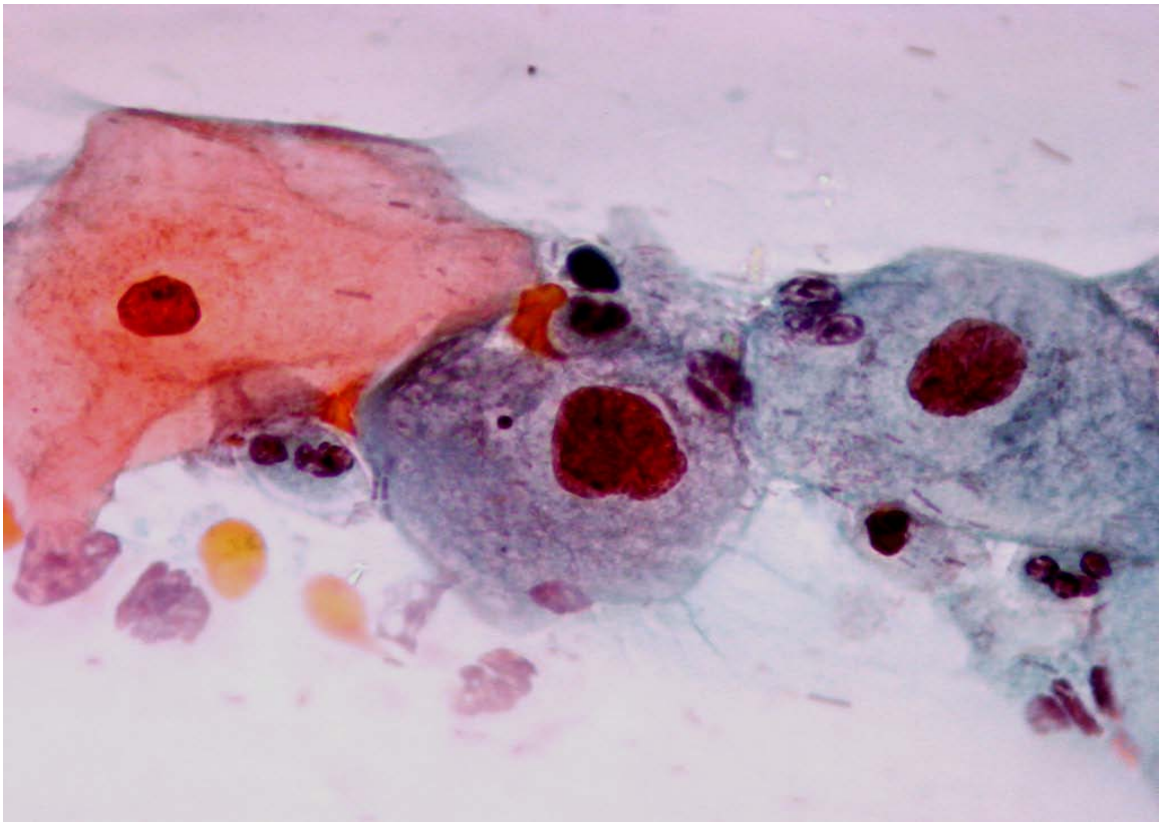

Essentials of Gynecologic Cytology

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2011

Essentials of Gynecologic Cytology

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Preface

Essentials of Gynecologic Cytology is written for practicing pathologists in private laboratories and community hospitals, residents in Pathology and cytotechnologists who want a quick review of the cytopathology of the uterus and its annexae. It consists of nine chapters and eight of them are devoted to the evaluation of the Pap smear; a test that has been regarded as the most complicated to interpret in medicine.

The text in this monograph is concise and contains relevant information for cytologic interpretation of cell samples from the above-mentioned anatomic sites. Lesions commonly encountered in day-to-day practice and lesions with distinct cytologic manifestations are briefly discussed and illustrated. The cytologic materials used for the illustrations were from two Canadian institutions: University of Alberta Hospital, Edmonton, Alberta and BC Cancer Agency, Vancouver, British Columbia. The Pap smears from the former were alcohol-fixed with commercial fixatives and those from the latter were air-dried, re-hydrated and then fixed in ethanol prior to staining. Therefore, some minor differences will be observed in some cell images. Most illustrations are taken from conventional Pap smears, with a few images taken from liquid-based preparations added for comparison. Some important differences in the cytologic findings between conventional and liquid-based preparations are mentioned elsewhere in the text. A small number of histologic images are also included for cytohistologic correlations.

For improvement of the future editions of this monograph, constructive comments from the reader will be highly appreciated.

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Related material by the same author

Essentials of needle aspiration cytology. Igaku-Shoin, New York, 1991
Essentials of exfoliative cytology. Igaku-Shoin, New York, 1992
Essentials of cytology, An atlas. Igaku-Shoin, New York, 1993
Critical issues in cytopathology. Igaku-Shoin, New York, 1996
Essentials of lung tumor cytology. UBC Pathology, Vancouver, 2008
Essentials of abdominal fine needle aspiration cytology. UBC Pathology, 2008
Essentials of head and neck cytology. UBC Pathology, 2009
Essentials of fluid cytology. UBC Pathology, 2010

Important remarks

In this monograph:

- Cytologic materials (conventional Papanicolaou smears, Liquid-based preparations and Fine-needle aspirates) were stained by the Papanicolaou method or by another staining method as otherwise indicated.
- Most cytologic images were taken under medium or high magnification.
- Histologic sections were stained with hematoxylin and eosin or with another staining method as otherwise indicated.
- Conventional Pap smear is abbreviated as CP smear.
- Liquid-based preparation is abbreviated as LBP
- The 2 terms “Cervical intraepithelial neoplasia” (CIN) and “Squamous intraepithelial lesion” (SIL) are used interchangeably in many sections:
    - Low-grade SIL (LSIL) as Low-grade CIN or CIN 1
    - High-grade SIL (HSIL) as High-grade CIN or CIN 2 and CIN 3

Chapter 1

Gynecologic Cytology: Historical development, Current status, Technical considerations and Reporting

Katherine M. Ceballos, Brenda Smith and Gia-Khanh Nguyen

THE PAP TEST

HISTORICAL DEVELOPMENT

George Nicholas Papanicolaou has been regarded as the father of modern cytopathology. He was born in Greece in 1883, studied medicine in Athens and immigrated to the United States in 1913. In New York he first used exfoliated cells to study the estrous cycle in guinea pigs, and then he subsequently worked on human cells. In 1943 Papanicolaou and Traut published their book *Diagnosis of Uterine Cancer by the Vaginal Smear* that has had a strong impact on clinical practice worldwide. Also emerging in the field was Ruth Graham, one of the earliest and most renowned cytologists. Her paper on radiation changes of cancer cells of the uterine cervix has been regarded as a classic study, and her 1950 monograph entitled *The Cytologic Diagnosis of Cancer* has greatly contributed to the field of clinical cytology. In the 1950s, the cytology literature began to proliferate and *Acta Cytologica* commenced publication under George L. Wied with an editorial board consisting of Graham, Papanicolaou, Pundell and Reagan. Early volumes consisted of symposia devoted to definitions and criteria for cytodiagnosis. Four other international cytology journals have subsequently appeared, attesting to a need for advancement of cytopathology: *Diagnostic Cytopathology* in 1985, *Cytopathology* in 1990, *Cancer Cytopathology* in 1997 and *Cytojournal* in 2005.

The practice of gynecologic cytology in the United States in the past 10 years has undergone important changes that have happened as a result of the publication of the article entitled “Lax laboratories” by Bogdanich in the *Wall Street Journal* on November 13, 1987, and the subsequent introduction of Bill HR 5471, the Clinical Laboratory Improvement Amendments of 1988 (CLIA ’88). As a result, numerous cytology laboratories in the United States have lost their licenses, and several professional organizations have concentrated their efforts to evaluate the value of the Pap smear in cervical cancer screening.

CURRENT STATUS

1. THE BETHESDA SYSTEM

The first Bethesda conference met on December 12-13, 1988 at the National Institute of Health in Maryland to consider methods of reporting gynecologic cytology in meaningful diagnostic terminologies. The second Bethesda conference was held on April 29-30, 1991 to discuss the problem of implementation, clarification of terminologies, the "less-than-optimal" category, adequacy criteria, and reporting. The third Bethesda conference held from April 30 to May 2, 2001 further revised the terminology. The Bethesda System has enjoyed widespread popularity, and it has been adopted almost worldwide to replace the outdated Papanicolaou numerical class system for reporting Pap smears.

2. LIQUID-BASED PREPARATIONS

These preparations have been developed to improve preservation of cytologic detail and thus the diagnostic sensitivity of cervico-vaginal cytology. The ThinPrep™ Pap Test (formerly Cytyc Corp., now Hologic™ Inc) and the SurePath™ Pap test (BD Diagnostics) were approved by the Food and Drug Administration (FDA) of the United States. The FDA has approved ThinPrep as an alternative for the CP smear and approved claims that it is more effective than the CP smear in detecting cervical low-grade epithelial lesions. At the present time the ThinPrep Pap Tests has replaced nearly 100% of the cervical screening market in the United States. The morphology of squamous and glandular cells is preserved but the cellular patterns or arrangements associated with some lesions are altered, requiring slight modifications of cytodiagnostic criteria. Attaining proficiency in the interpretation of liquid based cytology will require a lot of time and effort from the cytology community worldwide.

3. CYTOLOGY AUTOMATION

Automation has been in development since the 1950s. The Neopath AutoPap® 300 QC and the Neuromedical System PapNet were the first two devices that were approved by the FDA for quality control rescreening of negative Pap smears. The AutoPap System was the only device that was approved for primary screening. Recently, the Neuromedical Systems, Inc. has declared bankruptcy, and its intellectual property was sold to AutoCyte, Inc., now part of BD Diagnostics. Current FDA-approved devices for automated screening are the BD FocalPoint™ Slide Profiler, and the ThinPrep Imaging System. These new technologies have increased the cost of medical care, and at this moment they are not considered to be cost-effective.

4. CERVICAL CANCER SCREENING

Cervical cytology screening has been credited to the 70% decrease in cervical cancer mortality in the United States and Canada in the past 50 years. Prior to 2002 various organizations generally recommended that screening should start at the beginning of sexual activity. It should continue annually throughout life, and women with several prior negative Pap smears may have the test repeated at longer intervals.

The revised cervical cancer screening guidelines of the American Cancer Society and those of the American College of Obstetricians and Gynecologists were published in 2002 and 2003.

- Both organizations recommended that screening should start 3 years after sexual debut or by the age of 21, whichever comes first.
- If a woman has 3 consecutive negative tests in the preceeding 10 years the screening may stop at age 70.
- For women aged 30 and older dual screening by Pap smear and HPV testing to triage equivocal cases is an option. If both tests are negative, screening should not be repeated for 3 years.
- Both organizations do not recommend cytology screening for hysterectomized women without a cervix, unless the surgery was performed for cervical premalignant or malignant lesions.

Most European countries have established cervical cancer screening programs. Their recommended screening starts between the ages of 20 and 25 years and continues every 3 to 5 years until age 60 to 65.

Many developing countries do not currently have any cervical cancer screening programs. For those countries, it has been estimated that the lifetime risk of cervical cancer could be reduced by up to 30% if a screening program uses a combination of Pap smear and HPV testing in women aged 30 to 59 at least once per lifetime.

The prophylactic HPV vaccines for unexposed girls and women will likely to have an impact on the future cervical cancer screening. Currently, Gardasil, a vaccine targeting HPV types 6, 11, 16 and 18, and Cervarix, a vaccine targeting HPV types 16 and 18, are the two FDA-approved vaccines for females aged 9 to 26. The two HPV types 16 and 18 are responsible for about 70% of cervical cancers, but other HPV types that are not included in Gardasil and Cervarix are still responsible for the remaining 30% of cervical cancers. Therefore, women should continue to have cervical cancer screening by Pap smear regardless of their vaccination status.

5. QUALITY ASSURANCE QUALITY

Quality assurance has always been an integral part of gynecologic cytology practice. It encompasses laboratory procedures, diagnostic accuracy and reliability with the goal to reduce false-positive and false-negative results.

False-negative results may be due to:

- Sampling error in:
  - collection device that does not sample lesional cells or
  - failure of transfer of lesional cells to the glass slide
- Laboratory error in:
  - detection or
  - interpretation

To minimize the laboratory component of these errors, a workload limit for each cytotechnologist screener and a re-examination of Pap smears is required:

- Workload limit: 90 and 100 slides per 24 hours in Canada and The United States, respectively (minimum amount of time required to screen those slides is 8 hour).
- Re-examination of cytologic materials:
  - prospective re-screening of 10% of all negative cases.
  - retrospective review of all negative Pap smears during 5 years before the diagnosis (5-year lookback) from patients with a newly diagnosed high-grade squamous intraepithelial lesion or cancer.
  - review of all cases having cytologic-histologic discrepancies.

Results of re-examination of cytologic materials are statistically documented annually for evaluation of the laboratory performance.

