be distinguished from reserve cells.





Reserve cells in LBC preparations

Lower uterine segment (LUS) cells

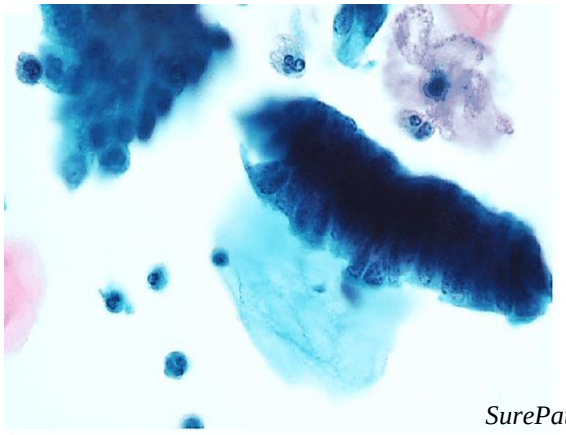
The lower uterine segment or uterine isthmus is the narrower lower region of the uterine corpus where the upper endocervical canal ends and the body of the uterus begins. The demarcation between endocervical and endometrial cells in this region is not sharply defined histologically and LUS cells can show elements of both types of epithelia. The cells in the lower uterine segment do not respond to menstrual cycle hormonal fluctuations and are not shed at menstruation.

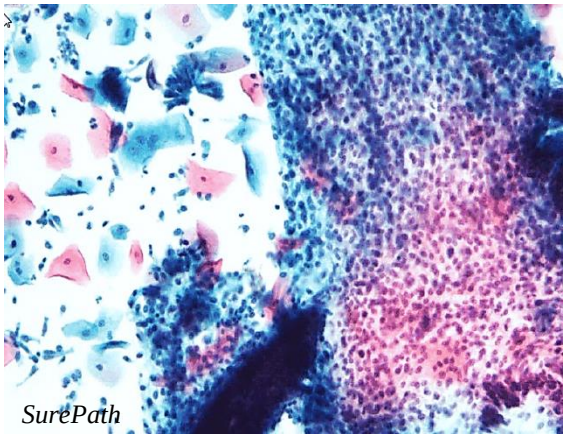
LUS cells have become relative common in cervical cytology samples since the cytobrush has been used. Sampling from high in the endocervical canal is sometimes referred to as “high scrape” sampling.

LUS cells usually appear in large multi-layered fragments rather than individual cells or small groups.

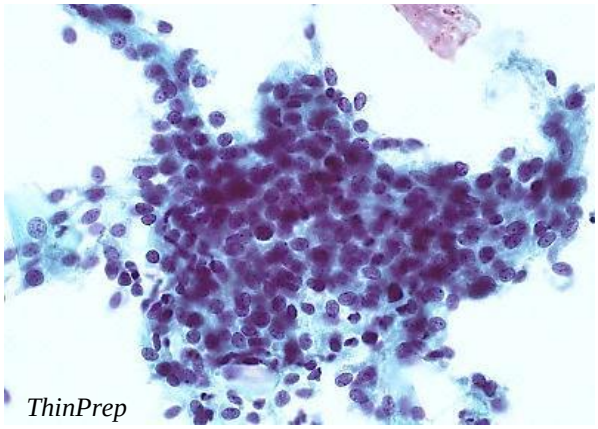
- May see tubular glands and densely packed spindled stromal cells. Finding the typical biphasic pattern of epithelial cells and stromal cells is very helpful in making a correct interpretation of high sampling but stromal cells are not always present.
- nuclei overlap and are small. Chromatin is evenly distributed with a uniform granularity.
- Occasional mitotic figures may be observed if the sampling is high enough to include functioning endometrial cells, but these should be normal proliferative mitoses.

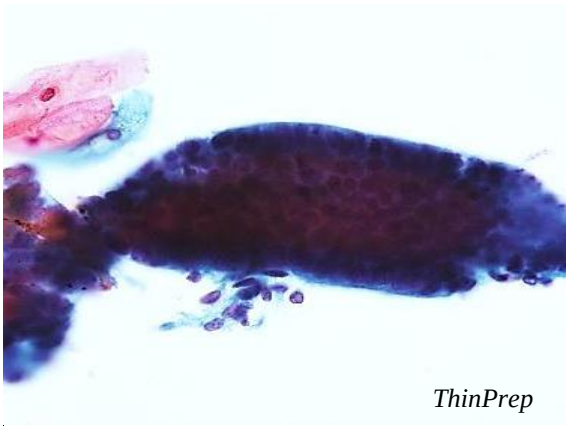
When LUS cells form crowded hyperchromatic cell groups they can be of diagnostic concern at low power. The very uniform and predictable nature of the nuclei in these large cohesive sheets identifies the benign nature and origin of the cells.





