# Cervical smears made easy

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## Dedicated to

- My parents Daya and Daisy, for paving the way,
- Anura, Nipuna and Narada, for the love and support,
- Dilrukshi for the encouragement,

  and

- Department of Pathology, Sultan Qaboos university Hospital, Muscat, Oman, for the happy times and the opportunities.

## Contributors

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## Content

***Atypical squamous cells of uncertain significance (ASCUS - chapter 9) and Atypical glandular lesions of uncertain significance (AGUS) in Chapter 11***

# Foreword

It is an honor to write a foreword for Professor Dilani Lokuhetty's book "Cervical cytology made easy". The clarity of expression of ideas and the succinct way in which they have been put forward in this book are remarkable. The book works its way up brick by brick. Every part of the procedure and common mistakes are dealt with in great detail. The ample use of images of great quality adds to its clarity and makes this a very user-friendly book. The 'bullet-point' style of writing of Dilani adds very much to this. Indeed, the book lives by its name and makes cervical cytology 'simple'.

Gynecologists often feel intimidated by the newer classifications of cervical cytology. Speaking for them, I see this book as an attempt to build-up cervical cytology from it foundations. Dilani has intended this as one that is aimed at cytologists and trainees in the specialty. Undoubtedly it will be invaluable for gynecologists who are looking for easy answers to their questions regarding cervical cytology as well. As one who has been working towards improving the standards of care of cervical pathology I found this book invaluable.

Professor Lokuhetty is a reputed cytologist who has been working in the specialty for almost two decades, both in Sri Lanka and abroad. This book was written while she was working at the Sultan Quaboos University Hospital where she spent her sabbatical. Her years of experience are shown in the book, which addresses cervical cytology from a very practical viewpoint. The pitfalls and inadequacies of cytology are highlighted from its beginning.

I congratulate Professor Lokuhetty on writing a book that presents facts so succinctly and in a way that helps the practicing cytologist and cytoscreener enormously.

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# Preface

The aim of this book 'Cervical smears made easy' is to make screening and diagnosis of cervical smears a less tedious and a pleasant task for both cytoscreeners and cytopathologists engaged in cervical smear diagnosis. It will also serve as a guide to the postgraduate trainees in pathology, in their quest to master diagnosis of cervical smears.

Both Bethesda system terminology and the British Society for Clinical Cytologists (BSCC) terminology is used in the book to describe entities. The use of dual terminology is expected to enhance its' user friendliness across borders of countries, based on the terminology used in the setting of the reader. Abbreviations used are explained at the beginning of the book. Throughout the book the entities described are accompanied by relevant images, enabling a pictorial library to be built up in the mind of the reader. The accompanying images are those that were encountered on conventional smears on routine practice. This is appropriate as most of us yet encounter only conventional smears. These images were captured using a DP71 digital camera. The differential diagnosis of important entities encountered during screening is emphasised in the text, highlighting helpful differential diagnostic criteria with supporting images. In the latter part of the book comparative images of similar entities are provided and few real life challenging cases are presented for self assessment.

I am grateful to my colleague and friend, Dr Anna Saparamadu for her contribution and encouragement in compiling the book. The Senior Biomedical Scientists Ms Usha Rani Bai, and Ms Amal Al Maashari, and Biomedical Scientists Ms's Najath Al Dairi and Amna Al Shukeili, of the Cytology Division, Sultan Qaboos Univeristy Hospital (SQUH), Muscat, Oman, contributed significantly and most willingly. I wish to place on record my gratitude to them and to Prof Anand Date, Head of the Department and the consultants and staff in the Pathology Department at the SQUH for the encouragement received. Mr Amal Ranawaka of the audiovisual unit of Faculty of Medicine, University of Colombo is acknowledged for the help rendered with the images. This book would not have been possible without the encouragement and guidance extended to the novice author by Prof Colvin Gooneratne a veteran editor. I am indebted to him. DL. February 2012, Colombo, Sri Lanka.

# Content

# Abbreviations

| | | |
|---|---|---|
| **ASCUS** | - | Atypical squamous cells of undetermined significance |
| **ASCUS-LG** | - | Atypical squamous cells of undetermined significance, cannot differentiate from low grade |
| **ASCUS-HG** | - | Atypical squamous cells of undetermined significance, cannot differentiate from high grade |
| **AGUS** | - | Atypical glandular cells of undetermined significance |
| **BSCC** | - | British Society of Clinical Cytologists |
| **CIN** | - | Cervical intraepithelial neoplasia |
| **CGIN** | - | Cervical glandular intraepithelial neoplasia |
| **HCG** | - | Hyperchromatic crowded cell groups |
| **HGSIL** | - | High grade squamous intraepithelial lesion |
| **HPV** | - | Human papilloma virus |
| **IUD** | - | Intrauterine device |
| **LGSIL** | - | Low grade squamous intraepithelial lesion |
| **SIL** | - | Squamous intraepithelial lesion |
| **SCC** | - | Squamous cell carcinoma |
| **IAC** | - | Invasive adenocarcinoma |

# Chapter 1 – General information

**History of the cervical smear:**

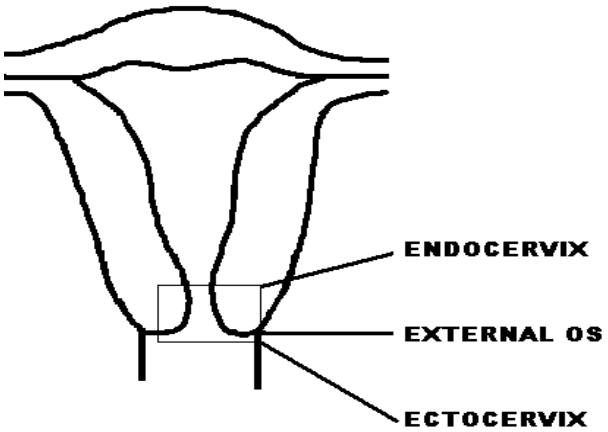
Prof Aurel Babes, a Romanian discovered that cervical cancer could be recognized by the presence of malignant cells in samples obtained from the vagina as far back in 1927. The work on cervical smears was pioneered by George Papanicolaou and the cervical smear was introduced in late 1940 – early 1950.

**The utility value of the cervical smear:**

The cervical smear or the Pap test is only a screening tool to detect the early cytological changes of cervical cancer and shouldn't be considered a diagnostic test. It is also not a fool proof test with false negatives occurring due to sampling, screening and interpretive errors.

**The sampling area:**

The junctional zone between the ectocervix and the endocervix is sampled, yielding both squamous cells from the ectocervix and glandular cells from the endocervix in cervical smears.




**Papanicolaou stain (Pap stain) is used to stain cervical smears.**

The Papanicolaou stain is routinely used on alcohol fixed cervical smears, as it provides good nuclear detail and allows differentiation of superficial from intermediate squamous cells leaving mucin unstained. The stain includes Haematoxylin and Orange G6 (OG6) and EA50 stains.

- Haematoxylin stains the nuclei.
- Orange G6 stains the cytoplasm for keratin.
- EA50 stain consists of eosin and light Green, and counter stains the cytoplasm blue, green, or pink.
- Pap stain stains the metabolically inactive cells pink (E.g. Superficial cells) and keratinized cells orange. Thick cellular areas in the smear are also stained orange.

**The recommended approach to cervical smear screening:**

- Approach screening and assessment of cervical smears with an open mind with out any preconceived ideas.
- Adopt a systematic approach to screening. Place the slide on the microscope stage with the label on the right side. Start from one end of the slide and progress from top to bottom to the other end systematically with out missing any areas using the 10X objective. Place a dot to the left of any abnormality detected during screening. Any abnormal cells detected should be further analyzed under high power objectives (20X and 40

**Beware …**

- Not to assess the smear based on the pattern alone. Look at the cytological features of individual cells.
- Never base your diagnosis on a single criterion alone.
- Assess the worrisome cells in relation to all other cells in the smear.
- Try not to focus on one entity because this may lead to missing other entities.

**How would you assess the adequacy of the smear?**

- An adequate sample will have at least 10,000 cells covering at least 10% of the slide.
- Take a random check across the slide under 20X objective to assess this.
- Identify at least 10 fields with 30 well-preserved cells of epithelial type, to ascertain the number of cells present on the slide as adequate.

**Satisfactory and unsatisfactory smears:**

**Satisfactory**

- The smear is labeled appropriately with the correct person identification details.
- All relevant clinical information is provided.
- Cells cover more than 10% of the slide surface.
- At least 75% of cells are available for cytological evaluation.

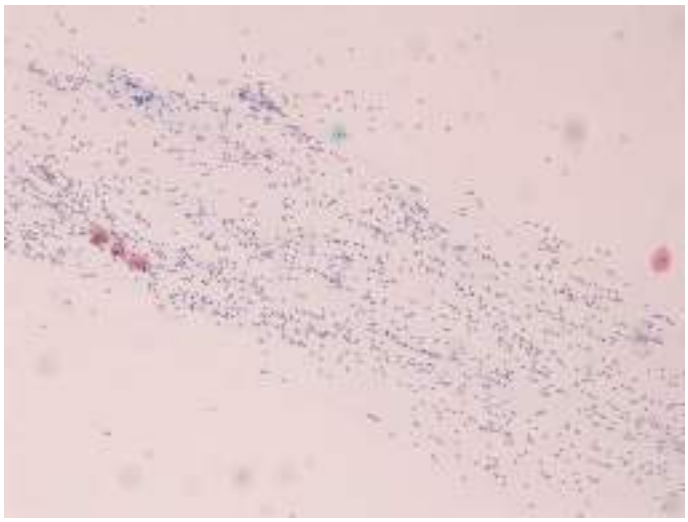
  **Note:** It may still be necessary for endocervical/metaplastic component to be present for a smear to be considered satisfactory in some settings,.

**Unsatisfactory**

- Person identification is lacking or confusing.
- The slide is technically unacceptable E.g. Broken.
- Smear is scanty with squamous cells covering < 10% of slide.
- >75% of cells show air drying artifact, contaminants, thick areas, blood and inflammation obscuring interpretation.

**Smears that fall under unsatisfactory category:**

- **Sparsely cellular smears (Figure 1.1)**

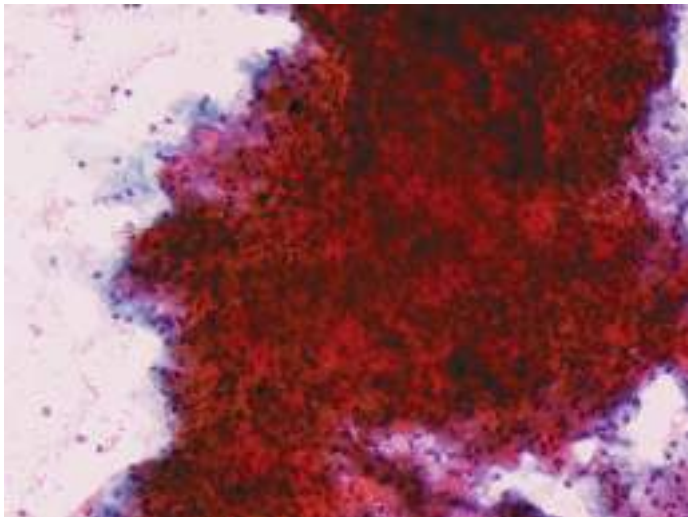


An adequate number of squmaous cells are not present in the smear to make a conclusive diagnosis.

- **Smears with mainly of endocervical cells**

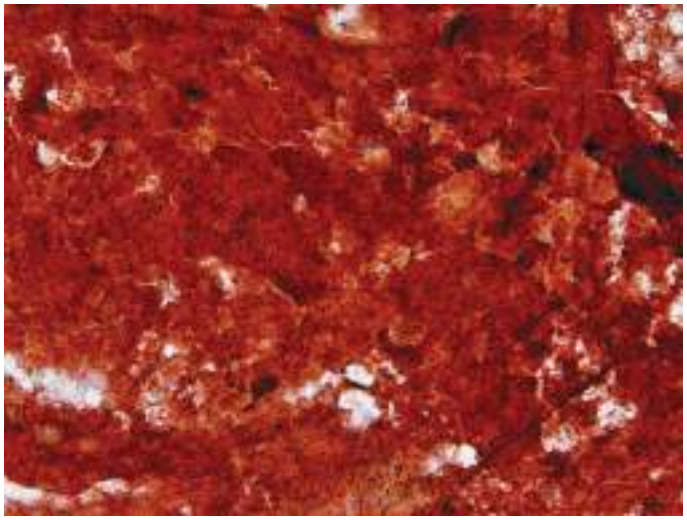
An adequate number of squmaous cells are not present in the smear to make a conclusive diagnosis.

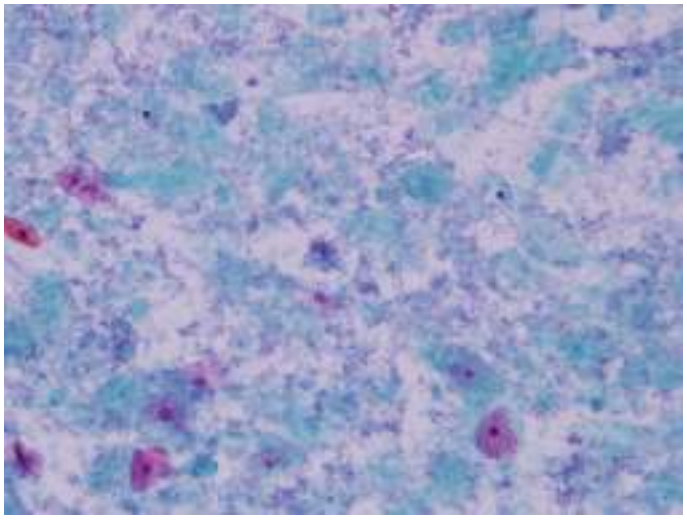
- **Thick smears (Figure 1.2)**

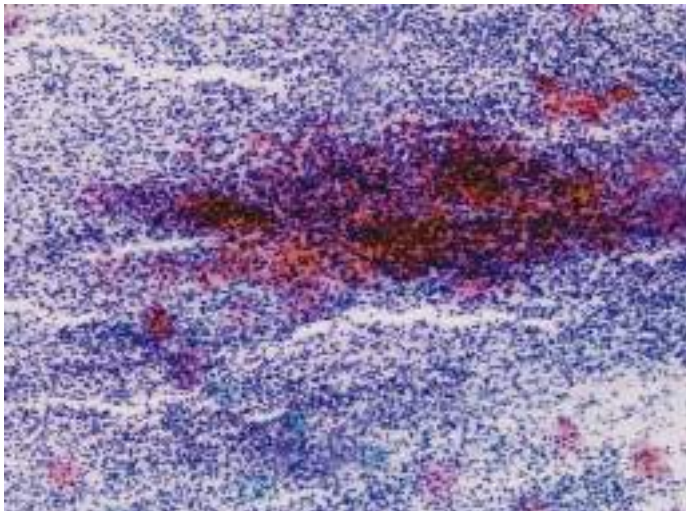


Thick areas with overlapping cells appear as orangiophilic areas. Assessment of cytological details is precluded in these areas.

- **Cells overshadowed by blood, (Figure 1.3a) bacteria (Figure 1.3b) or inflammatory cells (Figure 1.3c)**

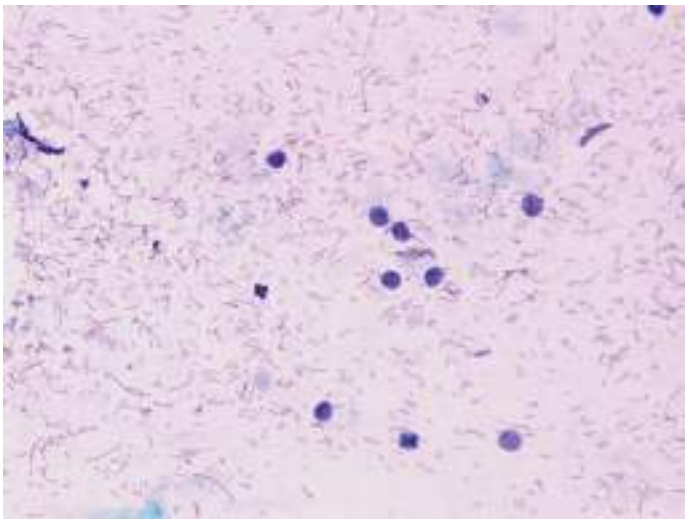






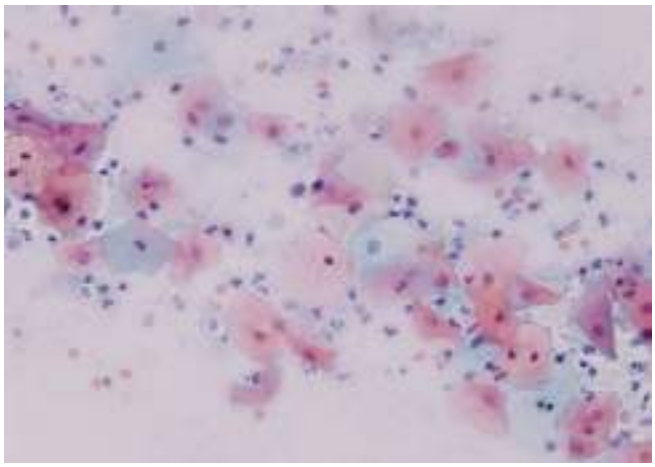
Blood, bacteria or inflammatory cells obscure squamous cells in the smear preventing assessment of cytological details.

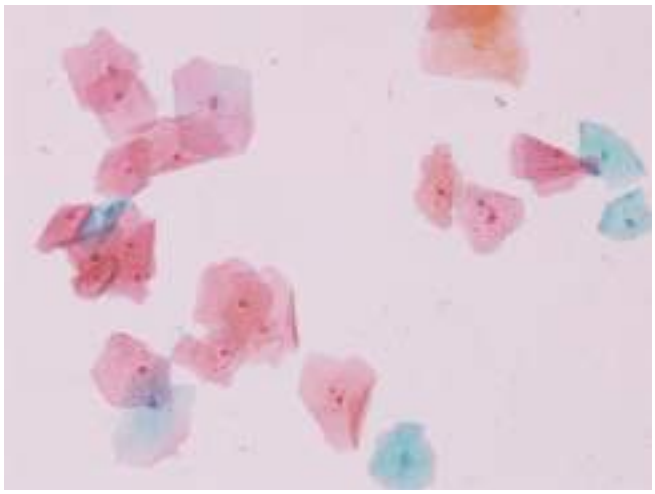
- **Smears with marked cytolysis (Figure 1.4)**



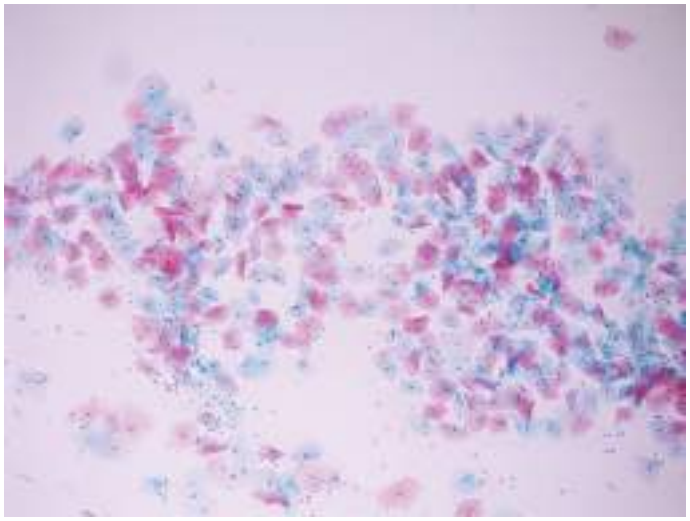
Lactobacilli act on cytoplasm of intermediate squamous cells converting glycogen to lactic acid. This leads to lowering of the pH and lysis of the cell cytoplasm. Naked nuclei in cytolysis prevent assessment of accurate cytological details (E.g.The nuclear cytoplasmic ratio). Lactobacilli are abundant in these smears. Inflammatory cells are absent. Superficial and parabasal cells are sparse.

- **Smears with air drying (Figure 1.5a) and suboptimal staining (Figure 1.5b)**



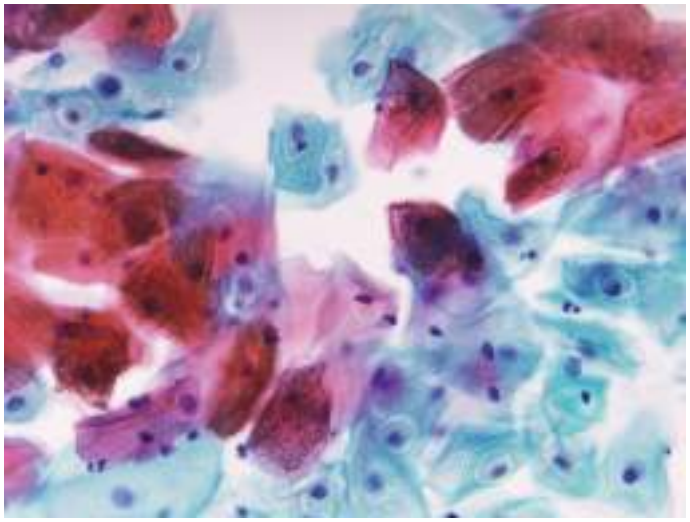


Air dried cells are lightly stained and hazy and have artificially bloated nuclei. The nuclei are also pale and lack chromatin detail precluding assessment of accurate nuclear details. (Compare with cells in the well-preserved, well-stained smear **– Figure 1.5c**)



Squamous cells that are exposed to formalin vapor also do not take up the nuclear stain (Haematoxylin) precluding assessment of nuclear features.

- **Corn flaking (Figure 1.6)**



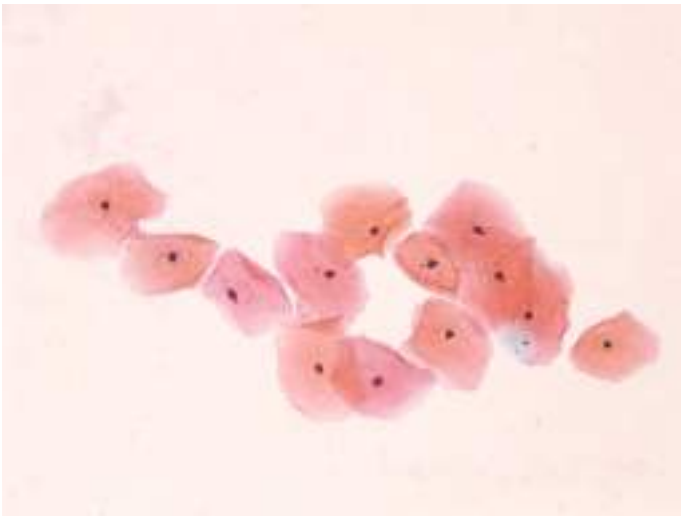
When the mounting agent dries on the slide before cover slipping air bubbles get trapped on top of the cells resulting in brownish areas covering the nuclei. This interferes with assessment of nuclear details. Re cover slipping is necessary.

# Chapter 2 – Normal cells found in cervical smears:

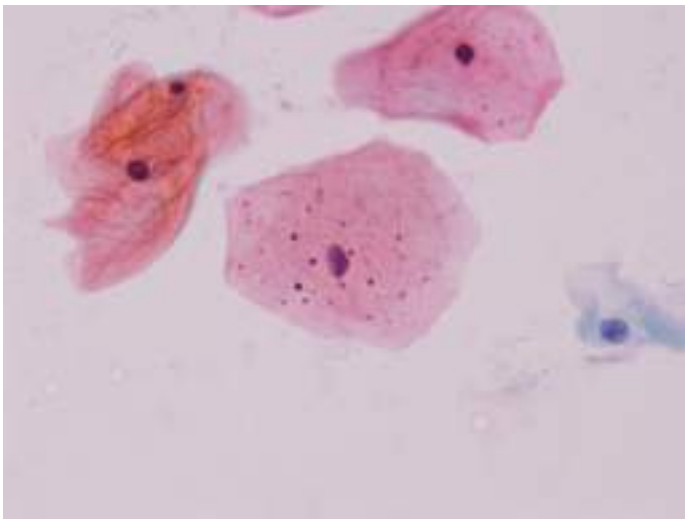
**Squamous cells**

Squamous cells line the ectocervix. The basal/reserve cells differentiate to parabasal cells to intermediate cells and superficial cells.

**Superficial squamous cells (Figure 2.1a)**

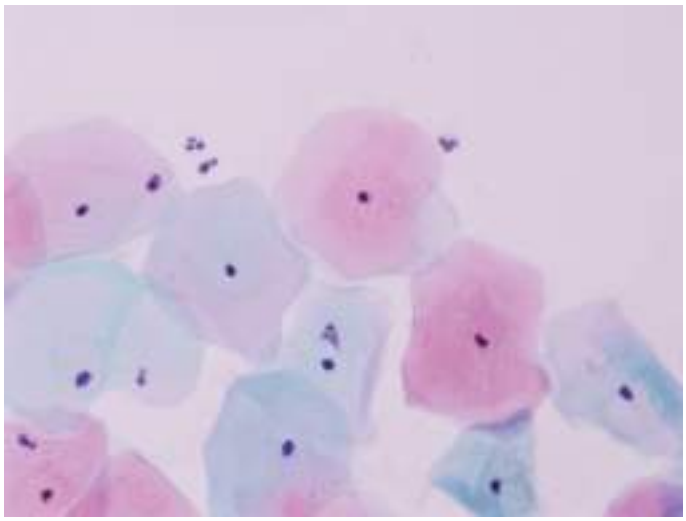


- Large flat polygonal cells (40-60 μm in diameter), occurring singly or in clusters.
- The nucleus is small, central and pyknotic with no discernible chromatin structure.
- The cytoplasm is abundant, pinkish to orange and is occasionally folded.
- Rarely a granular layer is formed in the epithelium. Then cytoplasmic granules may be found in the constituent cells. **(Figure 2.1b)**

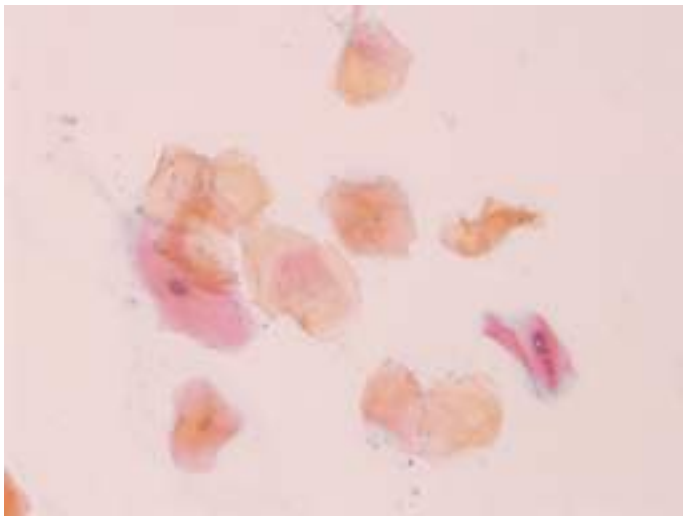


**Note:** Colour is not a good criterion to differentiate superficial from intermediate squamous cells, as at times the cytoplasm of superficial cells stain blue. Look for nuclear details.

**(Figure 2.1c)**

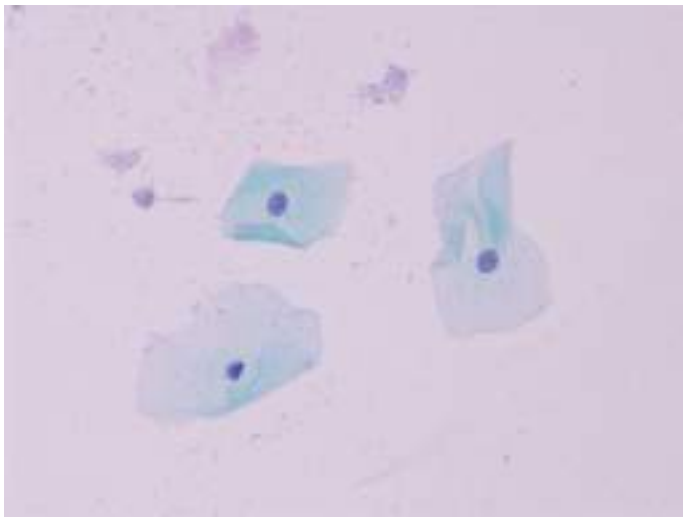


**Anucleate squames (Figure 2.1d)**



- These are mature squamous cells with orange or yellow cytoplasm lacking nuclei.
- They are encountered with hyperkeratosis and as a vulval contaminant.

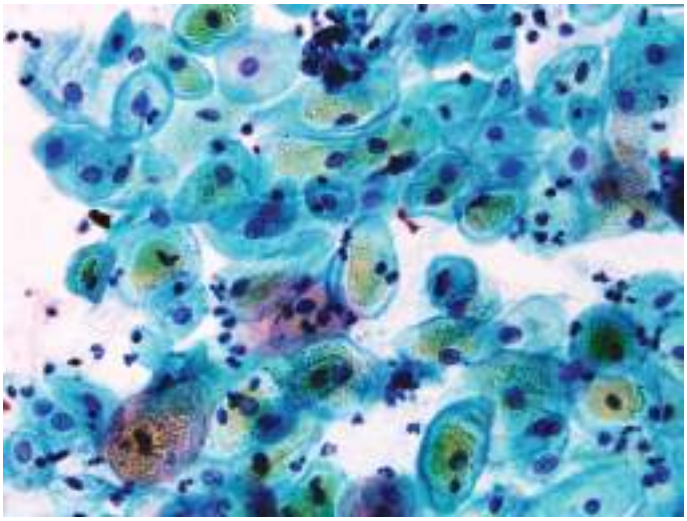
**Intermediate squamous cells (Figure 2.2a)**



- Large polygonal shaped cells (30-60 μm in diameter), occurring singly or in sheets.
- Round/oval centrally placed vesicular nucleus. (same size as that of a neutrophil)
- Fine, even, granular, chromatin.
- Abundant, translucent, green cytoplasm, usually folding at the periphery.
- Poorly fixed cells may stain pink/orange while retaining other characteristics.

Note: Intermediate cell nucleus serves as the key reference for size, chromatin quality and staining. In comparison, dyskaryotic cells/cells from squamous intra epithelial lesions (SIL) appear larger and darker with coarser chromatin.

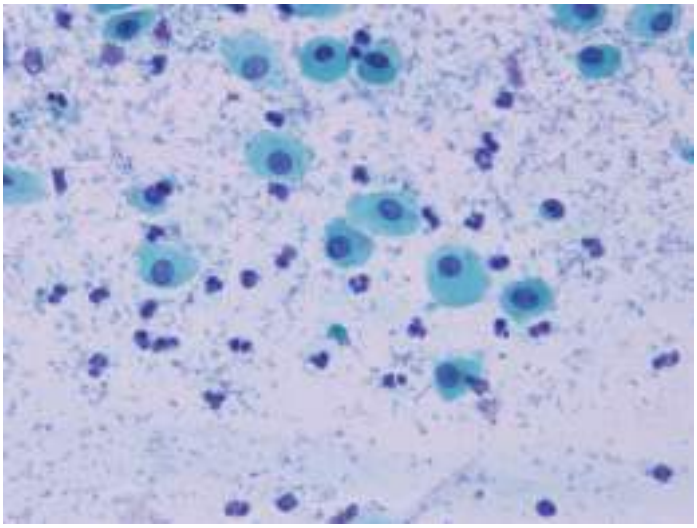
**Glycogenated squamous cells (Figure 2.2b)**



These cells are seen during the secretary phase, pregnancy and in diabetes.

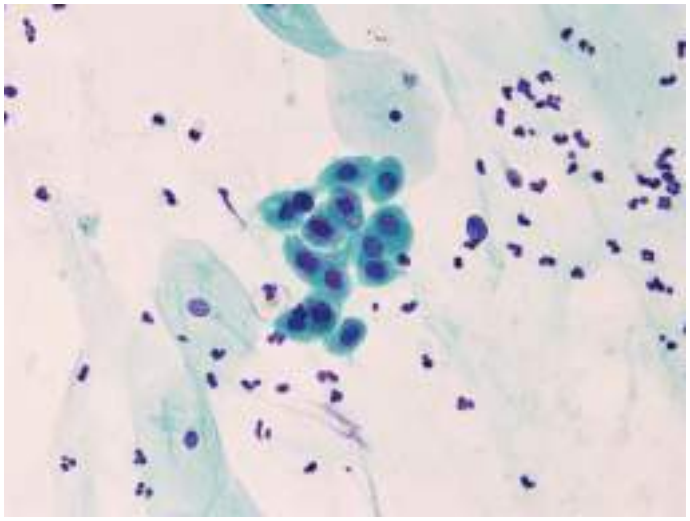
- Due to progesterone effect the cytoplasm is filled with glycogen appearing as a central golden yellow area.
- Surrounded by lactobacilli.
- The cytoplasm eventually undergoes cytolysis.

**Parabasals (Figure 2.3)**

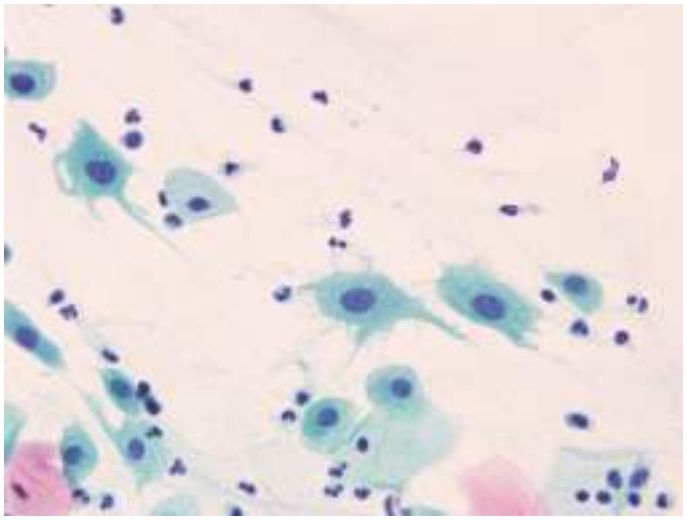


- Occur singly or in sheets.
- Round/oval in shape.
- Round/oval, centrally placed, vesicular nucleus occupying about half of the cell.
- Fine evenly granular chromatin.
- Dense green cytoplasm.

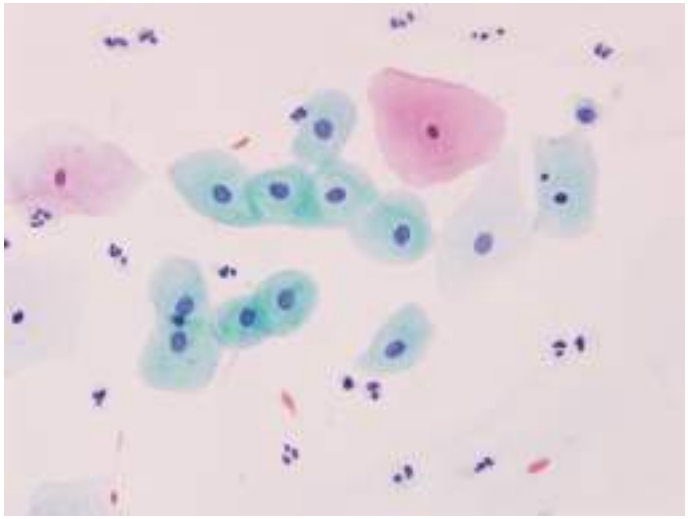
**Metaplastic squamous cells in immature squamous metaplasia (Figure 2.4a)**



- Occur singly and in loose sheets.
- Shows a cobble stone pattern of arrangement.
- About the same size as parabasal cells (20-30μm) with rounded sharply defined cell borders with dense blue green cytoplasm. A denser ectoplasmic rim may be seen.
- May have cytoplasmic vacuoles.
- Round to oval centrally placed nuclei with smooth membranes and fine, evenly granular chromatin pattern.
- Nucleoli may appear in reactive cells.
- Scraped out cells have spidery cytoplasmic projections (spider cells) **(Figure 2.4b)**



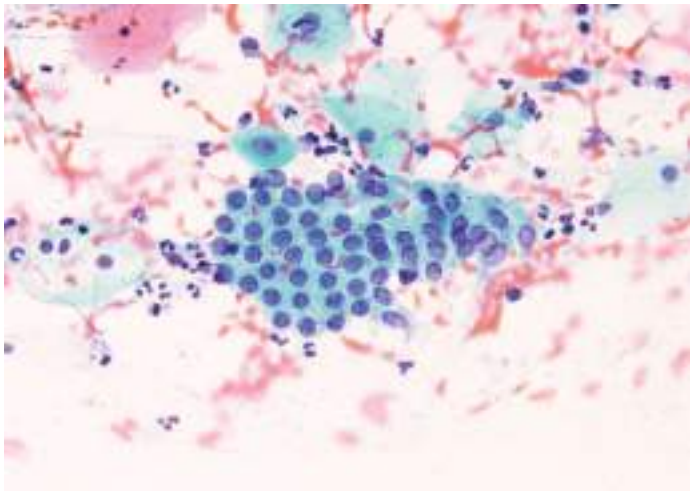
**Metaplastic squamous cells in mature squamous metaplasia (Figure 2.4c)**

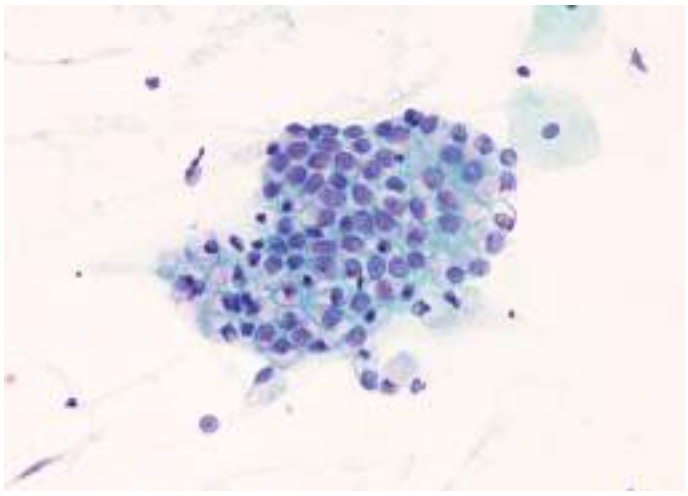


- A cobble stone arrangement may be seen.
- Resemble intermediate cells with more rounded cell borders and denser cytoplasm.