DIRECT ENDOMETRIAL CELL SAMPLES

In the past fifty years, efforts have been made to design a simple endometrial sampling device for use in the physician's office to obtain cells directly from the endometrium for cytologic examination. In 1943, Cary was the first investigator to report on the use of a metal canula and a syringe to aspirate endometrial cells. Morton et al. modified the Cary technique by including in the procedure an endometrial lavage with normal saline under positive pressure. The saline was then re-aspirated and processed for cytologic study. This sampling technique was never adopted because of the fear of seeding endometrial cancer cells into the peritoneal cavity. In 1955, Ayre promoted endometrial brushing to obtain endometrial cells. Butler and associates subsequently modified the Ayre brush and the technique for removing endometrial cells from the bristles of the brush. Despite a high diagnostic accuracy for endometrial carcinoma, the brushing technique was not widely

adopted. In 1964, Dowling and Gravlee reported on the combination of endometrial aspiration with uterine lavage under negative pressure to obtain endometrial cells. This technique did not produce consistently acceptable results because of the poor preservation of cell morphology, and it was subsequently discontinued. In 1974, Isaacs and co–workers promoted a relatively simple endometrial aspiration device to sample endometrial cells. This sampling technique was widely used and achieved a high diagnostic accuracy rate for endometrial cancer. In 1973, Milan and Markley reported on the use of a plastic helix to sample the endometrium by scraping. The Mi–Mark helix was widely adopted for many years, but it was subsequently found to be less efficient in detecting endometrial lesions. In recent years, a few endometrial scraping devices have been introduced to clinical practice. Of these, Endocyte and Endopap samplers have been the most popular ones. They are simple to use and cause little or no discomfort to patients and the cell samples obtained have a low rate of cellular inadequacy.

FINE NEEDLE ASPIRATION

Fine needle aspiration (FNA) during laparoscopy for cytologic evaluation of ovarian cystic lesions was originally performed by Mintz and associates in 1967. In 1971, Kjellgren and coworkers utilized this technique to classify ovarian cancers and have published the results in their 1972, 1974 and 1979 publications. In 1979, Ramzy and Delaney reported on the cytologic features of epithelial ovarian tumors, and Sevin and his group reported on their FNA experience with gynecologic malignancies. In the early years of 1990s Trimor-Tritsch have developed a transvaginal sonographic scorering system to distinguish a benign from malignant ovarian adnexal cysts, and a transvaginal FNA has been used to diagnose cystic lesions with low and intermediate scores. FNA of small benign-appearing uterine adnexal masses detected during laparoscopy has now become a more routine procedure for patient care, and it proved to be a safe and economical method for diagnosis of those lesions.

TECHNICAL CONSIDERATIONS

A. CONVENTIONAL CERVICAL/VAGINAL SMEARS

The most commonly used cell collecting devices for CP smears are the modified Ayre spatula and the cytobrush. Cell samples from the uterine cervix and posterior vaginal fornix are usually collected, respectively, by the pointed end and the blunt end of an Ayre spatula. They are either deposited onto 2 glass slides or mixed together on one glass slide. The use of a cytobrush in conjunction with a spatula can help ensure an adequate representation of the squamocolumnar junction. Once the samples have been evenly spread on the glass slide, they are immediately fixed with a commercial spray fixative. To prevent the formation of thick cellular ridges, the slide should be held about 25 cm from the spray nozzle or floated within the fixative for 15 to 30 min. The slides are then air-dried and placed in a rigid

container for mailing to a referral cytology laboratory. If the slide is broken during transportation, the cytologic material may be transferred to a new glass slide for examination by using a special technique.

B. LIQUID-BASED PREPARATIONS

Collecting devices for liquid-based preparations may be either a broom-like device or a plastic spatula in combination with a cytobrush. The ThinPrep Pap Test (Hologic™ Inc) consists of rinsing the collection device into a vial of a methanol-based fixative media (PreservCyt®). The TP 2000 or 3000 processor uses a semi-automated technique for preparing monolayer cell samples. After gentle dispersion to homogenize the specimen, the solution is aspirated through a membranous filter which is subsequently pressed against a glass slide. Negative pressure and surface tension allow for transfer of the cells to the slide, which is then automatically placed in 95% ethanol for fixing before staining. After cell transfer, the filter assembly is discarded.

The BD SurePath™ Pap test requires collection devices (broom, or plastic spatula and cytobrush) with detachable heads which are dropped into the ethanol-based collection vial. The automated processor homogenizes the specimen and dispenses it onto a density gradient reagent where cells are separated from interfering cell debris and inflammatory cells. Automated pipetting transfers the cell concentrate to small plastic chambers for gravity sedimentation onto glass slides and then subsequent Pap staining within each chamber.

C. DIRECT ENDOMETRIAL CELL SAMPLES

Materials procured by Endopap or Endocyte samplers are spread on a glass slide and fixed in 95% ethanol or with a commercial spray fixative. The smears are then stained by the Papanicolaou method. Any minute tissue fragments obtained are fixed in 10% neutral buffered formalin for supplementary histological examination.

D. FINE NEEDLE ASPIRATES

Cytologic material obtained by fine needle aspiration is used to prepare direct smears and/or cytospin smears. The cell films are either wet-fixed with 95% ethanol for staining with the Papanicolaou technique or hematoxylin and eosin, or air-dried for staining by the May-Grünwald-Giemsa technique or by one of its modified methods (Giemsa and Diff-Quik methods).

E. PAPANICOLAOU STAIN

STAINING TECHNIQUE

The Papanicolaou stain is the standard method for staining cervicovaginal cell samples worldwide. For optimal cytologic interpretation, an adequate well-prepared, well-stained and well-preserved cell sample is mandatory. The preparation of cell samples for cytodiagnosis consists of 3 basic steps: fixation, staining and mounting of slides.

For Papanicolaou staining, fixation of cell samples with alcohol is mandatory. If the smear is air-dried, it must be rehydrated with either an isotonic saline solution or with a solution consisting of 50% glycerol and water prior to fixation in 95% ethanol and staining with the Papanicolaou method. This procedure satisfactorily preserves the morphology of squamous cells in cervicovaginal smears but the morphology of endocervical glandular cells is less satisfactorily preserved.

The usual fixative is 95% ethanol, however substitutes such as 100% methanol, 80% isopropanol or denatured alcohol are suitable. The cell samples must be fixed for at least 15 to 30 minutes. If the slides are to be sent to a distant laboratory for cytologic evaluation, they should be air-dried after the fixation and carefully packed in a rigid container for mailing. At the laboratory, the slides will be re-immersed in 95% ethanol prior to staining. Papanicolaou stained smears may subsequently be stained with antibodies by routine immunocytochemical techniques without prior destaining of the cells with an acid-ethanol solution.

Papanicolaou staining consists of two main consecutive steps: nuclear staining with hematoxylin and cytoplasmic staining using Orange G and EA 36 or 50 polychrome. For nuclear staining two techniques are used, depending on the laboratory preference: progressive and regressive methods. In the progressive method the cell nuclei are stained to the desired intensity with hematoxylin. In the regressive method the nuclei are over-stained with hematoxylin and then excess hematoxylin is removed with diluted HCl. The cytoplasm is then stained with Orange G and EA 36 or 50 polychrome. To obtain good cellular detail the Papanicolaou staining solutions should be changed after staining 2000 slides or every 6 to 8 weeks, which ever comes first. They should be filtered daily to eliminate precipitates and "floaters".

TROUBLESHOOTING

Troubleshooting may be encountered in any aforementioned steps of cell sample preparation but they are most frequently encountered during the preparation and staining of the specimen. Common problems in Papanicolaou staining are summarized in Tables 1.1 and 1.2.

Table 1.1. Nuclear staining problems*

Problem	Possible Reason(s)
Nuclei too dark	1. Overstaining with hematoxylin 2. Excess stain not adequately removed by rinsing with tap water 3. Inadequate rinsing in HCl solution 4. HCl concentration too weak 5. Ammonium chloride solution (or other bluing agent) too strong
Nuclei too pale	1. Diluted hematoxylin solution 2. Inadequate time in hematoxylin 3. Polyethylene glycol coating not adequately removed prior to staining with hematoxylin 4. HCl not completely removed by tap water 5. HCl solution too concentrated 6. Slide dipped too long in HCl solution 7. Ammonium hydroxide solution too weak 8. Excessive time in chlorinated tap water 9. pH of tap water after hematoxylin not alkaline enough

Table 1.2. Cytoplasmic staining problems*

Problem	Possible Reason(s)
Inconsistent cytoplasmic staining	1. Air-drying prior to fixation 2. Polyethylene coating not adequately removed prior to staining 3. Slides left too long in ethanol rinses or clearing solution following OG/EA staining 4. Time in hematoxylin too long 5. Excess hematoxylin not removed prior to OG/EA staining 6. Inadequate rinsing between solutions 7. Inadequate rinsing following staining with dyes 8. Inadequate draining of slides between rinses 9. Inappropriate pH of tap water or EA solution 10. Change of cell pH by bacterial infection 11. Variable thickness of smear
Cytoplasm too green	Green dye too strong in EA solution
Lack of contrasting cytoplasmic stain	Exhausted hematoxylin and EA dye
Hazy grey appearance of cells	1. Dehydrating & clearing solutions contaminated with water 2. Incomplete removal of polyethylene glycol coating prior staining
Opaque white color on the back of slide	Inadequate rinse after Scott tap water substitute
Pink, orange or yellow slides	Oven temperature too high
"Cornflake" artifact	Air bubbles entrapped on cell surfaces

** These 2 Tables are adapted with modifications from: Holmquist MD, Keebler CM. Cytopreparative techniques. In: Manual of Cytotechnology, Keebler CM, Somrak TM, eds. 7th ed, 1993. Chicago, ASCP Press, p 410-448.

REPORTING CERVICAL CYTOLOGY

Since the introduction of cervical cancer screening programs in the 1940s, different classification systems have been used to report Pap smears. Prior to The Bethesda System (TBS), which was developed to standardize nomenclature and establish more consistent reports, the Papanicolaou numerical classification, the Reagan classification of "mild, moderate, severe dysplasia" and "carcinoma in situ", and Richart's concept of "cervical intraepithelial neoplasia" (CIN) have been used extensively to report cervicovaginal cytology worldwide. The original Papanicolaou system consisted of 5 classes: I (no atypia), II (atypia but no malignancy), III (suggestive of, but not conclusive for malignancy), IV (strongly suggestive of malignancy) and V (conclusive for malignancy) has proven to be difficult to reproduce. As a result, the dysplasia and CIN classification systems were more widely used. With the elimination of the Papanicolaou classification in 1988 by the first Bethesda Conference, the other two above-mentioned classifications are often still used in conjunction with TBS, as they provide histologic equivalents in cervical pathology.

The 1988 TBS has been revised twice. The first review was in April 1991 and the second was in April/May 2001. When compared to the 1991 TBS, the latest TBS (2001) has several modifications, and the most important of which are listed below:

- The type of the specimen, the utilization of an automated screening device and HPV testing.
- Any preparation containing abnormal cells (Atypical squamous cells of undetermined significance (ASC-US), Atypical glandular cells (AGC) or worse is classified as satisfactory.
- Cellularity criteria are given: if >75% of epithelial cells are obscured by blood or inflammatory exudates, the specimen is considered as "unsatisfactory".
- The ASC category is divided into 2 subtypes with more precise morphologic definitions and criteria for ASC-US and ASC, cannot rule out HSIL (ASC-H).
- Individualization of Endocervical adenocarcinoma in situ and elimination of Atypical glandular cells of undetermined significance (AGUS), favor neoplastic and AGUS, favor reactive; and addition of atypical glandular cells, NOS, and AGC, favor neoplastic.

Shortly after its introduction in 2001, the majority of cytology laboratories in the United States had adopted TBS 2001 for reporting Pap smears (85.5% by 2003). It is now used extensively around the world to report cervical cytology.

THE BETHESDA SYSTEM - 2001

The Bethesda System-2001 consists of several components, as outlined below, and it is recommended for reporting cervical cytology.

SPECIMEN TYPE

Indicate conventional (Pap smear) vs. liquid-based preparation versus other

SPECIMEN ADEQUACY

- Satisfactory for evaluation (describe presence or absence of endocervical or transformation zone component and other quality indicators, e.g., partially obscuring blood, inflammation, etc.)
- Unsatisfactory for evaluation… (specify reason).
- Specimen rejected/not processed (specify reason).
- Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason).

GENERAL CATEGORIZATION (Optional)

- Negative for Intraepithelial Lesion or Malignancy.
- Epithelial Cell Abnormality: See Interpretation/Result
- Other: see Interpretation/Result (e.g. endometrial cells in a woman ≥40 yr of age).

INTERPRETATION/RESULT

A. Negative for Intraepithelial Lesion or Malignancy

When there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report - whether or not there are organisms or other non-neoplastic findings.

1. Organisms:

- *Trichomonas vaginalis*
- Fungal organisms morphologically consistent with *Candida* spp.
- Shift in flora suggestive of bacterial vaginosis.
- Bacteria morphologically consistent with *Actinomyces* spp.
- Cellular changes consistent with herpes simplex virus.

2. **Other Non-neoplastic Findings** (Optional to report; list not inclusive):

- Reactive cellular changes associated with
  - inflammation (includes typical repair)
  - radiation.
  - intrauterine device (IUD).
- Glandular cells status posthysterectomy.
- Atrophy.