SurePath preparations showing LUS cells





ThinPrep preparations showing LUS cells

Normal endometrial cells
These cells are common in cervical cytology samples as they exfoliate from the endometrial cavity, pass down the endocervical canal and are picked up by the cervical sampling device. Endometrial cells will be discussed in a separate educational module.

Other cellular and non-cellular constituents

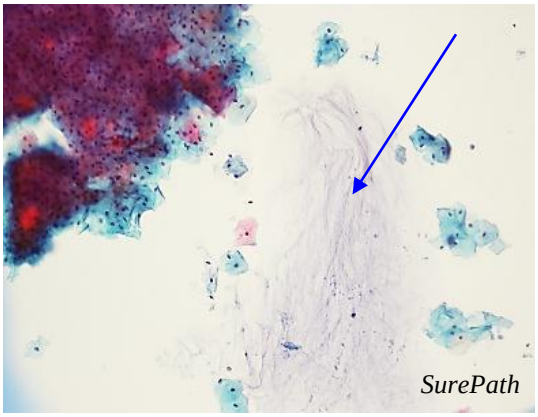
Leucocytes and Erythrocytes
Leucocytes and erythrocytes are both are very common. Blood in the sample occurs if taken during menstruation and may also occur if the sample is taken vigorously or if the epithelium is fragile.

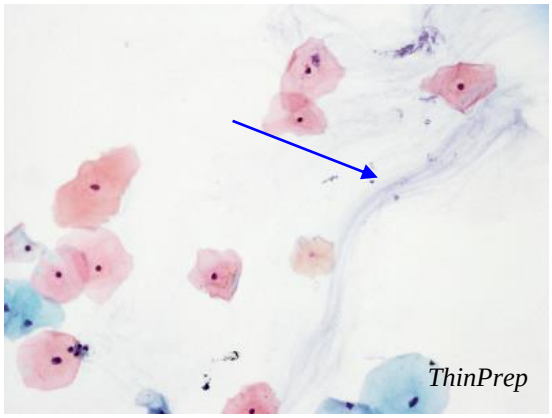
ThinPrep: Haemolytic agents in the ThinPrep fixative fluid usually remove red blood cells prior to slide preparation. For samples containing excessive blood, an acetic acid wash can be used to further lyse red blood cells before ThinPrep slide processing.

- Inflammatory material is mostly removed by ThinPrep processing but will be seen when large numbers of inflammatory cells are present

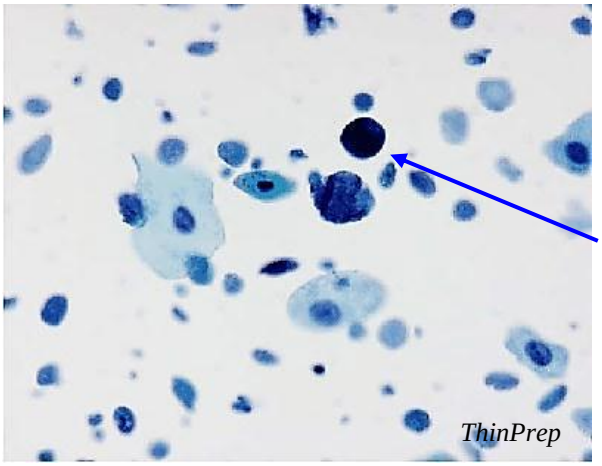
SurePath: Non-diagnostic debris and excess inflammatory cells are largely removed from the sample. As with ThinPrep, the number of leucocytes is significantly reduced, allowing easier visualization of epithelial cells, diagnostically relevant cells and infectious organisms.

Mucus
Mucus produced by endocervical cells is present in small quantities in LBC preparations as a natural entity. It appears amorphous and sometimes "stringy". In atrophic women mucus may also appear as amorphous blobs, sometimes called “blue blobs.” These are an amorphous grey colour, are the size of a small parabasal cell and may have a darker central core. They can be caused by inspissated mucus or degenerating parabasal cells.