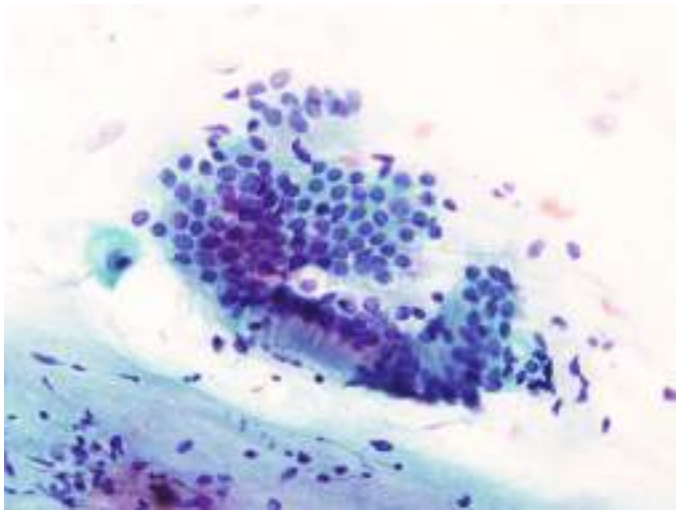
**Endocervical cells**

- Occur singly or as flat monolayers **(Figure 2.5a)** with an en face honeycomb appearance due to prominent cell borders. **(Figure 2.5b)**

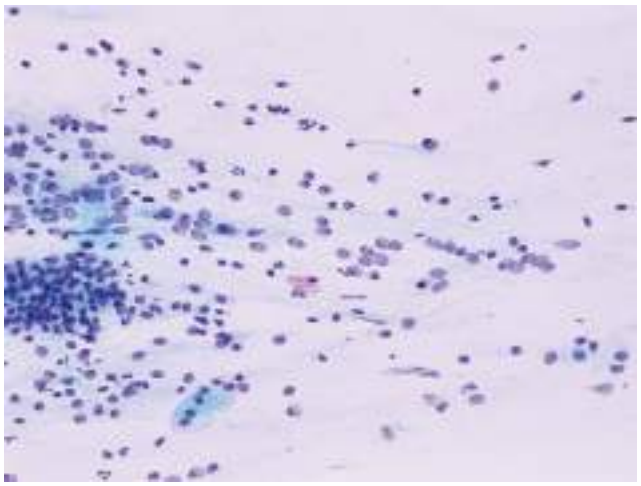


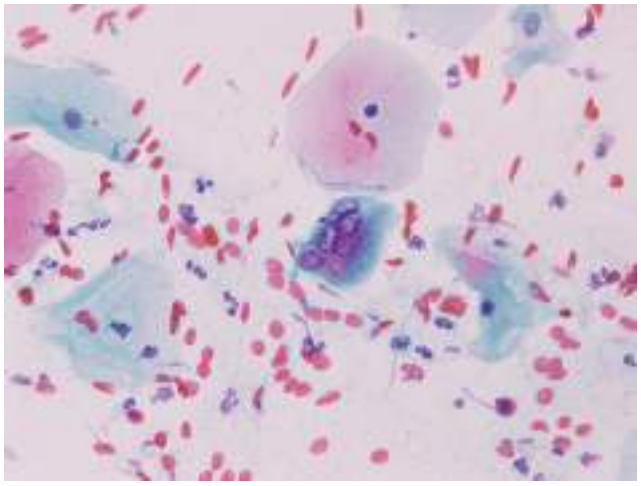


- On side profile, columnar endocervical cells palisade with orderly basal, uniform nuclei with resembling a picket fence. **(Figure 2.5c)** Nuclear stratification is not obvious.



- The cytoplasm is cyanophilic, translucent or vacuolated.
- Cytoplasm may be distended by a secretary vacuole (goblet cells).
- Nuclei have fine evenly granular chromatin.
- May have nucleoli.
- Bare stripped nuclei **(Figure 2.5d)** and occasional giant cells **(Figure 2.5e)** may be seen.

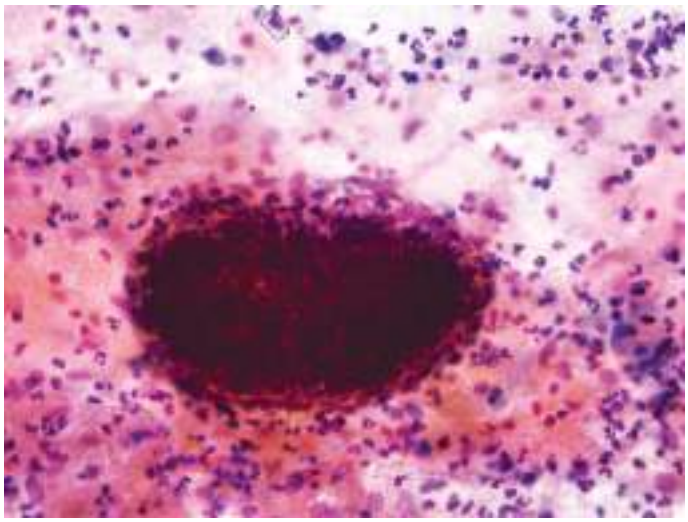




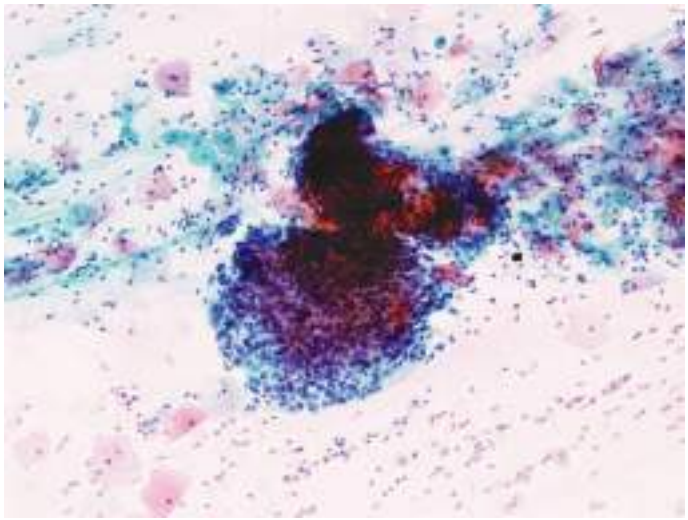
**Endometrial cells**

Appearance of endometrial cells in smears varies with the stage of the menstrual cycle as well as on the degree of cell preservation.

- Exodus pattern is seen immediately after menstruation.
- These are tight three dimensional cell clusters with an inner core of stromal cells with outer epithelial cells. **(Figure 2.6a)**

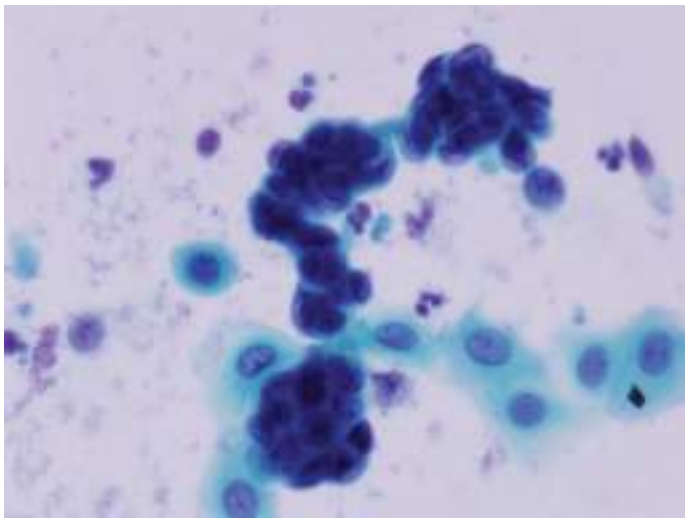


- Later, densely hyperchromatic, disorganized crowded cells groups are formed with the onset of degenerative changes. **(Figure 2.6b)**



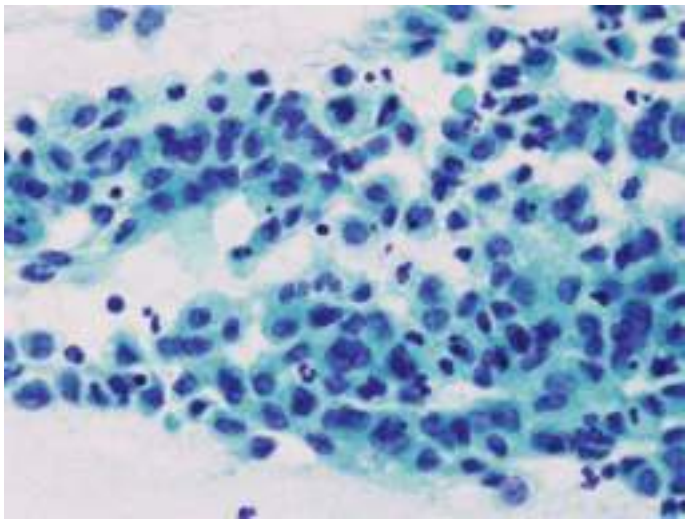
- Neutrophils and histiocytes are often present.

Epithelial cells **(Figure 2.6c)**



- Nuclei are round to oval or angular showing minimal size variation.
- Nuclear size is equivalent to an intermediate squamous cell nucleus or a neutrophil.
- Chromatin is finely granular, evenly distributed and generally hyperchromatic.
- Nuclear borders may be pronounced.
- Cell borders may be indistinct in groups.
- Cytoplasm is generally cyanophilic and scanty and may show discrete vacuoles.

**Stromal cells (Figure 2.6d)**



- Similar in appearance to small histiocytes and form loose aggregates (sticky histiocytes).
- Present singly or in large groups or streaks.
- Minor variation in cell size.
- Nuclei may be round, oval, reniform (bean-shaped) or angular.
- Chromatin is finely granular with several small chromocentres.
- Cytoplasm is cyanophilic and is finely vacuolated with ill-defined borders.

### Deep stromal cells

- Nuclei are oval to spindle-shaped.
- Chromatin is finely granular and evenly distributed.
- Nuclear membrane may be infolded producing a longitudinal nuclear groove.
- Wisps of cyanophilic cytoplasm are present at either end of the nucleus.

## Other cellular components

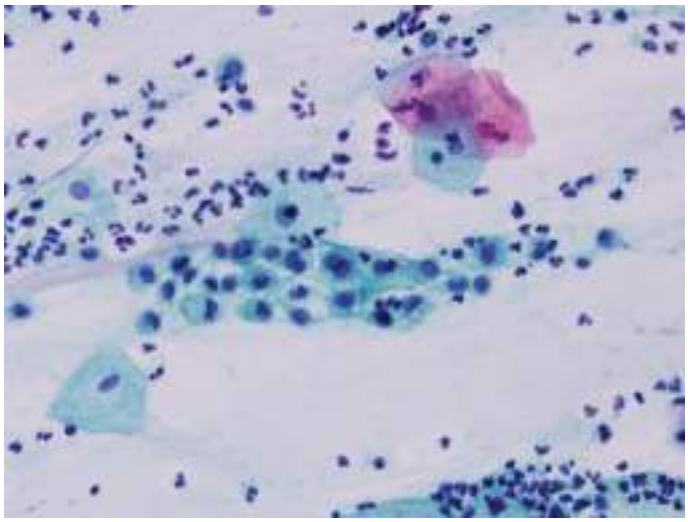
### Reserve cells

- Difficult to identify in cervical smears. Small crowded cells with round darkly stained nuclei. Form hypercellular crowded cell groups. (HCG)
- Nuclei are twice the size of a neutrophil (larger than endometrial cell nuclei), spindle to oval in shape with granular chromatin and nuclear grooves.

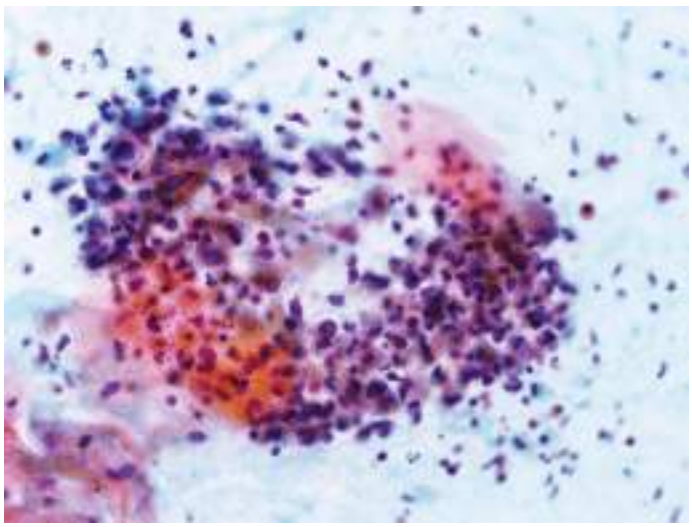
### Neutrophil polymorphs

### Macrophages/histiocytes

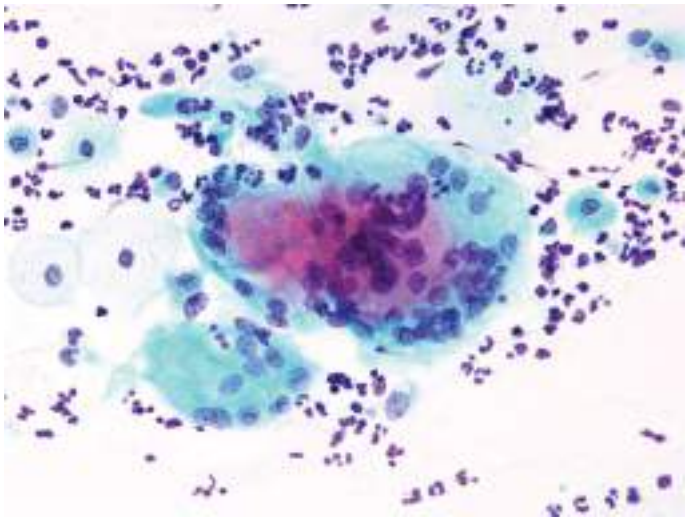
- Usually occur as single cells or in clusters **(Figure 2.7a)**. Seen in the late



menstrual exodus **(Figure 2.7b)**, pregnancy and in inflammation.



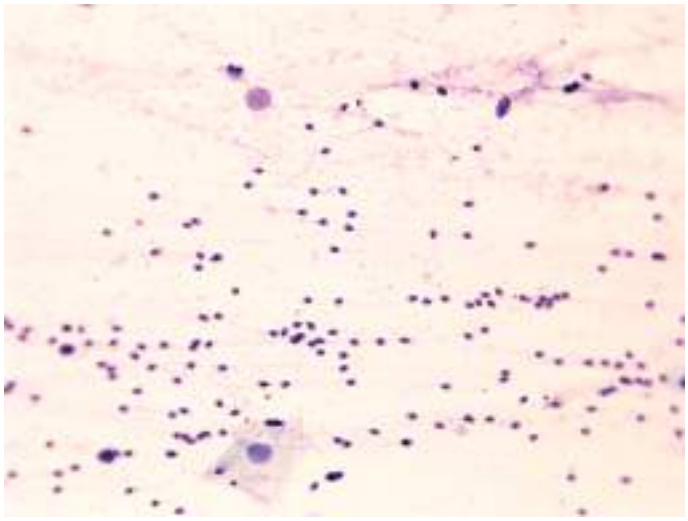
- Reniform nuclei and foamy cytoplasm are characteristic. Nuclear moulding is absent.
- Aggregated histiocytes form giant cells. **(Figure 2.7c)**



**Other inflammatory cells**

- Occasionally eosinophils and plasma cells may be present.

**Spermatozoa (Figure 2.8)**

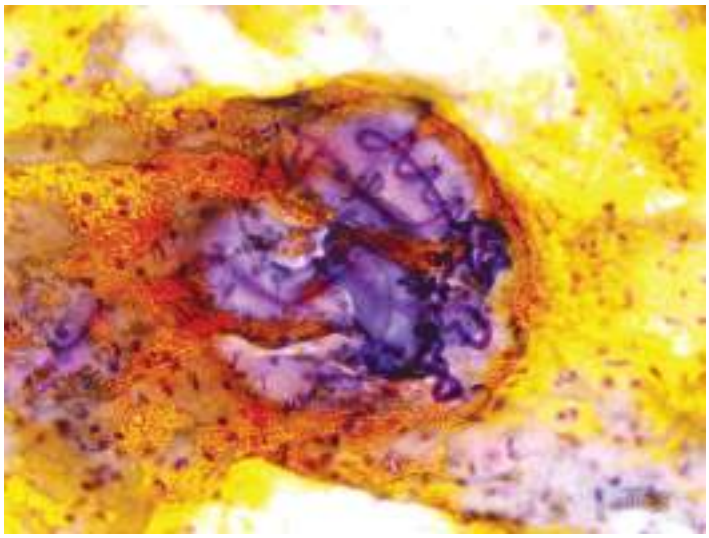


- Present in post coital smears. Basal part of the head of the spermatozoa stains more darkly.

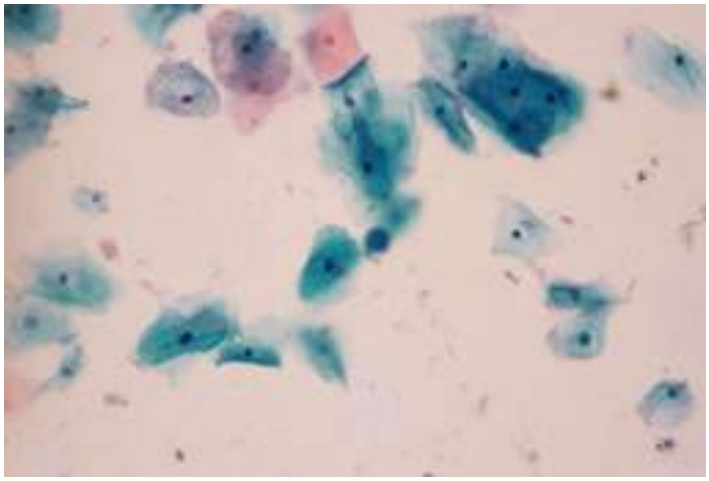
  The tails degenerate early.

**Other findings**

- Curschmann spirals **(Figure 2.9a)** are uncommon. Formed due coiled cervical mucous.

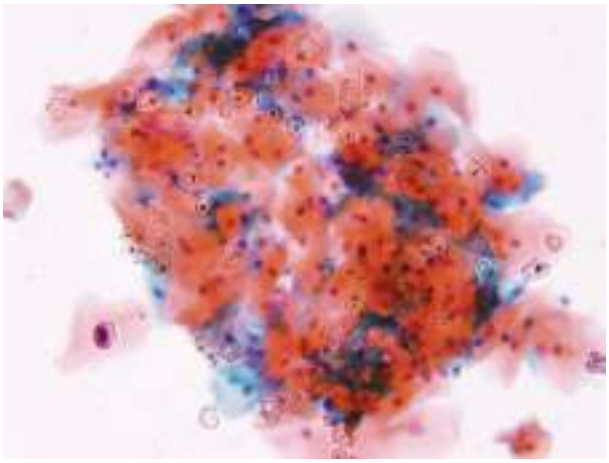


- Ferning of cervical mucous is associated with high oestrogen levels during the mid cycle.
- Worm eggs. E.g. Enterobius vermicularis eggs
- Parasites. Microfilaria of Wuchereria bancrofti
- Amoebae **(Figure 2.9b) –** Identified rarely in women wearing IUD's

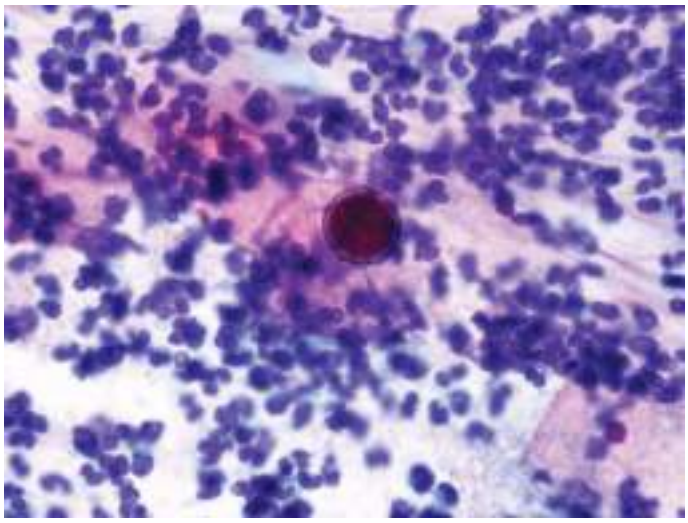


**External contaminants**

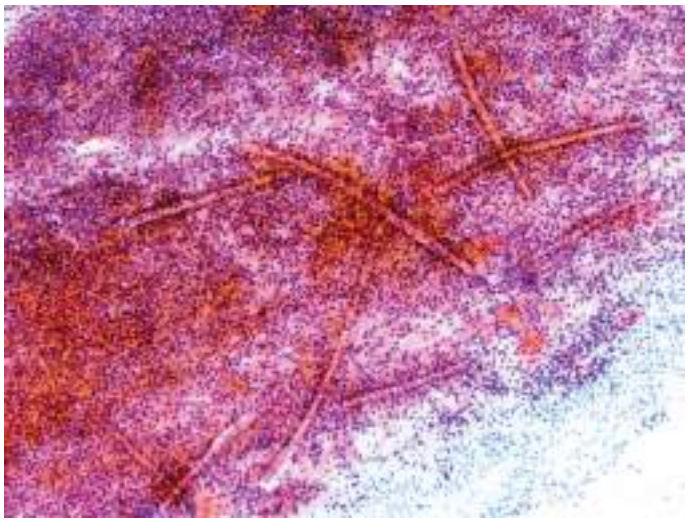
- Glove powder **(Figure 2.10)**



- Pollen grain **(Figure 2.11)**



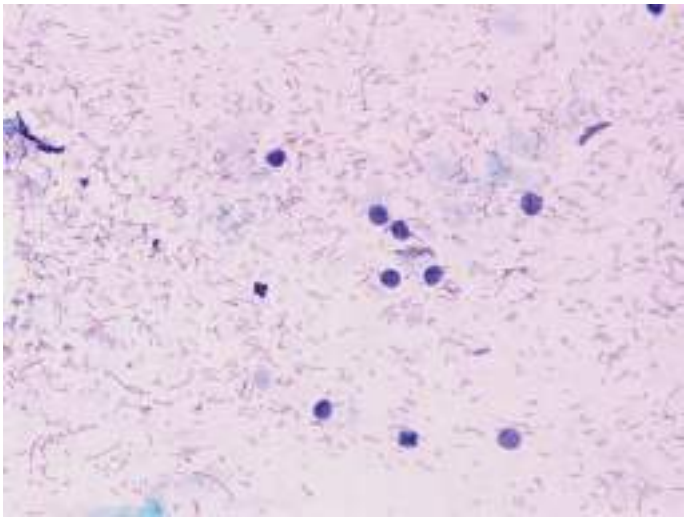
- Suture material **(Figure 2.12)**



**Normal flora**

- Normal flora consists of numerous aerobic and anaerobic bacteria.
- Common organisms are Lactobacillus and Staphylococcus epidermidis.
- Lactobacilli cause cytolysis of intermediate squamous cells during pregnancy and during the secretary phase of the cycle when the progesterone levels are high.
- Cytolysis is characterised by bare intermediate cell nuclei in a back ground of lactobacilli.

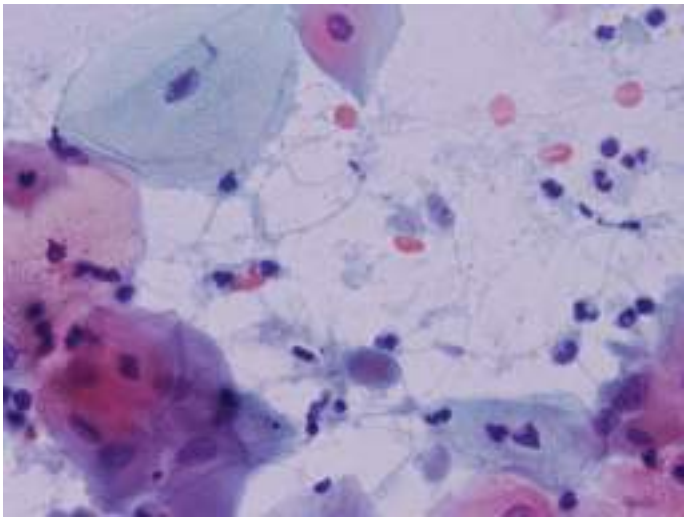
**(Figure 1.4)**



**Leptothrix**

- Non-pathogenic thread like filamentous bacteria are present in loops or pairs.
- Clinically unimportant.
- Look around for trichomonas when leptothrix is present, as they like each other's company

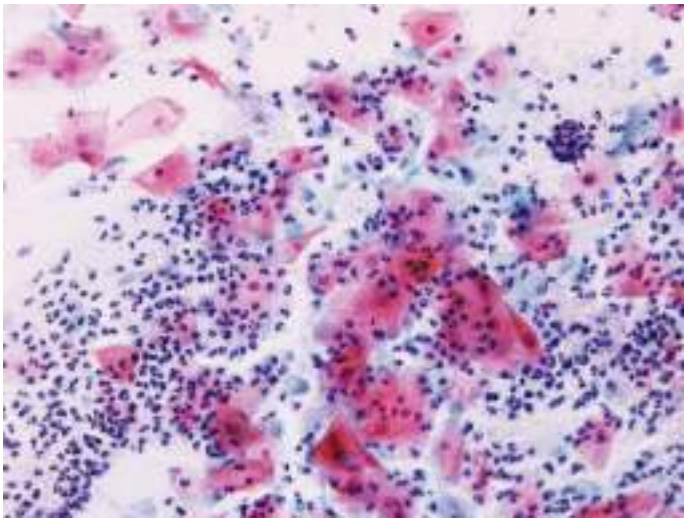
**(Figure 2.13)** Trichomonas however may also be found alone.



# Chapter 3 – Infections

**Changes in the smear pattern**

- Heavy inflammation **(Figure 3.1)** and debris

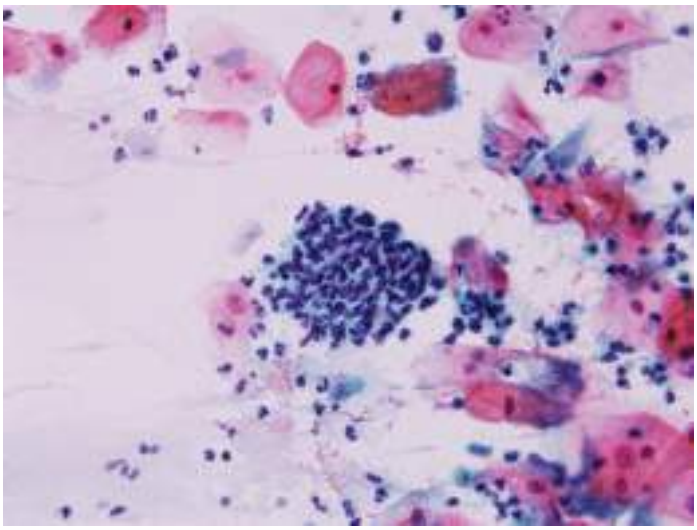


- Excess eosinophilic staining reaction
- Increased maturation pattern in postmenopausal women
- Decreased maturation pattern in young women

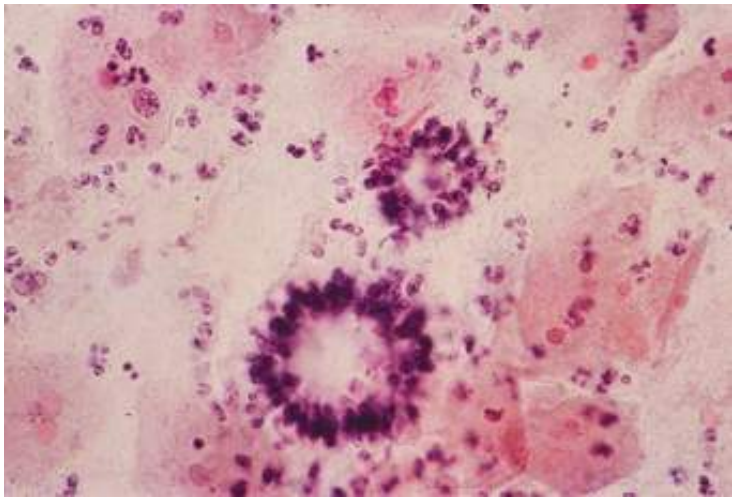
**Note:** When then the background has sheets of neutrophils, look carefully for infectious agents.

**Clues to look for**

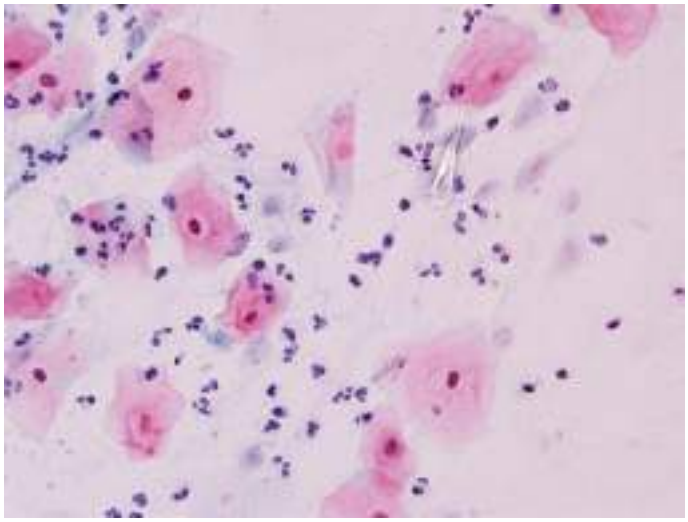
- Poly balls / Cannon balls **(Figure 3.2a)**



These are squamous cells covered with neutrophils. Poly doughnuts **(Figure 3.2b)** are squamous cells with neutrophils attached to the peripheral border. Look around for trichomonas and candida when these are detected.



- Perinuclear clearing of squamous cells **(Figure 3.3)**



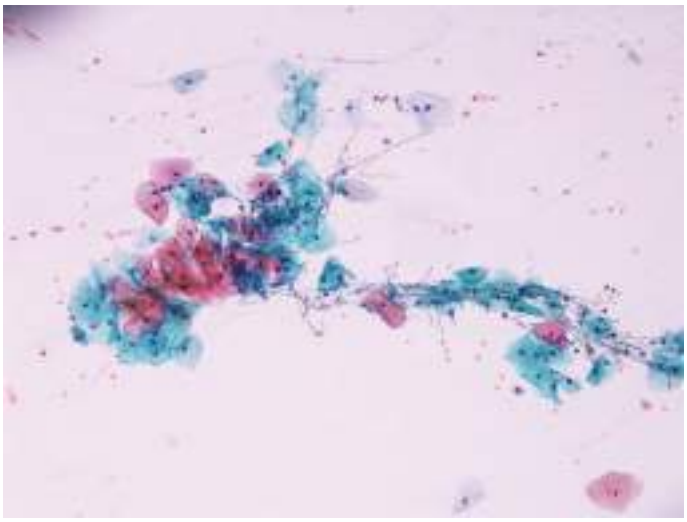
Perinuclear clearing may be seen with trichomonas (Trich halos) and candida infections. In koilocytic change the halos are larger, better defined with condensed cytoplasm at the periphery, with associated nuclear changes.

## Specific infections

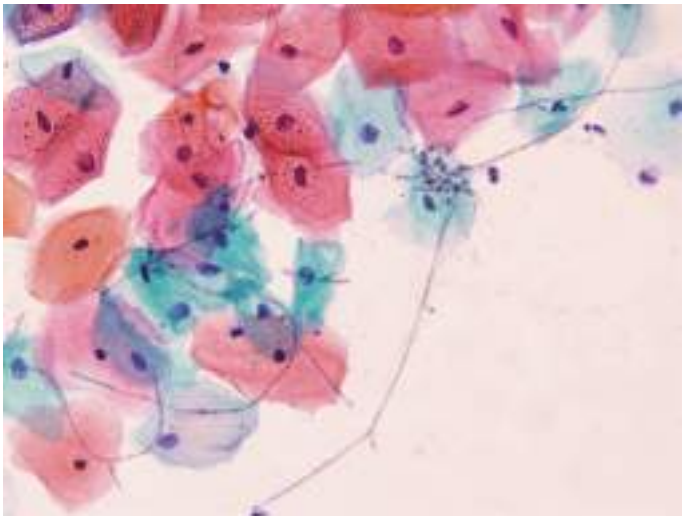
### *Candida albicans* infection

Predisposing factors include pregnancy, diabetes mellitus, and treatment with broad-spectrum antibiotics, immune suppression and debilitating diseases.

- **(Figure 3.4a)** – Squamous cells are attached to candida hyphae giving rise to a linear flower garland /chicken kebab) appearance.



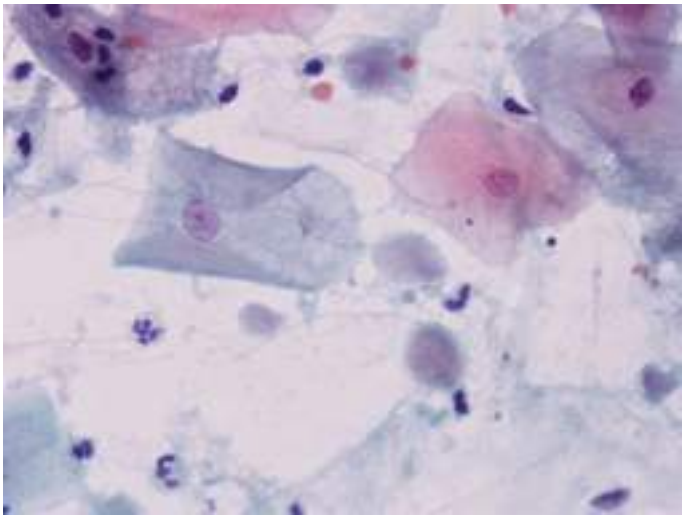
- **(Figure 3.4b)** - Pseudo-hyphae (appear septate) and yeast forms of candida, stain red with the Papanicolaou stain.



***Trichomonas vaginalis* infection**

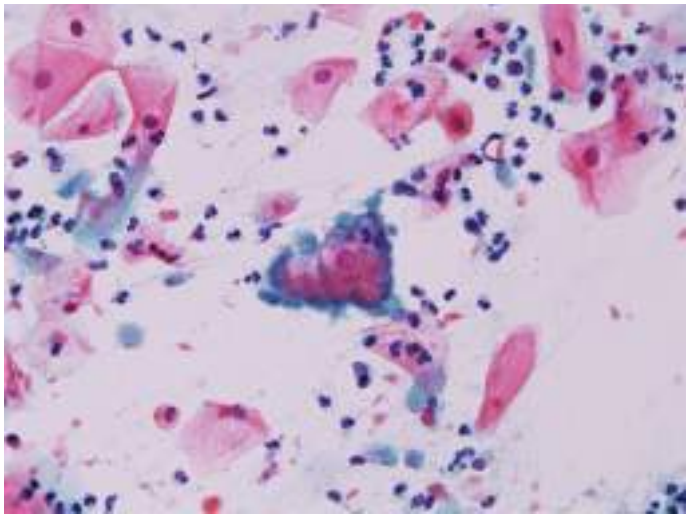
This is a sexually transmitted unicellular protozoan. Infestation can be asymptomatic with no inflammatory response. Symptomatic infection is associated with inflammation.

- Oval to pear-shaped organisms varying in size from 8 to 40 µm. **(Figure 3.5a)**

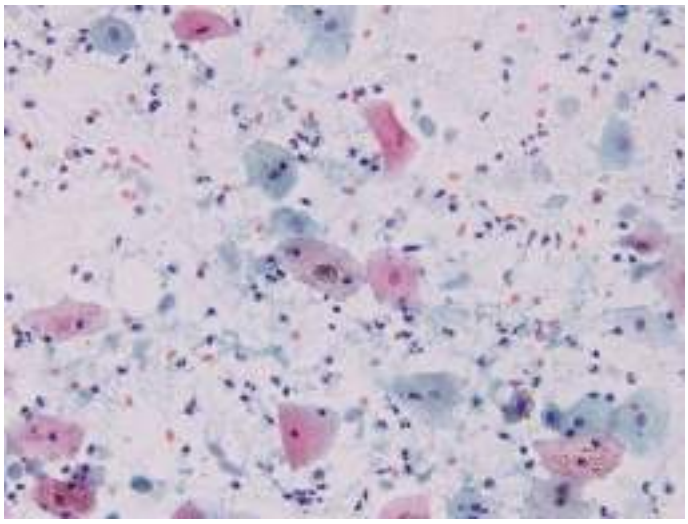


- Cytoplasm stains green to grey-green with central reddish granules.
- It is essential to see oval to crescent shaped, eccentric and hypochromatic nuclei to distinguish from non specific cytoplasmic fragments.
- The flagella are not usually seen in conventional smears.
- In heavy infections, trichomonas may be found attached to the surface of squamous cells.

**(Figure 3.5b)**



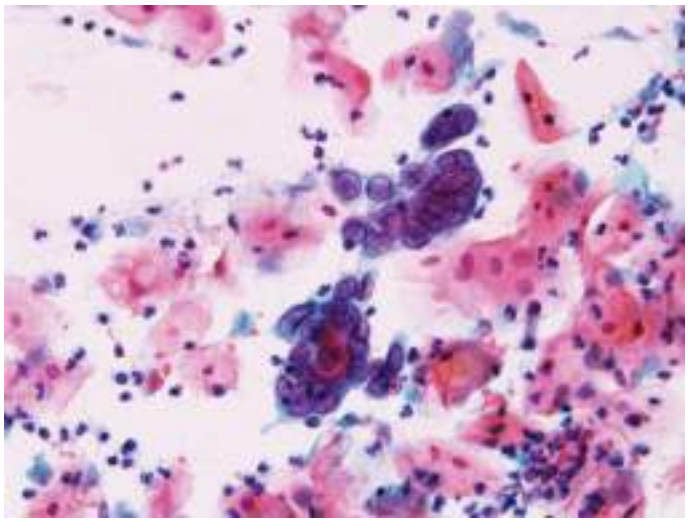
- The background may be dirty (benign diathesis) with inflammation and leptothrix **(Figure 3.5c).** Other features include poly balls, overall eosinophilia of squamous cells with perinuclear clearing (trich halos) and anaerobic flora. Lactobacilli are generally absent.



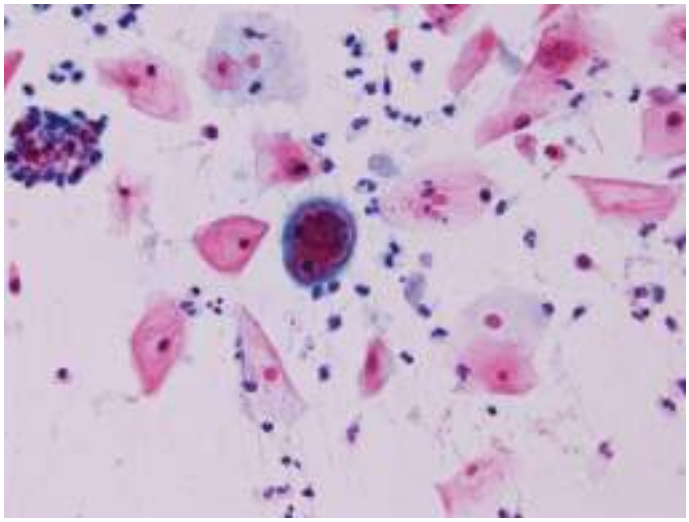
**Genital *Herpes simplex* infection**

This is a sexually transmitted infection caused by either type 1 (HSV-1) or mostly by type 2 (HSV-2) Herpes simplex virus. It infects both squamous and endocervical cells. May be asymptomatic or may present with painful and recurrent blisters.

- Infected cells show viral cytopathic effects with cytomegaly, multi nucleation, nuclear moulding, a thick nuclear membrane, ground-glass chromatin and a thickened cytoplasm. **(Figure 3.6a)**

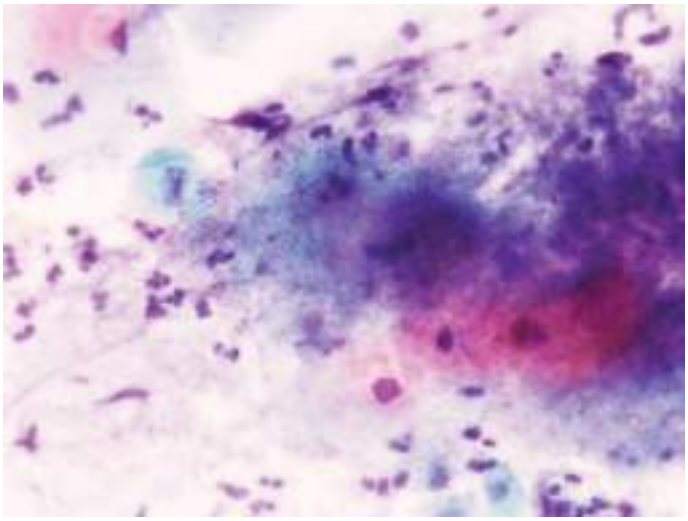


- Large eosinophilic, less commonly basophilic inclusion bodies (Cowdrey body inclusions) surrounded by a clear halo may be seen. **(Figure 3.6b)**

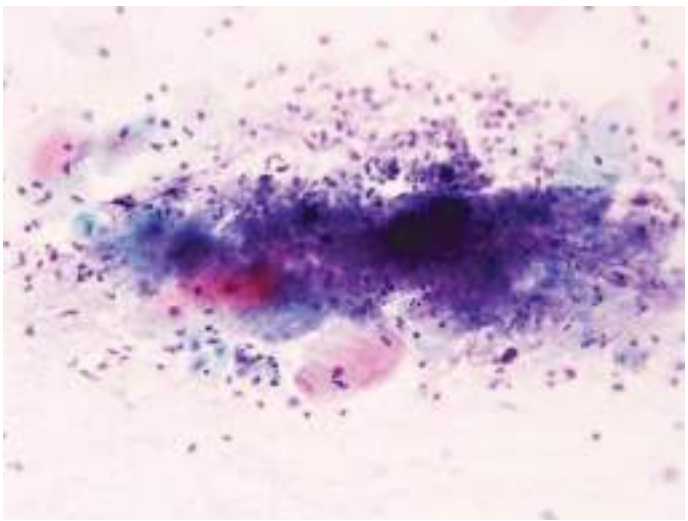


***Actinomyces* infection**

Actinomyces are Gram positive bacilli found mostly in women who have an IUD in-situ. They may be symptomatic or asymptomatic.