3. Other

- Endometrial cells (in a woman ≥40 years of age) (specify if “negative for squamous intraepithelial lesion”)

B. Epithelial Cell Abnormalities

1. Squamous cell:

- Atypical squamous cells
  - of undetermined significance (ASC-US).
  - cannot exclude HSIL (ASC-H).
- Low-grade squamous intraepithelial lesion (LSIL)
  (encompassing: HPV/mild dysplasia/CIN1).
- High-grade squamous intraepithelial lesion (HSIL)
  (encompassing: moderate and severe dysplasia, CIS, CIN 2 and CIN 3).
- with features suspicious for invasion (if invasion is suspected).
- Squamous cell carcinoma

2. Glandular Cell:

- Atypical
  - endocervical cells (NOS or specify in comments)
  - endometrial cells (NOS or specify in comments)
  - glandular cells (NOS or specify in comments).
- Atypical
  - endocervical cells, favor neoplastic.
  - glandular cells, favor neoplastic.
- Endocervical adenocarcinoma in situ.
- Adenocarcinoma
  - endocervical
  - endometrial
  - extrauterine
  - not otherwise specified (NOS)

C. Other Malignant Neoplasm: (specify)

ANCILLARY TESTING

Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician.

AUTOMATE REVIEW

If specimen was examined by automated device, specify the device and the result.

EDUCATIONAL NOTES AND SUGGESTIONS (optional)

Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included).

SPECIMEN ADEQUACY

Evaluation of specimen adequacy is important. There are 2 categories of specimen adequacy in The Bethesda System 2001:

- Satisfactory for evaluation
- Unsatisfactory for evaluation (lack of patient identification or unacceptable specimen due to slide broken beyond repair...)

Depending on the specimen type, the estimated minimum numbers of well-preserved squamous cells required for a specimen to be regarded as adequate or satisfactory for cytologic evaluation are different:

- 8,000 to 12,000 for a CP smear, and
- 5,000 cells for a LBP.

The numbers of squamous cells constitute an additional criterion besides the presence of at least 10 well-preserved endocervical or metaplastic squamous cells. Any specimen with abnormal cells is, by definition, satisfactory for evaluation.

For obscuring factors, if a specimen has more than 75% of squamous cell nuclei obscured by white blood cells, blood, drying artifact, other, it should be termed unsatisfactory, assuming no abnormal cells are identified. (Fig.1.1). When 50% to 75% of the epithelial cells are obscured, a statement describing the specimen as partially obscured should follow the satisfactory term. Abundant cytolysis does not qualify the specimen as “unsatisfactory” unless nearly all nuclei are devoid of cytoplasm. (Fig.1.2).

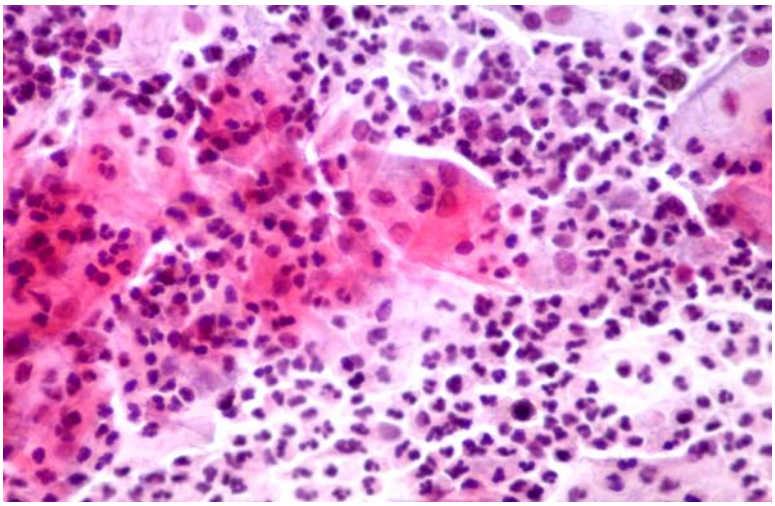

Fig.1.1. An “unsatisfactory” CP smear showing abundant polymorphonuclear leukocytes obscuring over 75% of the squamous cell nuclei.

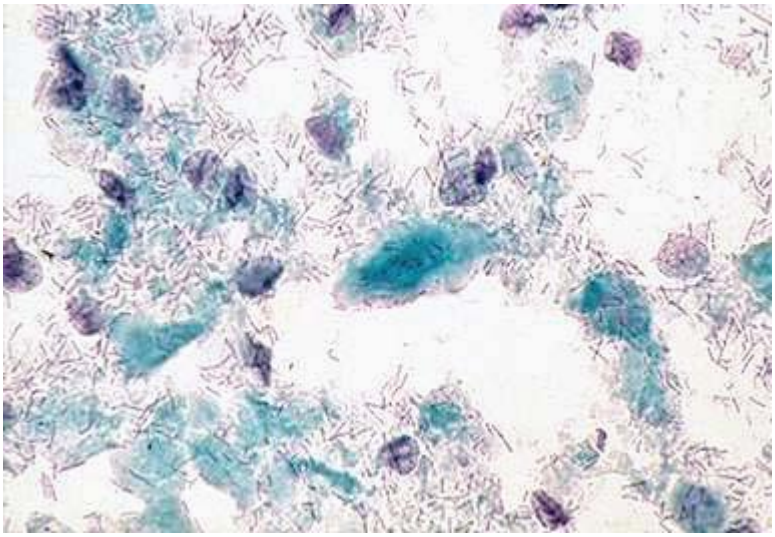

Fig.1.2 A CP smear with extensive cytolysis by Döderlein bacilli but still showing fairly well-preserved nuclei, some of which are surrounded by a small amount of cytoplasm.

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Chapter 2

Normal Uterus and Vagina

Katherine M. Ceballos, Brenda Smith and Gia-Khanh Nguyen

THE PAP TEST

Cell samples from the uterine cervix are usually obtained with an Ayre–type spatula with a longer cervical tip, or with a cervical brush. Many heathcare providers use a combination of a spatula and an endocervical brush to sample the exocervix, cervical canal and T zone. To avoid air-drying artifactual changes that interfere with the cytologic evaluation, the cervical cell sample is immediately spread onto a glass slide and fixed with a commercial spray fixative. Cervical smears may also be wet-fixed with alcohol and are stained by the standard Papanicolaou method. In the Canadian province of British Columbia, cervical cancer screening is performed in one central cytology laboratory. All cervicovaginal smears are air-dried and submitted to the laboratory where they are rehydrated with 50% glycerol and water before staining with the Papanicolaou method. This technique satisfactorily preserves the morphology of cervical squamous cells but has more limited value with endocervical glandular cells.

The **normal uterine cervix** consists of an exocervix and an endocervical canal. The exocervix is covered by a nonkeratinizing, stratified squamous epithelium and the endocervix is lined by a single layer of columnar epithelium with complex folding and a layer of reserve cells. Two types of glandular cells are identified: non-ciliated, mucous-secreting cells and ciliated cells that are very few in number. With advancing age, the distal part of the cervical canal is replaced by metaplastic squamous cells. The squamocolumnar junction is on the exocervix and the transformation zone (T-zone), also known as ectropion, is located between the original squamocolumnar junction and the inner border of metaplastic squamous epithelium. (Fig.2.1). Endocrine and melanotic cells that can be visualized by immunohistochemistry are rarely observed in the cervix. For cervical cancer screening the T-zone should be sampled, as it is the site of origin of > 90% of cervical cancer and its precursor lesions. A representative cell sample from the T-zone commonly contains abundant squamous cells, metaplastic squamous cells and many glandular cells from the endocervical canal.

The **vagina mucosa** is also covered by a layer of nonkeratinizing squamous epithelium that is similar to that of the exocervix and yields abundant squamous cells similar to those of the exocervix.

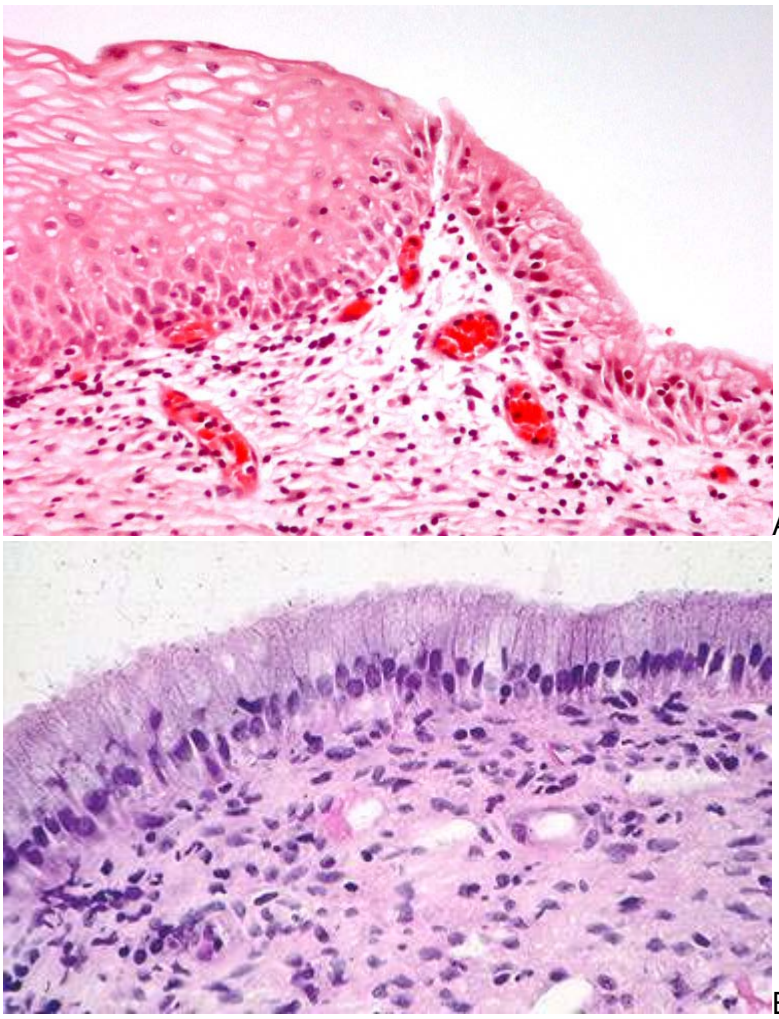


Fig.2.1. Histology of normal uterine cervix in women in reproductive ages.
A. Squamocolumnar junction showing an abrupt change between the nonkeratinizing squamous epithelium of the exocervix and the endocervical glandular epithelium.
B. Endocervical mucus-secreting glandular epithelium with basally located nuclei.

REPRODUCTIVE AGE

A cervical smear from a **non-pregnant woman of reproductive age** is cellular and shows numerous epithelial cells. Normal squamous cells exfoliate predominantly singly and endocervical glandular cells usually exfoliate singly or in sheets of different sizes. Vaginal smears show only squamous cells that are similar to those of the exocervix. Squamous cells of the cervix and vagina are classified as superficial, intermediate and parabasal, according to their characteristic features described below. A maturation index is expressed as a ratio of different types of squamous cells (parabasal: intermediate: superficial).

During the **proliferative phase** of the menstrual cycle, the number of superficial cells increases gradually under the influence of increased levels of serum estrogen. Prior to ovulation the serum estrogen reaches its peak and superficial cells predominate in the smear with the maturation index shifting to the right (for example 0:20:80). After **ovulation** and under the influence of an increasing level of serum progesterone, the intermediate squamous cells predominate in the smear, shifting the maturation index to the middle (for example 0:80:20). With the availability of sophisticated biochemical methods used for measurement of serum hormonal levels, a cytologic evaluation of maturation index by vaginal smear is currently no longer requested by endocrinologists or gynecologists. Normal endometrial cells may also be seen in normal Pap smears, depending on the phase of the menstrual cycle. Normal endometrial cells are described below.

Main cytologic features of normal cervical cells are summarized as follows:

- *Superficial squamous cells* are polygonal in shape with translucent, eosinophilic thin cytoplasm that may contain brownish keratohyalin granules. Their nuclei are pyknotic, centrally located and measure 16 to 20 μm^2 in area. These cells are seen singly and in loose clusters. (Fig. 2.2).