Mucus in SurePath and ThinPrep samples



"Blue blob" in an atrophic LBC preparation. These are globular collections of basophilic amorphous material reflecting either degenerated parabasal cells or inspissated mucus.

Histiocytes

- are single cells that are common and may clump together giving an epithelial-like appearance.
- The nucleus is usually eccentric and round to oval when in the resting (non-active) state
- The cytoplasm is either foamy or vacuolated which can be confused with secretory activity. The vacuoles are usually multiple and of various sizes. Ingested debris may or may not be present.

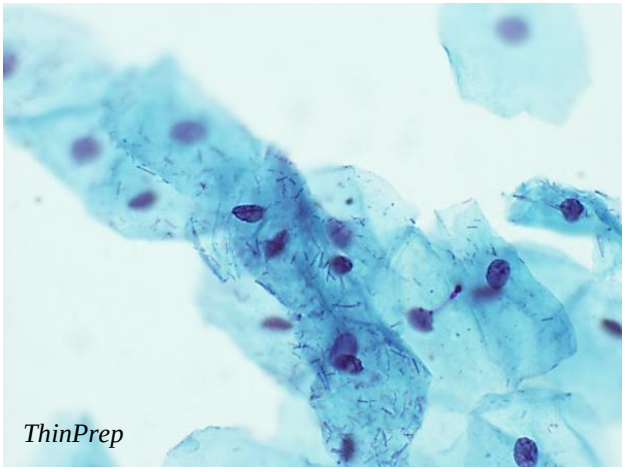
Normal flora

A variety of organisms are present in the cervix/vagina in the absence of disease as natural flora. Some organisms cause an epithelial reaction under conditions which allow their numbers to increase. Döderlein bacilli (*Bacillus vaginalis*) are the most common organisms found in the normal vagina and belong to the lactobacilli group. In the normal cycle they proliferate mid-cycle and if particularly abundant, can cause cytolysis in the luteal phase of the menstrual cycle.

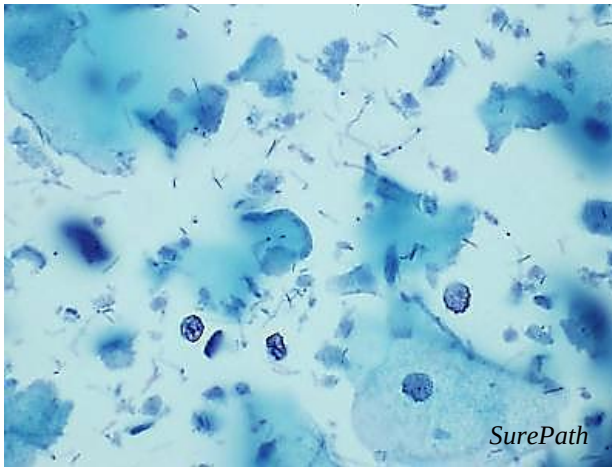
Lactobacilli

Döderlein bacilli are a heterogenous group of commensal bacilli.

- are slender, gram-positive rods (2-6 microns in length) which stain blue.
- They help maintain the acid pH of the vagina by hydrolysing glycogen from the cytoplasm of intermediate squamous cells into lactic acid (called glycolysis). This is a natural regulatory process and aids in defence against infections by maintaining a constant pH.
- When lactobacilli multiply rapidly, as often occurs in the secretory luteal phase of the menstrual cycle, they can have a cytolytic effect on epithelial cells (without causing inflammation). Cytolysis may be so marked that the sample may be unsatisfactory due to insufficient viable intact cells.