- Branching filaments of Actinomyces staining red, is covered with colonies of blue powdery bacteria. **(Figure 3.7a)** They appear as bluish wispy clusters of cotton wool under low power. **(Figure 3.7b)**



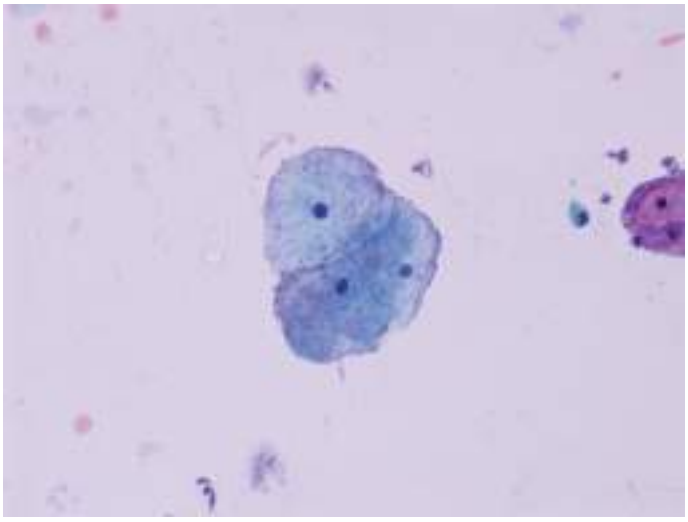
- The background shows an inflammatory response.

**Differential diagnosis -**
Anaerobic bacteria colonies appear similar; however lack branching filaments of Actinomyces.

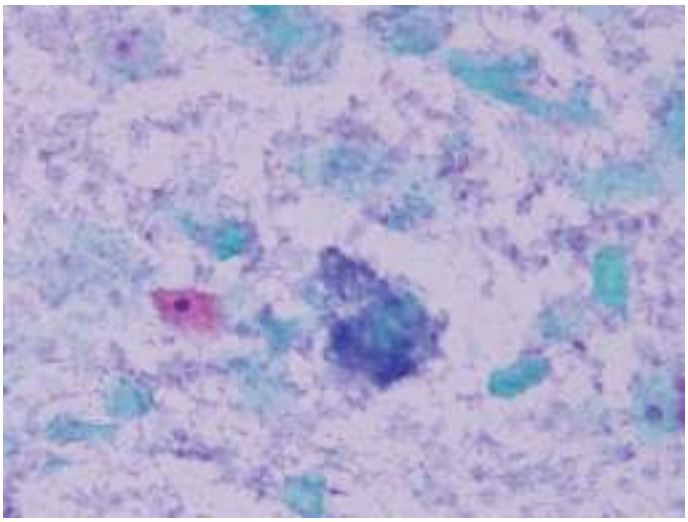
**Bacterial vaginosis / coccobacillary infections**

Bacterial Vaginosis consists of a combination of coccobacilli including Gardnerella vaginalis, Bacteroides species etc. Infection is usually not associated with complications. It may pose an increased risk of developing pelvic inflammatory disease following surgical procedures and increases the susceptibility to other sexually transmitted infections.

- Clue cells are mature squamous cells coated with powdery material comprising cocco-bacillary organisms. **(Figure 3.8a)**



- A haze of coccobacilli forms a fine powdery background. The background is usually non-inflammatory. Lactobacilli are absent. **(Figure 3.8b)**



# Chapter 4 - Hormonal patterns

## Oestrogenic pattern

This is seen immediately prior to ovulation (proliferative phase), with oestrogen therapy and hyper oestrogenic states such as obesity or polycystic ovarian syndrome, rarely with hormonally active ovarian tumours and medications including Tamoxifen and digitalis.

Oestrogen influences full maturation of squamous cells in to superficial cells.

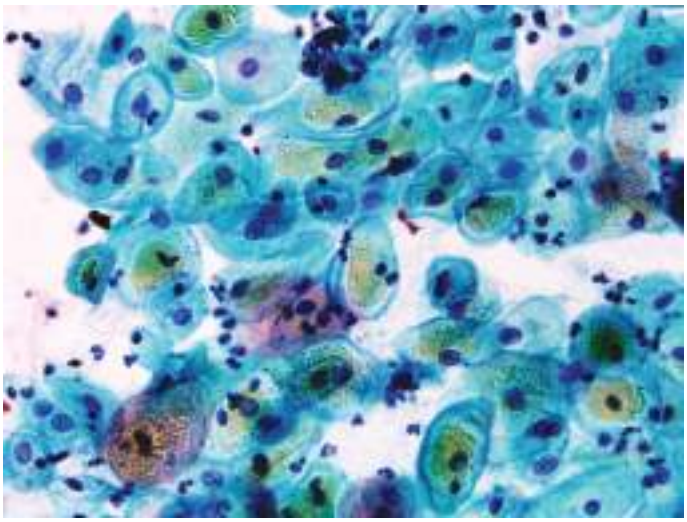
- Smears contain mainly large, flat, dissociated superficial squamous cells in a clean back ground free of neutrophils. **(Figure 4.1)**

## Non oestrogenic pattern

Non oestrogenic pattern is seen during the secretary phase of the cycle, menarche, early puberty, pregnancy and in the premenopausal age group. This pattern is also associated with progesterone therapy.

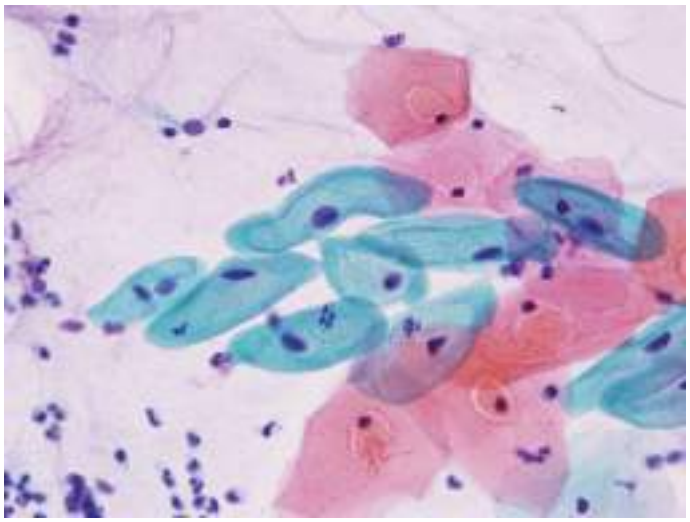
Full maturation of the squamous cells is inhibited under progesterone influence, resulting in mainly intermediate cells.

- Smears comprise mainly compact clusters of intermediate cells with cytoplasmic glycogen and curled edges. **(Figure 2.2b)**



- Lactobacilli appear with cytolysis on further exposure to progesterone.

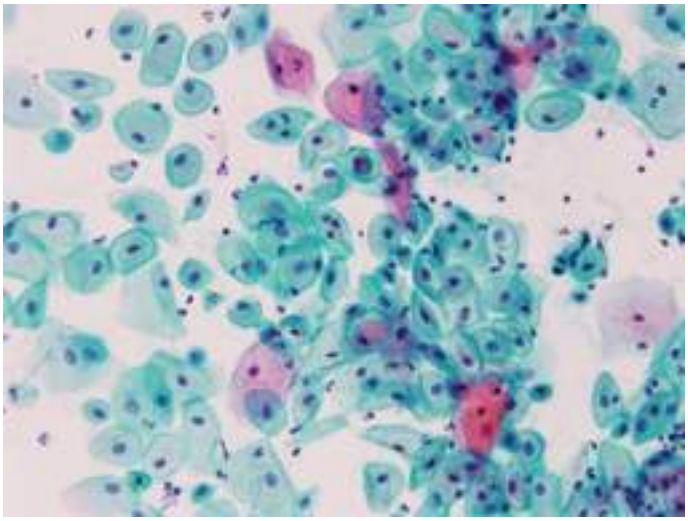
- Navicular cells are seen in pregnancy. These are boat shaped intermediate cells, distended with yellowish glycogen, with the nucleus pushed to the periphery. The thickened rim of cytoplasm folds at the edges. **(Figure 4.2)**



**Atrophic pattern**

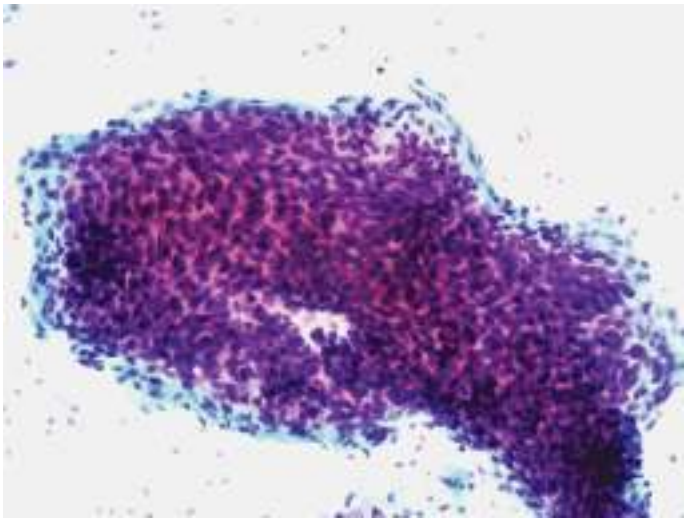
Atrophic pattern is seen during the postpartum period, lactation, in oestrogen deficiency states (such as Turner syndrome, hypopituitarism) and in radiation induced artificial menopause and post menopause.

- The smears contain mainly parabasal cells. **(Figure 4.3a)**

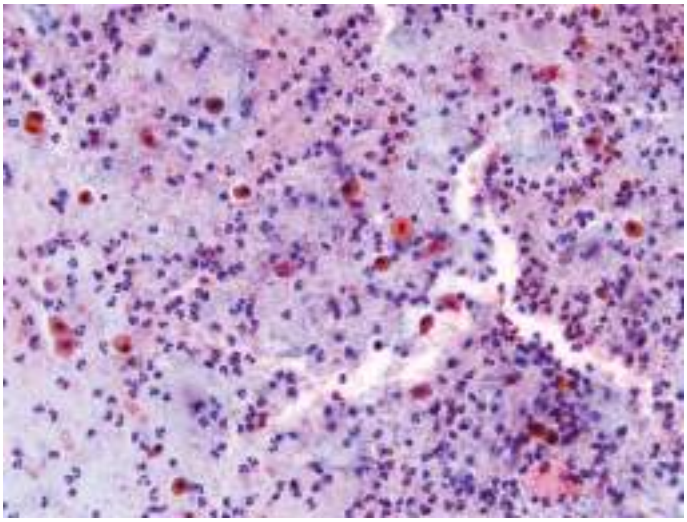


- Postmenopausal atrophy may show atrophic hypercellular cell groups shed in sheets.

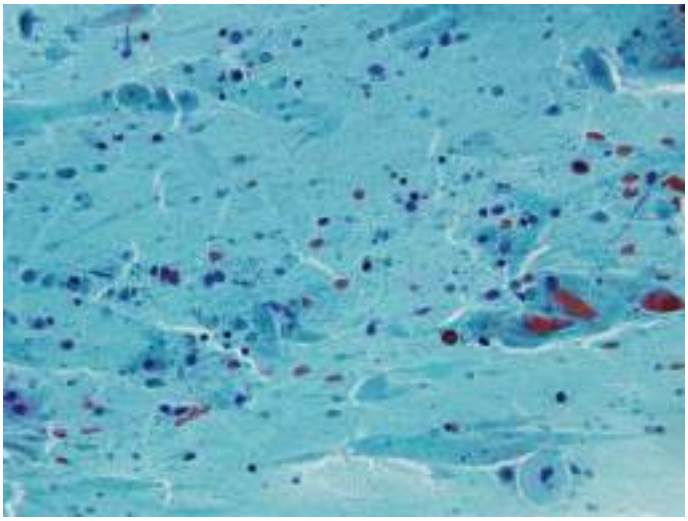
**Note** that there are no atypical nuclear features. **(Figure 4.3b)**

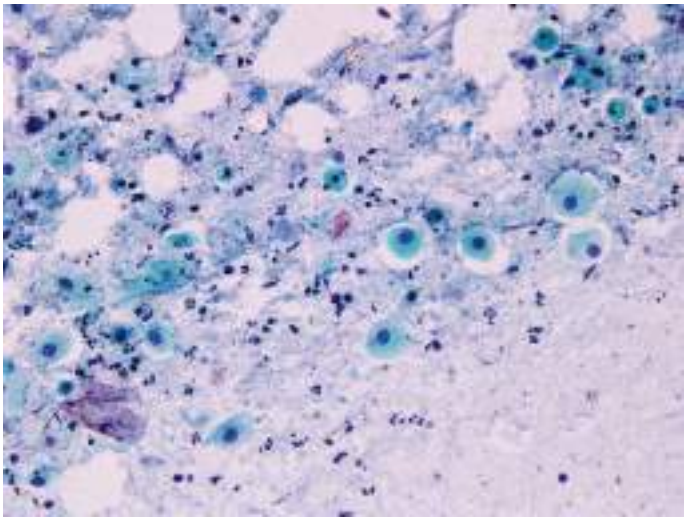


- Red atrophy is characterized by dissociated parabasal cells with pyknotic nuclei and a reddish orange cytoplasm **(Figure 4.3c)**

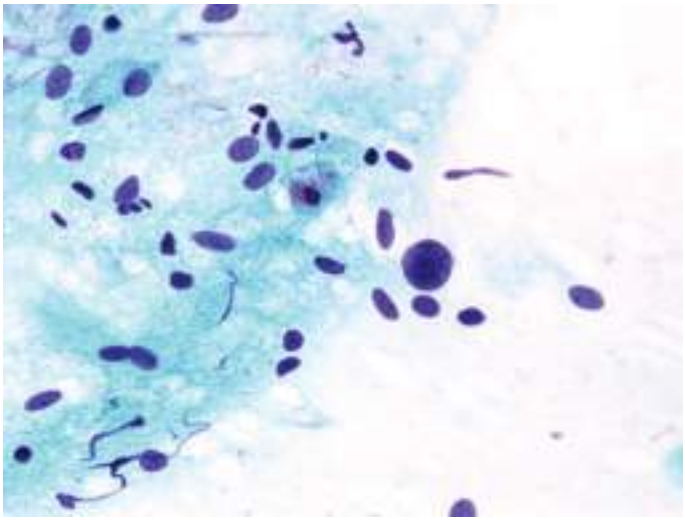


- The background may have a granular appearance **(Figure 4.3d,e)**. It is thought that the disintegration of blue blobs is responsible for this.

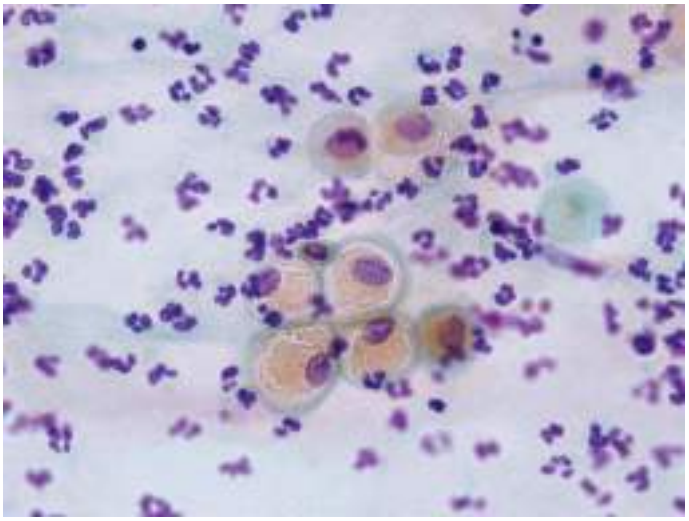




- Blue blobs are the same size as parabasal cells and differ from dyskaryotic nuclei by lack of discernible chromatin pattern. **(Figure 4.3f)**



- Glycogenated parabasal cells are seen in androgenic atrophy due to excess of either exogenous or endogenous androgens in postpartum/lactational smears. **(Figure 4.3g)**



**Note:**

- Physiological cytohormonal patterns are best evaluated in smears obtained from the upper third of the lateral vaginal wall, as these smears are devoid of glandular and metaplastic cells, anucleate squames and inflammatory changes.
- The classical changes expected may not be seen during post partum and lactation period and in menopause due to residual effects of oestrogen.

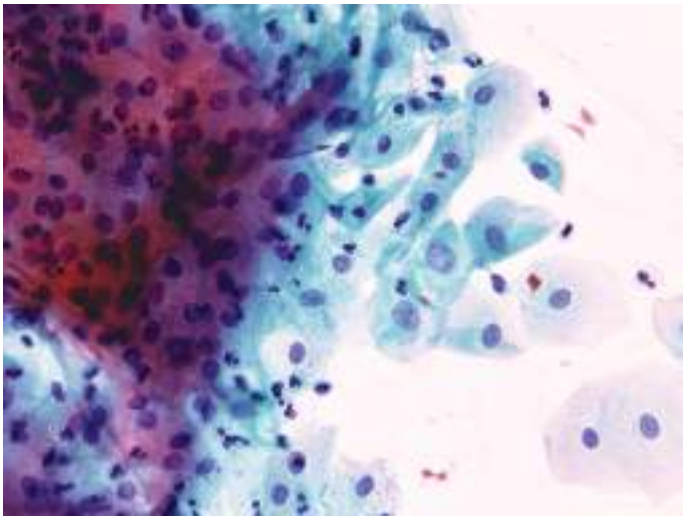
# Chapter 5 - Benign cellular changes:

**Reactive changes associated with inflammation**

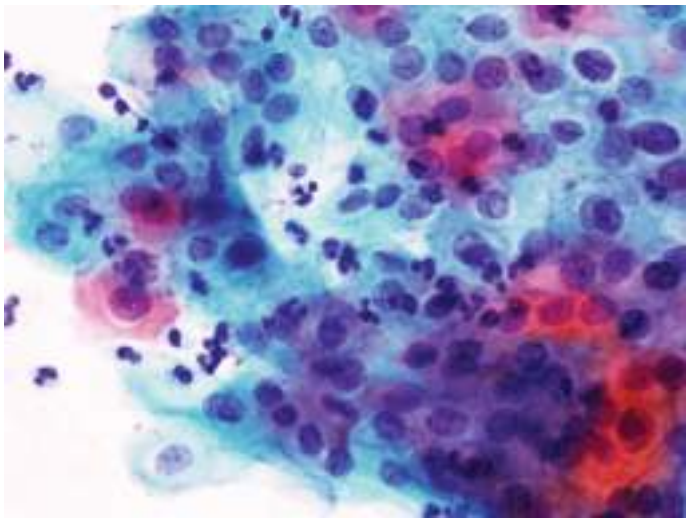
- Dense inflammation in the background (mainly neutrophils with or with out lymphocytes and plasma cells)
- Epithelial cells are densely admixed with neutrophils with some permeating the epithelial cell cytoplasm.
- Epithelial cell may show altered maturity, exhibited by changes in the staining reaction of intermediate, parabasal and metaplastic cells. (increased metaplastic cells in younger women and increased maturation pattern in older women)

**Reactive changes in squamous cells**

- Nuclear and cytoplasmic enlargement with bland nuclear hypochromasia due to absorption of water **(Figure 5.1).** Nuclear enlargement is less than twice the size of an intermediate cell nucleus. Other changes include multi nucleation, wrinkling of the nuclear membrane, chromatin degeneration, prominent and multiple nucleoli.

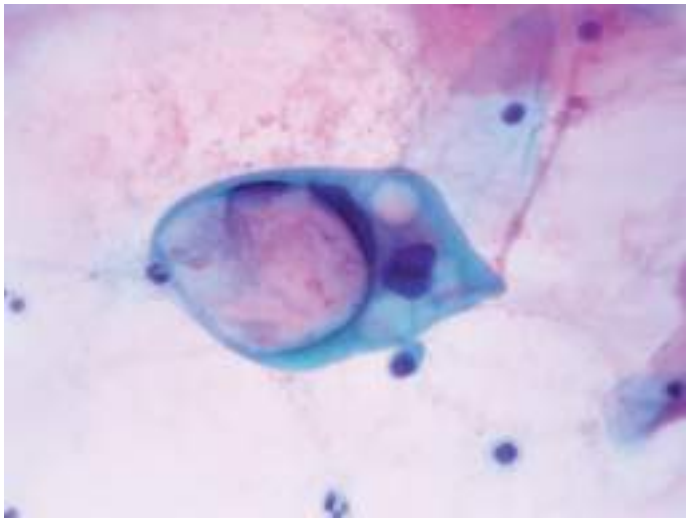


- Reactive changes may closely mimic squamous intraepithelial lesions (SIL)/dys-karyosis when the nuclei are larger and are more hyperchromatic. **(Figure 5.2).**

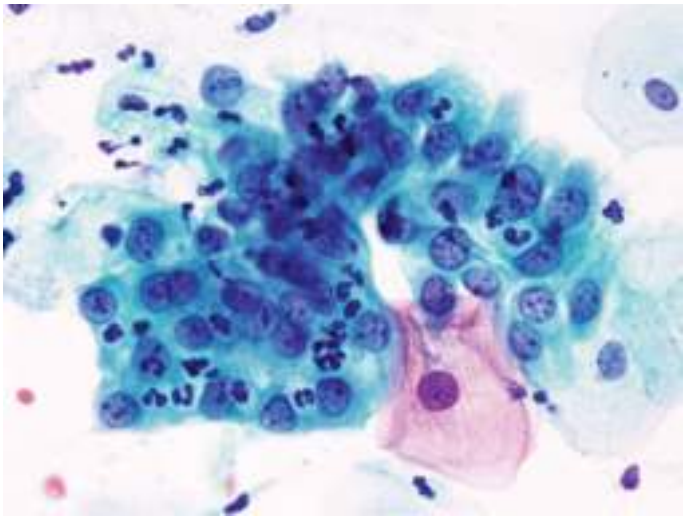


**Differential diagnosis –** Nuclei of SIL/dyskaryosis are irregularly arranged and are larger, more pleomorphic and hyperchromatic with irregular borders and abnormal chromatin.

- Cytoplasmic changes of reactive cells include vacuolations, perinuclear halos, **(Figure 5.3)** altered/two tone staining and abnormal keratinisation
  E.g. parakeratosis.

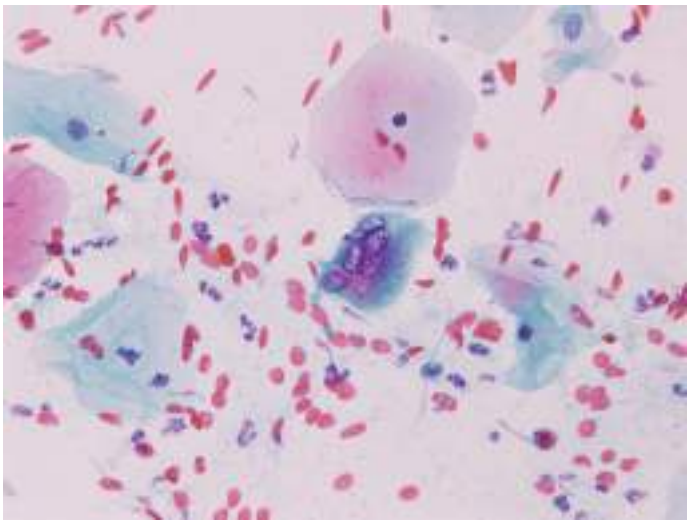


**Reactive changes in endocervical cells (Figure 5.4)**



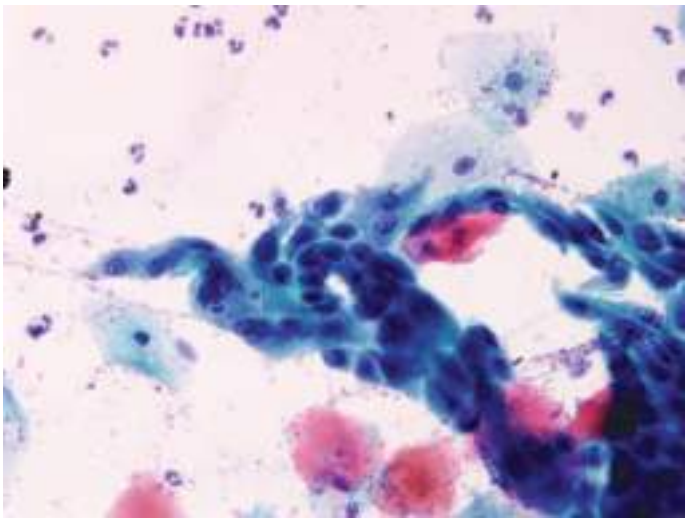
- The nuclei vary in size maintaining its shape.
- Nucleoli may be present with minimally coarse chromatin. Cytoplasm has mucous vacuoles with entrapped neutrophils.

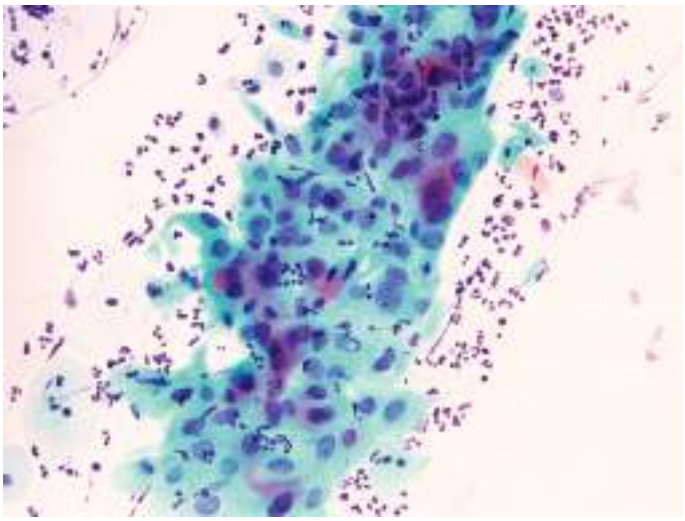
- Occasional multi nucleation. **(Figure 2.5e)**



**Repair / regeneration**

Seen in endocervical **(Figure 5.5)**, and squamous metaplastic cells **(Figure 5.6)** during healing following surgical procedures, radiation therapy etc.



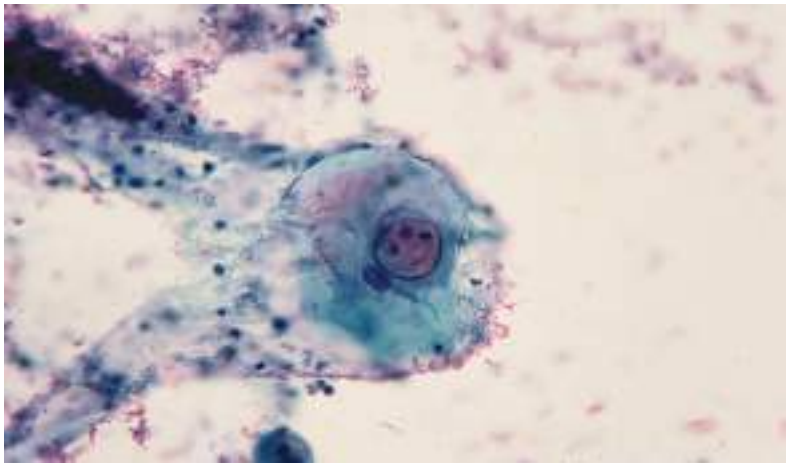


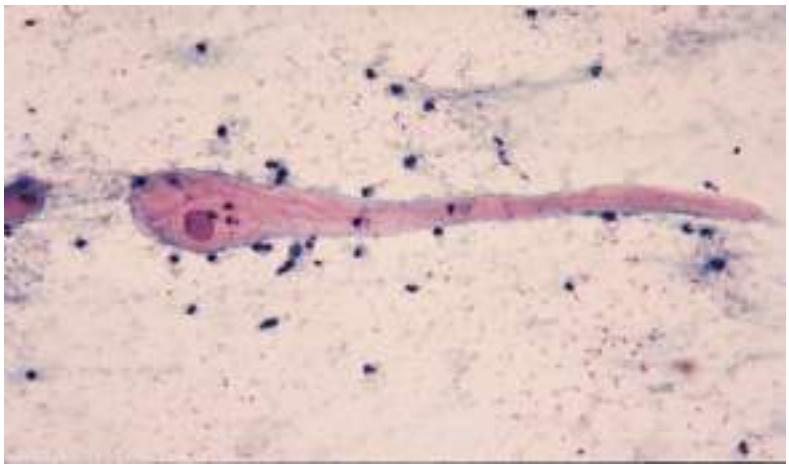
- Orderly monolayered cohesive sheets of cells. There may be a streaming pattern with retained polarity.
- Nuclei are usually enlarged, hypo/hyperchromatic, uniform or pleomorphic with prominent nucleoli.
- Vesicular and finely granular evenly distributed chromatin.
- Scattered mitotic figures.
- Admixed with neutrophils.
- May be multinucleated.
- Fibroblasts may be seen.
- Additionally lack of tumour diathesis differentiates repair from glandular carcinoma.

**Radiation/chemotherapy changes**

Cytological effects of radiation may persist for many years. It is important to distinguish this from a recurrent malignancy. The chemotherapy affects are essentially similar to radiation changes. Thus an adequate clinical history is essential!

- Cytomegaly and nucleomegaly, marked pleomorphism with bizarre cell shapes, bi- and multi-nucleation, granular to smudged chromatin, cytoplasmic amphophilia with two tone cytoplasm (light green at the periphery with pink around the nucleus), Cytoplasmic vacuolisation +/- ingested neutrophils. **(Figure 5.7a,b)**

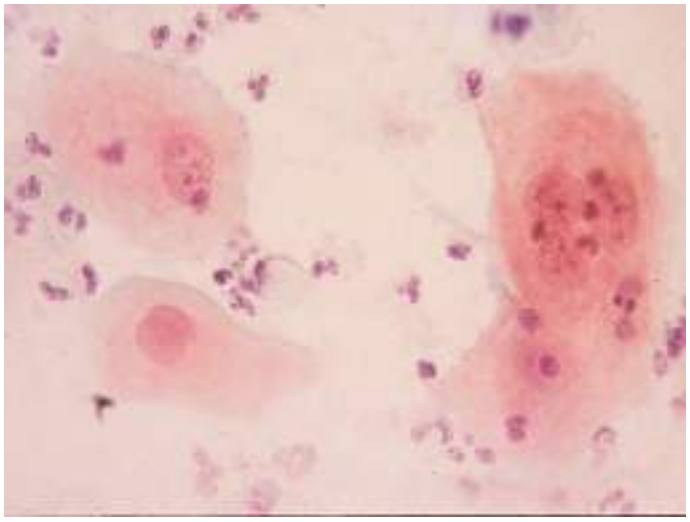




- Other changes include inflammation, multi-nucleated macrophages and repair/ regeneration changes.

**Note:** It is very important to appreciate the unaltered nuclear /cytoplasmic ratio.

**Vitamin deficiency states (Figure 5.7c)**

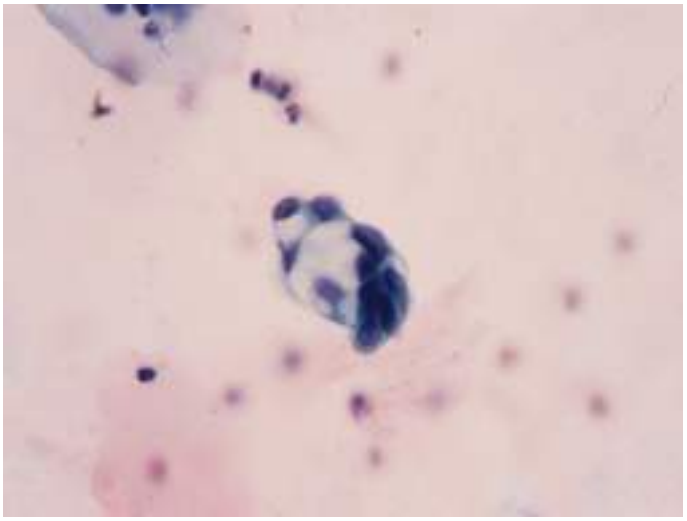


Similar changes to those observed with radiation changes occur with Vitamin B12 and Folate deficiency and disappear with replacement therapy.

**IUD changes**

Normal endometrial cells are shed throughout the cycle in IUD wearers, together with large fragments of endocervical and metaplastic cells. The main changes are seen in endometrial cells. Endocervical and metaplastic cells may also show reactive changes.

- Endometrial cells shed singly or as small groups and may appear atypical **(Figure 5.8).**

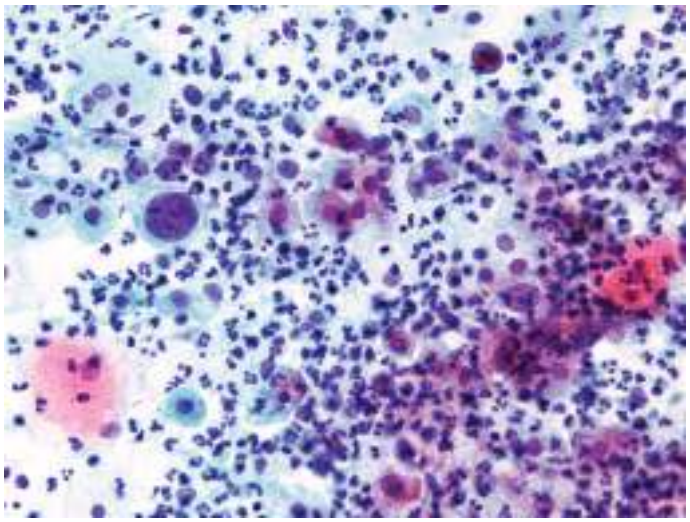


- Slight nuclear enlargement with scant vacuolated cytoplasm +/- ingested neutrophils. Within the cell group there is only slight variation nuclear size and shape.
- Single endometrial cells are small with a high N/C ratio, hyperchromasia, bland chromatin, regular nuclear outline, a small nucleolus and thickened cytoplasm.
- Actinomycotic colonies may also be present.

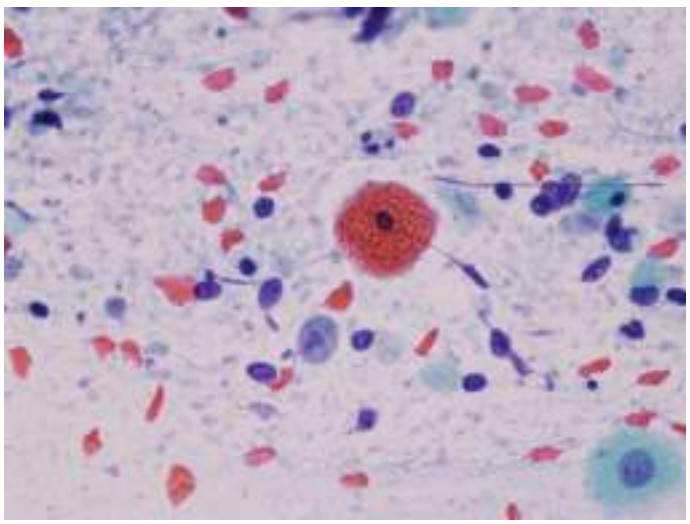
**Differential diagnosis:** Cell groups in endometrial adenocarcinoma show more variation in nuclear size and shape, chromatin clearing, nucleoli and a watery tumour diathesis in the background. Presence of nucleoli help to differentiate singly shed atypical endometrial cells from SIL/dyskaryotic cells. Most helpful in the differential diagnosis is the knowledge that an IUD is in situ.

**Atypia of atrophy (Atrophic vaginitis)**

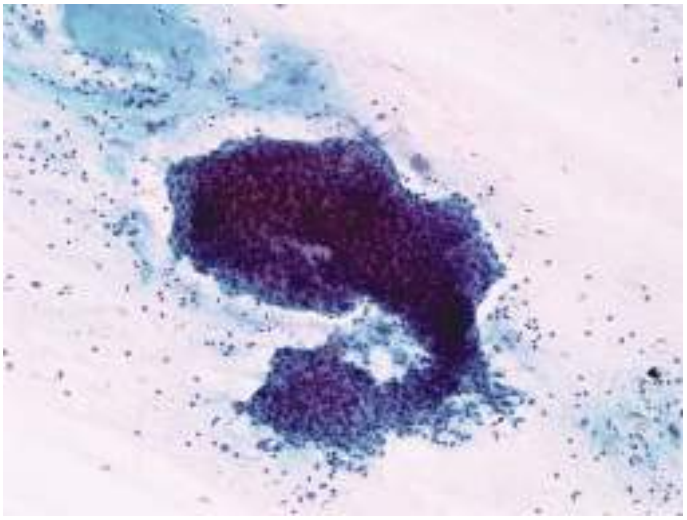
- Para basal cells with blue/gray cytoplasm appear degenerated with pleomorphic, hyperchomatic enlarged nuclei. **(Figure 5.9)**



- Cytoplasm may be orangeophilic due to degeneration. (pseudokeratinisation) **(Figure 5.10)**

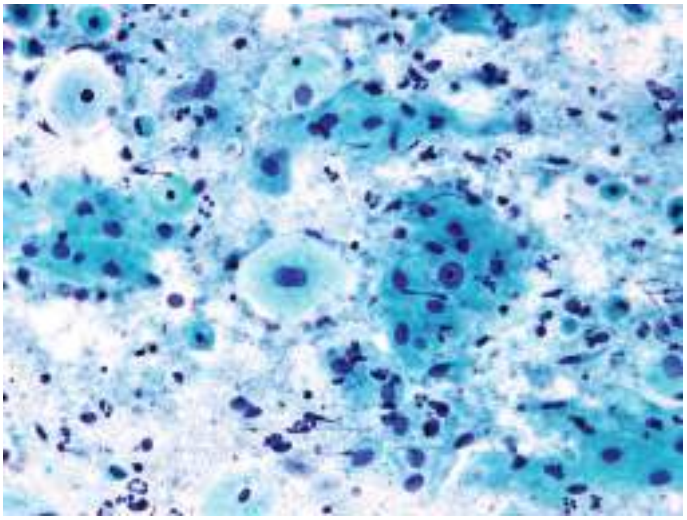


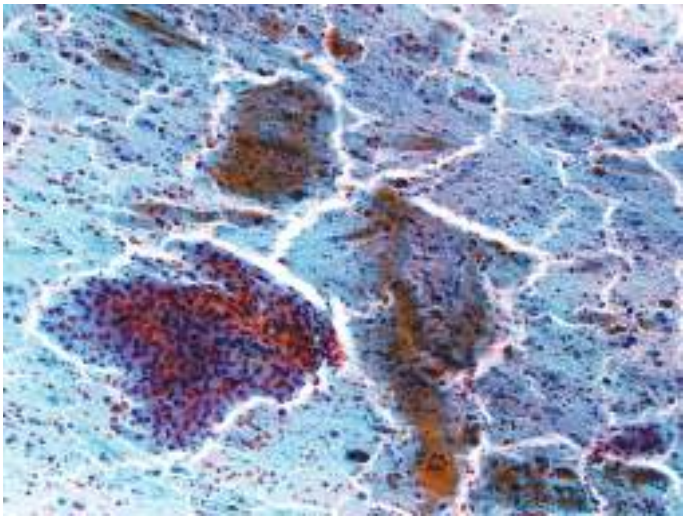
- Hyperchromatic, syncitial and crowded cell groups may be present. **(Figure 5.11)**



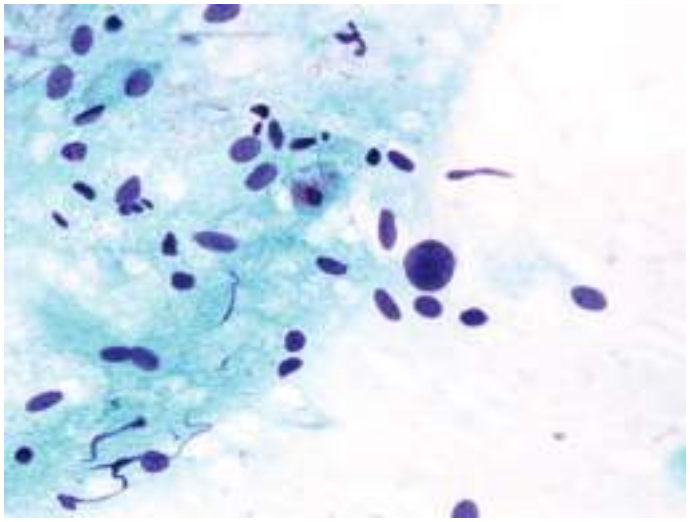
- Background may be dirty with necrosis (benign diathesis). Inflammation may be present.