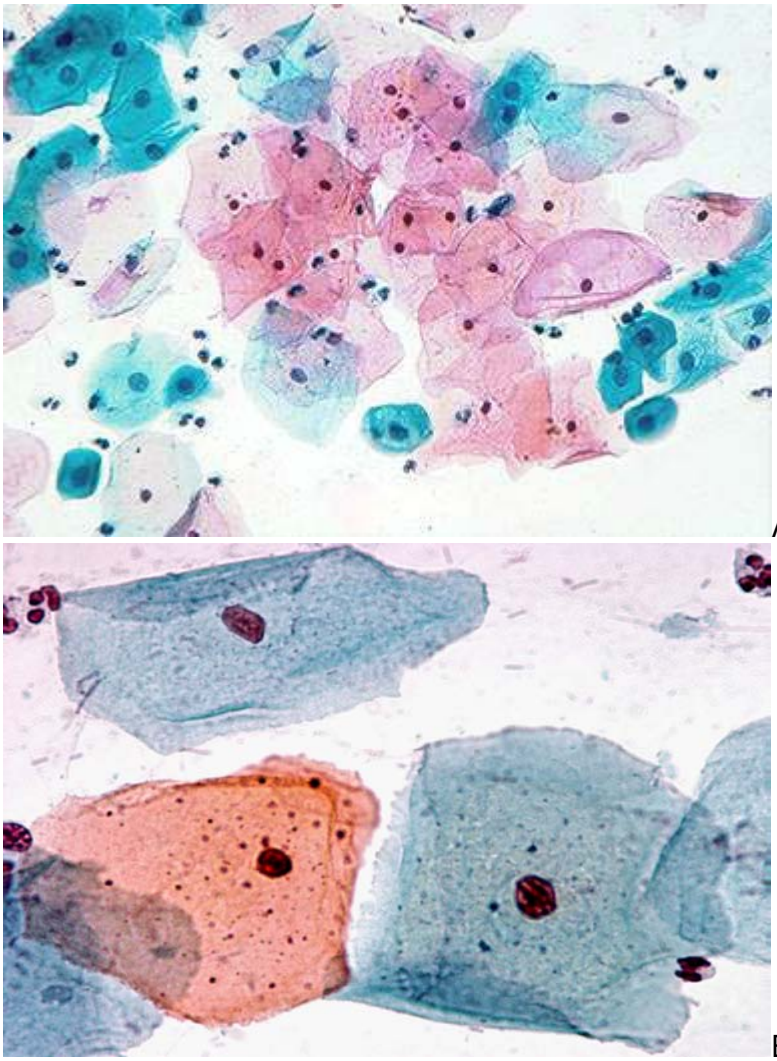
- *Intermediate squamous cells* are oval or polygonal in shape with translucent, eosinophilic or basophilic cytoplasm that commonly shows folding. Their nuclei are vesicular, have a fine chromatin and measure about 35 μm^2 in area. Occasionally, superficial and intermediate squamous cells have a spindle-shape or display a long cytoplasmic extension or “tail” that is a rare collection of different types of intracytoplasmic filaments called Herxheimer’s spirals.

- *Parabasal squamous cells* are rarely encountered in a smear from a premenopausal woman unless she is in the post partum period. They are commonly seen in post menopausal atrophy. They are seen singly and are oval in shape with opaque, basophilic cytoplasm. They have centrally located vesicular nuclei with fine chromatin which measure about 50 μm^2 in area.

- *Endocervical glandular cells* are columnar in shape with pale, abundant, mucinous cytoplasm and basally located vesicular nuclei displaying a granular chromatin and micronucleoli. A few are ciliated. In conventional Pap smears, endocervical cells are present singly or in monolayered sheets with characteristic honeycomb and picket-fence arrangements. Naked nuclei within mucus are a common finding, but they are not regarded as an evidence of Pap smear adequacy. Endocervical glandular cells commonly present singly in liquid-based preparations. (Fig.2.3).

- *Metaplastic squamous cells* arise from the reserve cells of endocervical columnar epithelium. They exfoliate singly or in pavement-like sheets and are polygonal or

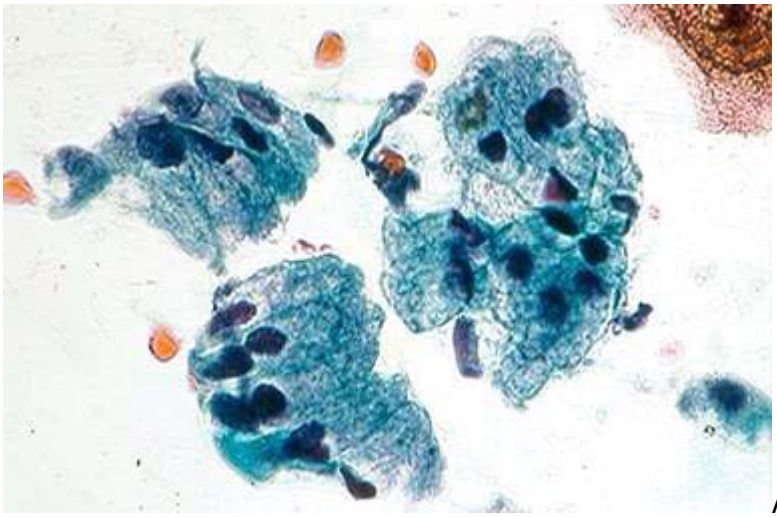
oval in shape. Their cytoplasm varies with the level of cell maturation and in immature cells it may be thin and vacuolated and with cytoplasmic extensions. It is waxy, basophilic or eosinophilic in mature cells. Their vesicular nuclei have granular chromatin and have an area of about 50 μm^2. (Fig.2.4).



A

B

Fig.2.2. Normal cervical and vaginal squamous cells in a CP smear:
A: Superficial, intermediate and metaplastic cells seen singly and in aggregates. A few smaller, oval parabasal cells are also present.
B: One superficial cell with pyknotic nucleus and intracytoplasmic keratohyaline granules and 2 intermediate cells with vesicular nuclei.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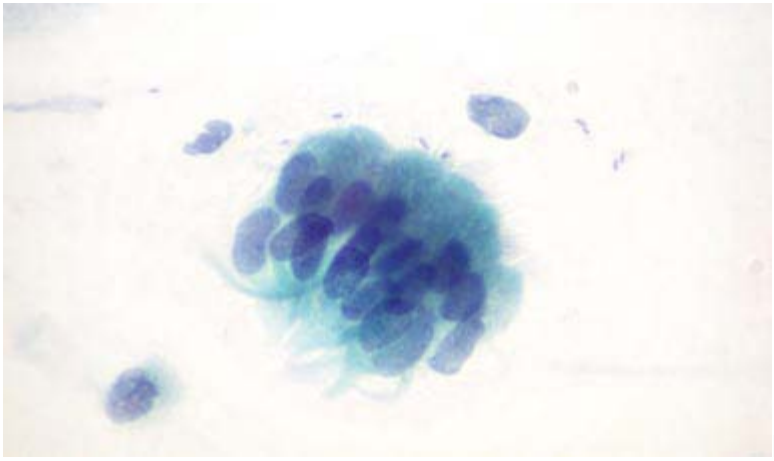
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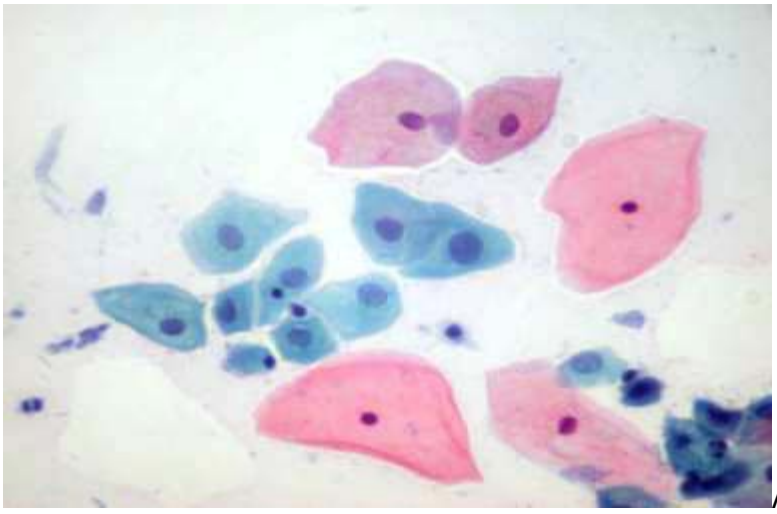
Fig.2.3. Normal endocervical cells in a CP smear:
A. Mucus secreting endocervical columnar cells in small epithelial fragments.
B. Minute epithelial fragment consisting of ciliated glandular cells.

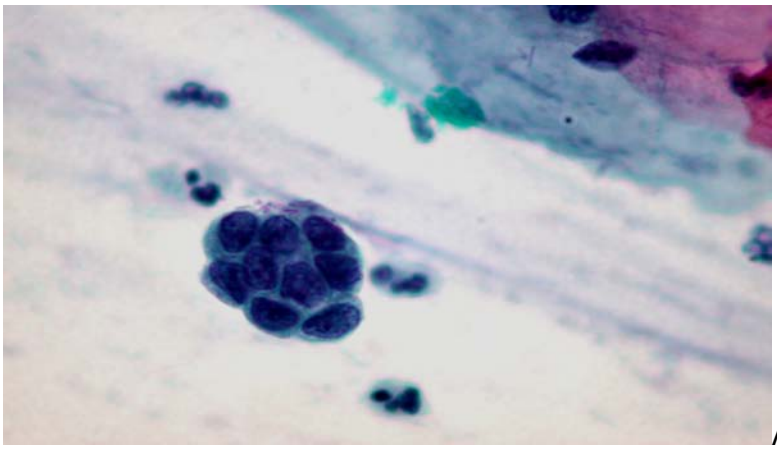

A

B

Fig.2.4. Metaplastic squamous cells in a CP smear:
A. A few smaller metaplastic cells admixed with superficial and intermediate cells.
B. Mature metaplastic cells and immature metaplastic cells with intracytoplasmic mucous vacuoles.

The **uterine corpus** consists of a thick smooth muscle wall and a triangular cavity lined by **endometrium** that is comprised of a columnar glandular epithelium supported by endometrial stroma, a specialized lamina propria. The endometrium consists of 2 layers: the basalis and the functionalis. The basalis layer is a thin deeper layer abutting the myometrium, and from this layer the endometrium regenerates after menstruation. The functionalis layer is located above the basalis layer and responds to ovarian hormones. It has a rapid growth during the first half of the menstrual cycle (proliferative phase) and it is characterized by straight and narrow glands with low columnar cells surrounded by spindle-shaped stromal cells with scant cytoplasm. During the second half of the cycle (secretory phase) it undergoes a maturation characterized by glandular secretion. The glands become more tortuous and the stroma is edematous. The superficial stromal cells, under the effect of progesterone, undergo a predecidual change that is characterized by an increased amount of cytoplasm and their nucleoli become visible. The endometrium in the lower uterine segment does not show the above-described cyclic changes. The length of the menstrual cycle varies considerably among female individuals, but it is 28 days long in most women. The proliferative phase is variable in length but the secretory phase is almost always 14 days long.

Exfoliated endometrial cells are more abundant during the first 10 to 12 days of the cycle, and they may be detectable in cervical vaginal smears. The normal endometrial cells are small, cuboidal cells with scant cytoplasm, round nuclei, chromatin clumping and small nucleoli, and they are commonly seen in cohesive clusters of different size. Superficial stromal cells resemble histiocytes and are present singly or in loosely cohesive sheets. Deep stromal cells appear as small loose clusters of spindle cells. Histiocytes and large masses of endometrial cells or wreaths (exodus) are more commonly observed on the above-mentioned days of the cycle. (Fig.2.5).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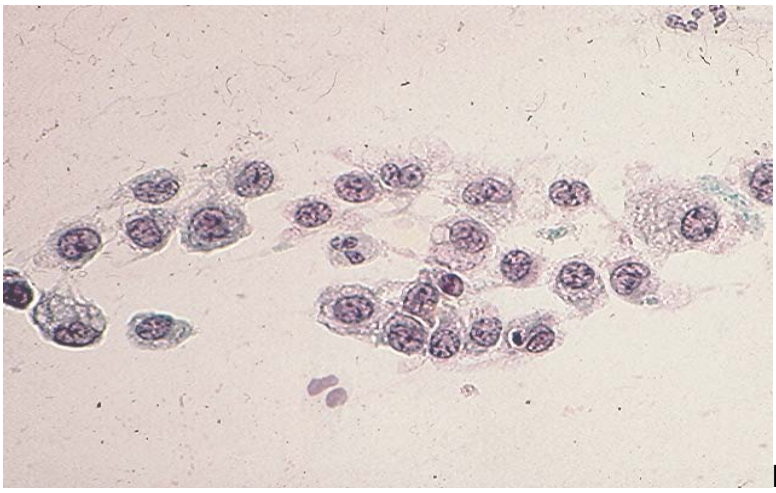
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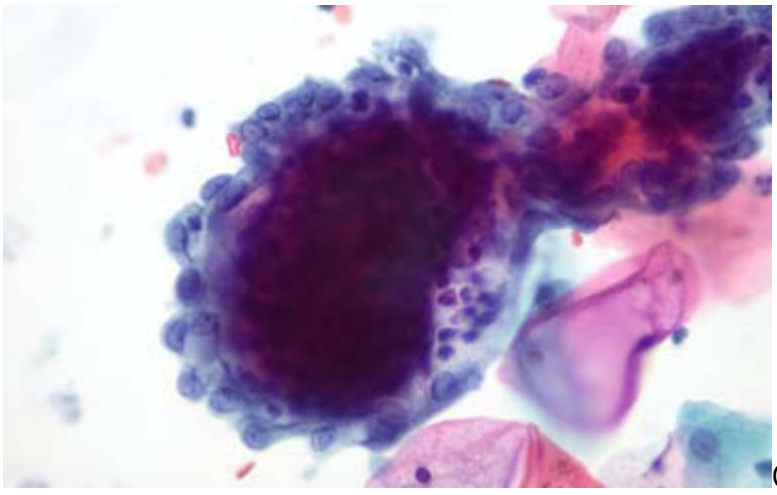
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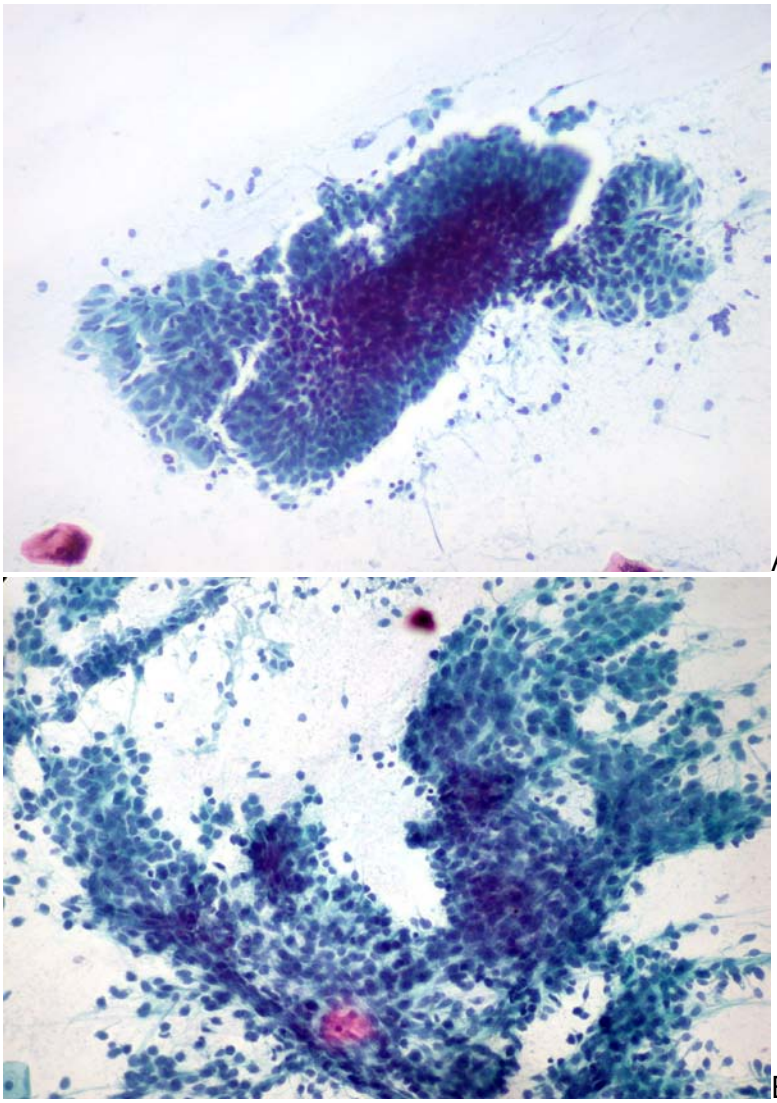
Fig.2.5. Normal, spontaneously exfoliated endometrial cells in CP smears:
A: A cluster of round endometrial epithelial cells showing scant cytoplasm.
B: Superficial stromal cells, resembling histiocytes, present singly and in loose clusters.
C. An endometrial wreath consisting of a large cluster of stromal cells (at the center) surrounded by a layer of endometrial epithelial cells.