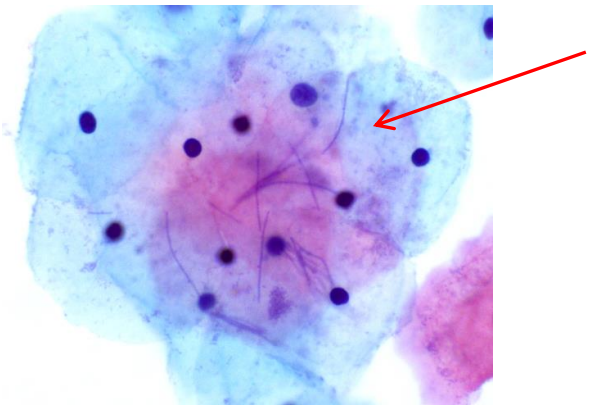
Normal squamous cells and lactobacilli



Cytolytic squamous cells associated with numerous lactobacilli

Leptothrix

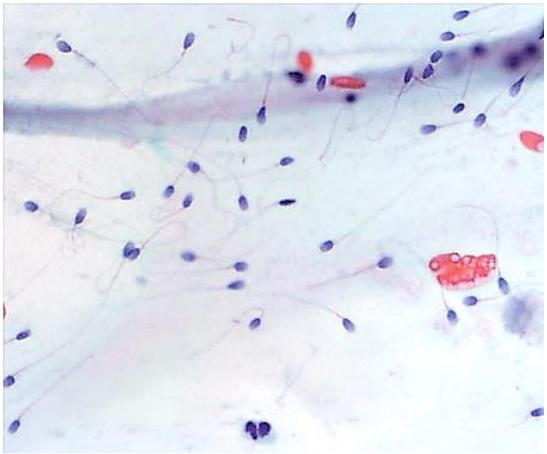
Leptothrix filaments are less commonly present than lactobacilli, are non-pathogenic and do not cause inflammation, although are often associated with Trichomonas vaginalis. The filaments form long or short, grey, thin thread-like structures which are segmented and may branch, but do not form spores.



Leptothrix: There is a mix of short and long rods. The characteristic curved and "S-shaped" forms of leptothrix are evident in the long rods (arrowed). The shorter forms are indistinguishable from long Doderlein bacilli.

Spermatozoa

Spermatozoa are easily identified in post-coital samples. They are seen as small round to oval sperm heads (about the size of a polymorph lobe) which stains with haematoxylin producing a two-tone effect. High power examination may or may not reveal the tail.

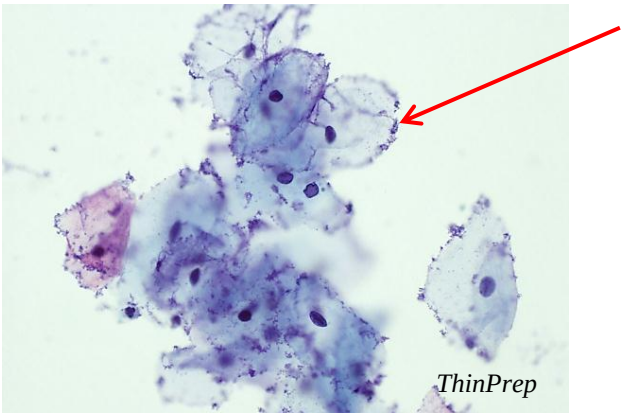


Spermatozoa showing the pale and dark zone of the sperm head when stained with haematoxylin.

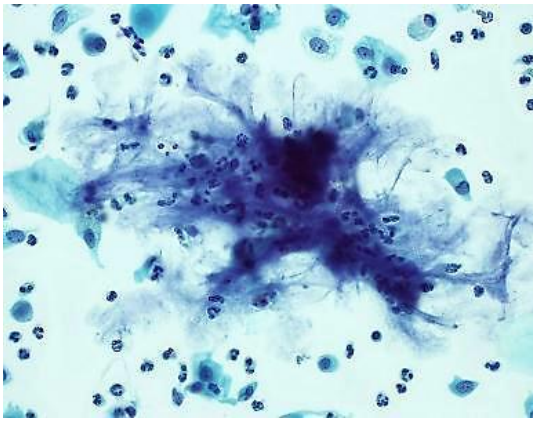
Contaminants and artefacts

Creams, lubricating jelly, talc/glove powder, and other introduced contaminants may be observed. Lubricants are used to allow easier insertion of the vaginal speculum. Problems that this can cause are:

- Abundant lubricant on the cervix requires removal prior to the sample being taken for cytology and this can remove exfoliated diagnostic cells.
- Residual lubricant can interfere with the endocervical brush or cervical broom in cervical cell sampling.
- Lubricant can clog up the filter in the ThinPrep processor if present in the sample, resulting in a less cellular slide preparation. Sample takers using ThinPrep vials need to be instructed not to use lubricant when taking cervical samples if possible, or to use it sparingly and only down the slides of the speculum to avoid getting lubricant into the ThinPrep vial. This is not a problem when using SurePath as the processing is different.



ThinPrep preparation showing excessive lubricant. Superficial and intermediate cells are clumped into a three-dimensional group and coated with a granular precipitate.



SurePath preparation containing lubricant