  **(Figure 5.12)** It may resemble tumour diathesis when admixed with blood. **(Figure 5.13)**





- Dark blue rounded bodies (blue blobs - possibly mummified atrophic squamous cells) resembling big dark nuclei of SIL/dyskaryotic cells may be present. However these lack a discernible chromatin structure. **(Figure 4.3f)**



**Note:** Do not diagnose SIL/dyskaryosis and squamous cell carcinoma (SCC) based on naked nuclei.

**Differential diagnosis from SIL/ dyskaryosis and SCC**

- Poorly preserved, dark, smudged chromatin in atrophy contrast with distinct abnormal chromatin in HGSIL/severe dyskayosis and SCC.
- Chromatin pattern in atrophy is similar to other parabasal/intermediate cells. It is different (coarser and darker) in HGSIL/severe dyskaryosis and SCC.
- Mitotic activity suggests neoplasia when reactive/ reparative processes are excluded.

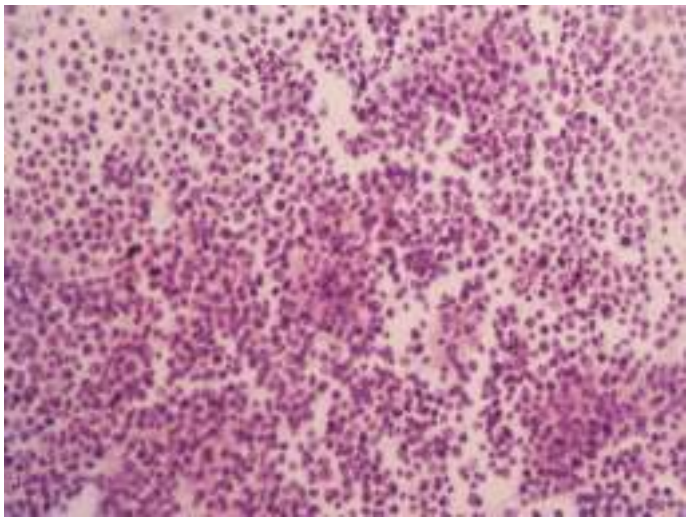
**Note:** The worrisome changes will disappear in repeat smears following local oestrogen therapy. Colposcopy should be suggested if it is uncertain whether the changes are due to SIL/dyskaryosis or SCC.

**Follicular cervicitis**

Mononuclear cells from sub mucosal lymphoid follicles may get dislodged during scraping.

- A loose cellular spread (rain drop appearance) of cells comprising a spectrum of mature and immature lymphocytes admixed with occasional plasma cells and macrophages.

- Presence of tingible body macrophages supports the diagnosis. **(Figure 5.14)**

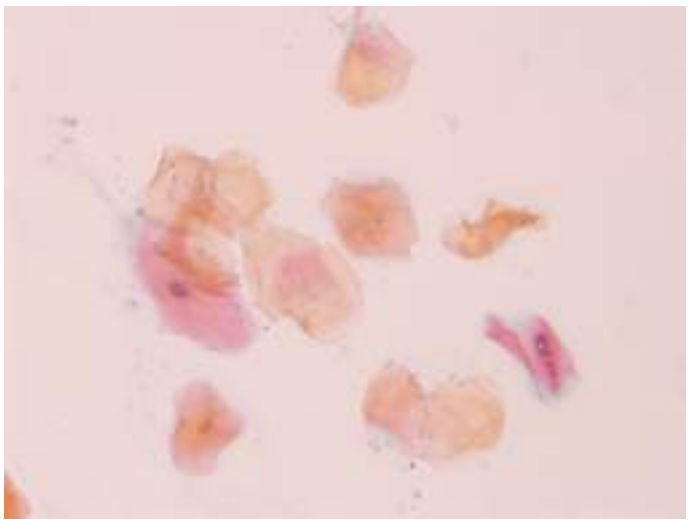


**Differential diagnosis:** The polymorphic cell composition and the localized spread of cells in the smear help to differentiate follicular cervicitis from lymphoma.

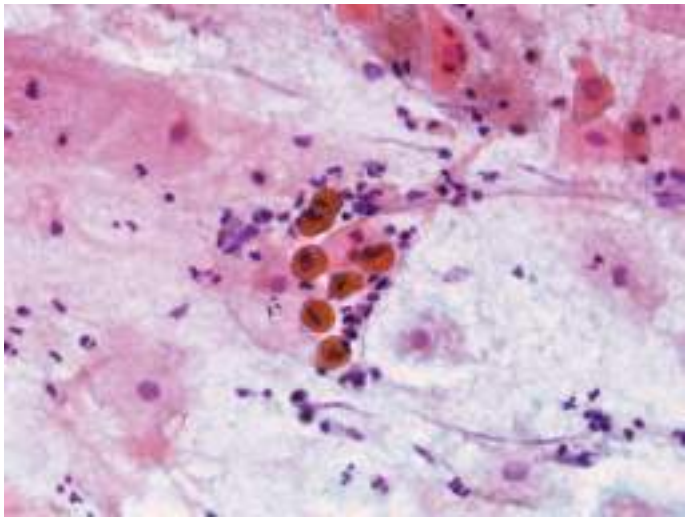
**Hyperkeratosis and parakeratosis**

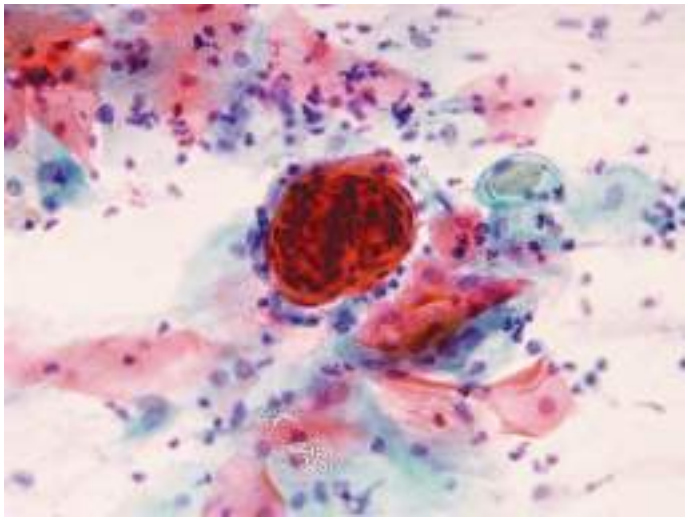
These represent abnormal maturation of the normal non-keratinised epithelium of the vagina and the ectocervix. Parakeratosis should be distinguished from dyskeratotic cells which have abnormal nuclei. Both hyperkeratosis/parakeratosis may be seen on the surface of a keratinising squamous dysplasia/carcinoma, however more frequently they are associated with chronic irritation and/or human papilloma virus.

- Hyperkeratosis - Anucleate squames occurring singly or in sheets **(Figure 2.1d)**

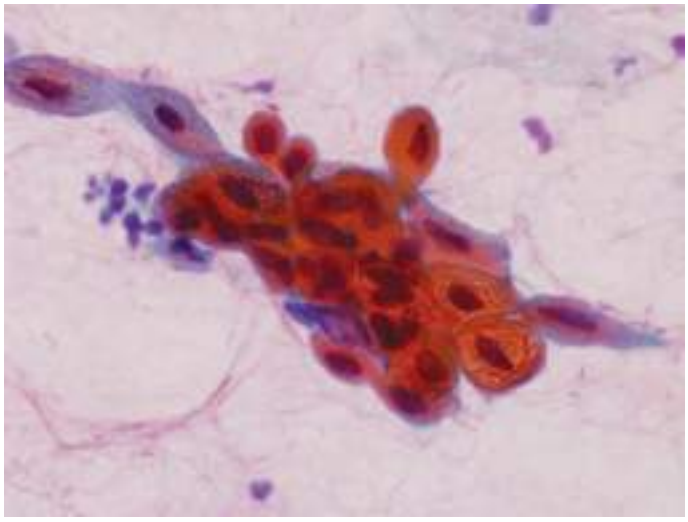


- Parakeratosis - Rounded or polygonal miniature superficial squamous cells with eosinophilic cytoplasm and dark pyknotic nuclei. They are arranged as single flat cells (Figure **5.15a)** or layered strips of cells or whorls. **(Figure 5.15b**)





**Differential diagnosis** – In dyskeratosis/atypical parakeratosis overlying more significant lesions like condylomas, SIL/dyskaryosis and SCC, the nuclei are atypical. **(Figure 5.15c)**



**Tubal metaplasia**

Recognition may be difficult.

- Arranged as flat sheets, three-dimensional clusters, small poorly cohesive groups and single cells that are smaller than endocervical cells. The nuclei are larger and more hyperchromatic. Nuclear spacing is regular but may show overlapping in three-dimensional groups and lack characteristic features of glandular neoplasms.
- The most helpful diagnostic feature is the presence of columnar cells with terminal bars and cilia.

# Chapter 6 - Abnormal squamous cells in cervical smears

Abnormal squamous cells are termed **squamous intraepithelial lesions (SIL)** in the Bethesda classification and **dyskaryosis** in the classification of British Society of Clinical Cytologists (BSCC).

**Squamous intraepithelial lesions (SIL)/Dyskaryosis**

**Some general features**

- Clean background with no tumor diathesis.
- Usually only few abnormal cells are present.
- Cells are isolated or arranged in a sheet.

**Cytomorphological features – Nuclear cytoplasmic ratio**

- Nuclear cytoplasmic ratio (N/C ratio) is increased.
- Low-grade lesions have a low N/C ratio. High-grade lesions are more undifferentiated with a high N/C ratio. The N/C ratio again drops in cells of squamous cell carcinoma.

**Cytomorphological features - Nucleus**

- Nuclei are round to oval in shape, not bizarre in low-grade lesions.
- Nuclear membrane is irregular, commonly in high-grade lesions.
- Occasional bi- or multi nucleation is seen in low-grade lesions.
- Hyperchromatic, finely granular and evenly distributed chromatin, becoming coarser as the lesion becomes more severe towards the high end of the spectrum (Vesicular chromatin, finely granular chromatin, coarsely granular chromatin – Diagram 1).
- Variability of nuclear features in size, shape, and chromatin pattern.
- Nucleoli are absent.

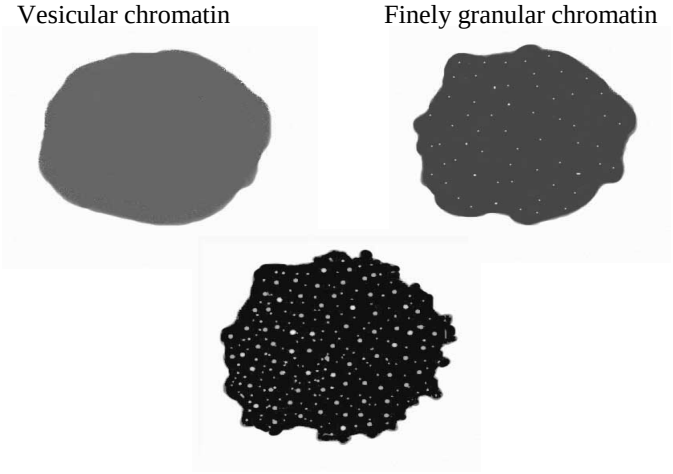



Diagram 1

**Note:**

- The gradually worsening irregularity of nuclear margin is associated with worsening chromatin abnormality.
- Changes in the N/C ratio are accompanied by other nuclear changes. Thus a cell with a high N/C ratio with a normally stained nucleus, vesicular chromatin and a smooth nuclear membrane is unlikely to be abnormal. Consider the possibility of immature metaplasia in this situation.

**Cytomorphological features – cytoplasm**

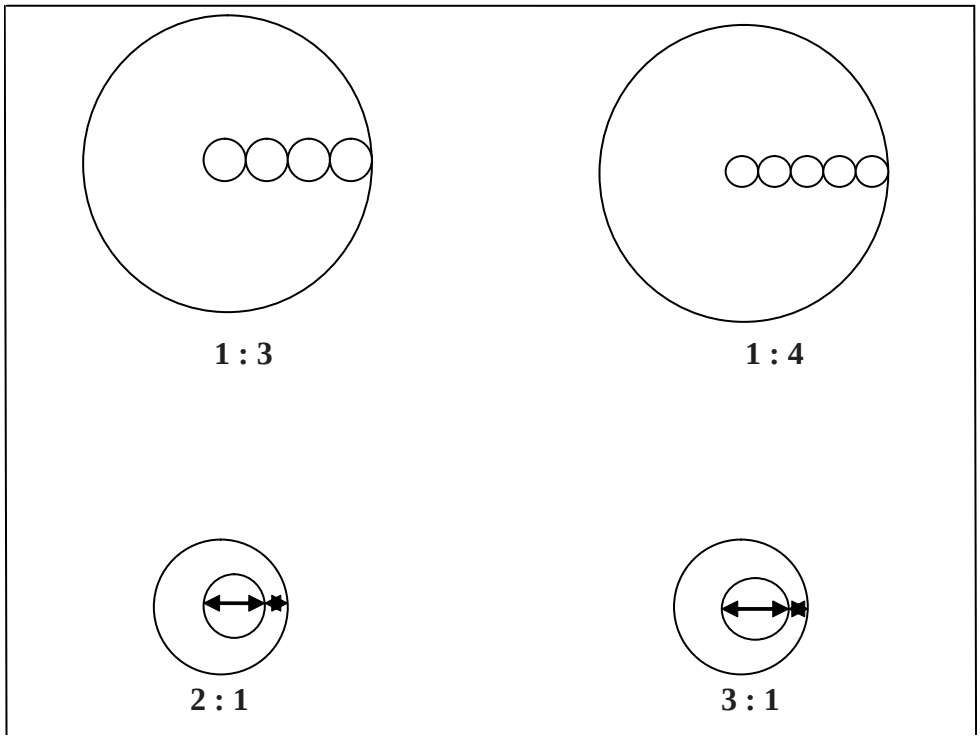
- Some degree of maturation is seen in all cells.
- Size varies from that of superficial cells in low-grade lesions to the size of para basal cells in high-grade lesions.
- Cell shape varies from polygonal to round to oval to spindle or elongated.
- Cell borders are well defined.
- Staining reaction varies from cyanophilic (blue/green) to eosinophilic (pink) to orangophilic (orange).

**Categorizing the abnormality**

- The worst cells present in the smear are taken in to account, irrespective of the number and the predominant cell type.
- Abnormal cells are categorized based on the N/C ratio and nuclear features (nuclear membrane, and chromatin pattern) in to low grade squamous intraepithelial lesions (LGSIL) or high grade squamous intraepithelial lesions (HGSIL) in the Bethesda classification, and in to mild, moderate and severe dyskaryosis in the BSCC classification.
- Nuclear features takes precedence over any degree of cytoplasmic maturation.
- Higher the nuclear cytoplasmic ratio more severe is the lesion.
- LGSIL/mild dyskaryosis is equivalent to cervical intraepithelial neoplasia 1 (CIN 1) on histology, with very mature superficial like cells with a N/C ratio of 1:3 or 1:4. (Diagram 2)
- HGSIL/severe dyskaryosis is equivalent to CIN 3/Carcinoma in situ on histology with very immature parabasal like cells with a N/C ratio of 2:1, 3:1 or greater (Diagram 2)
- HGSIL/moderate dyskaryosis is equivalent to CIN 2 on histology with cells that have morphology in between.

**Note:** Both moderate and severely dyskaryotic cells are included under HGSIL in the Bethesda classification.

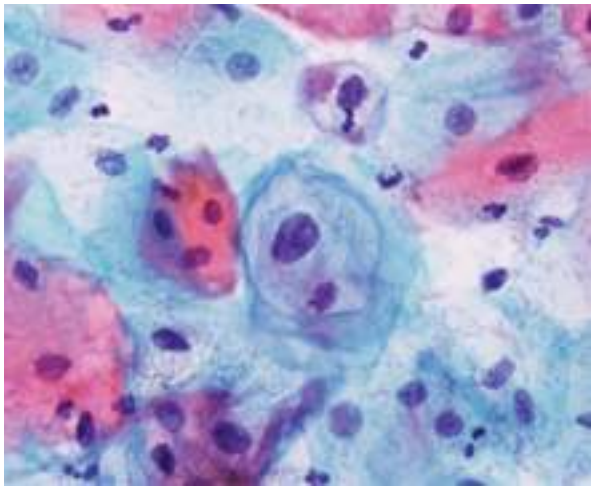
**Diagram 2 – Nuclear cytoplasmic ratio**

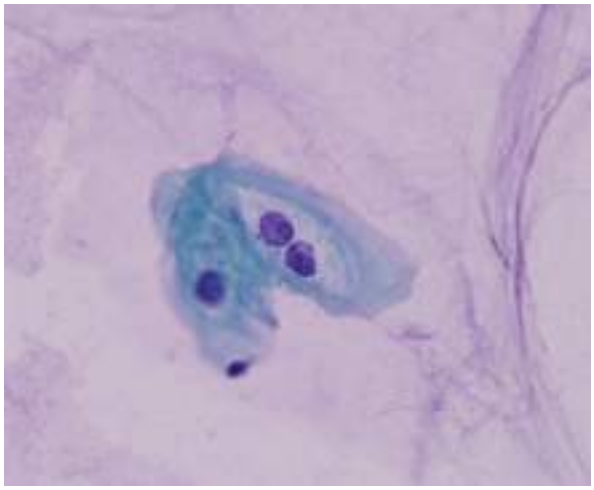



**Note:**

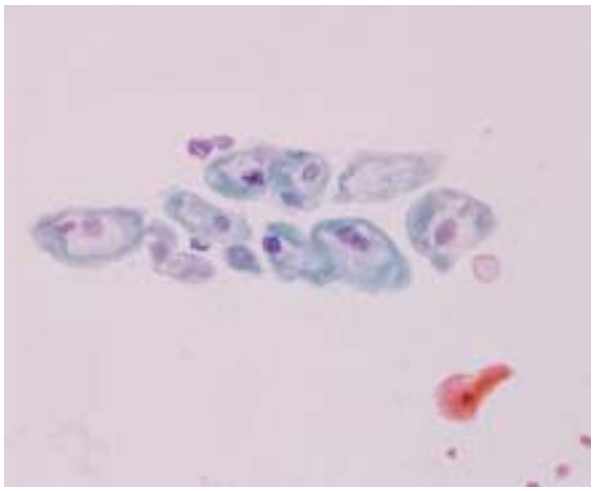
- LGSIL (mild dyskaryosis) resembles mature cells (intermediate and superficial cells) with large abnormal nuclei.
- Low end of the spectrum of HGSIL (moderate dyskaryosis equivalent to CIN2 on histology) resembles parabasal cells with abnormal nuclei.
- In high end of the spectrum of HGSIL (severe dyskaryosis equivalent to CIN3 on histology), most cells are even smaller resembling basal/reserve cells with abnormal nuclei.
- Thus LGSIL stands out in a cervical smear as they have the largest nuclei.

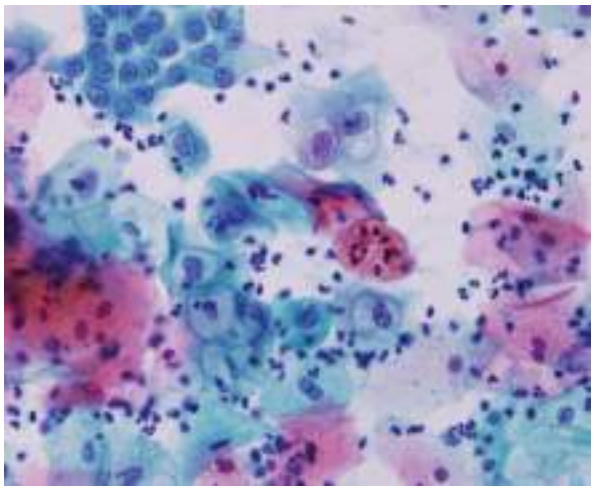
**Low-grade squamous intraepithelial lesions (LGSIL) with human papilloma virus (HPV) induced changes: koilocytotic change (koilocytes) (Figure 6.1 a,b,c,d)**





**(Figure 6.1 c,d)**





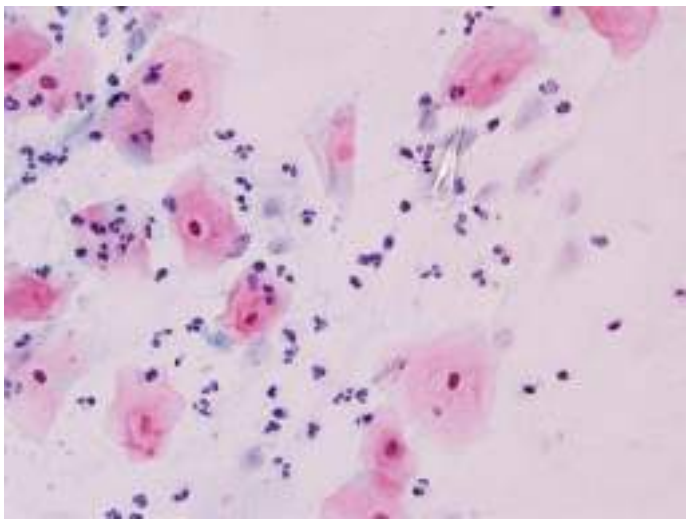
- HPV induced changes affect superficial or intermediate cells resulting in cells categorized as koilocytes.
- In the Bethesda Classification koilocytes are included under LGSIL.

  They are characterized by an abnormal nucleus, a perinuclear halo and a thickened uneven rim of dense cytoplasm.

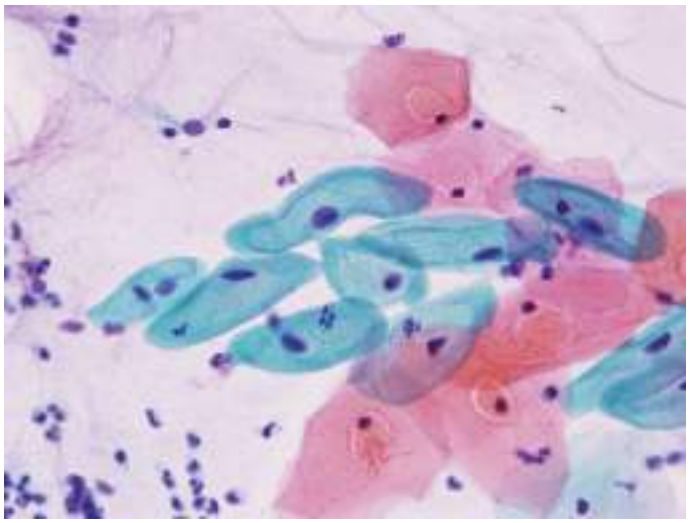
  **Cytoplasmic changes -** Shows a large clear perinuclear cavity with an irregular sharply defined border (perinuclear halo) and a dense polychromatic cytoplasm (blue or pink) at the periphery. Usually the cytoplasm is deeply eosinophilic.

  **Nuclear changes -** Include one, two or more enlarged hyperchromatic nuclei with wrinkled nuclear membranes and granular chromatin. Nucleoli are absent.

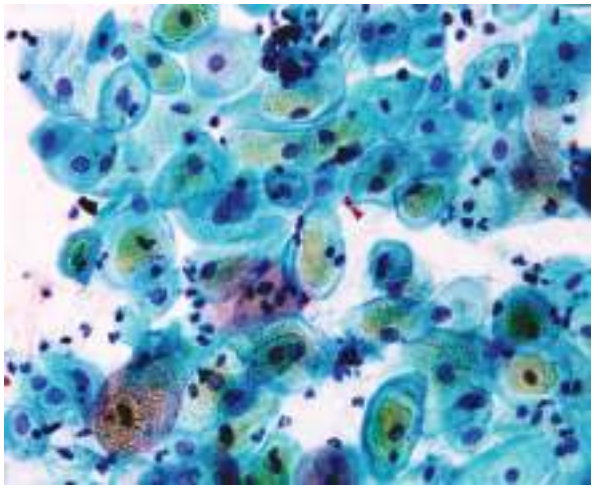
  **Note:** True koilocytes should be differentiated from pseudokoilocytes such as
- Trich halos – Width of the perinuclear clearing is less than the width of an intermediate cell nucleus. **(Figure 3.3)**

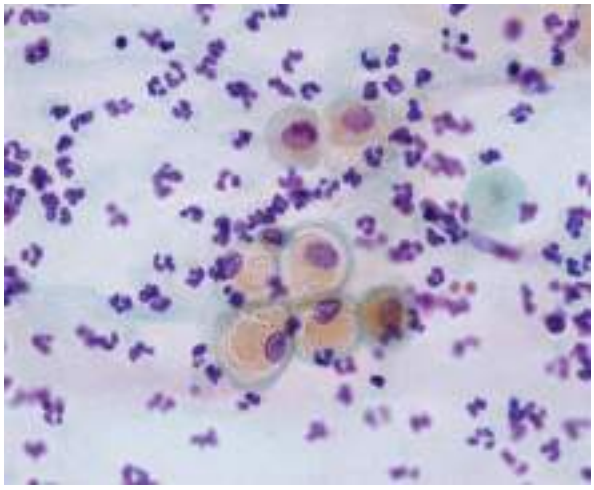


- Navicular cells **(Figure 4.2)**

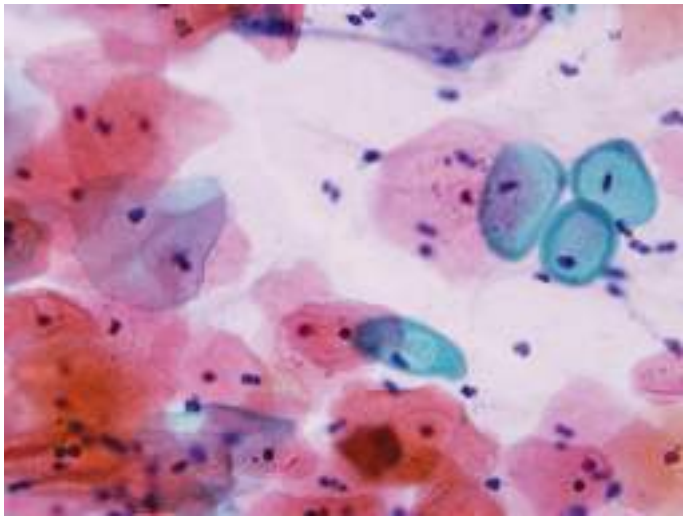


- Glycogenated cells – **(Figure 2.2b, 4.3g)**

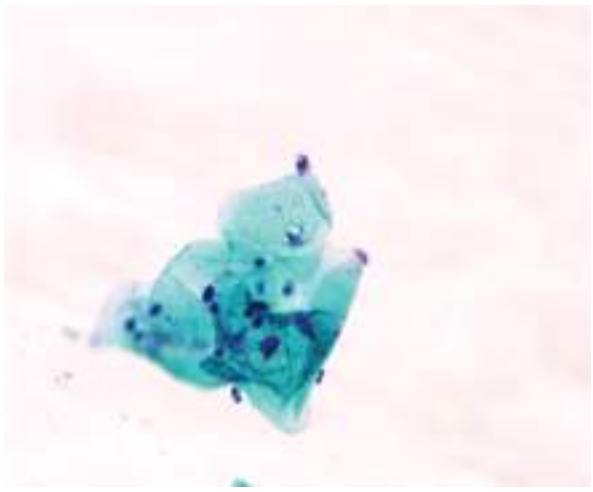


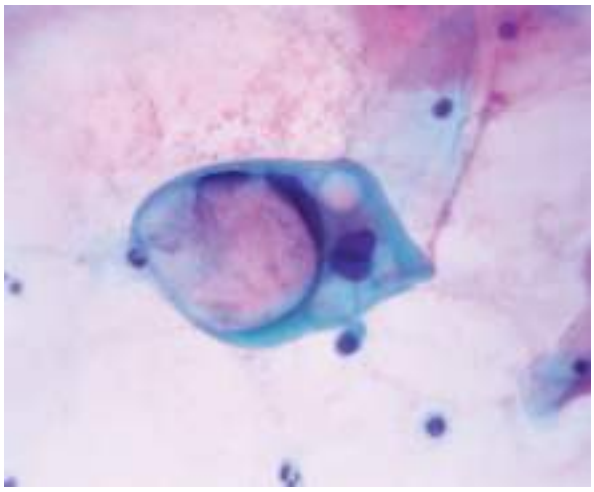


- Immature squamous metaplastic cells - **(Figure 6.2)** These are para basal size cells where as koilocytes are intermediate/superficial size cells. They have a pale endoplasm and a dense ectoplasm.



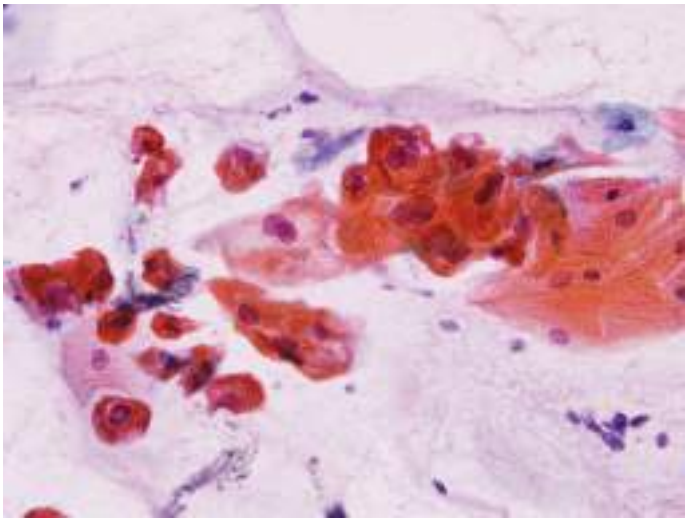
- Other non specific cytoplasmic clearing/vacuoles (Pseudokoilocytes with degenerative vacuoles) **(Figure 6.3 and 5.3)**





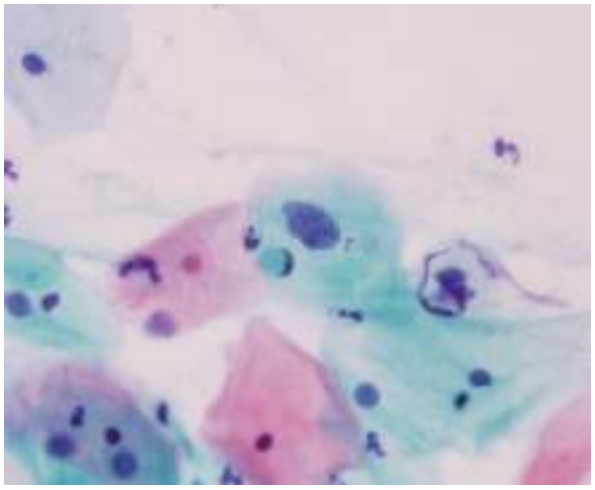
**Other HPV induced effects**

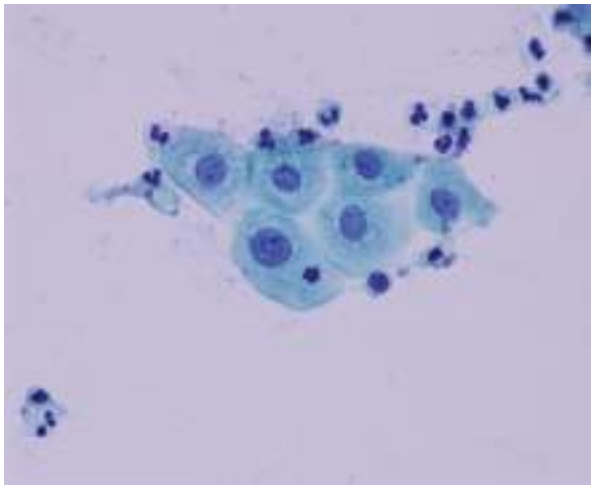
- Extensive cytoplasmic eosinophilia (keratinisation) **(Figure 6.4)**



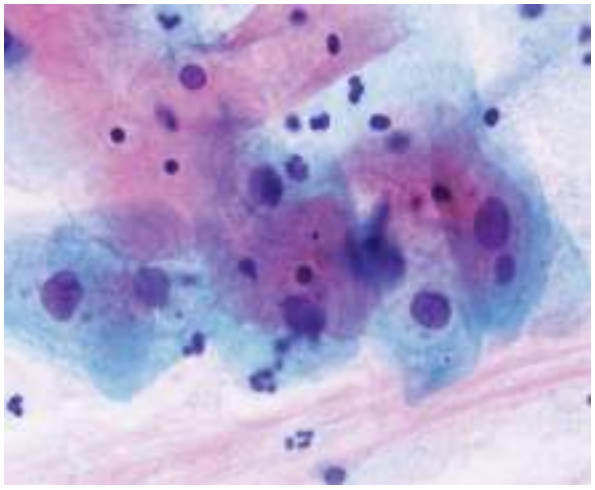
- Extensive parakeratosis and hyperkeratosis.

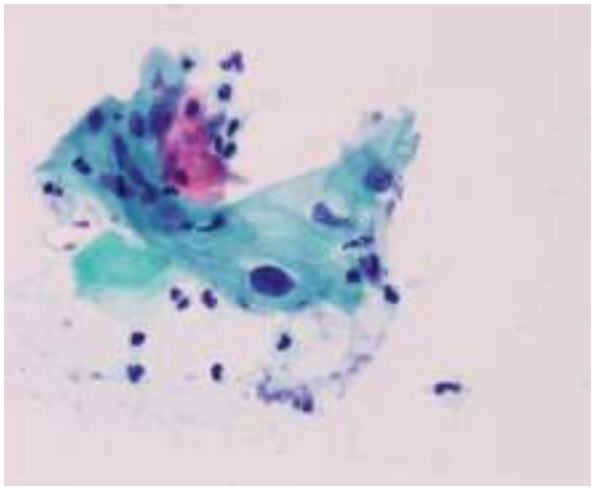
**Low-grade squamous intraepithelial lesions (LGSIL)/Mild dyskaryosis: (Figure 6.5 a,b,c,d)**





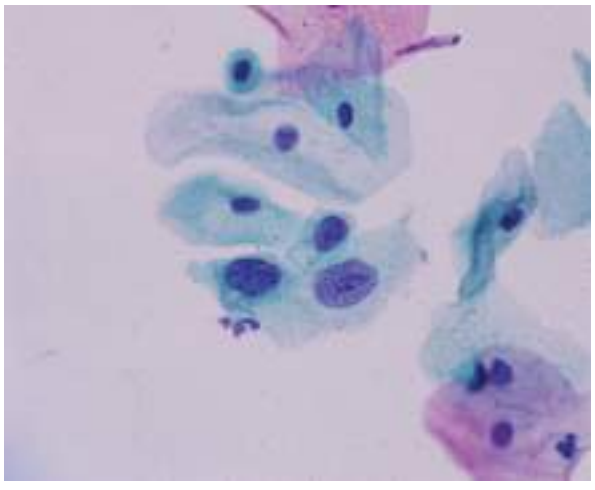
**(Figure 6.5 c,d)**

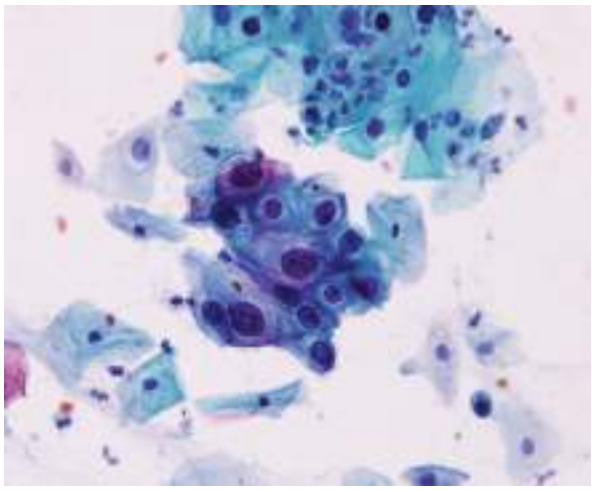




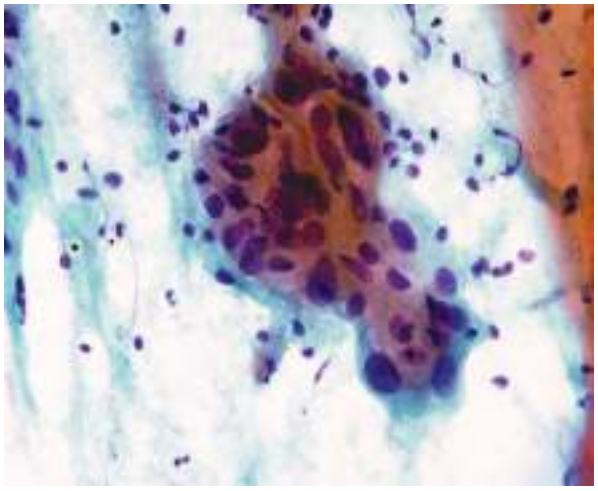
- Single cells and cells in clusters.
- Changes are usually confined to cells with mature superficial-type cytoplasm.
- Overall cell size is large, with fairly abundant, mature, well-defined cytoplasm.
- Cells have a thin transparent cytoplasm and distinct cytoplasmic borders, usually angular.
- Bi nucleation and multi nucleation are common.
- Nuclear enlargement is more than three times that of a normal intermediate cell nucleus (or a neutrophil), with a slightly increased nuclear to cytoplasmic ratio. Usually the nucleus occupies less than half the cell area.
- Nuclear hyperchromasia is variable accompanied by variations in nuclear size, and shape.
- Chromatin is uniformly distributed, may be coarsely granular or may appear smudged or densely opaque.
- Nucleoli are indistinct.
- Nuclear membrane contour is often slightly irregular, may be smooth.