An Ayre-type spatula with a longer tip or a cytobrush may inadvertently sample fragments of endometrium from the lower uterine segment (LUS). These LUS endometrial tissue fragments are more commonly seen in women with prior cervical cone biopsy and appear as large, thick cell sheets with folding. The epithelial cells at the periphery display nuclei in picket-fence arrangement. (Fig.2.6). Smaller endometrial cell clusters and aggregates of stromal cells are commonly present.



A

B

Fig.2.6. CP smear showing thick fragments and cell clusters of lower uterine segment endometrium scrapped by a cervical cell sampler with a longer tip.

During **pregnancy** the placenta secretes large amounts of estrogen and progesterone, and as a result, intermediate squamous cells predominate in the smear, accounting for at least 80% of the total cell population. Intermediate cells are rich in glycogen and display an elongated, boat-shaped configuration (navicular cells). Usually the smear consists entirely of intermediate squamous cells by the 4^{th} or 5^{th} month of pregnancy. (Fig.2.7).

In the **postpartum period** the Pap smear is predominated by parabasal cells. (Fig.2.8). This is due to placental parturition and suppressed ovarian functions.

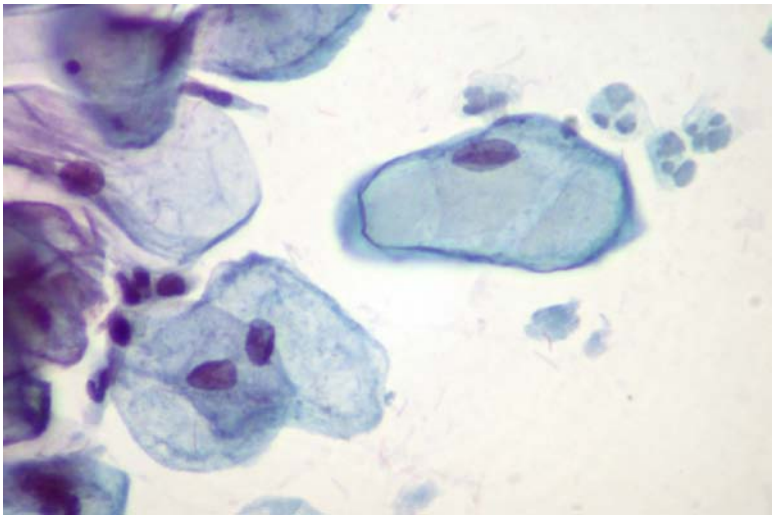


Fig.2.7. CP smear showing a few intermediate squamous cells and a navicular celll containing a large amount of intracytoplasmic glycogen that is yellowish in color.

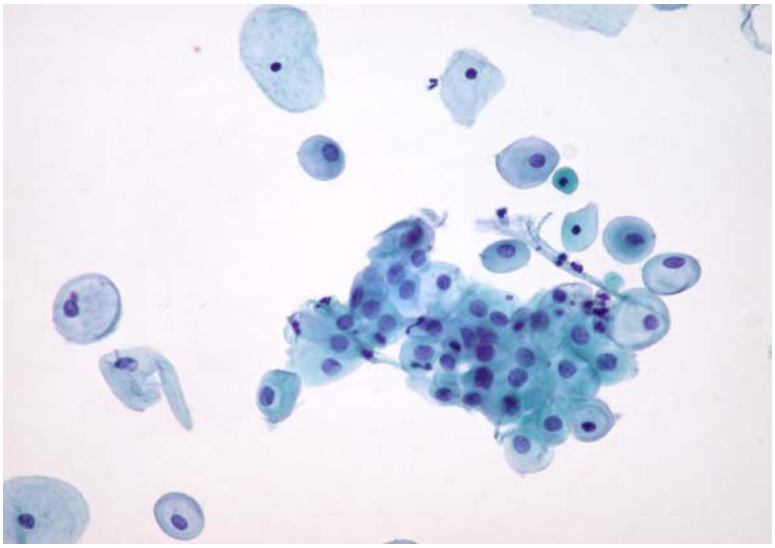


Fig.2.8. LBP from a woman in postpartum period showing parabasal cells singly and in a sheet.

During pregnancy the Pap smear may rarely show a few decidual cells. ***Decidual cells*** are of the same size as parabasal cells but occur in clusters and have thick, granular cytoplasm and larger oval or round nuclei without prominent nucleoli. (Fig.2.9). It is important to note that decidual nodules may occur in the cervix during pregnancy and in women taking progesterone-rich oral contraceptives. An Arias-Stella reaction affects endometrial cells in early pregnancy but it may also occur in endocervical canal. ***Arias-Stella cells*** are large cells with vacuolated cytoplasm, hyperchomatic multiple nuclei and prominent nucleoli.

They may mimic cells derived from a clear cell adenocarcinoma. ***Cytotrophoblasts*** and multinucleated ***syncytiotrophoblasts*** are rarely observed, except in patients with threatened abortion.

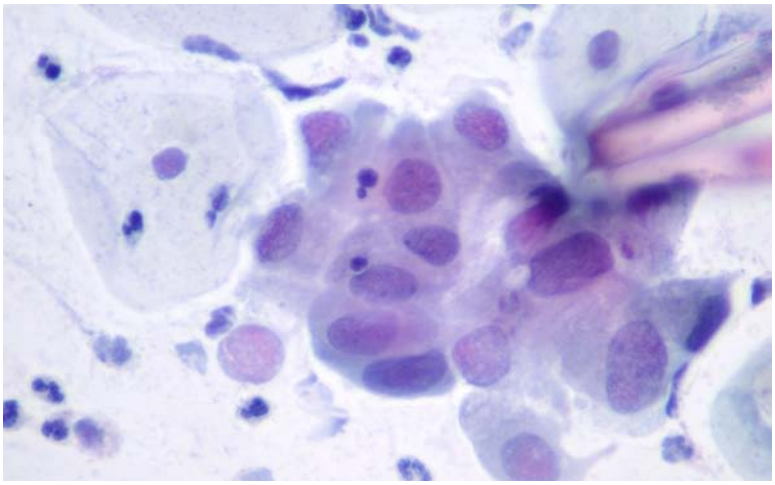


Fig.2.9. A group of decidual cells in a CP smear.

Cockleburrs are hematoidin crystal arrays that are often surrounded by histiocytes. They measure up to 100 µm in greatest dimension. They are more commonly found in pregnant women and rarely in nonpregnant women. (Fig.2.10).

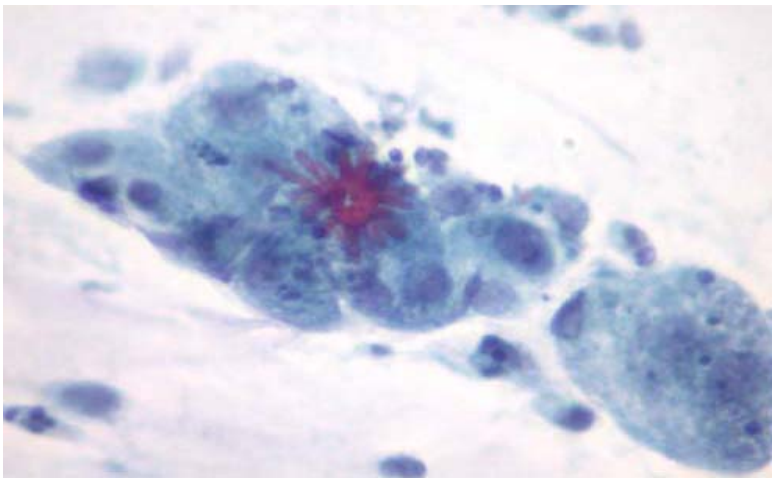
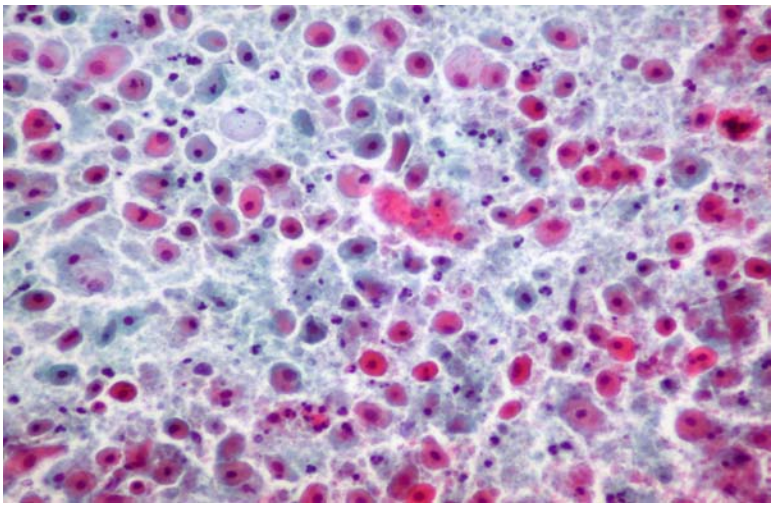


Fig.2.10. Cockleburrs surrounded by histiocytes in a CP smear.