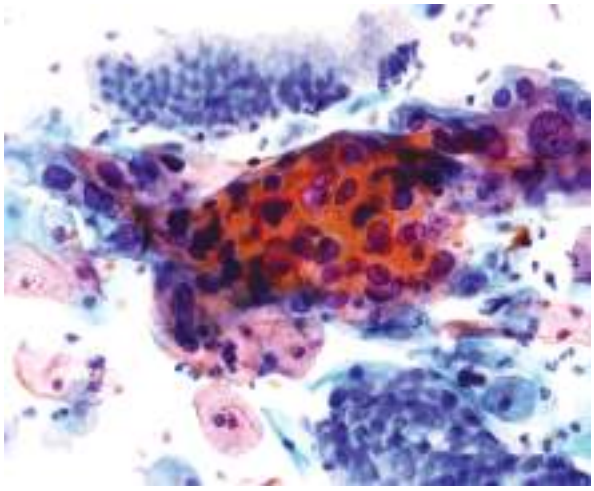
**High-grade squamous intraepithelial lesions (HGSIL)/Moderate and severe dyskaryosis: (Figures 6.6 a,b,c,d,e)**

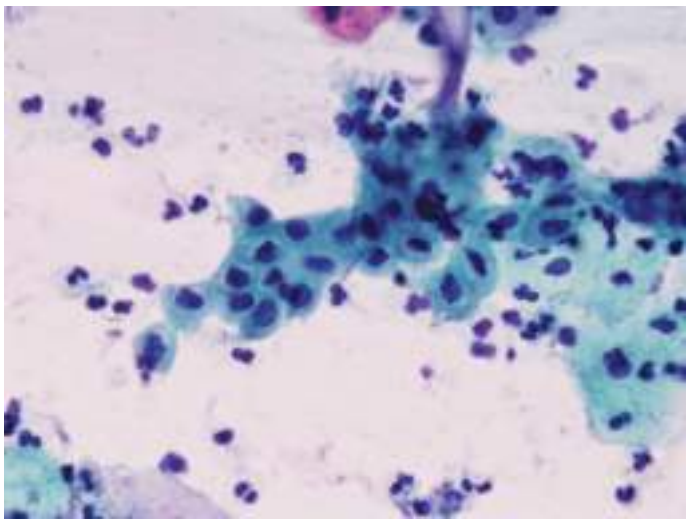




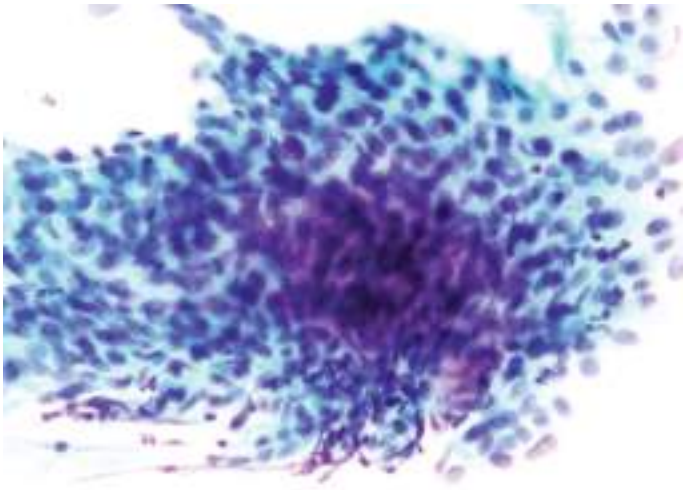
**(Figures 6.6 c,d,e)**





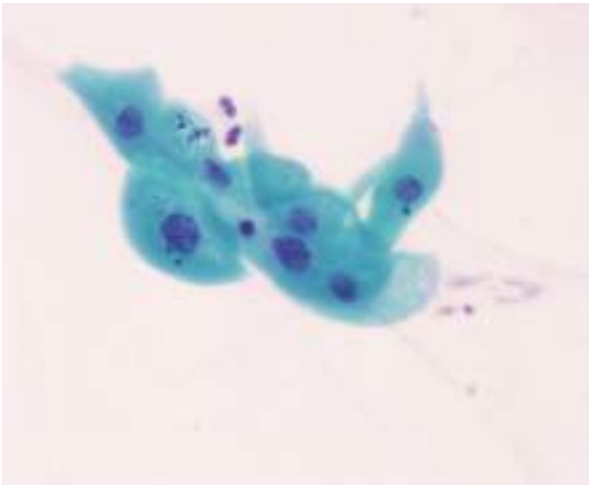


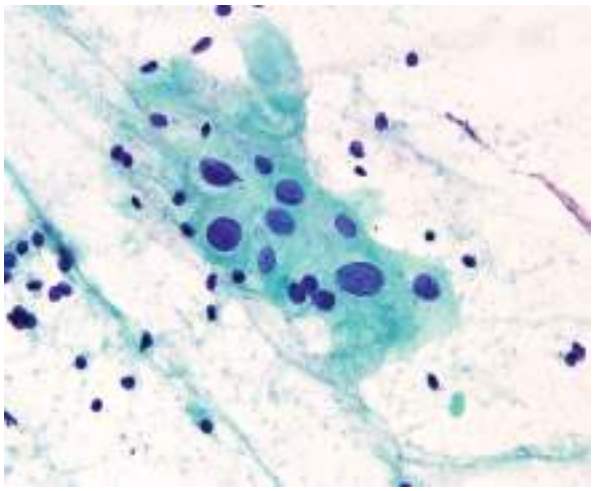
- HGSIL affect cells that are smaller and less mature than cells of LGSIL.
- Cells occur singly, in sheets, in syncytial aggregates or as hyperchromatic crowded cell groups (HCG). **(Figures 6.6f)**

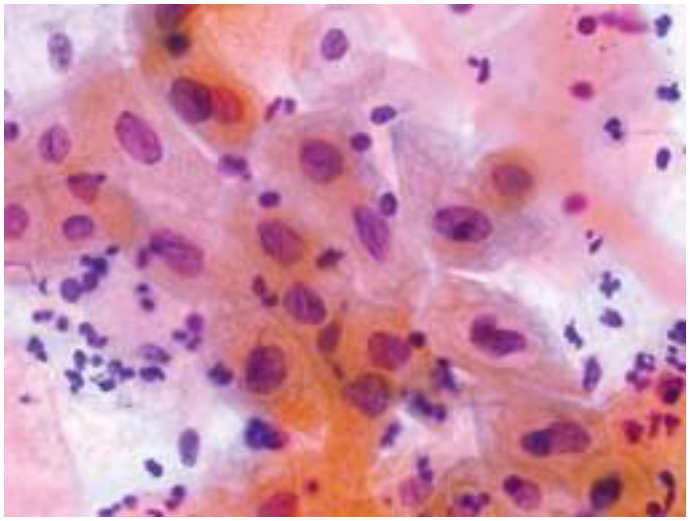


- Abnormal cells are characterized by disproportionate nuclear enlargement. Degree of enlargement is more variable than in LGSIL. HGSIL may have the same degree of nuclear enlargement as LGSIL but the cytoplasmic area is less, resulting in a higher nuclear cytoplasmic ratio. Other cells have very high nuclear cytoplasmic ratios, but with a nuclear size smaller than in LGSIL.
- Nuclear hyperchromasia is associated with variation in nuclear size and shape.
- Nuclear contour is quite irregular and frequently demonstrating nuclear indentations or grooves. (Raisinoid nuclei)
- Nuclear chromatin is fine or coarsely granular.
- Nucleoli are occasionally seen, particularly when HGSIL extends into endocervical gland spaces.
- The cytoplasmic appearance is variable. It can appear immature, lacy and delicate, densely metaplastic or mature and densely keratinized.
- The cell borders may be smooth or angular
- Bizarre cells and multi-nucleated cells may be present.

HGSIL-Moderate dyskaryosis **(Figure 6.7 a, b, c)**

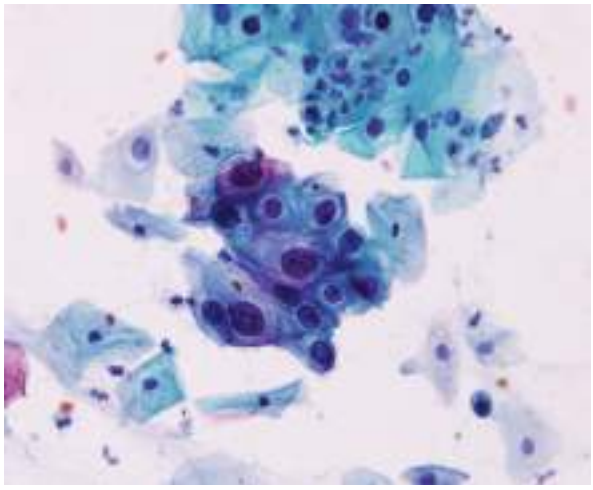


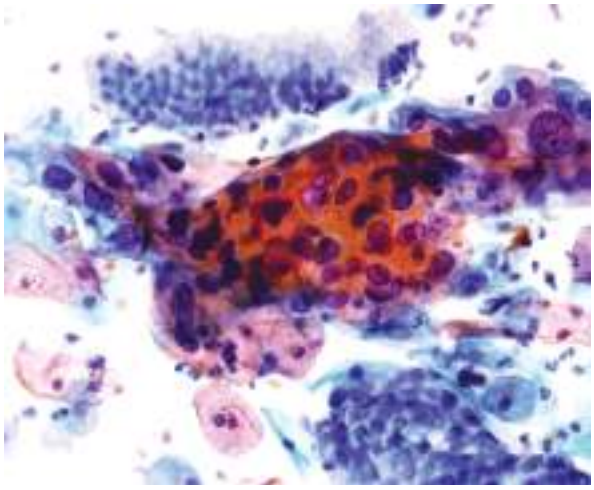




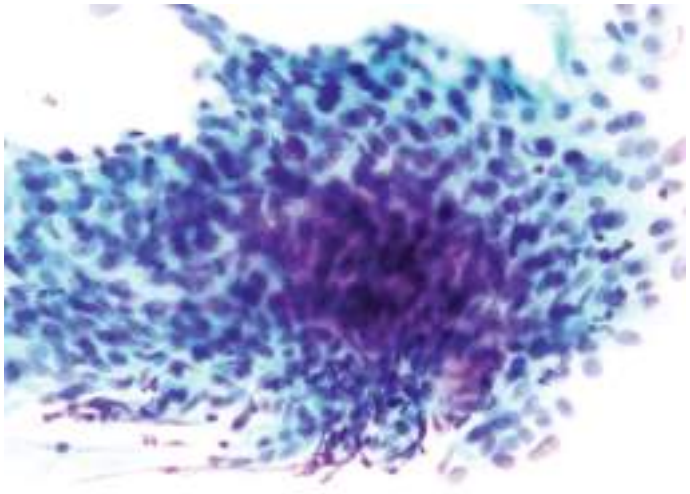
- Usually single cells with immature / dense cytoplasm.
- Nuclei are larger than in LGSIL (mild dyskaryosis), occupying half to two-thirds of the cytoplasmic area.
- Resemble parabasal size cells with increased nuclear size.

**HGSIL-Severe dyskaryosis (Figures 6.6 b,d)**





- Occur as single cells or as HCG and 3-dimensional clusters.
- The nucleus occupies more than 80% of the cell volume or more than two-thirds of the cytoplasmic area.
- The HCG of HGSIL-severe dyskaryosis has overlapping, disorderly arranged nuclei **(Figure 6.6 f)** and delicate cytoplasm. Abnormal chromatin can be seen at the edge. Mitotic figures are seen.

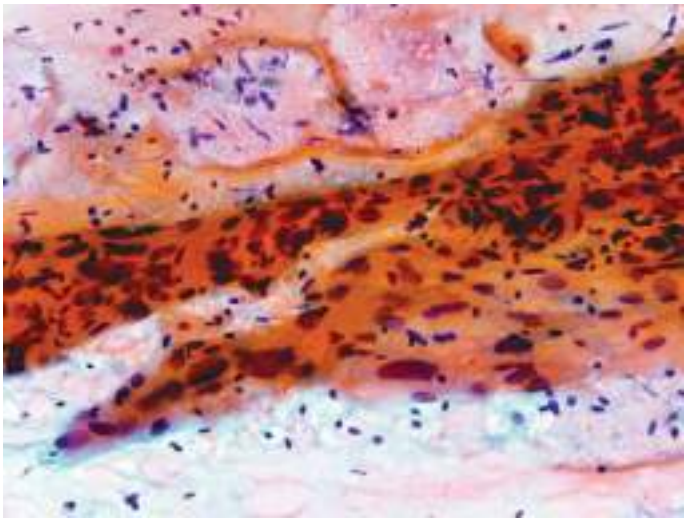


**Different morphologic patterns of SIL**

The different morphological patterns of SIL are not reported on a routine basis and are not included in the Bethesda system. However it may be clinically useful. E.g Non keratinising small cell lesions occur within the endocervical canal and may be missed on colposcopy.

- Keratinizing,
- Non keratinizing large (mature metaplastic dysplasia)
- Non keratinizing small (immature metaplastic dysplasia)
- Small cell

**Keratinizing type (Figures 6.8 a)**

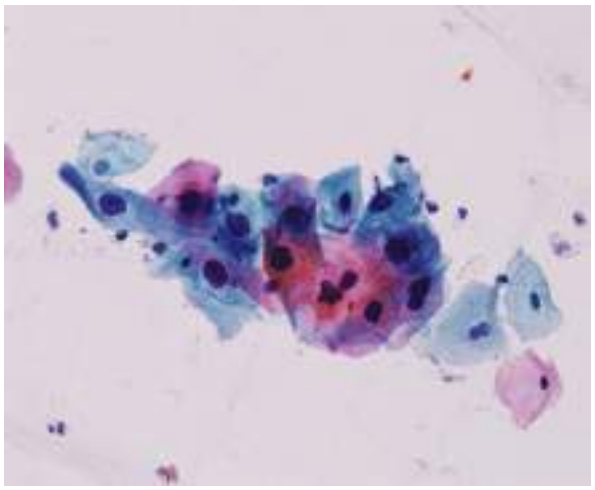


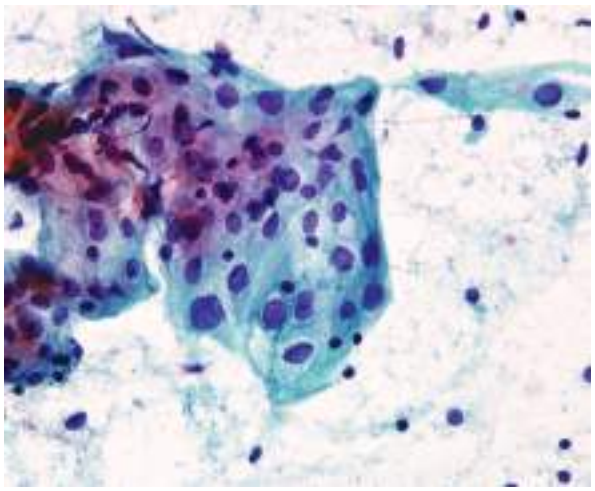
- Often found distal to the cervical os in the ectocervix.
- Lower grades resemble superficial cells with dysplastic nuclei. Higher grades show pleomorphic cells with spindle, tadpole and irregularly shaped cells.

  **Differential diagnosis:** Squamous cell carcinoma (SCC). Note lack of tumour diathesis in the back ground in SIL/dysplasia to differentiate.
- Thick, hyalinized (glassy), orangophilic cytoplasm.
- Hyperchromatic nuclei with very coarse or opaque chromatin.
- Nuclear pyknosis, karyorrhexis and karyolysis may be seen.
- Hyperkeratosis, parakeratosis and atypical parakeratosis are frequently present.

  **Differential diagnosis:** HPV changes. Note the absence of koilocytes in SIL/dysplasia to differentiate.

**Non-keratinizing large cell type (Figures 6.8 b, c)**



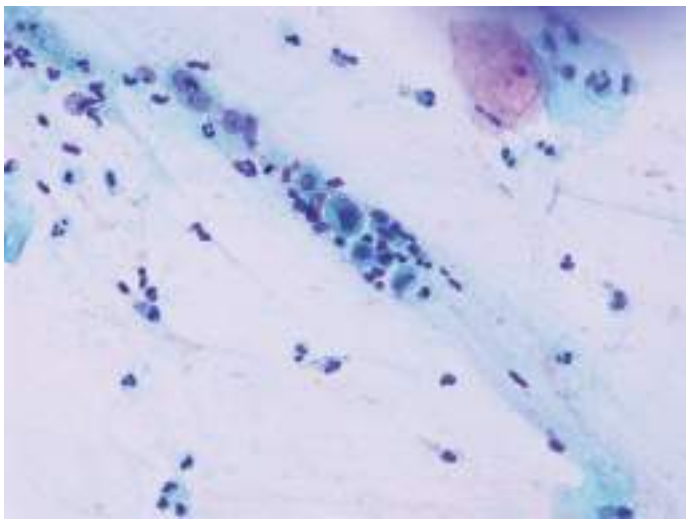


- Commonest type.
- Located proximal to keratinizing dysplasias, near the squamo-columnar junction.
- Large polygonal cells with basophilic cytoplasm and well defined borders (resemble intermediate cells with dysplastic nuclei) or with a cytoplasm that is dense and less than in normal intermediate cells (mimic mature squamous metaplasia).
- The nuclear chromatin is finely granular and hyperchromatic.

**Non-keratinizing small cell type**

- Uncommon.
- Arise from the immature squamous epithelium resembling immature squamous metaplasia with dysplatic nuclei.

  **Differential diagnosis:** Immature squamous metaplasia. N/C ratio is increased in both, look for atypical nuclear features to differentiate.
- Found at the upper limit of the transformation zone, high in the endocervical canal.
- Occur as single cells or as sheets with a cobble stone appearance.
- Small round or oval cells (parabasal size) with thick basophilic cytoplasm, distinct cell borders and enlarged and hyperchromatic nuclei.
- High nuclear cytoplasmic ratio and moderately granular nuclear chromatin with irregular borders.
- Very small cells could easily be missed. Look for strings of small dysplastic cells hiding among inflammatory cells in mucous. **(Figure 6.8d)**



**Small cell type**

- Atypical undifferentiated single small cells resembling reserve cells

  **Differential diagnosis: Small cell neuroendocrine carcinoma**
- Also occur as hyperchromatic crowded cell groups.

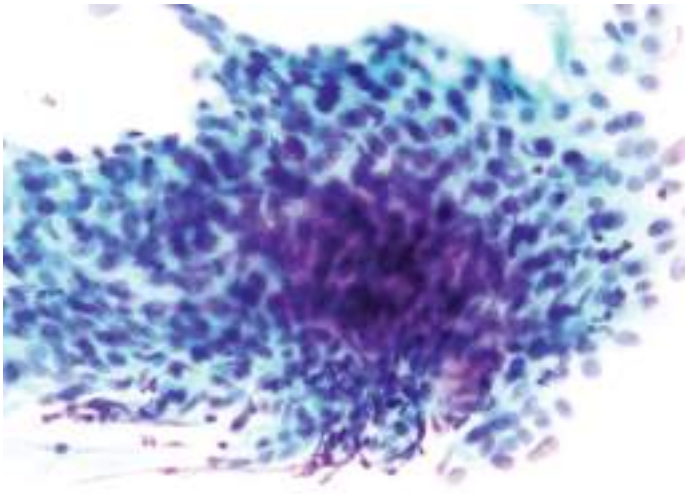
- Oval/spindly hyperchromatic nuclei with coarse or fine evenly distributed chromatin.
- Nucleoli are uncommon. The mitotic rate is high.

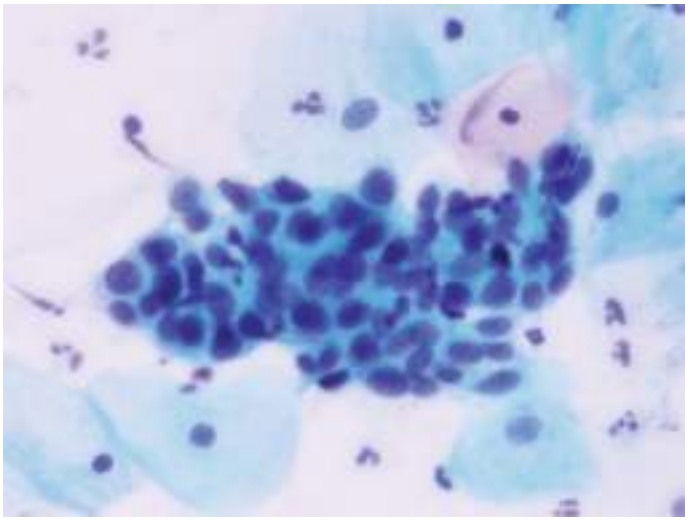
**Problems in diagnosing HGSIL/moderate and severe dyskaryosis**

- Detection is difficult when the cell number is sparse.
- Cells from malignant lesions may have a bland appearance.
- HGSIL/ moderate and severe dyskaryosis cells may get over shadowed by a LGSIL/ mild dyskaryosis or inflammation.
- May resemble other entities.

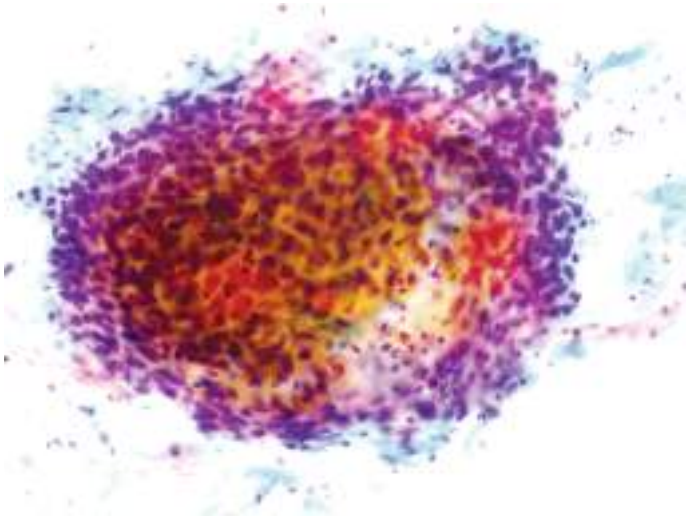
**HGSIL/severe dyskaryosis with gland duct involvement (HGSIL with GDI)**

- HGSIL/ severe dyskaryosis with GDI may be mistaken for a glandular abnormality (Adenocarcinoma in situ/AIS).
- May resemble glandular cells in a honeycomb/sheet arrangement, reminiscent of endocervical epithelium.
- Small gland-like spaces or lumina may be seen in cell groupings. **(Figures 6.6f and 6.9a)**





- The cell may ‘palisade’ at the edge resembling endocervical cells.
- Nucleoli may be present.
- HGSIL however reveal centrally located nuclei, spindling or whirling. Flattening of nuclei at the peripheral edge of the clusters gives rise to a smooth rounded border. **(Figures 6.9b)**



- The nuclear chromatin in HSIL is less coarse than in AIS.
- May be associated with focal epithelial cell necrosis and micro nucleoli even without invasion. The necrosis tends to be associated with the cell group in an otherwise clean background.

**Note:** It’s worthy to remember that HGSIL and AIS often can and do co-exist.

**HSIL/ severe dyskaryosis with features suspicious for invasion**

- The number of abnormal cells detected is large with tissue fragments.

  (Micro biopsies)
- Cells are pleomorphic with a keratinized cytoplasm, not accompanied by characteristic background features of necrosis or tumour diathesis.
- The cells will show more differentiation, more eosinophilia and more variation in nuclear size and shape.
- Micro nucleoli are present with slight chromatin clearing.
- A dirty or necrotic background may be seen when infiltration reaches a depth of 5.0 mm

# Chapter 7 – HGSIL/dyskaryosis: Other entities to be considered in the differential Diagnosis

**Histiocytes: (Figure 7.1)**

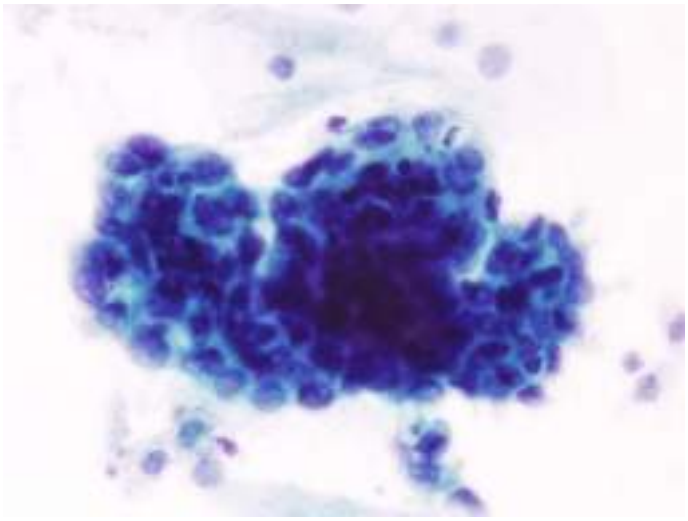
- May mimic HGSIL/dyskaryosis; however they vary in size, with the nuclei and cytoplasm showing degenerative changes.
- The reniform nuclei, pale and fine chromatin and the foamy cytoplasm, with the neighbouring cells displaying classical features of histiocytes help further in the differential diagnosis from HGSIL.

**Repair/regeneration: (Figure 7.2)**

**Differential diagnosis –** Glandular cells with reactive reparative changes vs. HGSIL/ dyskaryosis, non keratinizing, large cell type.

- Reparative cells are cohesive with preserved polarity and spatial orientation and prominent nucleoli.
- Atypical forms may occur with significant crowding and piling-up of cells.

**Exfoliated endometrial cells: (Figure 7.3)**



**Differential diagnosis –** Exfoliated endometrial cells vs. a small cell non-keratinising HGSIL/ dyskaryosis appearing as a HCG.

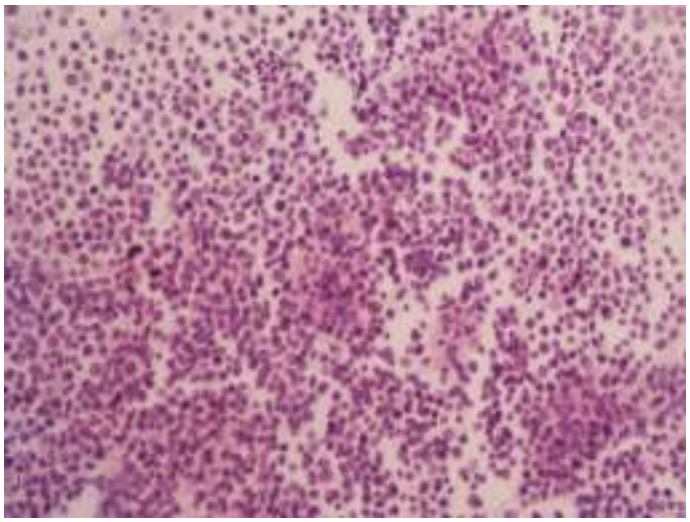
- Endometrial cells in clusters have small hyperchromatic nuclei and scanty cytoplasm.
- The biphasic appearance of endometrial cell groups when present (glandular epithelial cells surrounded by stromal cells, appearing as a cell cluster with a darker centre, Exodus pattern) helps in confirming them as exfoliated endometrial cells.

**Abraded lower uterine segment: (Figure 7.4)**

**Differential diagnosis –** Abraded lower uterine segment vs. HCG of HGSIL/dyskaryosis

- Commoner with endocervical brush and post conization specimens.
- Occur as large branched sheets with gland openings, strips and small round groups of cells
- Epithelial cell clusters may be mixed with endometrial stromal type cells.

**Follicular cervicitis: (Figure 5.14)**



**Differential diagnosis –** Follicular cervicitis vs. small cell HGSIL/ dyskaryosis

- A mixture of cells comprising streaks of dispersed small lymphoid cells, plasma cells with coarse chromatin, larger follicular centre cells and tingible body macrophages.

**IUD effect: (Figure 7.5)**

**Differential diagnosis –** Glandular cells with IUD effect vs. small cell HGSIL/ dyskaryosis

- Atypical endometrial cells with round enlarged nuclei, sometimes with visible nucleoli.
- Cytoplasm may be vacuolated. Mimic HGSIL-severe dyskaryosis when the cytoplasm forms a rim around the nucleus without any vacuolation.
- Clues to the diagnosis include admixed degenerating leucocytes and debris among endometrial material in mucous and the known use of an IUD.

**AIS/Adenocarcinoma: (Figure 7.6)**

**Differential diagnosis –** AIS/Adenocarcinoma vs. HCG of HGSIL/ dyskaryosis

- Difficult to distinguish from a HCG of HGSIL, especially when the glandular lesion is not well differentiated.
- Characteristic features of rosettes, nuclear pseudo stratification, palisading and feathering of the edges should be looked for in other areas.
- An adenocarcinoma is likely in the presence of prominent, multiple and irregular nucleoli.

**Compared to AIS, in HCG of HGSIL (usually in large cell non keratinising type) (Figure 7.7)**

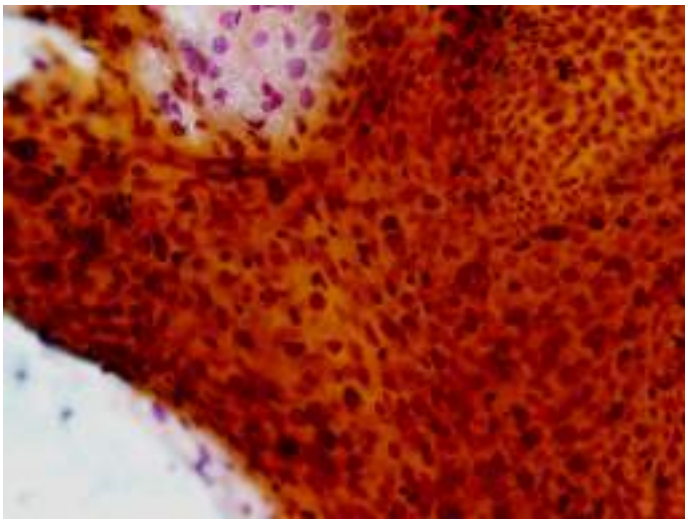
- Syncytial groups > 3 layers thick, are seen with no definite columnar shapes.
- Glandular architecture is absent in these groups.
- Palisading and pseudo stratification are absent.

- The nuclei are more disorganised and are seldom oval as seen in AIS, with finer chromatin.
- A higher proportion of bare nuclei with seldom nucleoli.

**Squamous cell carcinoma**

**Differential diagnosis –** SCC vs. Keratinizing HGSIL

Keratinising HGSIL **(Figure 7.8)** is often mistaken for SCC when abundant atypical keratinised squamous cells are present or when there is necrosis.



- Necrosis occurs in HGSIL with gland duct involvement adjacent to cell clusters in an otherwise clean background.
- Chromatin clearing, jagged irregular nuclear membranes and tumour diathesis in the background are not usual in HGSIL.

# Chapter 8 – HGSIL/dyskaryosis: Situations in which it could be easily overlooked.

**Small cell HGSIL/dyskaryosis**

- Small cells with irregular nuclear margins, irregularly dispersed chromatin, inconspicuous nucleoli and a high nuclear/cytoplasmic ratio may be thought of as histiocytes, lymphocytes, immature metaplastic cells or reserve cells.
- These are found hidden in mucous streaks mixed with inflammatory cells. Thus may be easily overlooked on screening.

  **Note:** Examine strings of cells in mucous carefully and look for other cells with lesser degrees of changes, so as not to miss this entity.

**Pale dyskaryotic cells**

- The nuclei of these cells are paler than surrounding neutrophil nuclei.
- However they retain their high nuclear/cytoplasmic ratio and irregular nuclear membranes.
- The pale chromatin pattern is fine, but irregularly distributed within the cells and between cells.

  **Note: Beware of this entity!** It is easily overlooked on screening, if only hyperchromasia is concentrated on.

**Sticky big bare nuclei**

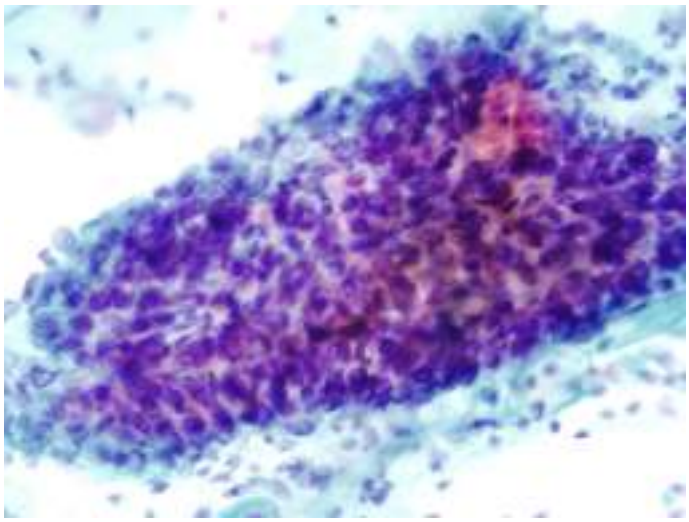
- Large bare nuclei originate from squamous and glandular high-grade lesions.
- The nuclei occur singly or in tightly cohesive groups of few nuclei.
- The nuclei are smooth and rounded. They are often moulded with finely granular chromatin and are hypo or hyper chromatic.
- These are particularly significant when there is variation in size.

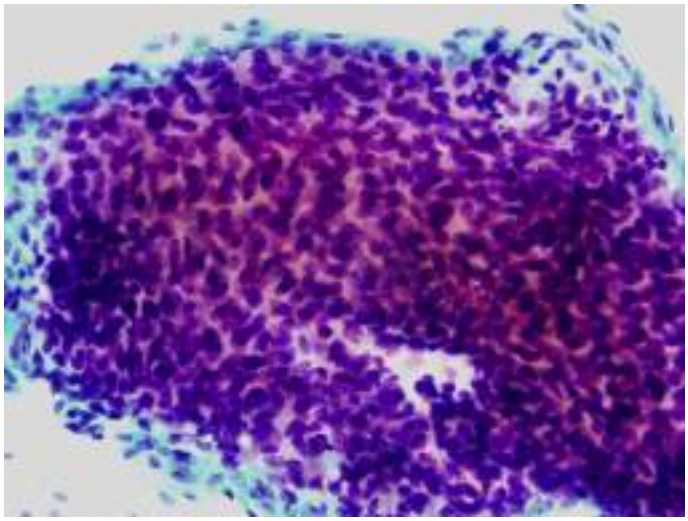
  **Note:** Look around carefully for atypical cells with intact cytoplasm.

**Hyperchromatic crowded groups/micro biopsies**

- HCGs may be benign or malignant.

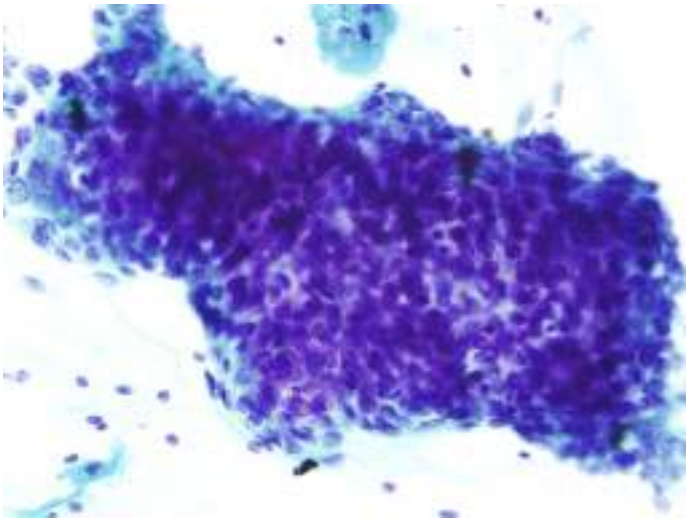
- Benign HCGs' include endometrial cells and severe atrophy. **(Figures 8.1 and 8.2)**



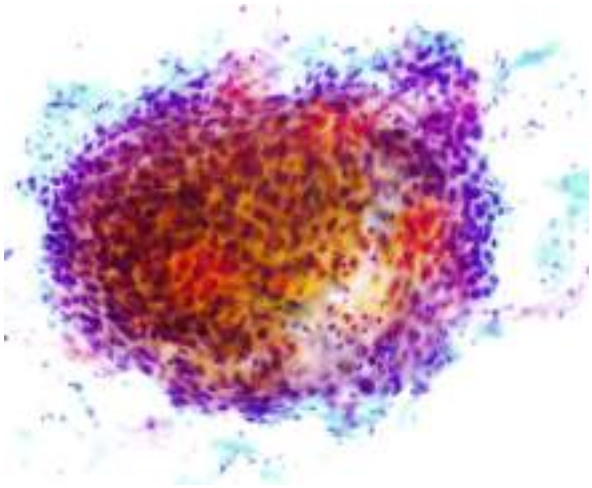


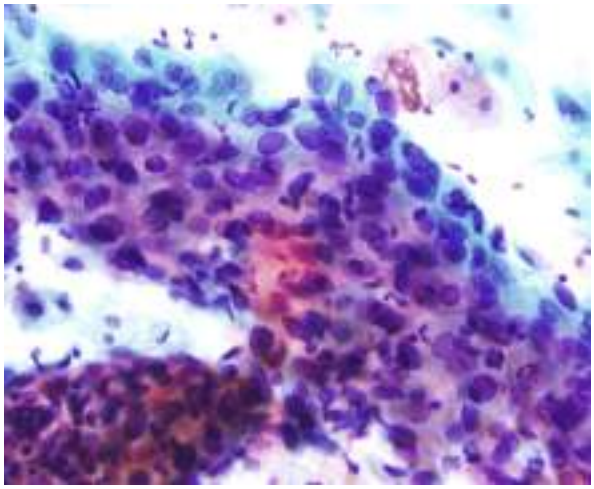
**Note:** bland, benign appearing nuclei. Look for individual parabasal cells in the background in atrophy.

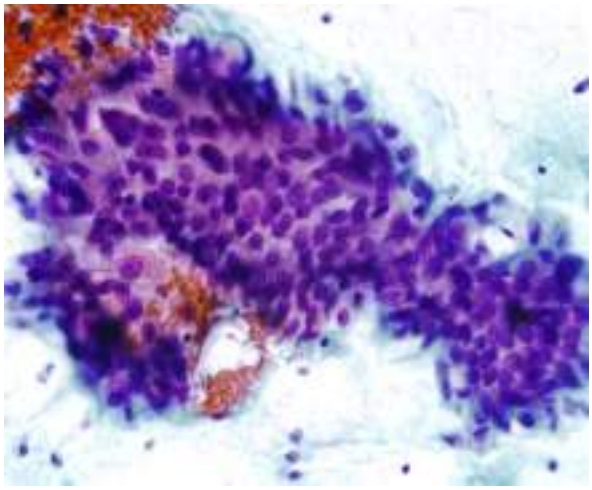
- Endocervical brush samples may also yield endocervical cells in groups (HCG) **(Figures 8.3).**

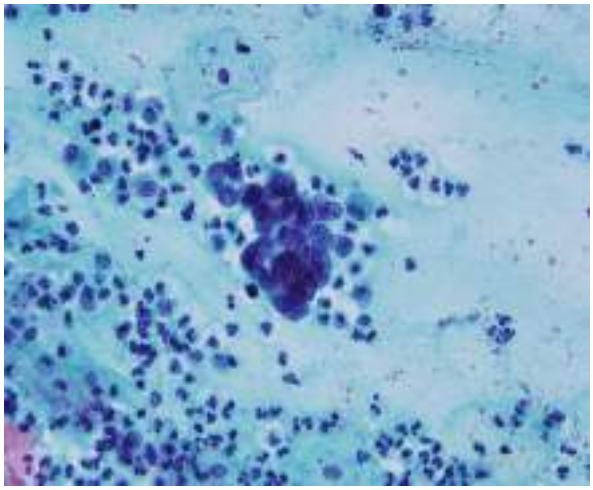


- Malignant or pre-malignant lesions with HCGs include HGSIL/severe dyskaryosis (**Figures 6.9b and 8.4),** SCC (**Figure 8.5),** endocervical AIS **(Figure 8.6)** and adenocarcinoma **(Figure 11.8b).**







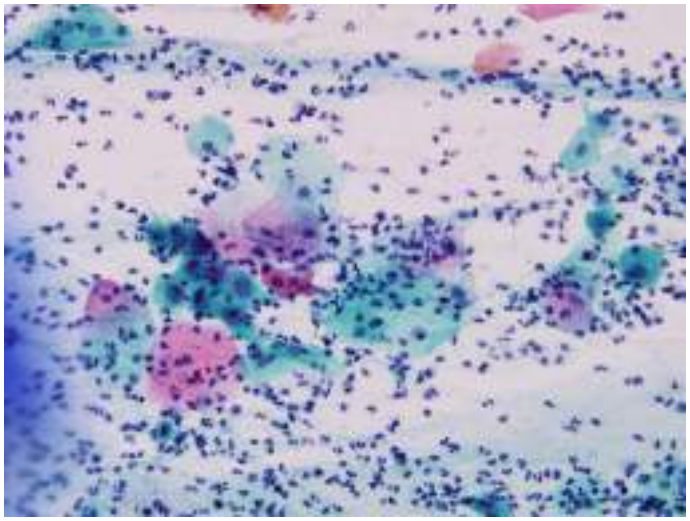


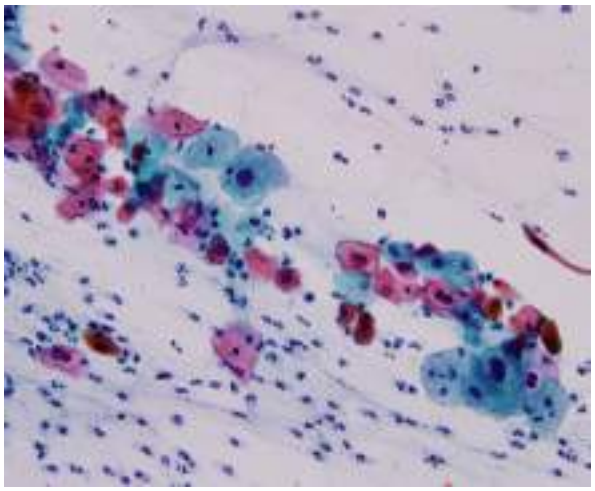
**Note:** Evaluate cell clusters for crowding, polarity, thickness of the cell groups, variability in the nuclear size, chromasia, chromatin structure and nuclear border to pick on malignant HCGs. Also search for mitotic figures and more typical areas of SIL/dyskaryosis.

- Differentiating between HCG of glandular and squamous origin has already been discussed.

**Inflammation/low cell yield:**

- SIL/Dyskaryotic cells may hide amidst the inflammatory exudate making detection on screening difficult. **(Figure 8.7)** Look for suspicious cells in streaks. (**Figure 8.8a and b**)





**Note:** Look for small, abnormal, keratinised, squamous cells in these cases.

**Squamous intra epithelial lesions/Dyskaryosis: Summary**

**Note:** Nuclear changes are the hallmark of squamous lesions. Pay attention to the nuclear size, shape, chromatin pattern, nuclear membrane and nuclear cytoplasmic ratio, to determine and type the lesion. Intercellular variability, cell size, cell arrangement, cytoplasmic qualities, the background appearance and being aware of the pitfalls and the patterns that could be easily overlooked in high grade lesions, help in making the correct diagnosis. In this process, the clinical context should also be given its due place and should not be forgotten.

# Chapter 9 – Atypical squamous cells of undetermined significance (ASCUS)/Borderline nuclear change

**When would you call a smear ASCUS?**

When squamous cells have nuclear changes that are more marked than seen in reactive cells, however falling short of the changes seen in SIL/dyskaryosis (either qualitatively or quantitatively), the smear is categorized as ASCUS. Thus the cellular changes are not clear cut and a more specific diagnosis cannot be made.

Equivalent BSCC terminology is **Borderline nuclear changes.**

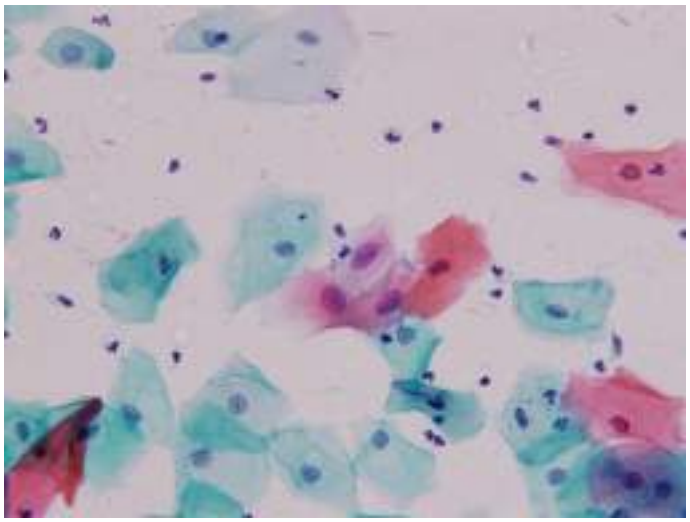
**Broad diagnostic criteria**

- The nucleus is enlarged to 2 1/2 to 3 times the size of an intermediate cell nucleus
- N/C ratio is only mildly increased
- Chromatin is finely dispersed
- Nuclear membrane is smooth or slightly wavy
- Normochromatic or mildly hyperchromatic
- Variation in cell size / shape and bi nucleation may occur
- Usually only a few cells are affected. (3 to 5 cells)

**Grades of ASCUS**

1. ASCUS – Low grade (ASCUS - LG)
2. ASCUS - High grade (ASCUS - HG)

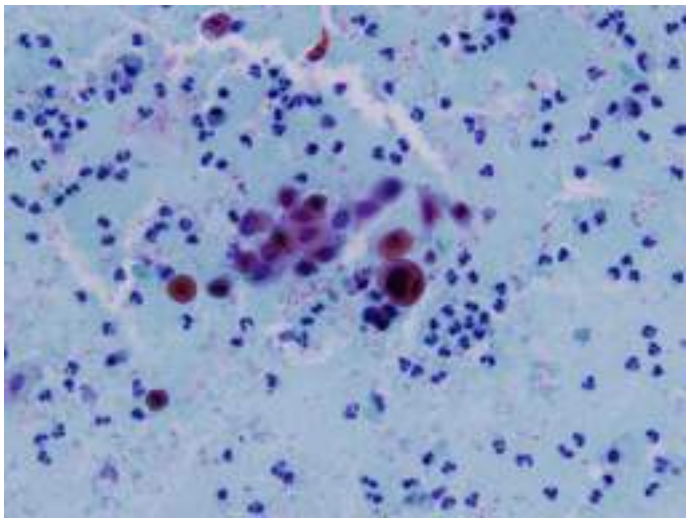
**ASCUS Low Grade (Figure 9.1)**



- Diagnostic criteria are as described above

**Recommendation for management – A repeat smear after an interval**

**ASCUS High Grade (Figure 9.2)**



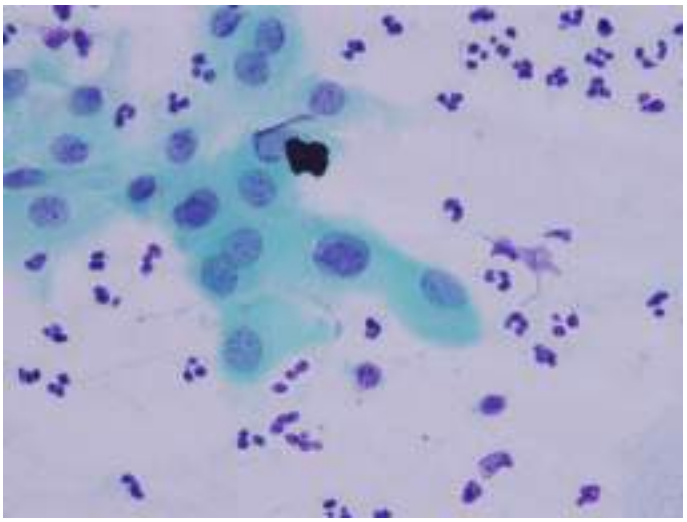
- Cells sparse and are small, the size of metaplastic cells.
- Nuclei are 1 1/2 to 2 1/2 times larger than normal and show uniform, dense chromatin; Therefore other nuclear details are not visible
- Nuclear cytoplasmic ratio may be high
- Cells occur singly or in small fragments of less than 10 cells.
- Cells may stream in mucous

**Differential diagnosis:** Degenerate cells with irregular hyperchromatic nuclei. In these, the nuclear membrane irregularity tends to involve the entire nuclear outline (a wrinkled appearance) and the chromatin is smudgy.

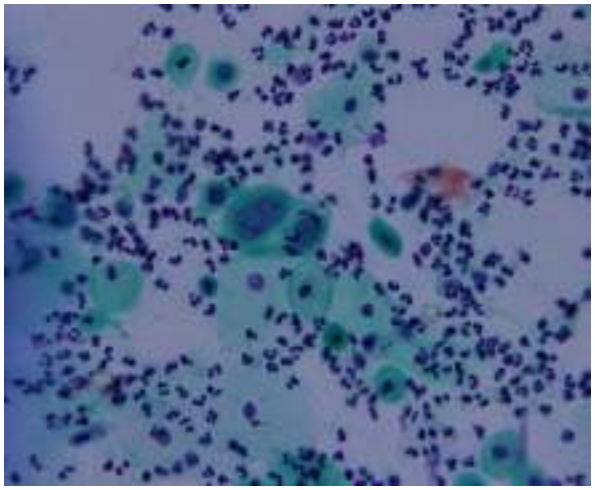
**Recommendation for management – Referred for colposcopy**

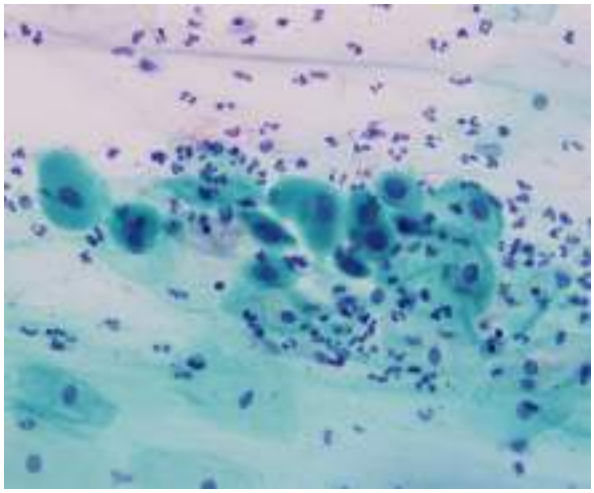
**ASCUS changes could be seen in**

- Intermediate squamous cells **(Figure 9.3)**

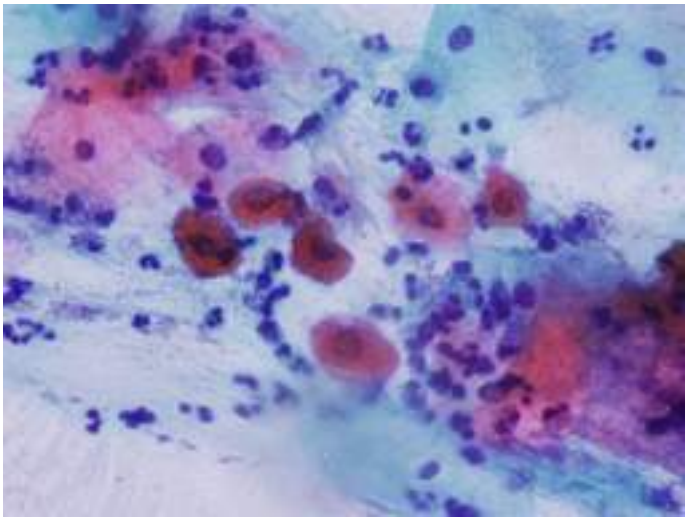


- Mature (non keratinized) metaplastic cells (**Figure 9.4a and b**)

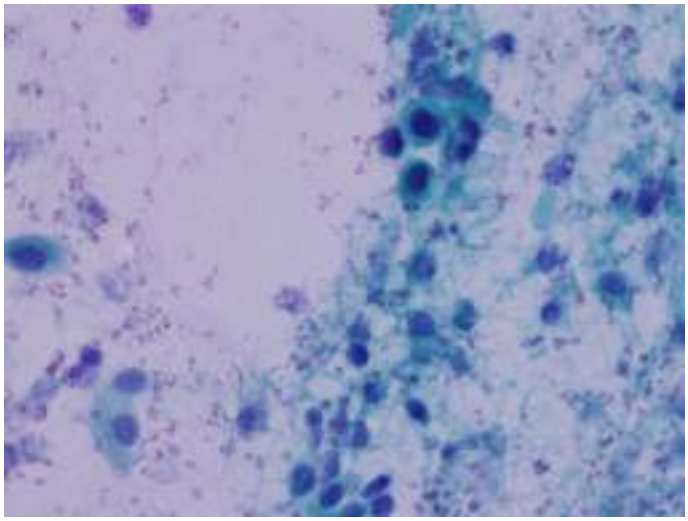




- Keratinized cells **(Figure 9.5)**



- Atrophic cells **(Figure 9.6)**



- Parakeratotic cells **(Figure 5.15d)**

**Note:**

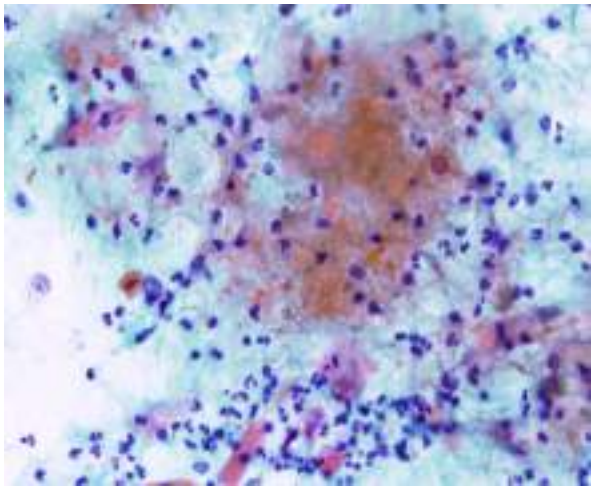
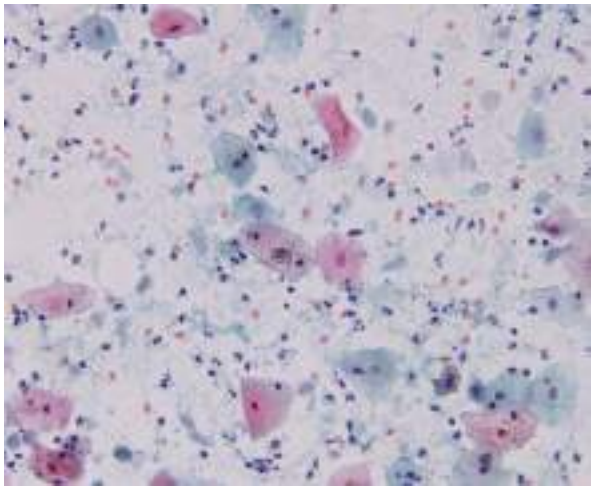
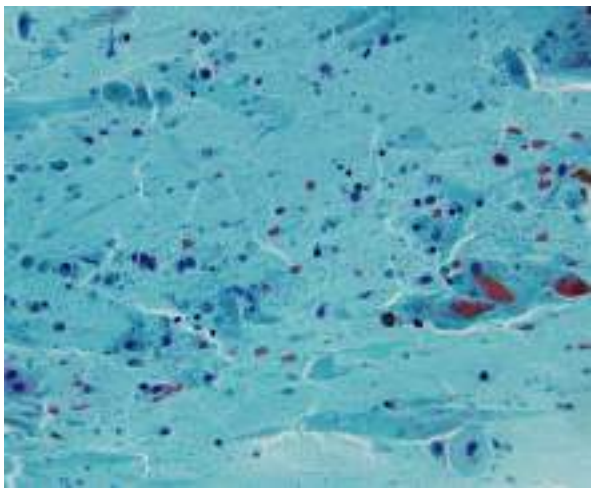
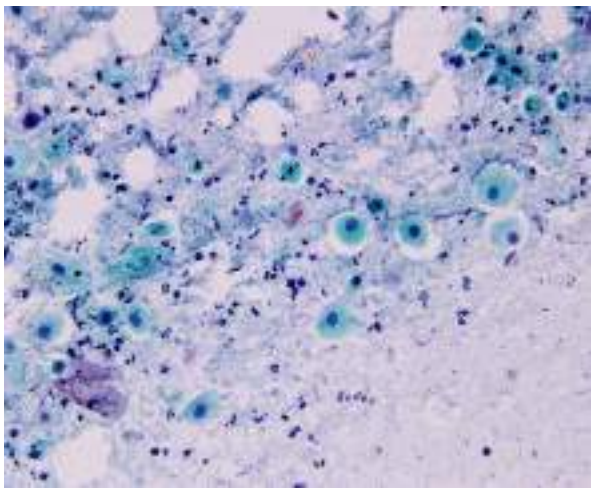
- Do not use ASCUS as a waste paper basket as the number of ASCUS and ASCUS/SIL ratio has implications for the reporting pathologist/laboratory quality. Thus ASCUS diagnosis should be based on strict criteria and the rate of diagnosis should be monitored.

  The Bethesda system suggests that the rate for ASCUS should not exceed 2-3X the SIL rate in any laboratory (ASCUS rate should only be about 50% of the reported abnormal rate).
- ASCUS-LG and ASCUS-HG have two different implications for patient management.

# Chapter 10 – Squamous cell carcinoma (SCC)

Most SCC can be diagnosed on cervical smears. Highly keratinized abnormal squamous cells and undifferentiated tumour cells suggest an invasive lesion, especially in the presence of tumour diathesis.

Tumour diathesis is a mixture of old and new blood, protinaceous and necrotic material with nuclear and cytoplasmic fragments. Neutrophils are less in number in contrast to inflammation. **(Figure 10.1)** Tumour diathesis should be differentiated from benign diathesis as seen in severe trichomonas **(Figure 3.5c)** and herpes infections and in atypical atrophy **(Figure 4.3d. 4.3e)**

False negatives diagnoses are often due to blood and necrosis.

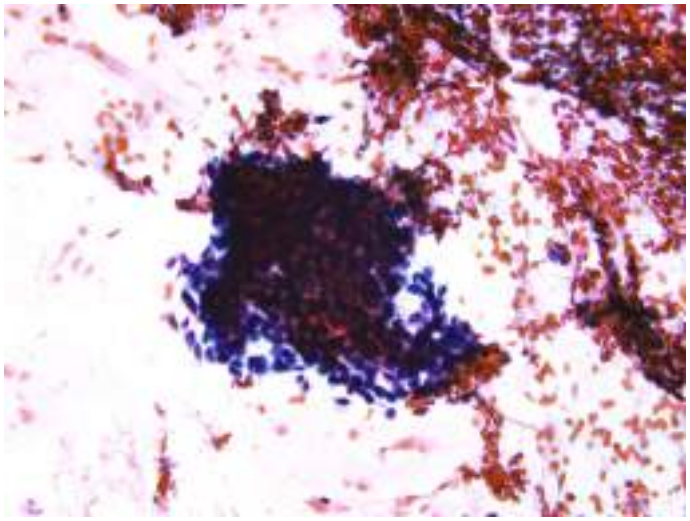
**General features**

- Tumour diathesis in the background.
- Isolated and syncitial sheets of cells that are smaller than superficial squamous cells.
- Irregular caudate or elongated cells.
- Nuclei are enlarged with high N/C ratio, but are smaller than in SIL/Dyskaryosis. Size and shape is variable. Usually cells are mononucleated.

- Nuclear membrane is irregularly thickened, wrinkled, angular and jagged
- Mostly coarse chromatin and hyperchromatic. Less commonly fine, hypochromatic transluscent or opaque.
- Nucleoli are enlarged and irregular, occasionally multiple.

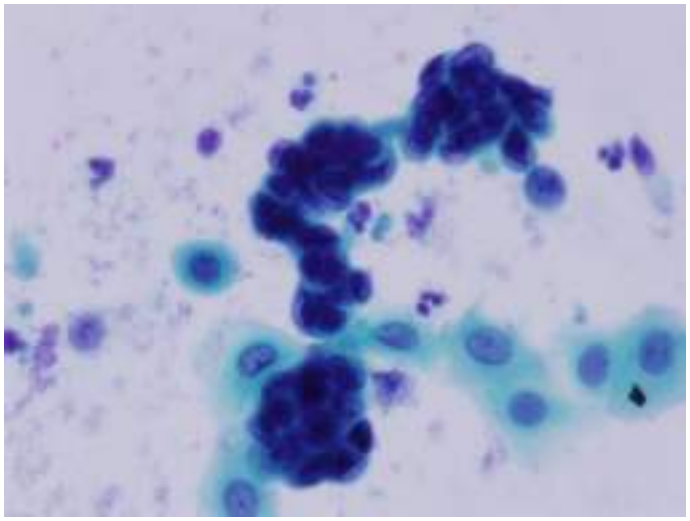
SCCs could be of small cell, keratinizing or non-keratinizing types.

**Small cell SCC, poorly differentiated (Figure 10.2)**



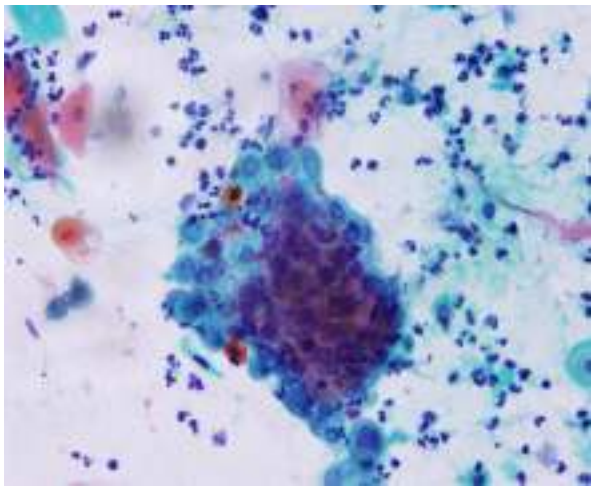
- Tumour diathesis in the background.
- Many small, oval to spindle pleomorphic cells with high N/C ratios in aggregates or as isolated cells.
- Nuclei are hyperchromatic with coarse chromatin. Nucleoli may be present, but are not prominent.
- Naked and pyknotic nuclei and mitotic figures may be seen.

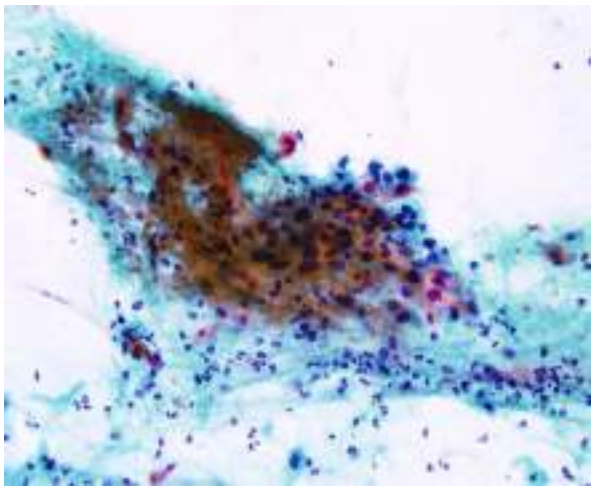
**Differential diagnosis:** Small cell SCC vs. endometrial cells **(Figure 2.6c)**



- Small cell SCC has cells with larger nuclei (1-2 times the size of intermediate cell nucleus. Endometrial nuclei are the same size as intermediate cell nucleus) with variable size shape and chromatin, in a background of tumour diathesis.

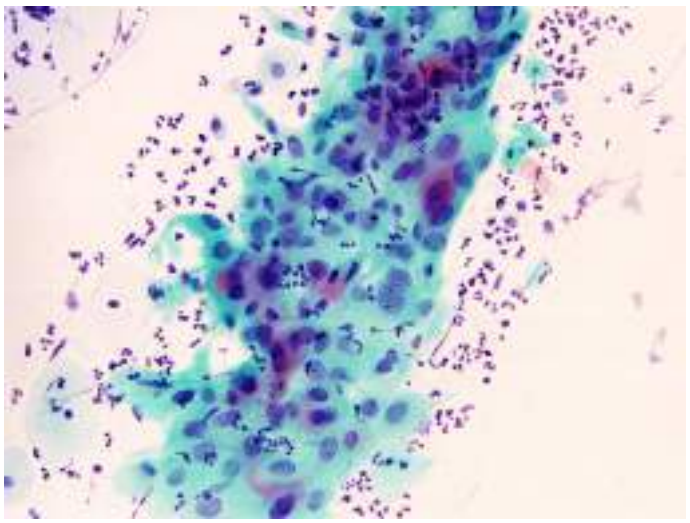
**Non Keratinizing SCC, moderately differentiated (Figure 10.3a,b)**





- The commonest type.
- Tumour diathesis is often present and prominent.
- Medium to large, relatively uniform cells.
- Occur singly or in syncytial aggregates with poorly defined cell borders.
- Frequently cells are smaller than those of HGSILs' however displaying most features of HGSIL.
- The nuclei demonstrate marked irregular distribution of coarsely clumped chromatin as a hallmark feature. Naked nuclei may be present due to cytoplasmic lysis.
- Large cell variants may show prominent macro nucleoli and a basophilic cytoplasm. Keratinisation is seen in occasional single cells (single dyskeratotic cells) with no epithelial pearls.

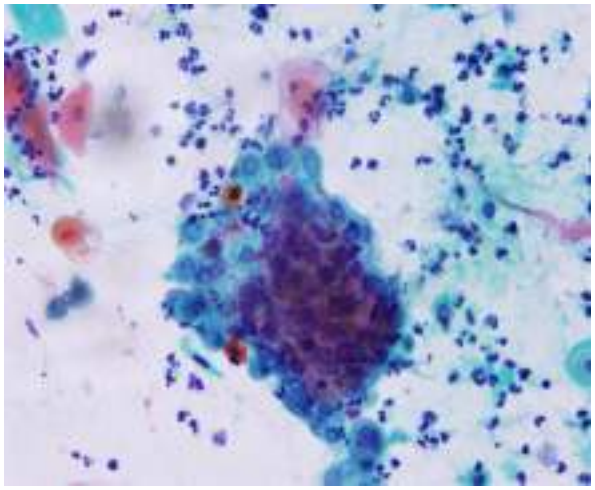
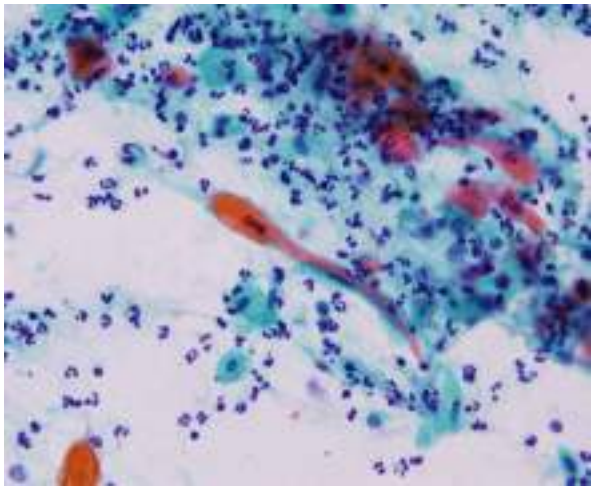
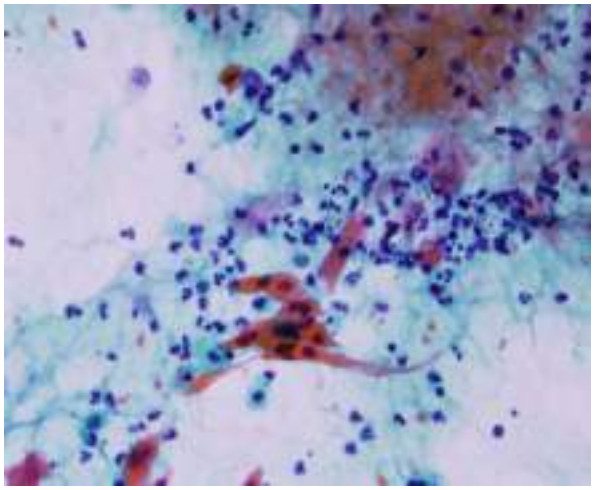
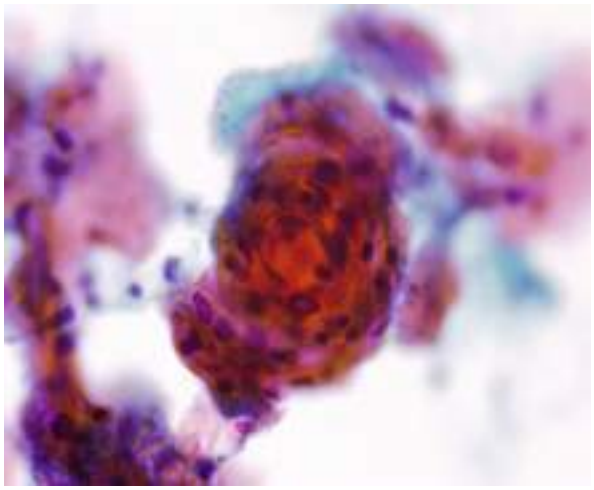
**Differential diagnosis:** Non keratinizing SCC vs. repair **(Figure 5.6)**



- Non keratinizing SCC has syncitial groups with overlapping, crowded cells and many single cells with tumour diathesis. More nuclear hyperchomasia is seen with granular and irregular chromatin.

**Keratinizing SCC, well differentiated**

- Clean background. (Tumour diathesis when present is usually less than in non-keratinizing SCC)
- Few to many abnormal cells, as isolated single cells or as syncytial aggregates.
- Relatively large cells with prominent pleomorphism. Bizarre cells (caudate, spindle, snake or tadpole shaped cells) containing dense orangeophilic cytoplasm. (Figure 10.4 a,b,c) Dyskeratotic epithelial pearls may be seen. (Figure 10.4d) The cytoplasm may be cyanophilic.

- Marked nuclear size variation with nuclear membrane irregularity. Numerous dense opaque nuclei may be present. Chromatin pattern is coarsely granular and is irregularly distributed with parachromatin clearing. Macronucleoli are less common than in non-keratinizing SCC. It may be difficult to appreciate coarse chromatin and nucleoli due to nuclear pyknosis**. (Figure 10.4e)**
- Associated with kerototic changes (hyperkeratosis, parakeratosis or atypical parakeratosis)

**Differential diagnosis of keratinizing SCC:** Keratinising SIL/dyskaryosis and atypical parakeratosis

**In keratinizing SCC Vs keratinising SIL/dyskaryosis**

- SCC is more cellular with more prominent plaeomorphism, coarse irregular chromatin, macro nucleoli, syncytial aggregates and tumour diathesis (when present).

**Keratinizing SCC Vs atypical parakeratosis**

- In atypical parakeratosis individual cells appear atypical but are small than carcinoma cells. **(Figure 5.15d)**

# Chapter 11 - Abnormal glandular cells

Abnormal glandular cells in a smear originate from the endocervix, endometrium or from the ovaries.

**Endocervical lesions:**

Abnormal changes of endocervical cells include,

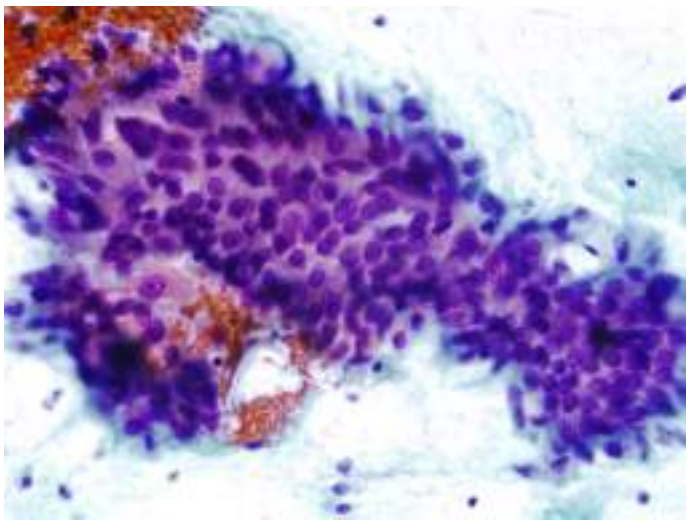
- Reactive changes – (discussed in chapter 5 under reactive changes in endocervical cells)
- Atypical changes (included under the category of atypical glandular cells of uncertain significance – AGUS)
- In situ endocervical lesions - Adenocarcinoma in situ (AIS) known as Cervical glandular intraepithelial neoplasia (CGIN) in the BSCC classification.
- Invasive endocervical adenocarcinoma

**Adenocarcinoma in situ (AIS/CGIN):**

**Architectural features**

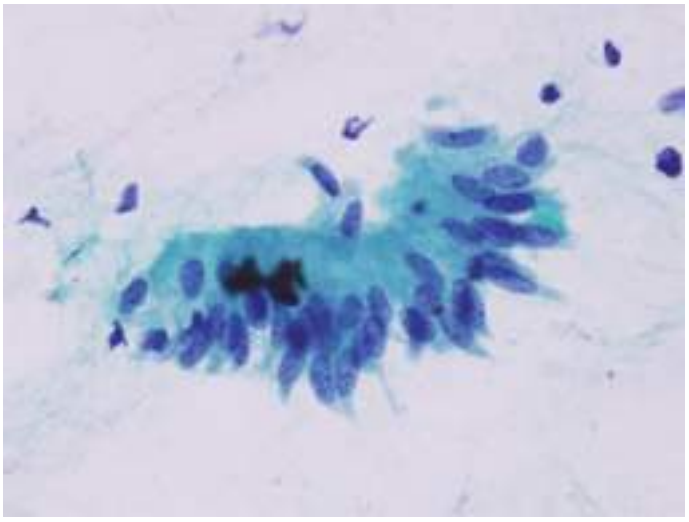
AIS/CGIN often associated with HGSIL/dyskaryosis

- Exfoliated pattern – Cohesive sheets and tissue fragments are present in a clean back ground. Tumour diathesis is rare.
- Dissociated cells are few unlike in HGSIL
- The honeycomb pattern is lost. Hyperchromatic crowded groups (2 layers thick) are present with cell crowding and nuclear overlapping. **(Figure 8.6)**

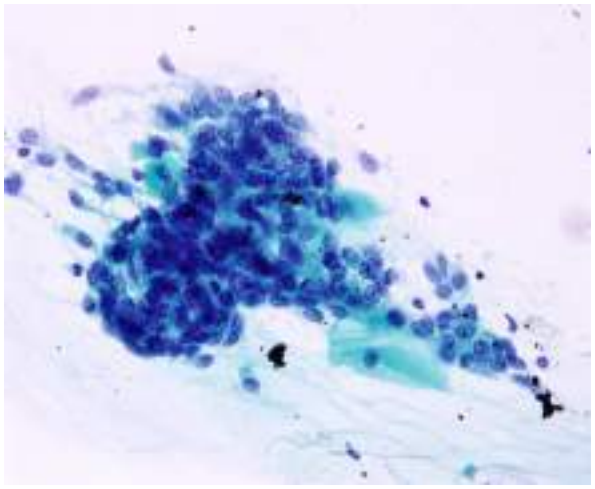


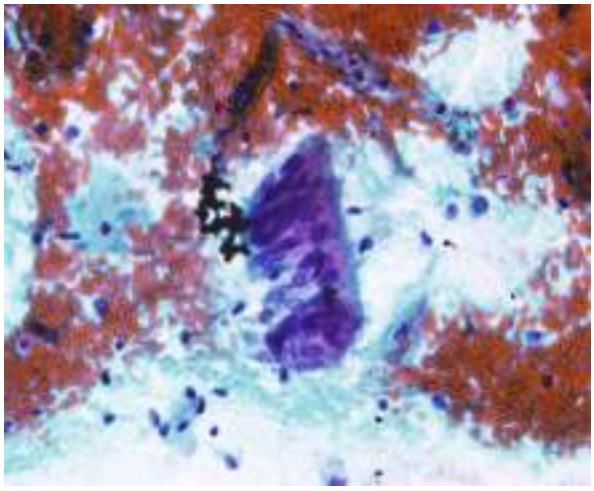
- Crypt openings are seen with in the cell groups.

- Edges of these cell groups show nuclear feathering, nuclear palisading and pseudo stratification. **(Figure 11.1)**

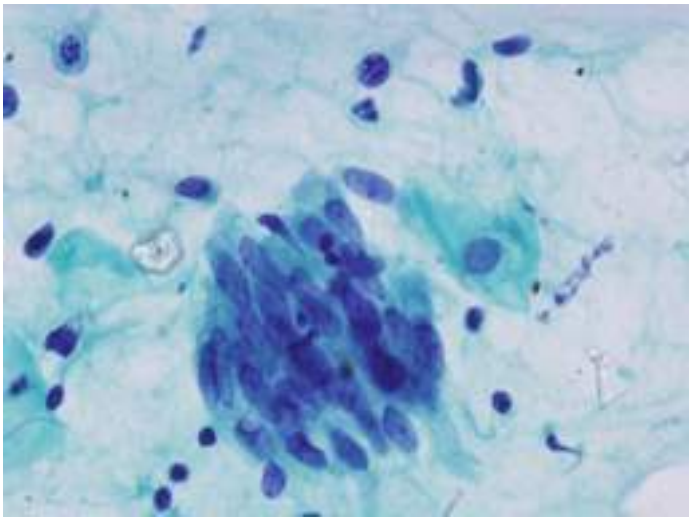


- Rosette formation **(Figure 11.2)** and strips of broken glands may also be seen. **(11.3)**

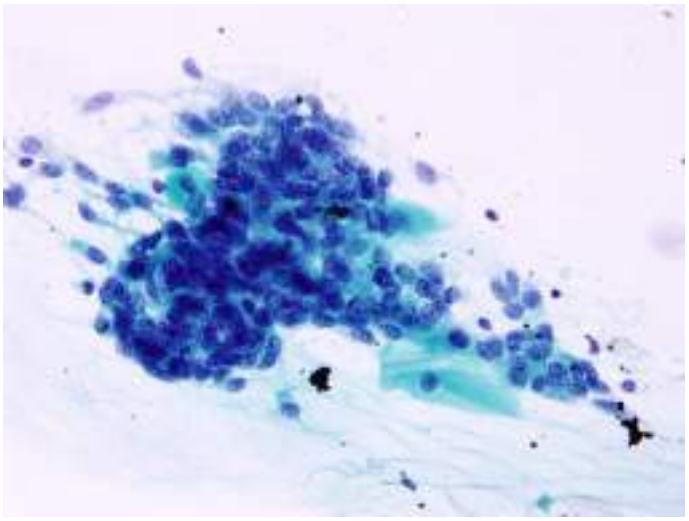




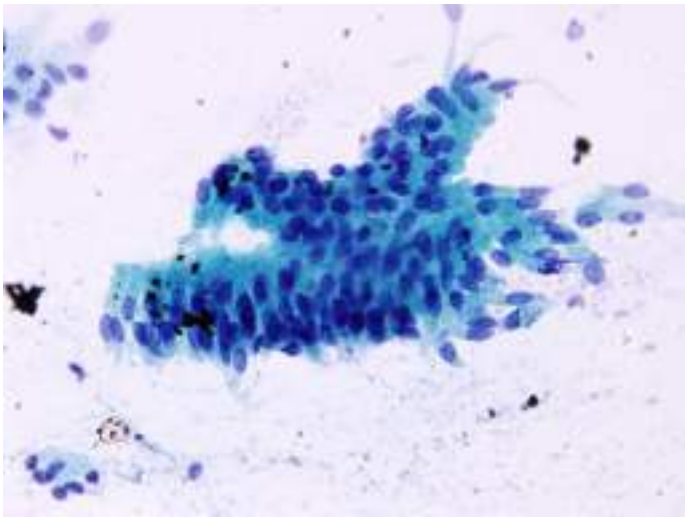
**Cytological features (Figure 11.4)**



- Nuclei are larger than in benign epithelial cells.
- Difficult to assess the N/C ratio in tissue fragments. The overall N/C ratio may be normal As the cytoplasm increases with the nuclear size.
- Elongated (oval)/spindle shaped nuclei. Variation in nuclear size between sheets of cells (with in a sheet nuclei are relatively uniform) with hyperchromasia and smooth nuclear membranes.
- Chromatin is often coarse/stippled giving rise to a speckled salt and pepper appearance **(Figure 11.2)**



- Inconspicuous nucleoli with normal mitosis.
- Scanty ill-defined cytoplasm is delicate with depleted mucin.
- Cytoplasmic tags may be seen at the edges of cell groups **(Figure 11.5)** with occasional goblet cells.