MENOPAUSE

In early **menopause** the Pap smear is predominated by superficial squamous cells. This is caused by the development of nonovulated graafian follicles. As menopause progresses the smear is predominated by either intermediate cells or parabasal cells. (Fig.2.11).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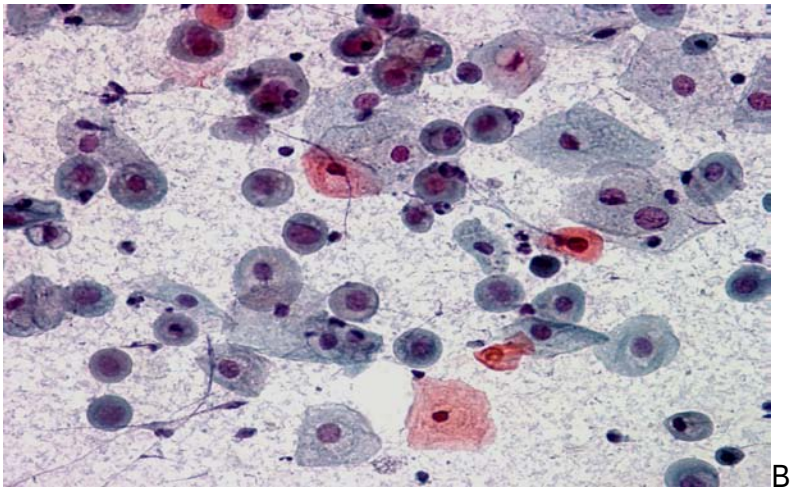
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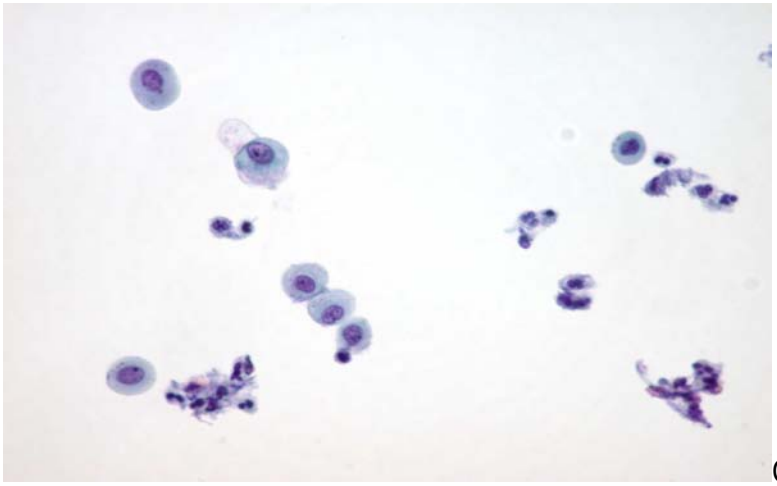

C

Fig.2.11. Atrophic cervix:
A, B. A CP smear showing a parabasal cell predominant pattern. Some cells display eosinophilic cytoplasm. Abundant necrotic debris is present in the smear background.
C. LBP showing parabasal cells in a clean background.

Parabasal cells from ***atrophic vaginitis*** may exhibit nuclear enlargement, mimicking dyskaryotic squamous cells. A repeat Pap smear taken immediately after a course of topical treatment with estrogen cream (to induce cell maturation) will be helpful to solve this diagnostic dilemma, as any dyskaryotic squamous cells will remain unchanged, and normal parabasal cells will have matured. (Fig.2.12)

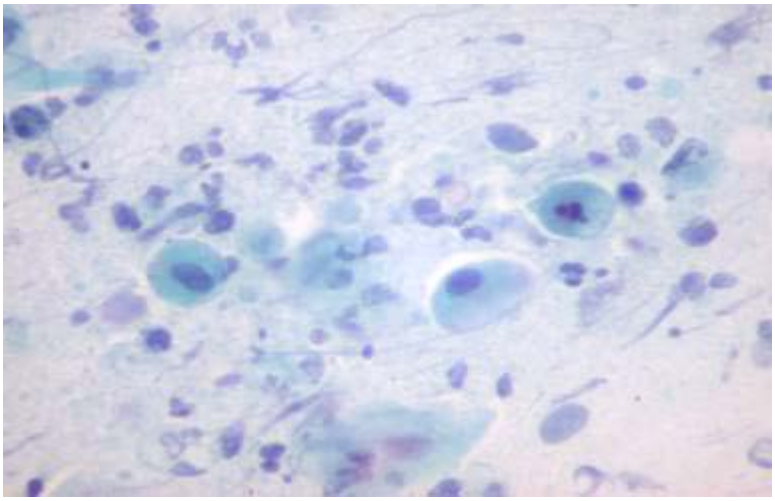


Fig.2.12. Atrophic vaginitis showing in a CP smear degenerated polymorphonuclear leukocytes and rare parabasal cells with slightly enlarged nuclei.

DIRECT ENDOMETRIAL CELL SAMPLES

Endocyte sampler is more commonly used to obtain endometrial cells. The cytologic material obtained is spread on glass slides and fixed in 95% ethanol or with a commercial spray fixative. The smears are then stained by the Papanicolaou method. Excess cytologic material is fixed in 10% normal buffered formalin for supplementary cellblock preparation. Properly collected material usually contains numerous glandular fragments and clusters of endometrial stromal cells. Criteria for cellular adequacy of endometrial samples vary among investigators. With Endocyte samplers Byrne required the presence of at least 15 endometrial fragments to consider a sample satisfactory for cytologic evaluation.

About 10% of all endometrial samples have been reported as inadequate for cytologic evaluation. Criteria for cellular adequacy vary with the types of endometrial lesions. For a nonmalignant lesion, the presence of at least 10 large fragments of endometrial epithelium, or clusters of endometrial cells on all available smears, is required for a confident cytodiagnosis. However, for an endometrial malignancy, the presence of 5 or 6 groups of well–preserved cancer cells with 5 to 10 cells in each group is adequate for a correct diagnosis.

The cytologic manifestations of a histologically normal endometrium in samples obtained by endometrial scraping with Endocyte and Endopap samplers are similar. About 4% to

10% of endometrial samples procured by these 2 devices show inadequate endometrial cells for evaluation.

Reproductive endometrium yields large sheets of surface endometrial epithelium with folded, endometrial gland openings and cells in a honeycomb pattern, as well as several endometrial glandular elements and loosely clustered stromal cells with oval nuclei and ill–defined cytoplasm. The endometrial glands from a proliferative endometrium are straight and tubular in shape and consist of polygonal cells with oval nuclei and scant cytoplasm. Epithelial and stromal cells with mitotic figures are commonly found. The glandular cells from a secretory endometrium are composed of irregularly open glandular segments showing larger epithelial cells in honeycomb pattern, with more abundant cytoplasm and perinuclear halos. Epithelial and stromal cells with mitotic figures are practically not observed. (Figs.2.13 to 2.17). Small endocervical epithelial sheets with mucus-secreting cells in honeycomb pattern are generally present

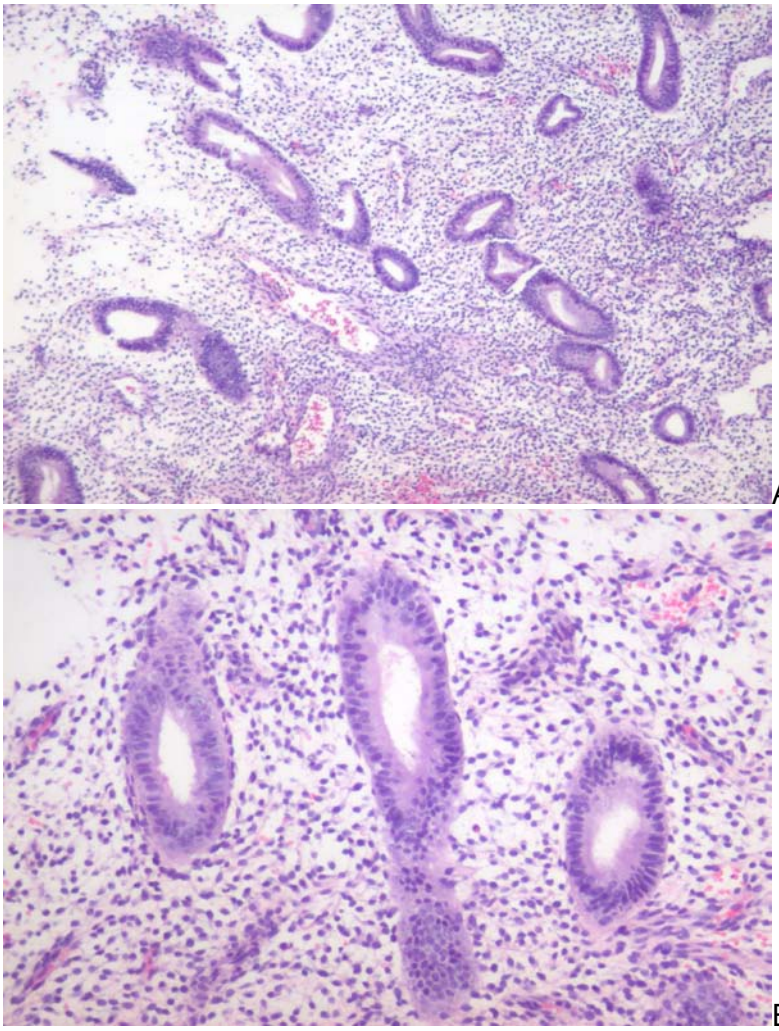
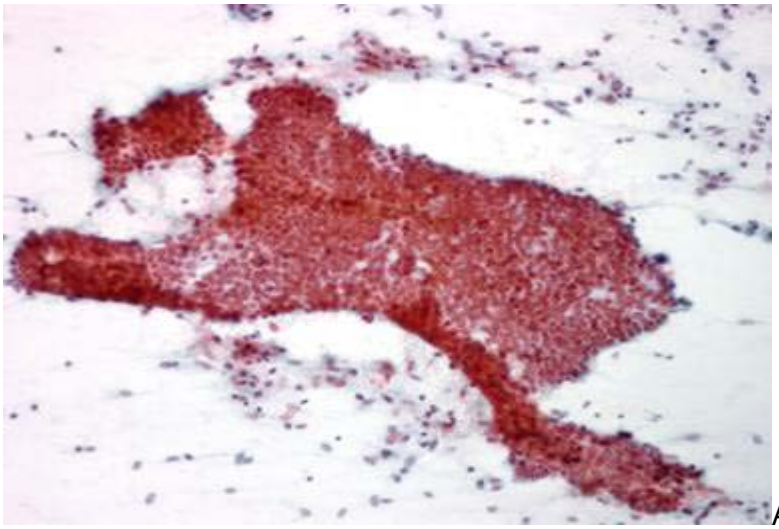


Fig.2.13. A, B: Histology of a proliferative endometrium showing tubular endometrial glands.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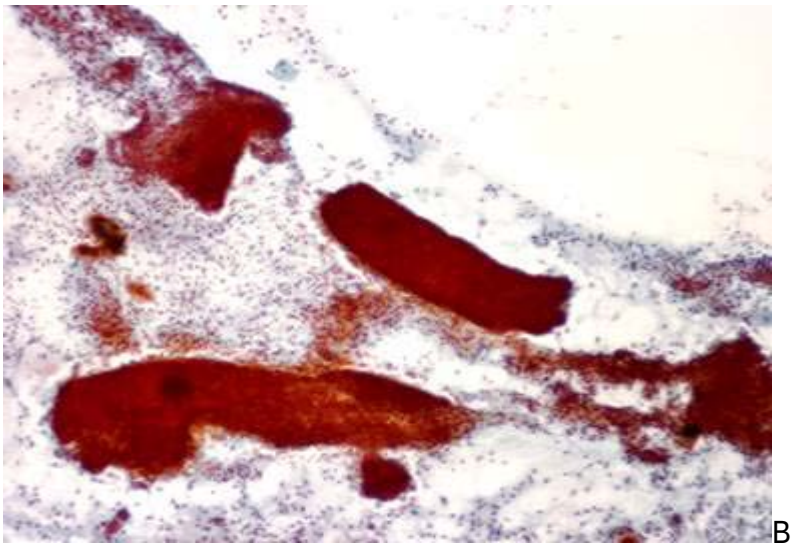
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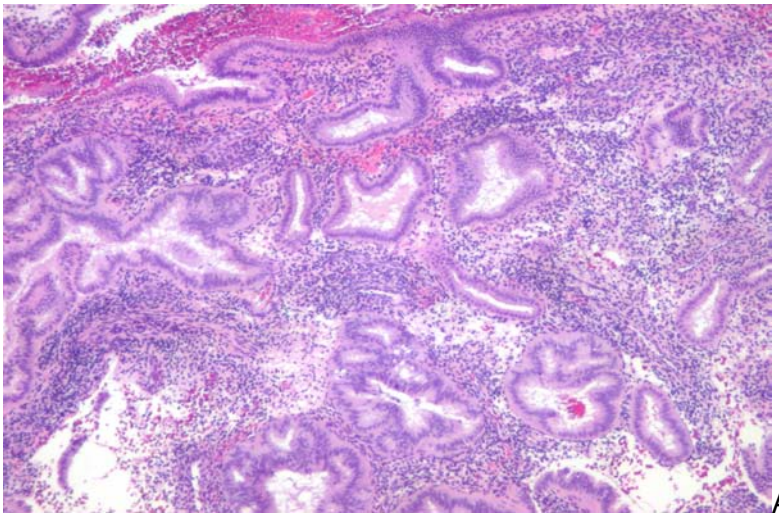
Fig.2.14. Proliferative endometrium yields in direct endometrial sample.
A. A monolayer sheet of endometrial surface epithelium with honeycomb pattern.
B. Proliferative endometrial glands with tubular configuration and stromal cells.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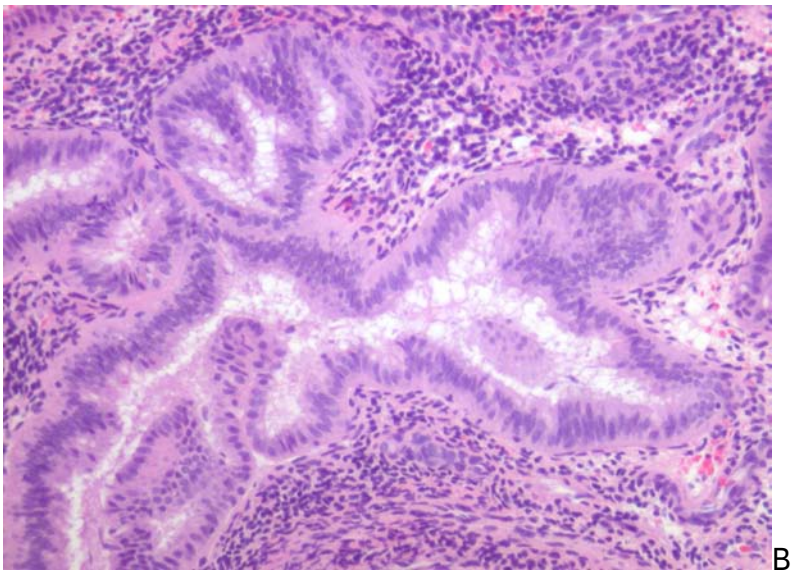
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Fig.2.15. A and B: Histology of a secretory endometrium showing irregular, tortuous endometrial glands with secretion.