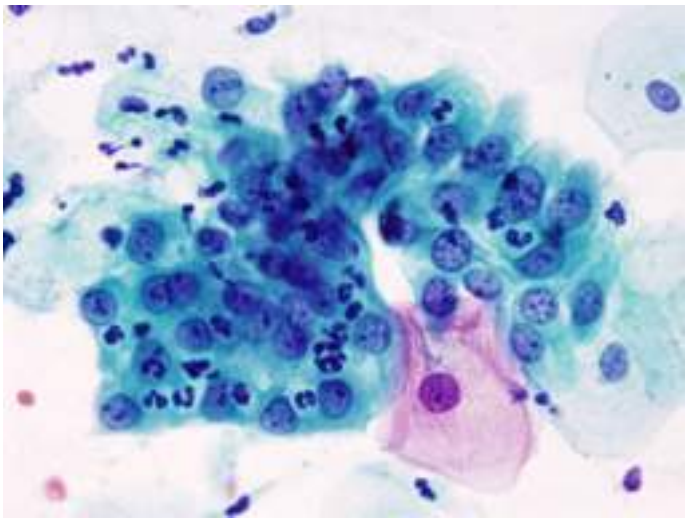


**Adenocarcinoma in situ (AIS) – Differential diagnosis**

- **Endocervical brush samples**

  Nuclear crowding is less marked in endocervical brush samples; however the size variation is greater. Generally, the nuclei are less hyperchromatic and do not line up at the edges of cell groups.

- **Cervicitis (Figure 5.4)**



  Cells are usually arranged as a monolayer and at times in small clumps, less than two cells in thickness. Neutrophils are often intermingled with the cell groups. Cytological differential diagnosis is usually with poorly differentiated adeno or squamous carcinoma.

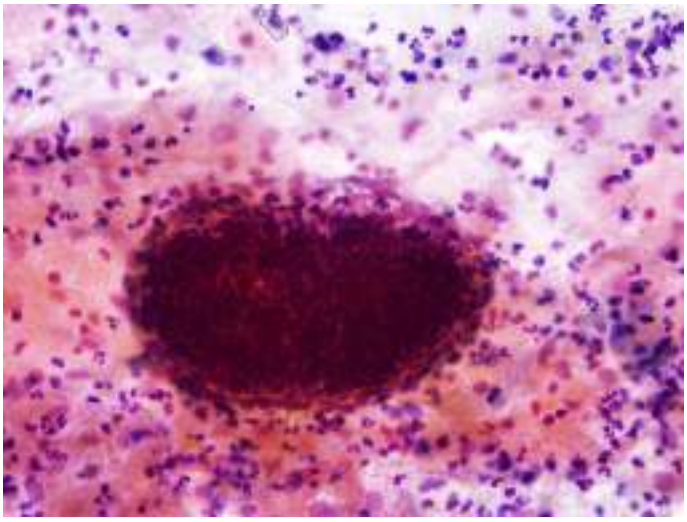
- **Lower Uterine Sample (LUS) (Figure 7.4)**

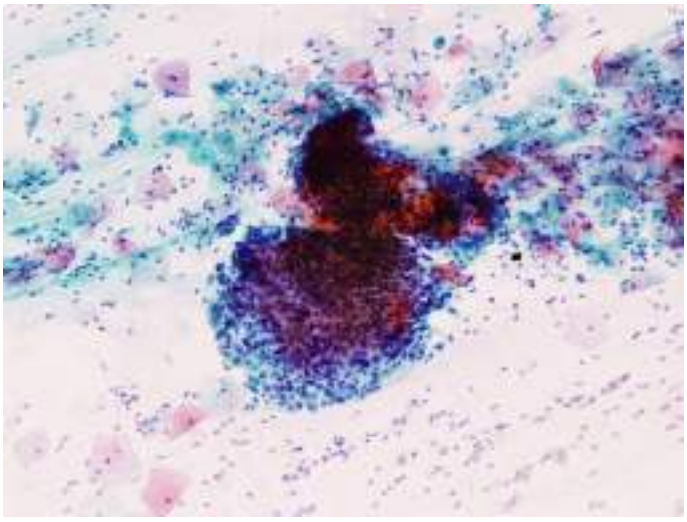
  Epithelial and stromal components form a biphasic pattern. Epithelial sheets have crowded round nuclei with tubular groups. Nuclei have characteristic coarse, open chromatin. Stromal groups have oval nuclei with capillaries within the cell groups. Stromal cells are often crushed with dissociated epithelial and stromal nuclei.

**Differential diagnosis:** AIS vs. LUS

| | AIS | LUS |
|---|---|---|
| Peripheral palisading | ++ | ++++ |
| Honeycomb sheet | ++ | ++++ |
| Nucleoli/mitosis | ++++ | ++ |
| Ciliated cells/terminal bar | +/- | ++ |
| Rosette | ++++ | + |
| Strips of broken gland | ++ | - |
| Nuclear feathering | ++++ | - |

- **Endometriosis**
- **Endometrial shedding (Figure 2.6a and b)**

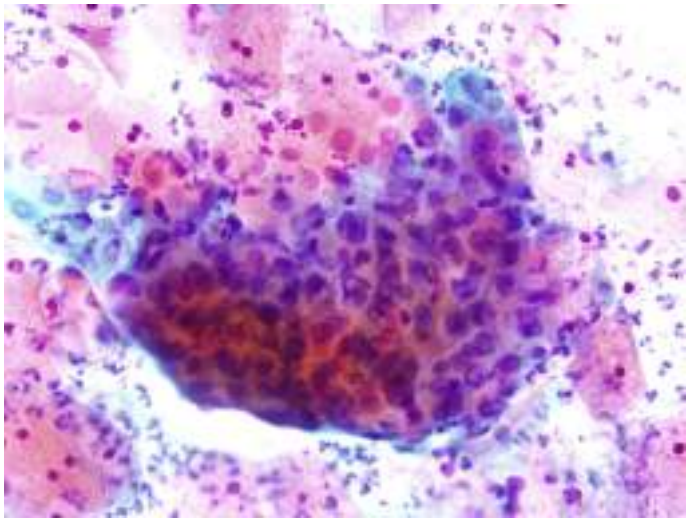




- **Tubal metaplasia**

  Form tight cell clusters with nuclear overlapping. Three cell types, ciliated columnar, goblet cells and intercalated cells may be seen with rosettes, nuclear stratification or nuclear feathering. Nuclei may be enlarged and hyperchromatic. Cilia appreciable in well preserved cells with terminal bars strongly favour a benign diagnosis.

**Atypical endocervical cells NOS/(AGUS endocervical) (Figure 11.6)**



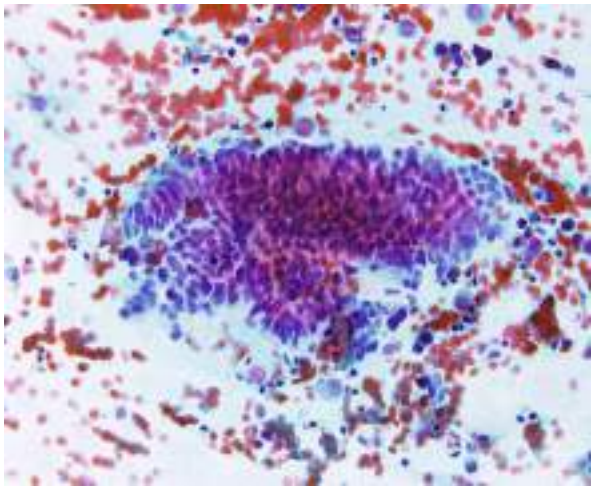
These are included under the category of Atypical Glandular cells of uncertain significance (AGUS) in the Bethesda system.

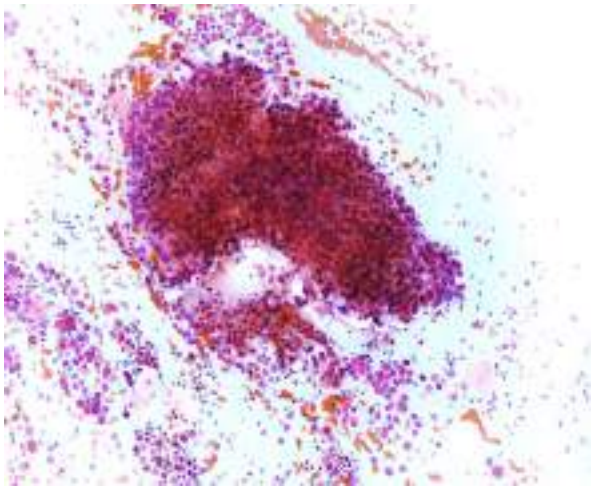
- Sheets and strips of cells with cell crowding and nuclear overlapping.
- Variation in nuclear size and shape with enlarged nuclei (up to 3-5 times), mild hyperchromasia and nucleoli.
- Mostly cohesive cells with uniform nuclei with smooth nuclear membranes and regular chromatin.

**Atypical endocervical cells NOS/(AGUS endocervical) - Differential diagnosis**

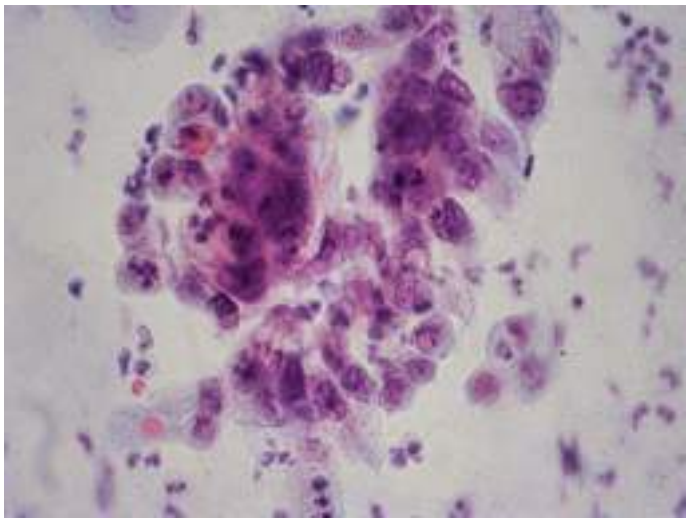
- Inflammatory changes in endocervical cells
- Squamous lesions involving the endocervix Eg. HGSIL extending in to gland ducts
- Tubal metaplasia
- Micro glandular hyperplasia
- Endometrial cells: LUS or endometriosis
- Radiation change
- Mesonephric duct remnants
- Aria stella reaction

**Invasive adenocarcinoma (IAC): endocervix (Figure 11.7a,b)**





- More material with super crowded sheets/clusters and single cells with a columnar configuration.
- Fewer rosettes and strips
- Super crowded sheets show nuclear crowding and irregular nuclear overlapping
- Similar cytological criteria as AIS, thus difficult to discriminate. However nuclei are more hyperchromatic and round. Presence of single cells suggest invasion.
- Nucleoli are often present
- Fine cytoplasmic vacuoles are present in low grade lesions.
- Inflammation and less often tumor diathesis is seen in the back ground.
- In later stages irregular sheets of pleomorphic cells with large nuclei with maldistributed chromatin, prominent nucleoli and frequent mitosis are found. Background consists of blood, inflammation and necrotic cellular debris.
  **(Figure 11.7c)**



**In situ vs. Invasive adenocarcinoma of cervix**

| | AIS | IAC |
|---|---|---|
| Presence of normal endocervical cells | ++++ | ++ |
| Necrotic background | - | +++ |
| Monolayer sheets | ++++ | + |
| Atypical single cell / naked nuclei | + | +++ |
| Nucleoli | - | +++ |
| An associated squamous lesion | +++ | - |
| Tissue fragments with irregular borders | + | ++++ |

**Invasive adenocarcinoma (IAC): endocervix – Differential diagnosis**

- **Poorly differentiated squamous cell carcinoma**

  Have a higher proportion of single cells with no evidence of glandular architecture. The nuclei are more pleomorphic, have a more disorderly arrangement of cells in groups, with coarser chromatin. Tumour diathesis is more commonly seen, than with poorly differentiated adenocarcinoma.

- **Endometrial adenocarcinoma**

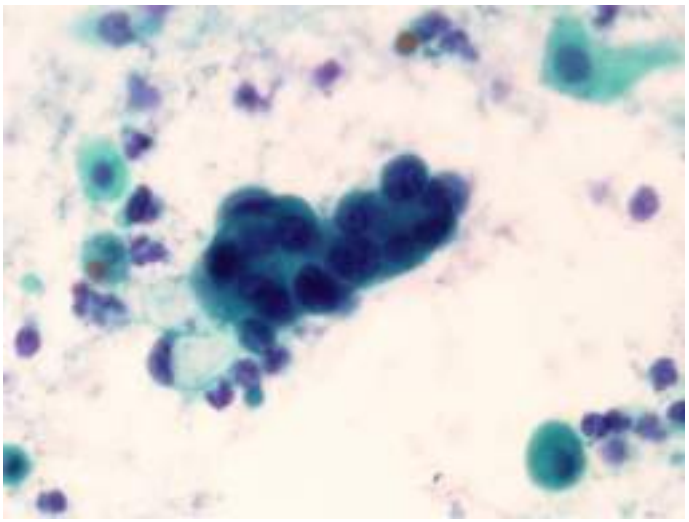
  Usually fewer exfoliated cells. Mostly in small aggregates of 4 to 6 cells, forming acinar or papillary groupings. The cells are smaller and are more hyperchromatic. Tumour diathesis is more frequently seen. Lesions involving the endocervical canal may be difficult to differentiate from cervical adenocarcinoma.

- **Small cell carcinoma of neuroendocrine type**

  Syncytial cell groups with marked crowding and disorderly arrangement with no glandular architectural features. Nuclei are hyperchromatic with very coarse irregular chromatin. Dissociated nuclei are common. Main differential diagnosis is with endometrial shedding and endometrioid variant of endocervical carcinoma.

## Endometrial lesions

### Atypical endometrial cells NOS/(AGUS endometrial) (Figure 11.8a)



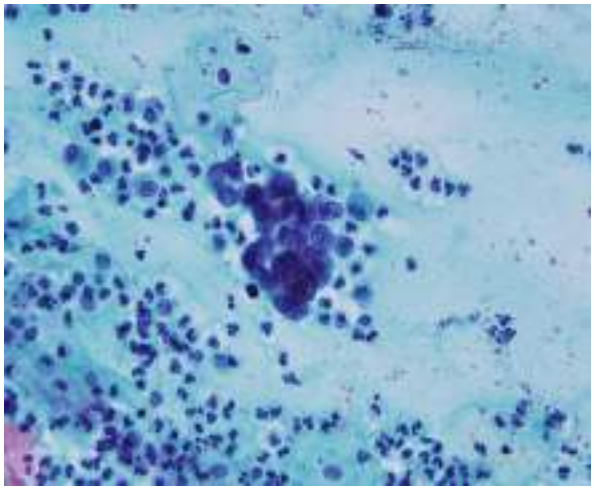
It is difficult to diagnose due to small numbers of cells and poor preservation following exfoliation. Similarities exist between benign, hyperplastic and well-differentiated carcinomas and correlation between cytology and histology are usually poor.

Endometrial cells when present in a cervical smear of a woman above 40 years of age should be reported.

### Endometrial adenocarcinoma

The sensitivity of detecting endometrial adenocarcinoma in a cervical smear is only about 50%

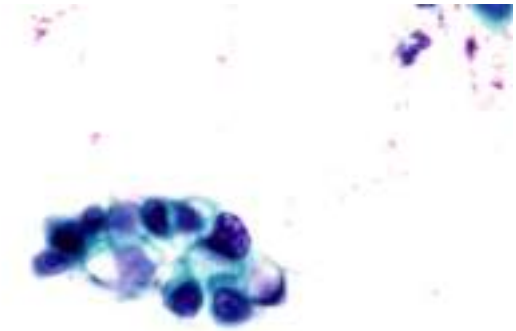
- Often of low cellularity.
- Background has a finely granular or watery tumour diathesis. (**Figure 11.8b**) (Compare with the coarsely granular necrotic tumour diathesis of SCC) **(Figure 52a)**



- Tight cell clusters or single cells with increase in cell and nuclear size. With increasing grade of the tumour the nuclei become larger.

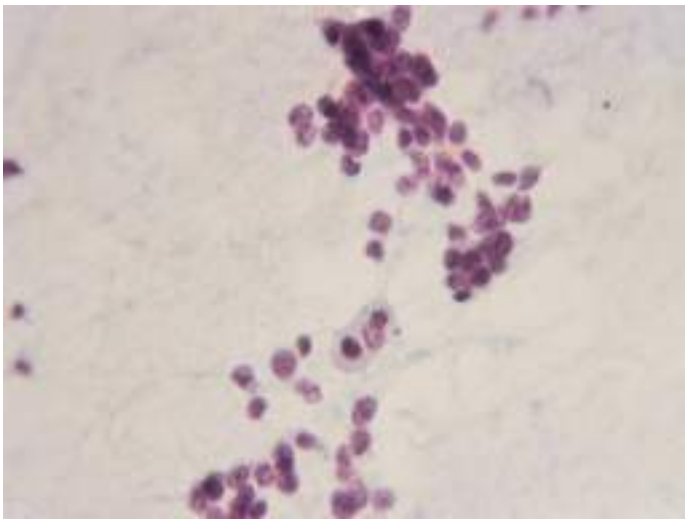
- Nuclei show size variation, loss of polarity, moderate hyperchromasia, irregular chromatin distribution and prominent nucleoli. They may be hypochromatic.

- Scant cyanophilic cytoplasm, often with vacuoles and intracytoplasmic neutrophils. **(Figure 11.8c)**

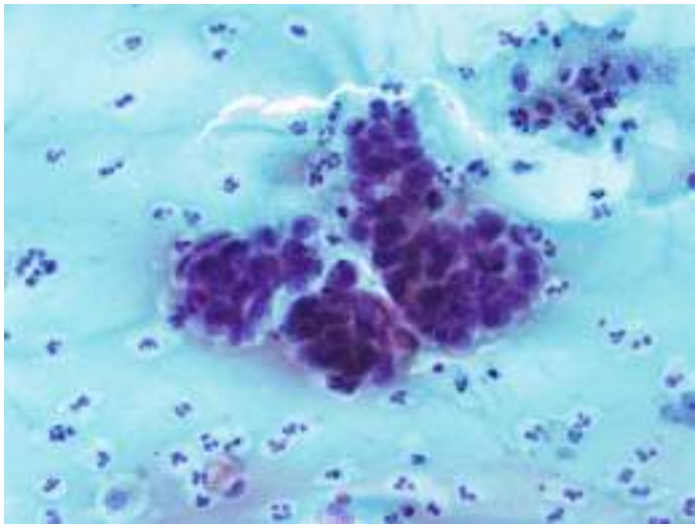


- Pyknotic debris with cyanophilic or eosinophilic cytoplasmic fragments.

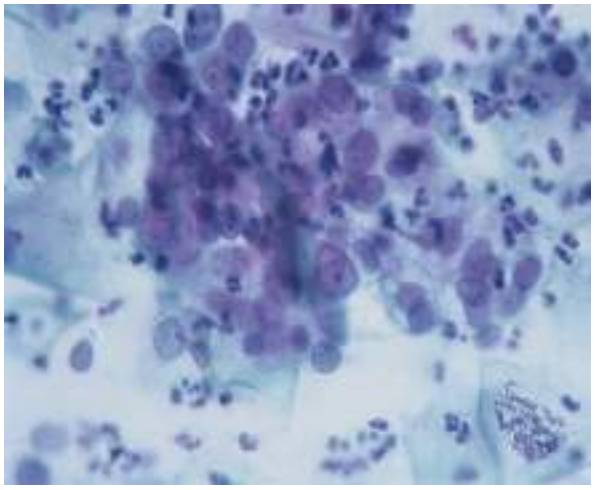
- Low-grade lesions **(Figure 11.8d)** – loose cell clusters, lightly staining nuclei, less prominent nucleoli.

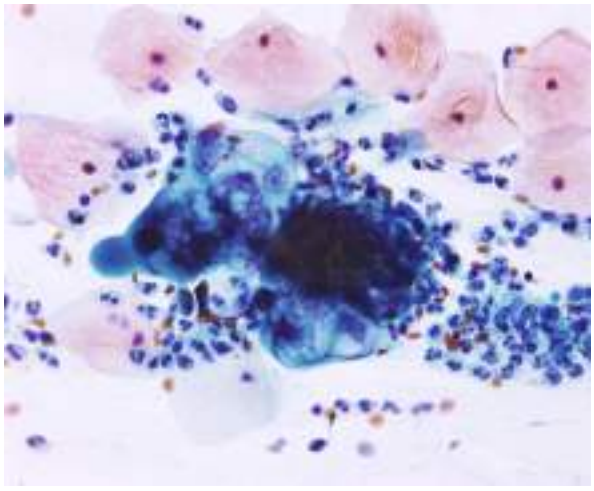


- High grade lesions **(Figure 11.8e)** - Tighter cell balls resembling exodus or sheets resembling SCC non keratinizing. Nuclei are more hyperchromatic with abnormal chromatin and multiple irregular macro nucleoli. (SCC non keratinizing has denser cytoplasm with better defined cell borders, coarser chromatin and less prominent nucleoli).



**Adenocarcinoma – Extra uterine (Figure 11.9a and b)**



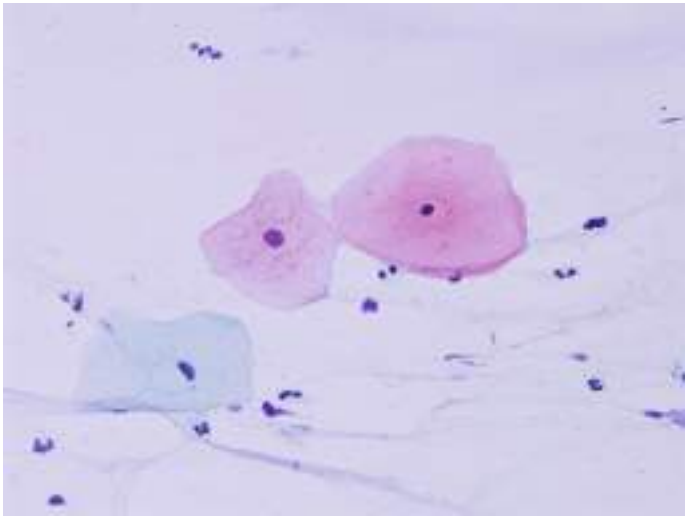


- When cells compatible with an adenocarcinoma occur in association with a clean back ground or with morphology unusual for tumours of the endometrium or cervix, an extra uterine neoplasm (ovary, fallopian tubes) should be considered.
- Cells are fewer in number.

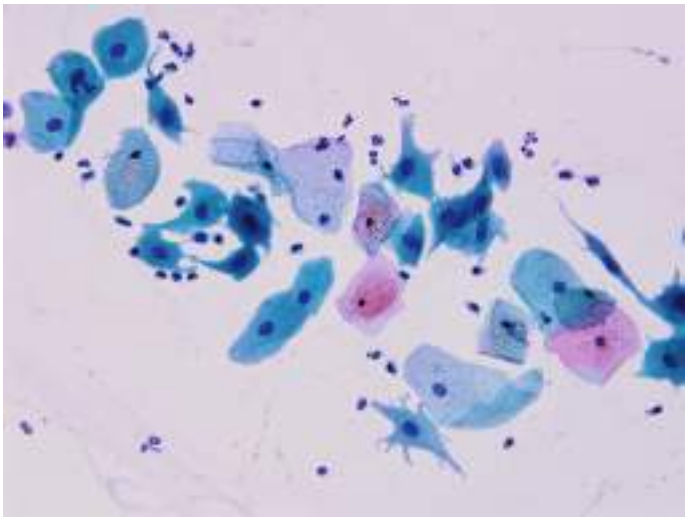
# Chapter 12

**Comparisons**

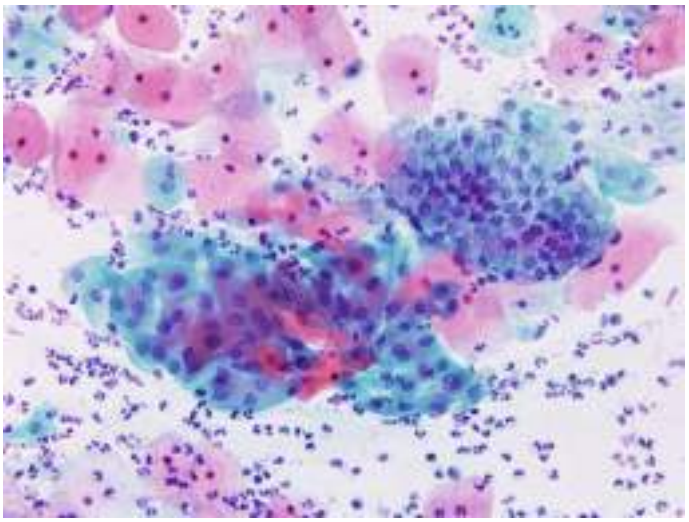
1. Intermediate and superficial squamous cell **(Figure 12.1)**



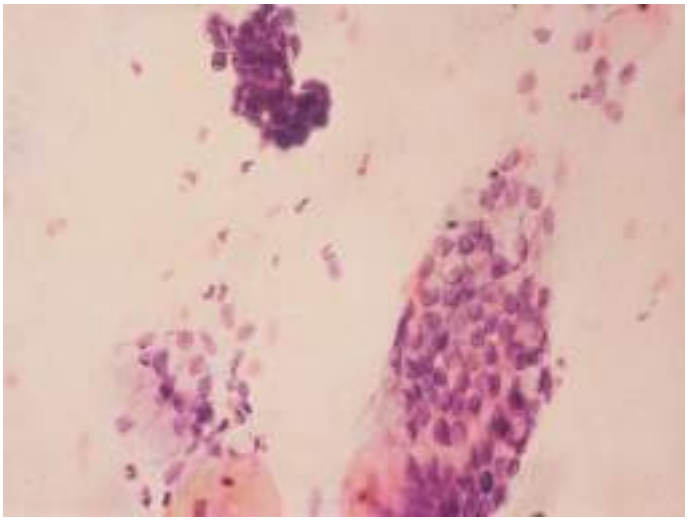
2. Superficial, intermediate and metaplastic squamous cells **(Figure 12.2)**



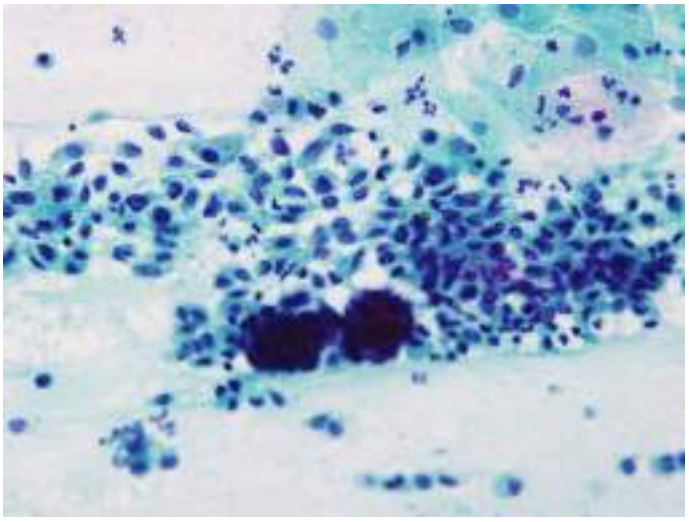
3. Endocervical and metaplastic squamous cell clusters **(Figure 12.3)**



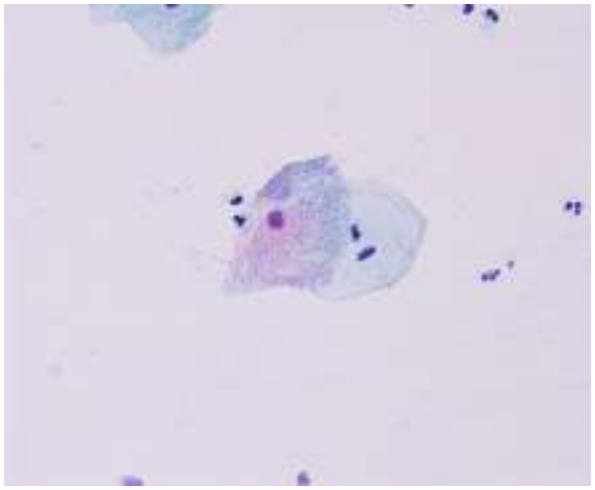
4. Endocervical and endometrial cells **(Figure 12.4)**

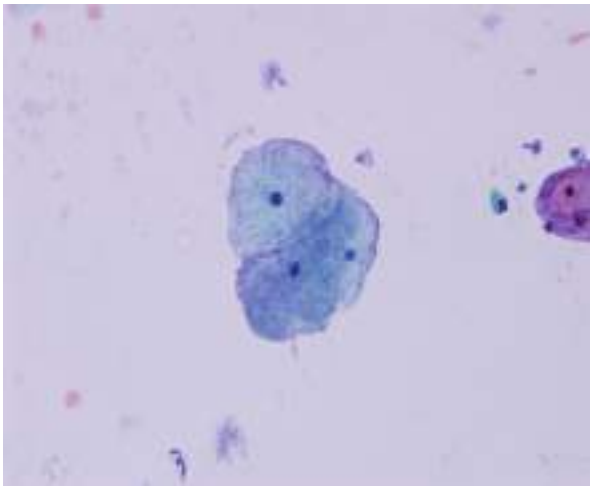


5. Endometrial glandular and stromal cells **(Figure 12.5)**



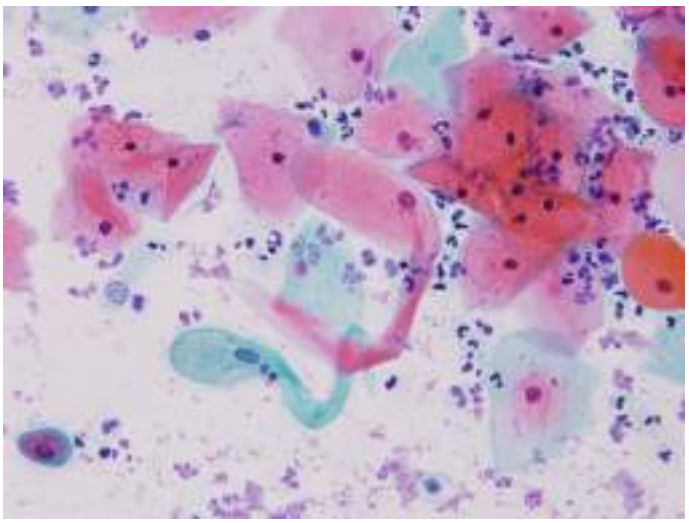
6. Bacteria coated cells **(Figure 12.6)**, compare with the clue cell in Figure 3.8a

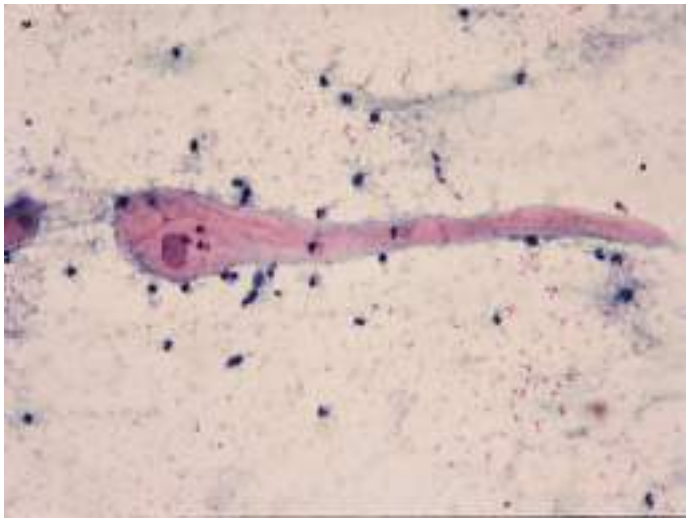




7. A benign elongated squamous cell **(Figure 12.7),** compare with malignant tadpole cells seen in squamous carcinoma **(Figures 14.4 b and c)** and post radiation **(Figure 5.7b) –** This was an isolated finding and probably due to mechanical distortion. In post irradiation smears and in smears with squamous carcinoma, the background cells show relevant radiation/dysplastic changes.

**Note:** Refer the appropriate text if you cannot identify the relevant cells/cell clusters separately.

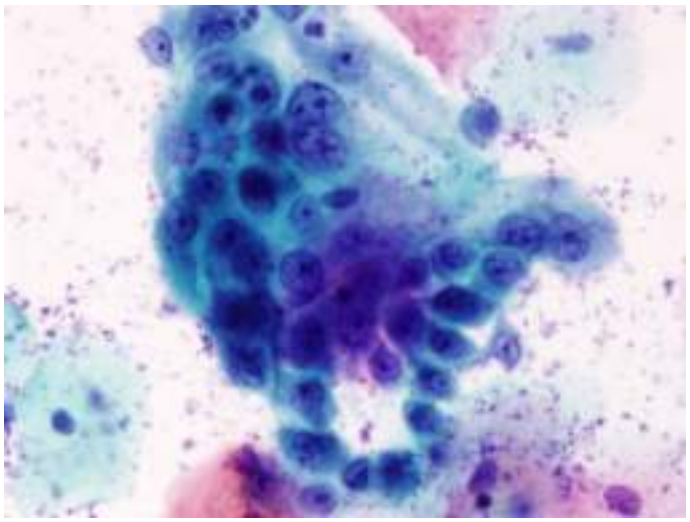




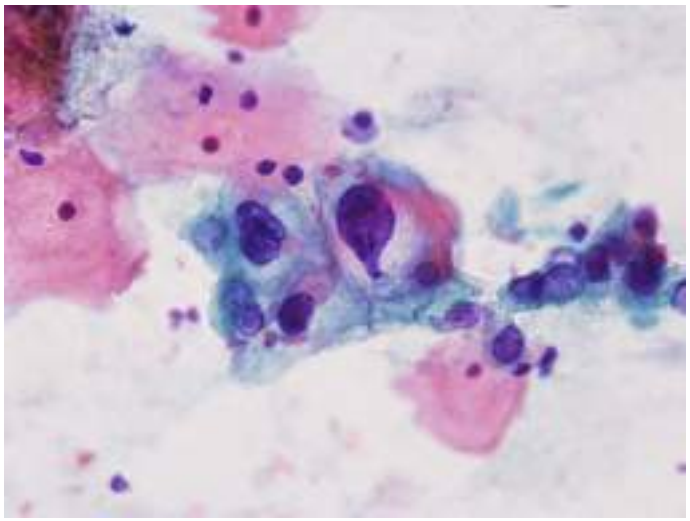
# Chapter 13

## Unknown cases for self assessment

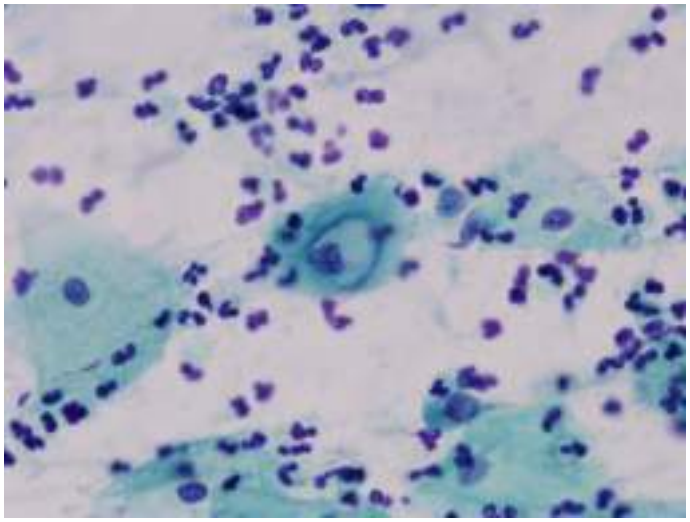
**Case 1 (Figure 13.1)** – Cervical smear



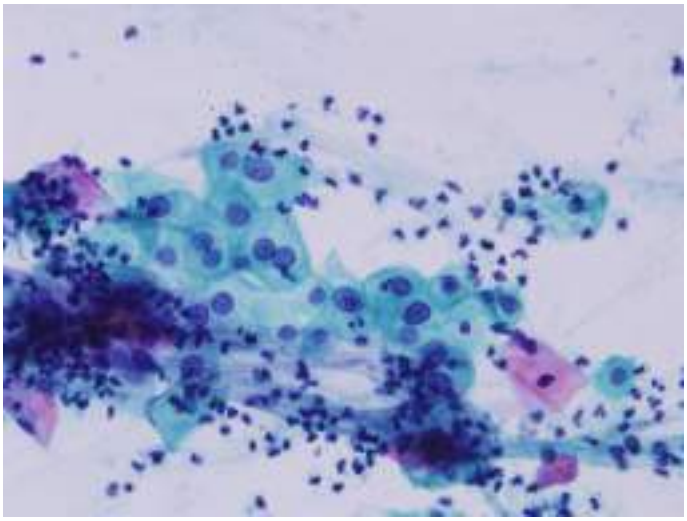
**Case 2 (Figure 13.2)** – Cervical smear



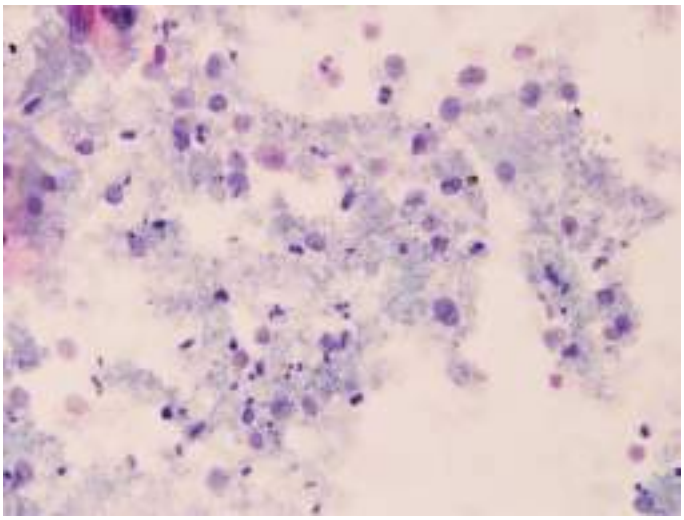
**Case 3 (Figure 13.3)** – Cervical smear



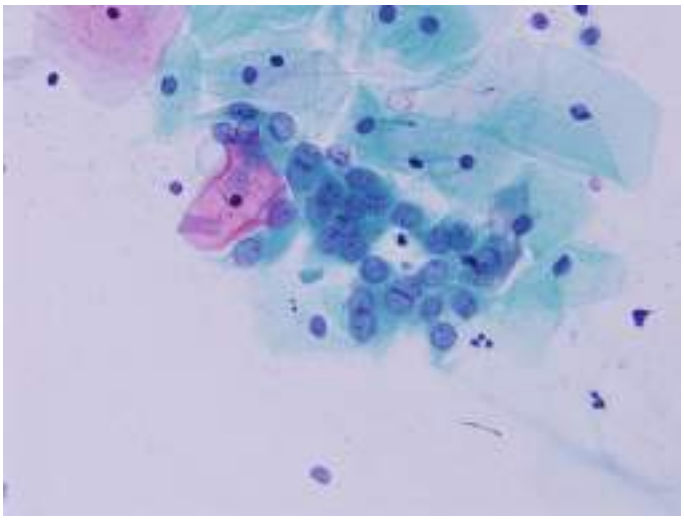
**Case 4 (Figure 13.4)** – Cervical smear



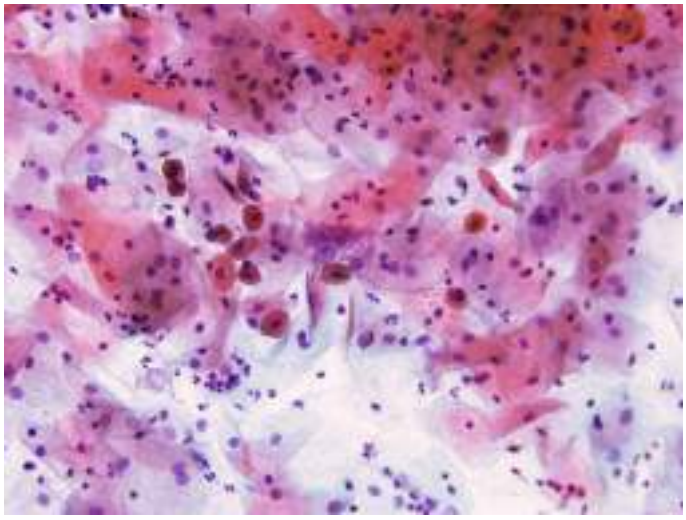
**Case 5 (Figure 13.5)** – Premenstrual cervical smear



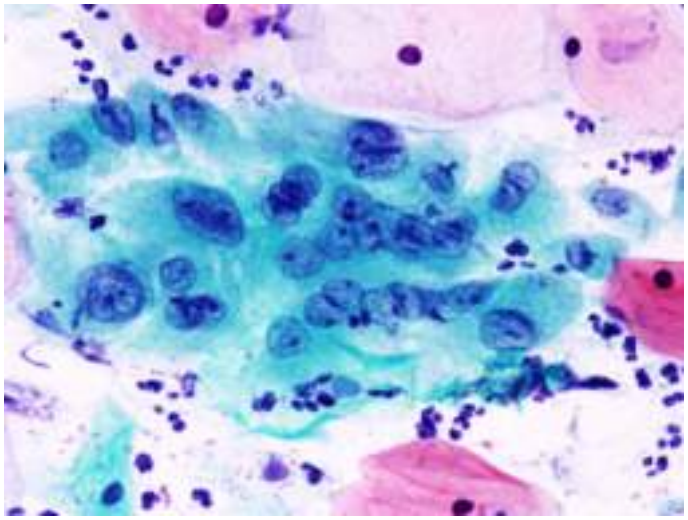
**Case 6 (Figure 13.6)** – Cervical smear



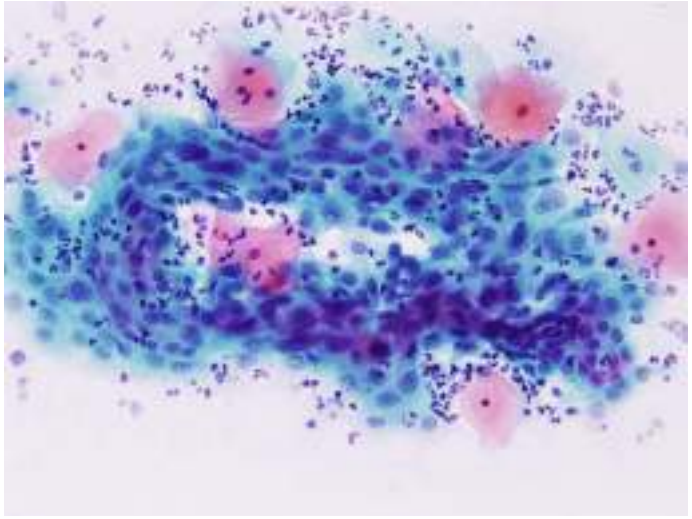
**Case 7 (Figure 13.7)** – Cervical smear



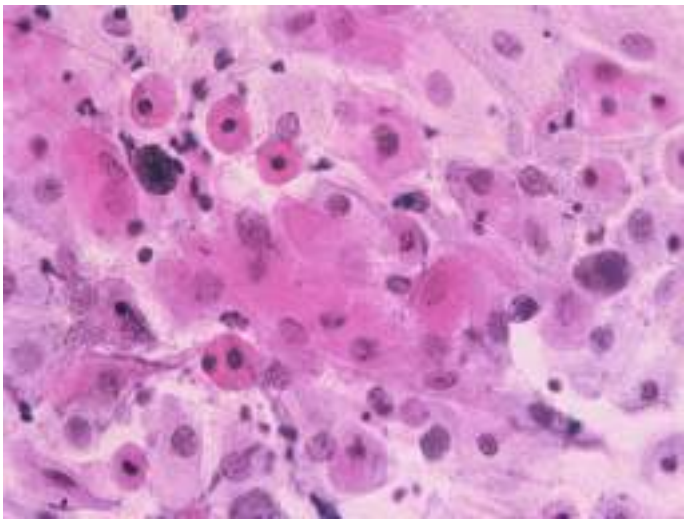
**Case 8 (Figure 13.8) –** Post partum smear at 7 weeks



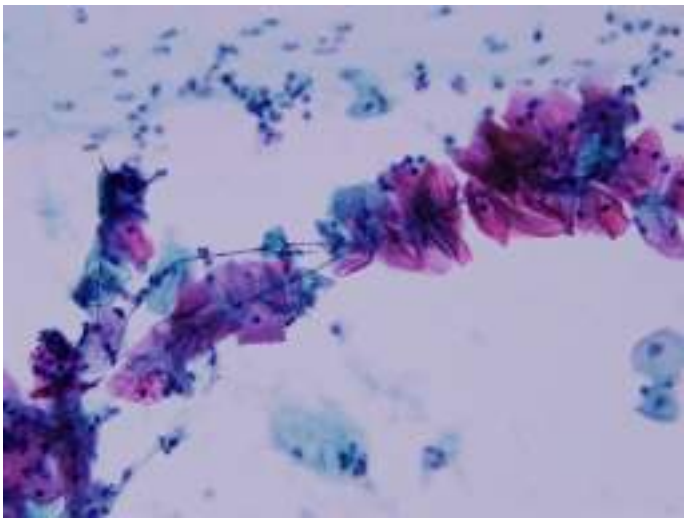
**Case 9 (Figure 13.9) –** Cervical smear following cone biopsy for HGSIL.