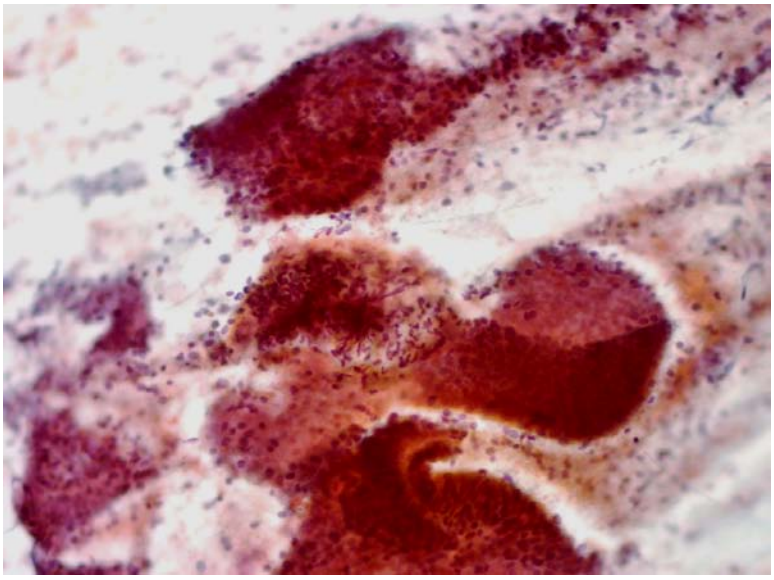

Fig.2.16. Secretory endometrium showing in direct sampling secretory endometrial glands with irregularly open glands and scanty stromal cells.

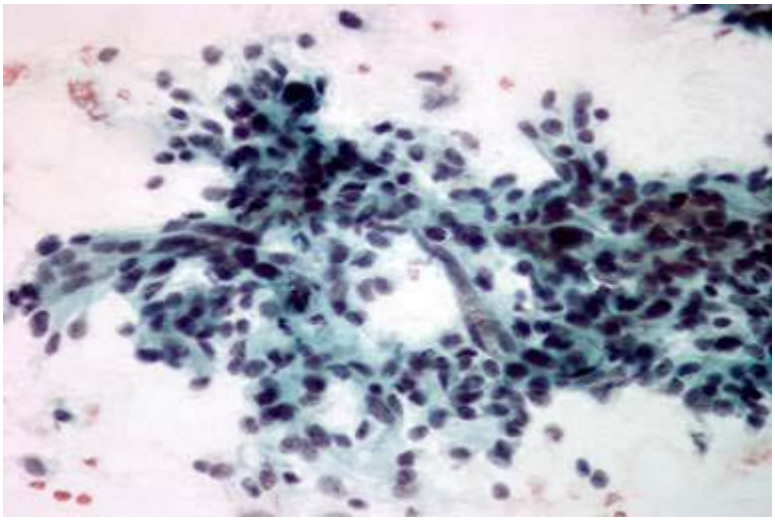

Fig.2.17. Clustered spindle-shaped stromal cells from a proliferative endometrium showing oval or elongated nuclei and ill-defined, slightly basophilic cytoplasm.

Atrophic endometrium yields scant cellular material containing a few small surface epithelial sheets, some straight and narrow tubular endometrial glands and scattered stromal cells. Epithelial and stromal cells with mitotic figures are not identifiable. (Fig.2.18).

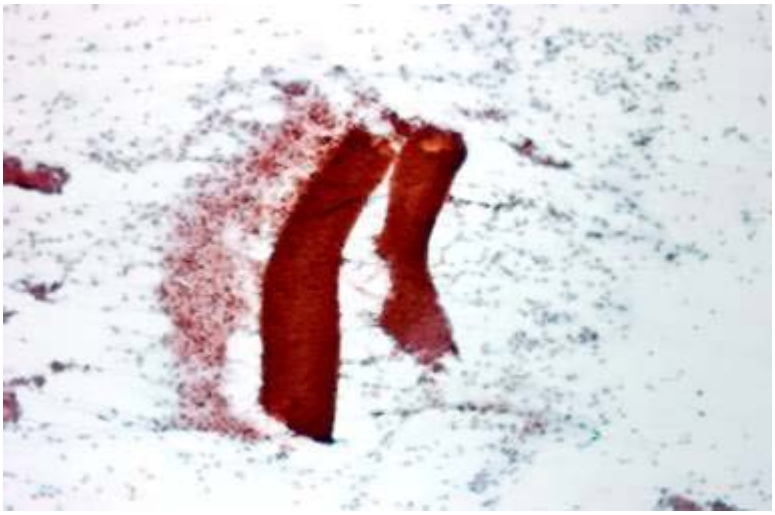

Fig.2.18. Atrophic endometrium showing in CP smear 2 short endometrial tubular glands and a loose aggregate of stromal cells.

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Chapter 3

Infections and Nonneoplastic Cellular Changes

Infections of the cervix and vagina are numerous and can be divided into those that are sexually transmitted and those that are not. Classic sexually transmitted diseases (STD) include syphilis, gonorrhea, chanchroid, lymphogranuloma venereum and granuloma inguinale; and material from these lesions are usually submitted to microbiology laboratories for culture and identification and not to cytology services. Other STDs are caused by Herpes simplex virus, Human papillomavirus, *Trichomonas vaginalis* and *Chlamydia trachomatitis*. These genital infections may be detected and/or suggested by routine cytologic examination of Pap smears. Nonvenereal infections are usually caused by an imbalance of the normal bacterial flora of the vagina. Only common infections with rather specific cytologic manifestations are discussed in this chapter.

BACTERIAL INFECTIONS

1. Gonorrhea is caused by *Neisseria gonorrhoeae*, a Gram-negative diplococci. It can be asymptomatic or manifested by purulent vaginal discharges associated with a burning sensation. The bacteria may be seen within the cytoplasm of neutrophilic polymorphonuclear leukocytes in Papanicolaou-stained smears, but the infection is confirmed by bacterial culture.

2. Bacterial vaginosis is also called a “shift in flora”. It is a common, nonspecific cervicovaginitis and is often asymptomatic. On Pap smears characteristic “clue cells” are present in a filmy background of small coccobacilli. These are superficial and intermediate squamous cells covered by a layer of bacteria (coccobacilli) that obscures the cell membrane. These cells should be differentiated from “false-clue cells” that are also squamous cells covered with bacillary organisms. An absence of lactobacilli is evident. (Fig. 3.1). The Pap smear has 80% sensitivity and 87% specificity in the diagnosis of bacterial vaginosis.

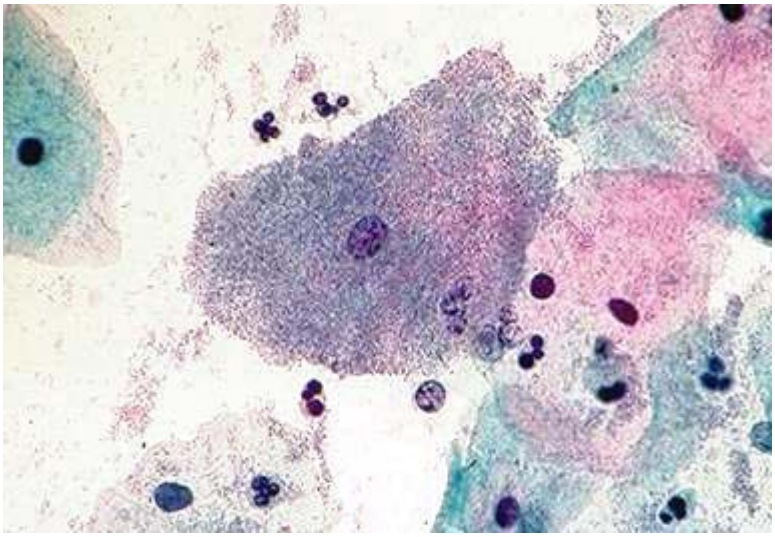


Fig.3.1. A “clue cell” covered with numerous coccobacilli in a CP smear.

3. Actinomycosis. Actinomyces normally reside in the female genital tract, so its presence is not an indicator of disease. Actinomycosis is characterized by a foul-smelling vaginal discharge containing sulfur granules. It is commonly caused by *Actinomyces israelii* in patients with IUDs or pessaries for contraception with a colonization rate of about 11% but this rate increases with the duration of use of the above-mentioned devices. These microorganisms are Gram-positive and present as irregular, thick bundles or clusters of filaments (Gupta bodies). The smear background shows numerous polymorphonuclear leukocytes. (Fig.3.2).

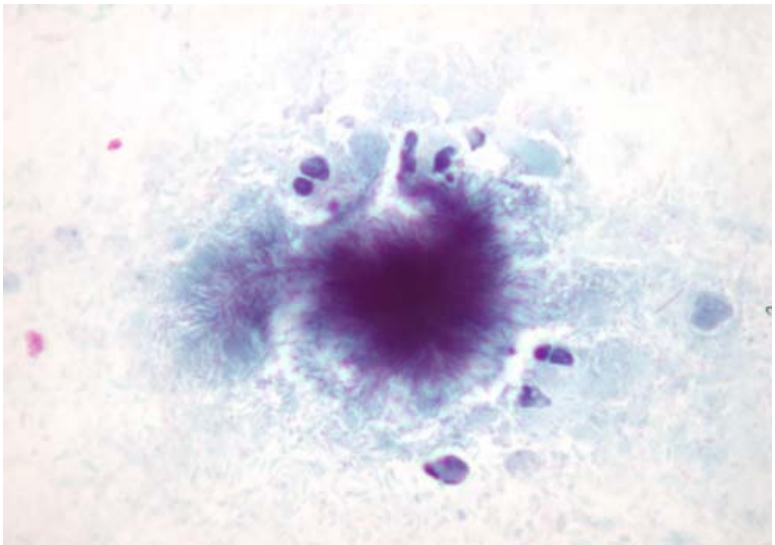


Fig.3.2. Actinomyces in a CP smear showing a thick cluster of filamentous elements.

4. Granuloma inguinale is an uncommon disease. Material scrapped from the ulcerated lesion reveal inflammatory exudates with vacuolated macrophages containing Donovan bodies (safety pin-shaped, gram-negative microorganisms) that are best demonstrated by Giemsa stain.

5. Chlamydia trachomatitis is the 2nd most common STD in the Western world after HPV infection. There are about 4 million new cases diagnosed annually in the United States. The infection is usually asymptomatic in females and affects the cervix, uterus and its annexae, but not the vulva or vagina. The agent is an obligate intracellular microorganism with 2 forms: the metabolically inactive form called the elementary body, and the metabolically active form called the reticulate body. The infection mainly involves the endocervical columnar epithelium but may spread to the endometrium and fallopian tubes. On Pap smears a Chlamedial infection may be suspected by the presence of intracytoplasmic vacuoles containing aggregates of small coccoid bodies within columnar or metaplastic squamous cells. (Fig.3.3). The diagnosis now is made by molecular testing. Metaplastic squamous cells with mucous globules may be mistaken for Chlamydial infected cells.