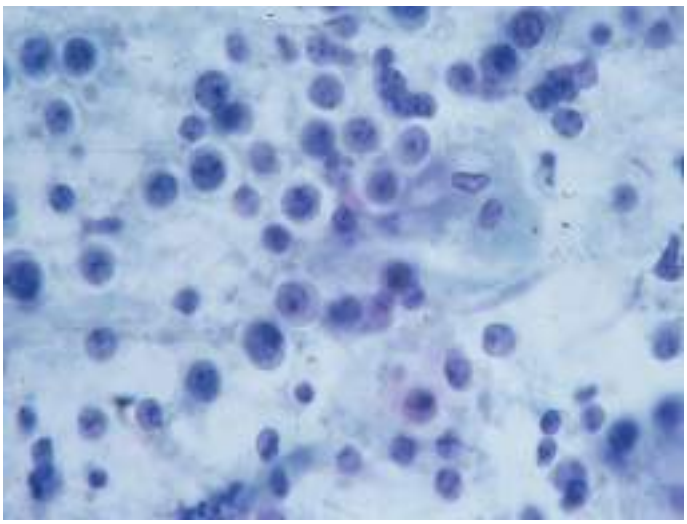
**Case 10 (Figure 13.10) –** Smear from a post menopausal woman



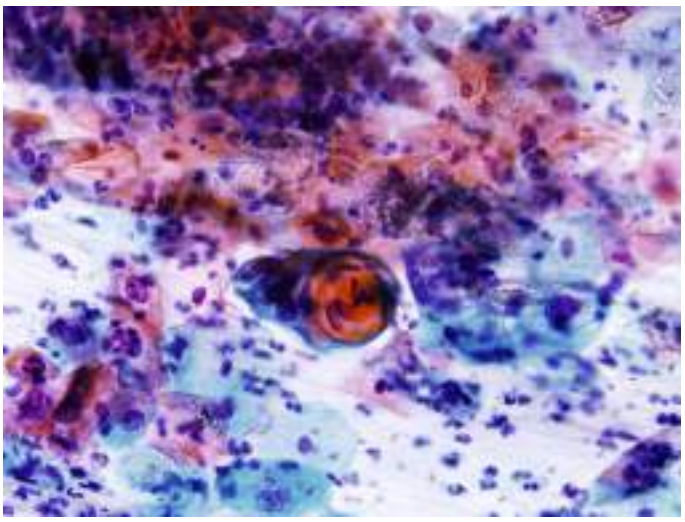
**Case 11 (Figure 13.11) –** Cervical smear in a woman with discharge



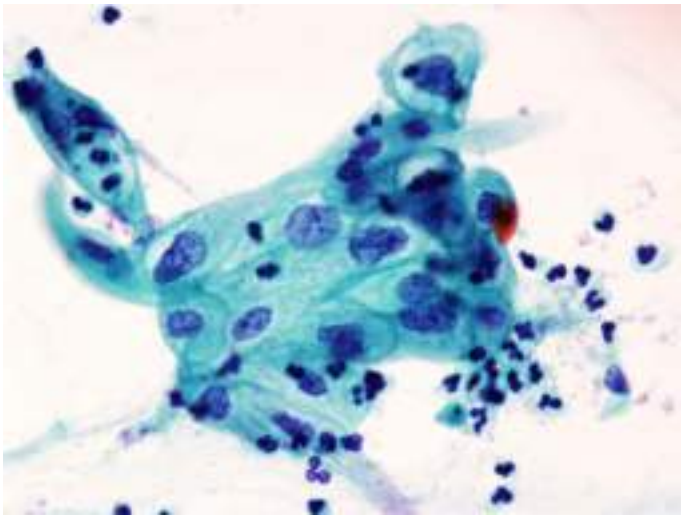
**Case 13 (Figure 13.12)** – Cervical smear



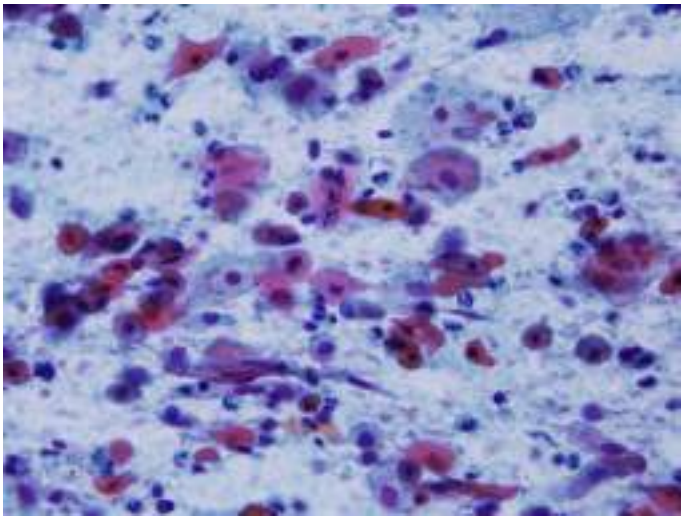
**Case 13 (Figure 13.13)** – Cervical smear



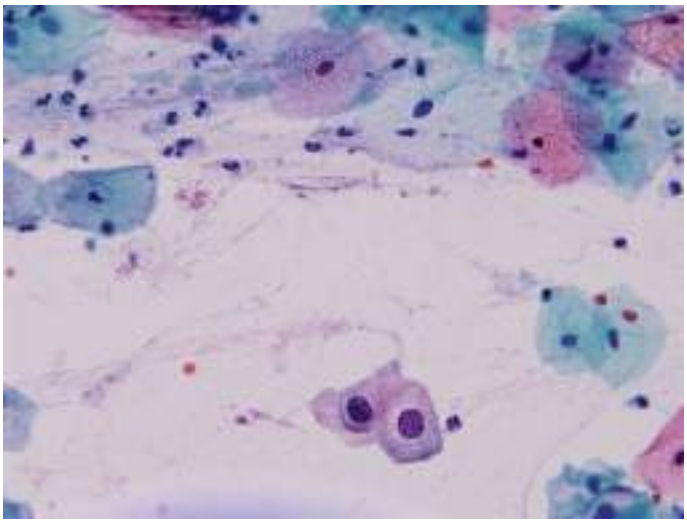
**Case 14 (Figure 13.14)** – Cervical smear



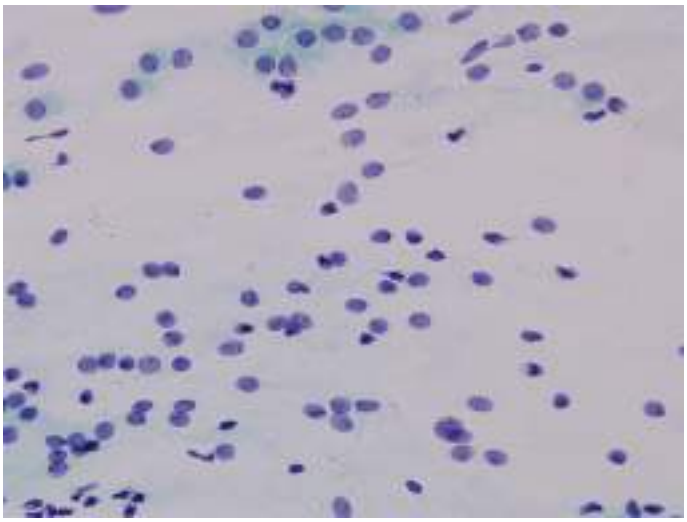
**Case 15 (Figure 13.15) –** Cervical smear from a post menopausal woman



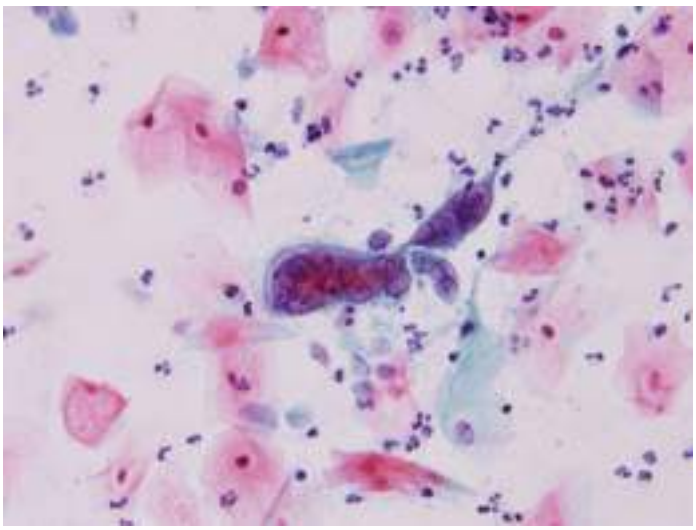
**Case 16 (Figure 13.16)** – Cervical smear



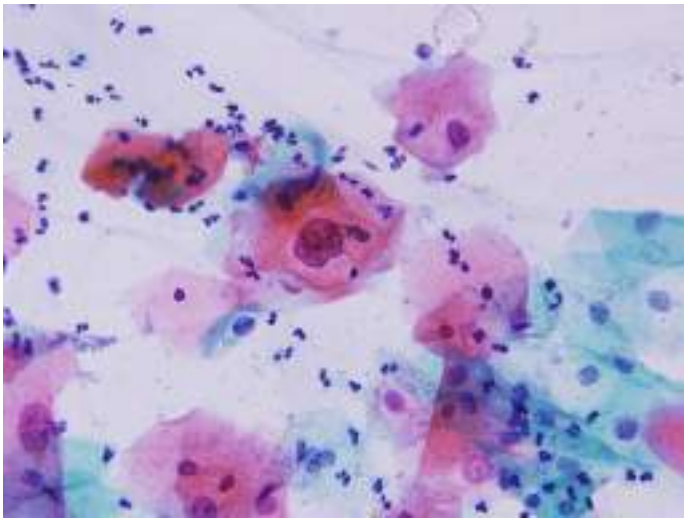
**Case 17 (Figure 13.17)** – Cervical smear



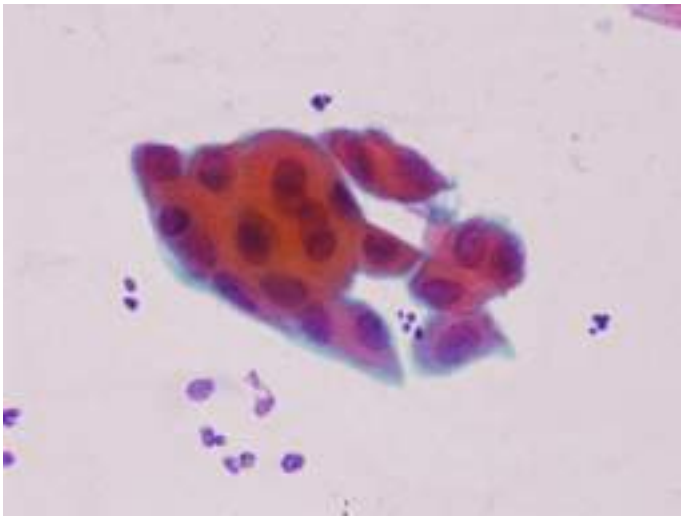
**Case 18 (Figure 13.18)** – Cervical smear



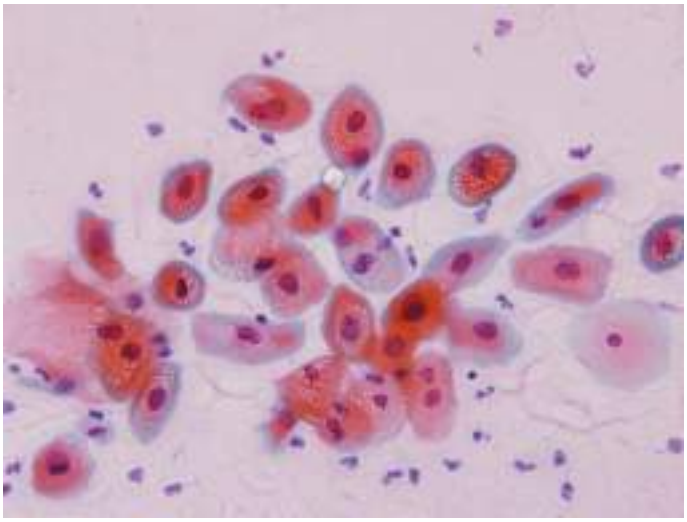
**Case 19 (Figure 13.19)** – Cervical smear



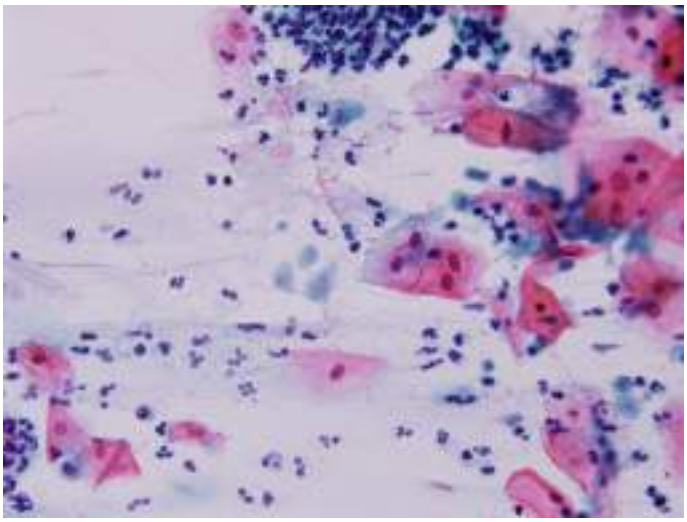
**Case 20 (Figure 13.20)** – Cervical smear



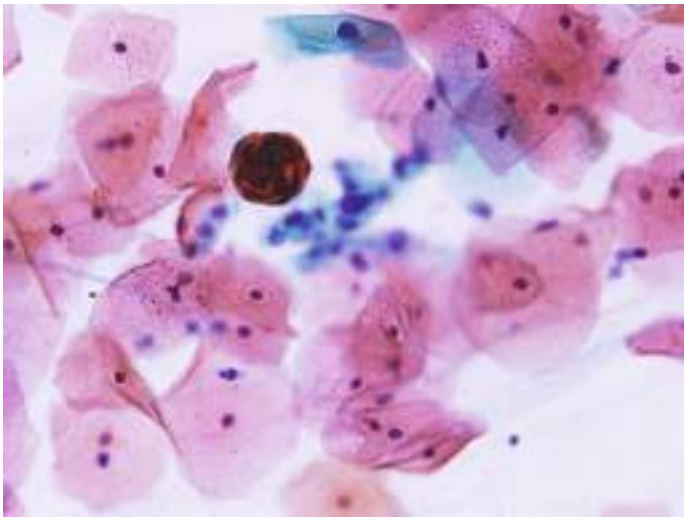
**Case 21 (Figure 13.21)** – Cervical smear



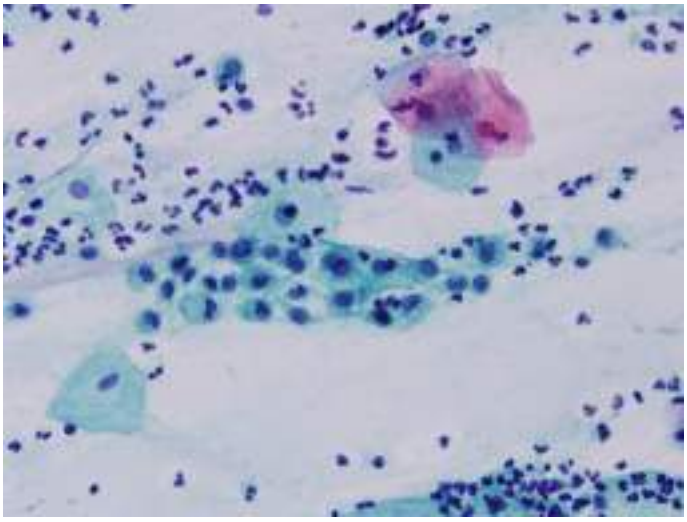
**Case 22 (Figure 13.22) –** Cervical smear in a woman with discharge



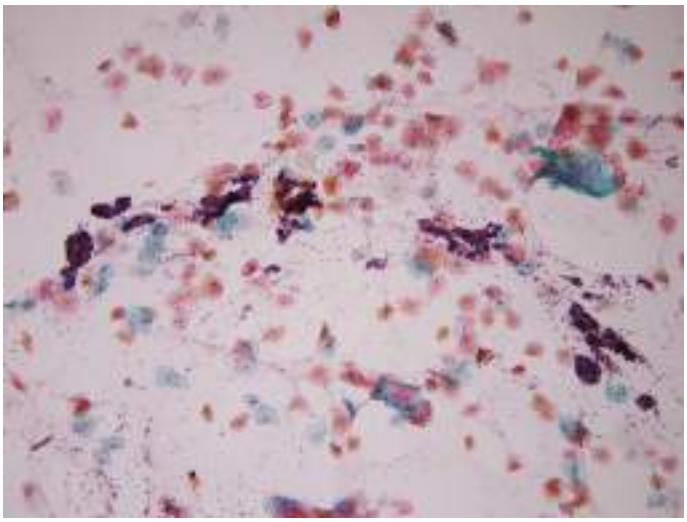
**Case 23 (Figure 13.23)** – Cervical smear



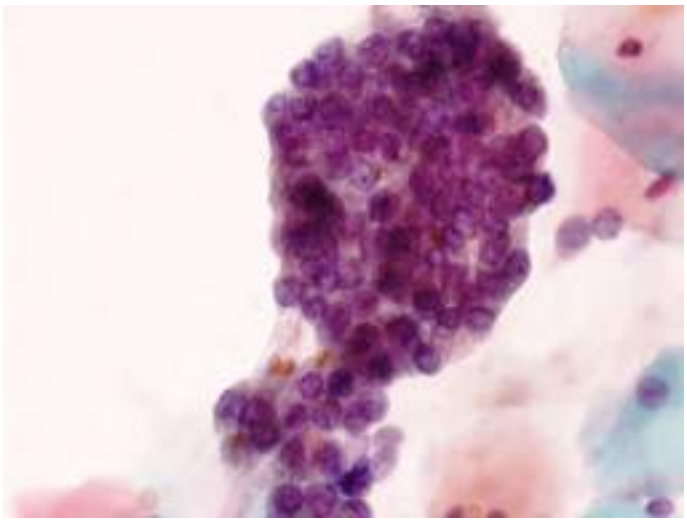
**Case 24 (Figure 13.24)** – Cervical smear



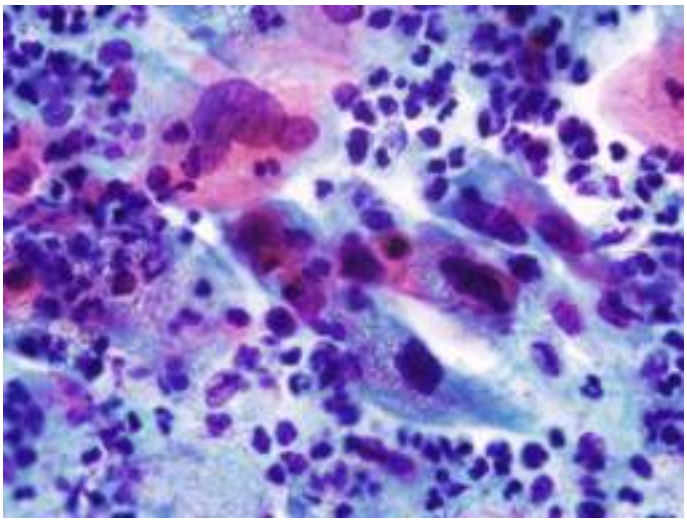
**Case 25 (Figure 13.25a)** – Cervical smear



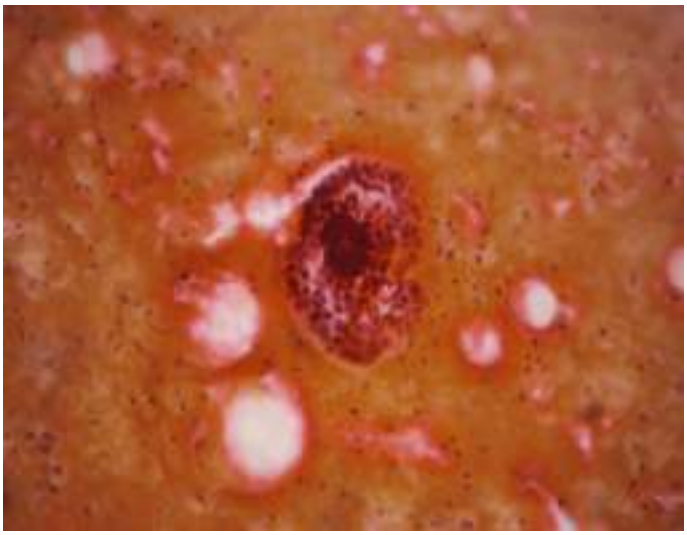
**Case 25 (Figure 13.25b)** – Cervical smear



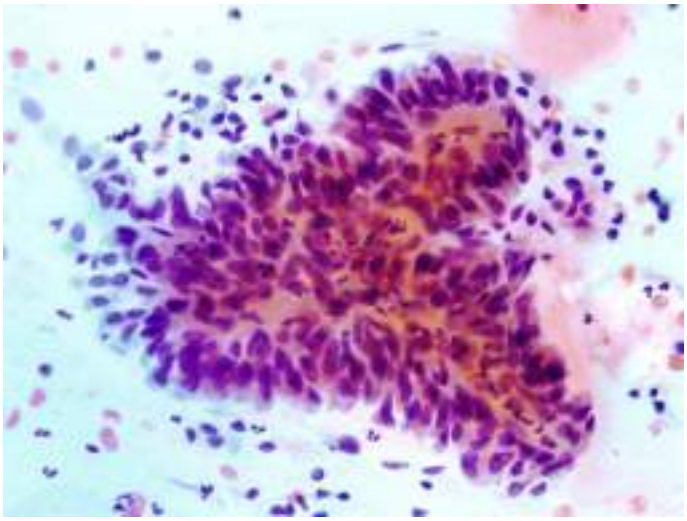
**Case 26 – (Figure 13.26) –** Post partum cervical smear at 6 weeks



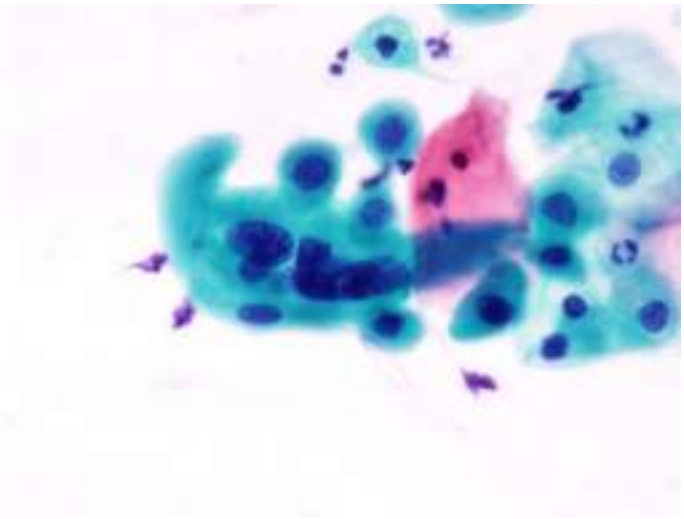
**Case 27 – (Figure 13.27) –** Cervical smear in a woman with uterine bleeding.



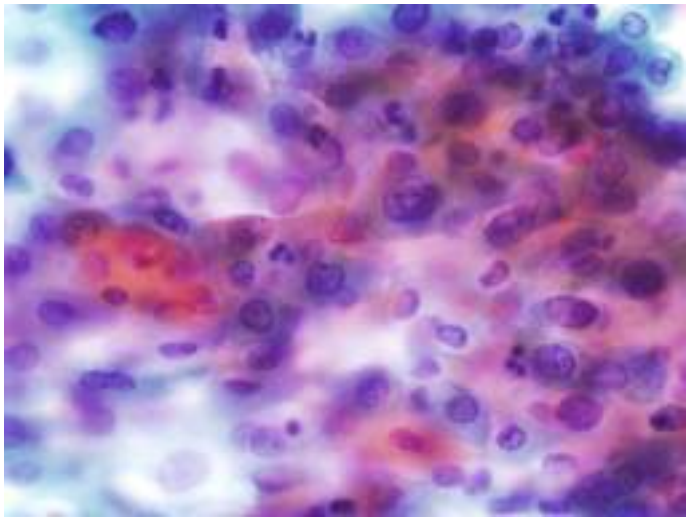
**Case 28 – (Figure 13.28)** – Cervical smear



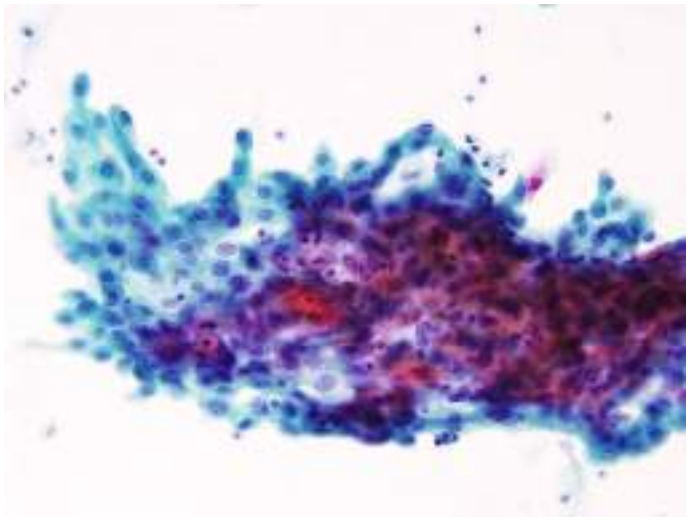
**Case 29 – (Figure 13.29)** – Cervical smear



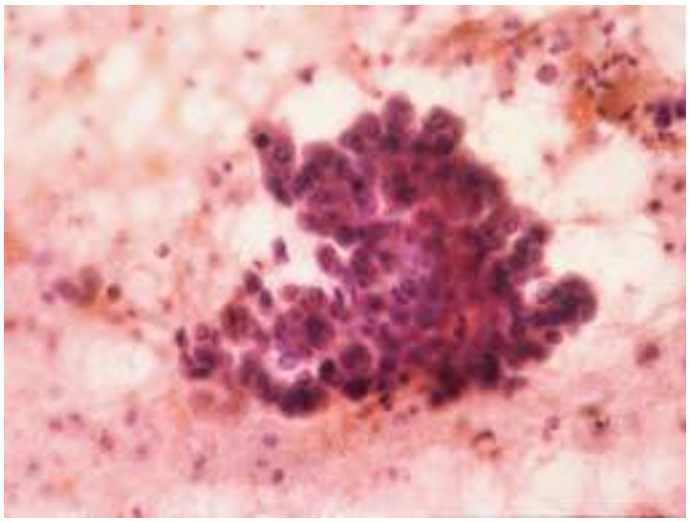
**Case 30 – (Figure 13.30)** – Cervical smear



**Case 30 – (Figure 13.30)** – Cervical smear



**Case 31 – (Figure 13.30)** – Cervical smear



# Answers

**Case 1 (Figure 13.1) – Reactive endocervical cells**

The nuclei vary size with nucleoli and minimally coarse chromatin, maintaining the columnar shape especially at the edge. The cell group is also not crowded.

**Case 2 (Figure 13.2) – HGSIL/Severe dyskaryosis**

Note the big dark nuclei as in LGSIL/mild dyskaryosis and vague perinuclear halos in the central cell cluster. Adjacent smaller cells however have a larger N/C ratio than would be expected in a LGSIL.

**Case 3 (Figure 13.3) – Koilocyte**

This koilocyte has a large clear well defined perinuclear halo, peripheral dense bluish cytoplasm and an enlarged nucleus with granular chromatin and a slightly wrinkled nuclear membrane. Nuclear hyperchromasia is not prominent.

**Case 4 (Figure 13.4) – LGSIL/Mild dyskaryosis**

The cells in the cluster are large, appear mature with abundant cytoplasm. Nuclei occupy less than half the cytoplasmic area with some binucleate forms. Nuclei are minimally hyperchromatic. Chromatin is fine with indistinct nucleoli.

**Case 5 (Figure 13.5) – Cytolysis**

Note the naked intermediate cell nuclei admixed with lactobacilli. Inflammatory cells are sparse in the background.

**Case 6 (Figure 13.6) – Reactive immature metaplastic cells**

A loosely cohesive sheet of cells with fairly well defined blue green, fragile cytoplasm. Nuclei are slightly enlarged, hypochromatic with fine chromatin and small nucleoli. Nuclear borders are regular

**Case 7 (Figure 13.7) – Parakeratosis**

Singly scattered and miniature polygonal to round squamous cells with dense orangeophilic cytoplasm and pyknotic nuclei with no atypia. Few elongated cells are present in the background, again without any nuclear atypia.

**Case 8 (Figure 13.8) – Possible decidual cells**

The follow up colposcopy and subsequent cervical smears in this female were normal. The possibility of decidual cells with abundant cytoplasm and vesicular nuclei was considered.

**Case 9 (Figure 13.9) – Endocervical repair**

Note the monolayered arrangement of cells with a streaming pattern. The polarity is retained. Nuclei are slightly enlarged and hypochromatic with uniform chromatin and prominent nucleoli. The background is clean with scattered neutrophils.

**Case 10 (Figure 13.10) – Atrophy with blue blobs**

Note the dark blue rounded bodies resembling big dark nuclei of SIL/dyskaryotic cells among para basal cells.

**Case 11 (Figure 13.11) – Candida infection**

Note the candida hyphae with squamous cells attached to it resembling a flower garland/ chicken kebab appearance

**Case 12 (Figure 13.12) - Lymphoma in a cervical smear**

Note the monomorphic cell population resembling lymphoid cells with atypical features. Tingible body macrophages as seen in follicular cervicitis are absent.

**Case 13 (Figure 13.13) – A dyskeratotic epithelial whorl in SCC**

Note the atypical nuclei in both the epithelial whorl and in the background squamous cells. This woman had a keratinising SCC.

**Case 14 (Figure 13.14) – HGSIL/Moderate dyskaryosis**

This cell cluster has enlarged nuclei with immature cytoplasm. Nuclei are larger than those of low-grade (mild dyskaryosis) cells, occupying half to two-thirds of the cytoplasmic area. Note the vague perinuclear halos in some cells, nuclear membrane irregularity and the coarse chromatin pattern. Nuclei are not that hyperchromatic.

**Case 15 (Figure 13.15) – Red atrophy**

Note small cells with reddish orange cytoplasm and pyknotic nuclei, in an atrophic smear with many parabasal cells in a bluish granular background. All cells lack nuclear atypia. Differential diagnosis: Parakeratotic cells.

**Case 16 (Figure 13.16) – HGSIL/Moderate dyskaryosis**

The two cells at the bottom of the field have enlarged nuclei occupying half to two-thirds the cytoplasmic area. The nuclei are hyperchromatic with coarse chromatin.

**Case 17 (Figure 13.17) – Bare endocervical cell nuclei**

Majority are stripped endocervical cell nuclei. Few cells on top still retain an intact cytoplasm assuming a columnar morphology.

**Case 18 (Figure 13.18) – Herpes simplex infection**

Note multi nucleation, nuclear moulding, thick nuclear membrane, ground-glass chromatin.

**Case 19 (Figure 13.19) – LGSIL/Mild dyskaryosis**

The single large cell in the centre has abundant mature superficial-type cytoplasm. Nuclear enlargement is over three times that of the adjacent intermediate cell nucleus, occupying less than half the cell cytoplsm. The nucleus is hyperchromatic with coarsely granular chromatin, indistinct nucleoli and a slightly irregular nuclear membrane.

**Case 20 (Figure 13.20) – Mature meplastic cells**

Note the monolayer of cells in a vague cobble stone arrangement. The cells have rounded cell borders and dense cytoplasm with regular nuclei.

**Case 21 (Figure 13.21) – Pseudokoilocytes**

Note immature squamous metaplastic cells of parabasal size, with well demarcated endo and ectoplasm. Koilocytes are intermediate/superficial size cells. Compare with the true koilocyte in case 3

**Case 22 (Figure 13.22) – Trichomonas infection**

Note the pear shaped trichomonas organisms in the centre. The cytoplasm is grey-green with central reddish granules and nuclei.

**Case 23 (Figure 13.23) – A parakeratotic whorl**

Note the tight squamous epithelial whorl with absent nuclear atypia. Compare with case 13

**Case 24 (Figure 13.24) – Histiocytes**

This cluster of histiocytes has reniform nuclei and foamy cytoplasm.

**Case 25 (Figure 13.25 a, b and c) –** This case was diagnosed as AGUS of endometrial origin in view of the enlarged nuclei with nucleoli. Evaluation however did not show any endometrial pathology. On review it was felt that these were probably abraded cell clusters from the lower uterine segment in view of the clean background and localised cell groups under 10X and branching sheets and large cell clusters with gland openings on higher power.

**Case 26 – (Figure 13.26) –** Similar to case 15, follow up colposcopy and subsequent cervical smears in this female were normal. It was felt that these are again decidual cells mimicking dysplatic cells or trophoblastic cells. Note the heavily inflamed background.

**Case 27 – (Figure 13.27) – Endometrial cells in exodus pattern –** The round cell cluster has darkly stainng epithelial cells in the centre and lightly staining stromal cells at the periphery. Note abundant blood in the background.

**Case 28 – (Figure 13.28) – AGUS/atypical endocervical cells NOS –** Nuclear stratification with a suggestion of featuring is seen at the edge of the cell cluster. However it is not a hyperchromatic crowded cell group and appears to have a vascularised core. Nuclear atypia is minimal. Features fall short of a diagnosis of AIS/CGIN.

**Case 29 – HGSIL/moderate dyskaryosis -** The cells have immature / dense cytoplasm and resemble para basal cells. Nuclei, occupy half to two-thirds of the cytoplasmic area with some coarse chromatin with no significant nuclear margin irregularity.

**Case 30 – HGSIL/dyskaryosis –** This crowded cell group resembles a group of reactive metaplastic cells on low power. Further evaluation under high power reveals enlarged atypical nuclei with coarse chromatin and irregular nuclear margins

**Case 31 – Endocervical adenocarcinoma –** This crowded cell group has overlapping, rounded nuclei with few single cells in a background of bloody tumour diathesis.

**Suggested further reading:**


1. DeMay R.M, The Art & Science of Cytopathology, Hong Kong: ASCP press, 1996

2. McKee G.T, Cytopathology, Spain: Mosby-Wolfe,1997

3. McKee G.T, Gray W, Diagnostic cytopathology,China:Churchill Livingstone, 2004

4. Solomon D, Nayar R Editors. The Bethesda system for reporting cervical cytology: Definitions, criteria, and explanatory notes. Springer 2004,2nd Ed.