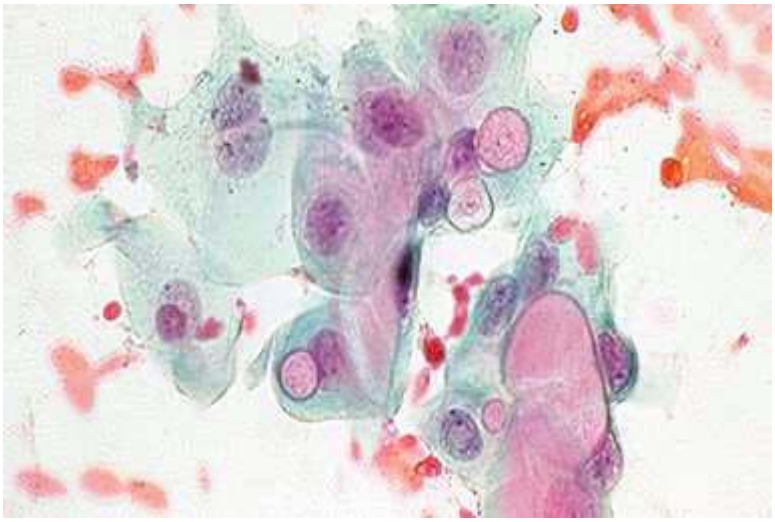
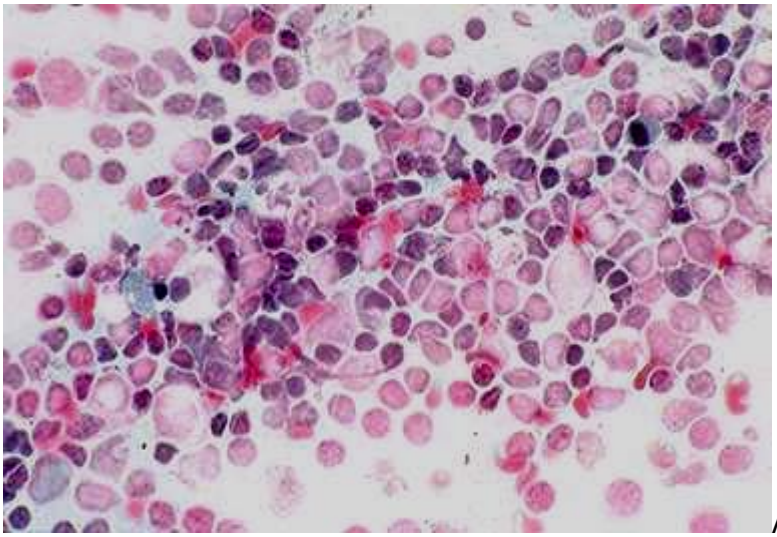


Fig.3.2. Chlamydial infection showing in a CP smear metaplastic squamous cells with vacuoles containing small coccoid bodies.

6. Follicular cervicitis is seen in about 50% of patients with Chlamydial infection, but the converse is not true. Numerous lymphoid cells at different stages of maturation and macrophages with tingible bodies are seen. (Fig.3.3).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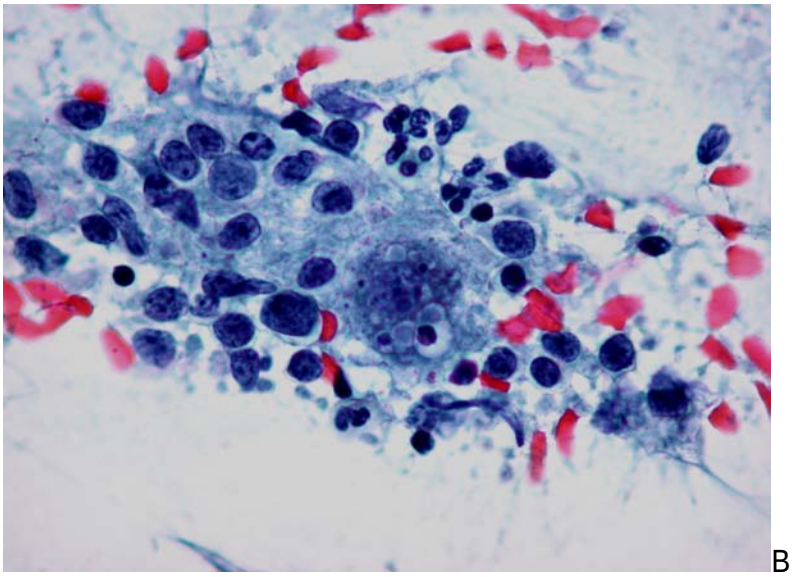
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Fig.3.3. Benign lymphoid cells at different stages of maturation and a large histiocyte with tingible bodies in a CP smear from a follicular cervicitis.

VIRAL INFECTIONS

1. HPV infection

Genital HPV infection is common, almost exclusively sexually transmitted and self-limiting in young women. HPV is a member of papovirus family. It consists of an icosahedral viral particle (virion) containing an 8000 base pairs of double-stranded circular DNA molecule surrounded by an icosahedral protein capsid. The HPV DNA strand contains 6 E genes and a controlling regulatory region that code for viral replication process, and 2 L genes that code for capsule proteins. These E and L genes are named as “E” or “early” and “L” or

“late” depending on the time of their expression in the course of the infection. The pathogenesis of HPV infection was summarized by Cibas as follows:

- HPV requires a human host cell to replicate.
- In the female genital tract, the virus affects the squamous cells of vulva, vagina and cervix and the glandular cells of the endocervix.
- However, it targets mainly the metabolically active metaplastic squamous cells in the transformation zone of the cervix.
- HPV infection begins in the basal layers of the epithelium in which the HPV genome is established, with the expression of E genes.
- As the cells mature and move toward the surface, L1 and L2 genes are expressed.
- And with the progress of the infection, the viral DNA becomes established throughout the entire thickness of the epithelium, but intact virions are found only in the upper layers of the epithelium.

HPVs are divided into low- and high-risk types: Low-risk HPVs are virtually never associated with cervical cancer and the most common types include 6, 11, 42 and 44. In these low-grade lesions the viral DNA does not integrate into the host genome and remains in the free episomal form. High-risk HPVs are the virus types that have been identified in CIN 2, CIN 3 and cancer, and the most common types are: 16, 18, 31, 33, 35, 45, 52 and 58. HPV types 16 and 18 usually integrate into the host genome and express large amounts of E6 and E7 proteins that block or inactivate tumor suppressor genes p53 and Rb (retinoblastoma), respectively: binding of E6 to p53 gene blocks the apoptosis of the cells, and binding of E7 to the Rb tumor suppressor protein pRb interferes with the cell cycle arrest. These bindings will lead to an uncontrolled cell proliferation. The transformed cells are capable of autonomous growth and susceptible to the acquisition of further mutations.

The overall prevalence of high-risk HPVs varies from continent to continent: 22.9% in Africa, 15.5% in the Americas, 6.6% in Europe and 8.3% in Asia. However, within the same continent, it varies from country to country. Its overall average prevalence in cytologically normal women is 10.4% worldwide. Young women have a high prevalence rate that is about 30% in the late teens and early twenties. It decreases through the reproductive years to as low as 5% and may rise again up to 10% to 20% in the late forties and fifties. The reasons for the second rise are not known with certainty. An increased number of new sexual partners in this age group or alteration in immune surveillance status has been suggested to explain this second rise.

As a high percentage of women harbor these viruses but only a small number of them develop cancer, there is a suggestion of additional roles of risk factors. The most important risk factors include early age of first intercourse, multiple sexual partners, a male partner with multiple previous sexual partners and persistent infection by high-risk HPVs. Other risk factors include cigarette smoking, immunodeficiency status and genetic vulnerability. The peak incidence of CIN is about 30 years and that of invasive carcinoma is about 45 years.

The precancerous changes referred to as CIN may begin as CIN 1 (flat condyloma) and progress to higher grade CINs 2 and 3 or they may begin at the outset as CIN 2 or 3, depending on the type of HPV infection (low- or high-risk virus) and other risk factors. According to some studies:

- CIN 1 lesions regress in 50% to 60%, persist in 30% and progress to CIN 3 in 20% of cases.
- Only 1% to 5% of CIN 1 lesions will eventually develop into an invasive cancer.
- CIN 3 lesions regress in about 33% and progress to invasive cancer in about 60% to 74% of patients.

Low-grade lesions are associated with replicative infections of HPV where the viral DNA is present as an episome outside of the host cell DNA. These reproductive infections allow completion of the viral life cycle and the production of whole infectious virions capable of infecting other cells. The koilocyte (characteristic for a CIN 1) is filled with complete virions ready to be discharged. Low-grade CINs typically run a benign course, resolve and clear to normal with nondetectable HPV in over 90% of cases. High-grade CINs and cancer may contain only the oncogenic portions of the HPV genome. These genes are most commonly integrated into the host's DNA that then allows for uncontrolled expression and cellular replication.

About 85% to 90% of CIN 1 lesions are caused by high-risk HPVs but they can also be caused by low-risk viral types. CIN 2 and CIN 3 are almost always caused by high-risk HPVs. CIN lesions have cytologic abnormalities that often reflect their severities. In Pap smears of patients showing only atypical squamous cells of undetermined significance, high-risk HPVs are found in about 50% of cases. In women with normal cervical cytology, 10% to 15% harbor high-risk HPVs, and of these, only about 10% will develop a high-grade CIN.

HPV type 16 is more commonly associated with squamous cancers and HPV type 18 is more commonly associated with adenocarcinoma and neuroendocrine carcinoma of the cervix. The incidence of these high-risk types detected in cervical cancer in the United States range from 0.05% for HPV 42 to 54.5% for HPV 16. HPV types 6 and 11 are the commonest low-risk types that are most commonly associated with condylomas or genital warts. (Figs.3.4 and 3.5). HPV types 16 and 18 are responsible for 70% of all cervical cancers; and together with the other high-risk types (45,31,33,35,52 and 58) are responsible for 87% of all cervical cancers. The reader is referred to Chapter 4 for illustrations of CINs and squamous cell carcinoma in Pap smears and biopsied tissues.

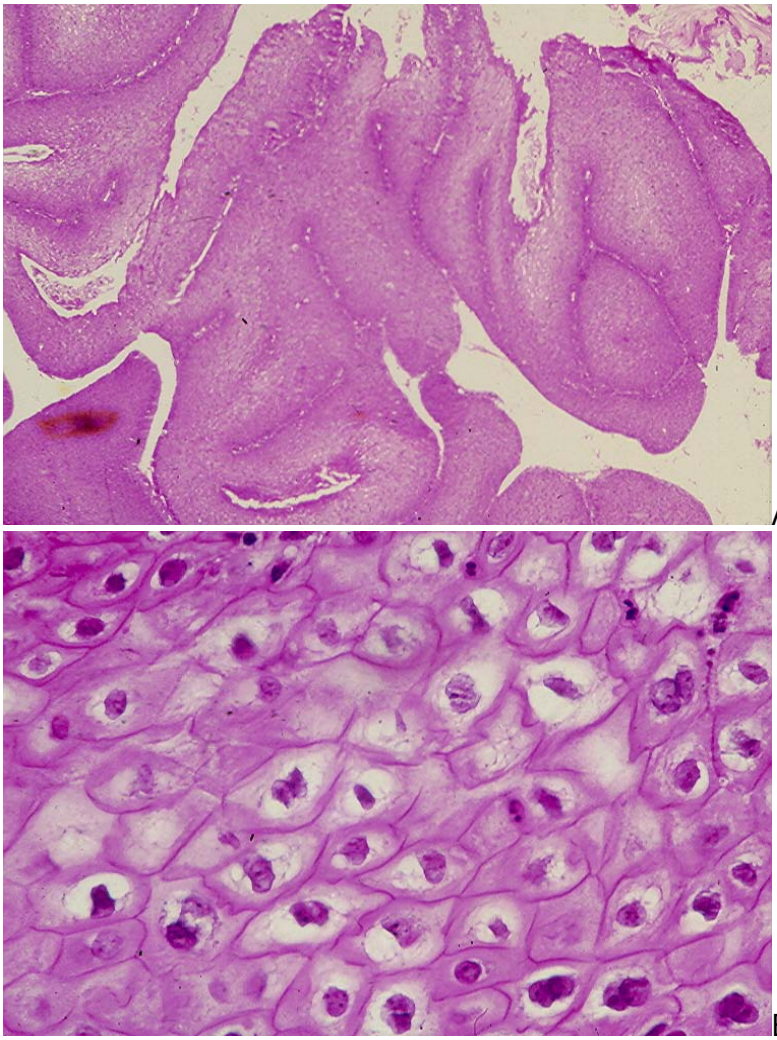


Fig. 3.4. Histology of a cervical condyloma.

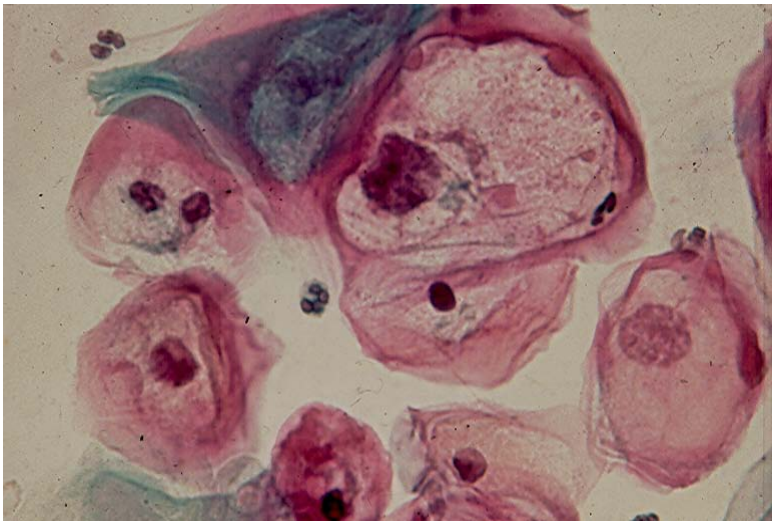


Fig.3.5. Koilocytes present in a CP smear of the lesion illustrated in Fig. 3.4.

The usefulness of HPV testing as a screening test for cervical cancer is limited. As most sexually active women will contract a cervical HPV infection at some point in their lifetime, and cervical cytology will remain as the main test for this purpose. In future years, with the introduction of HPV vaccines [Gardasil (targeting HPV types 6, 11, 16 and 18) and Cervarix (targeting HPV types 16 and 18)] administered to females aged 9 to 26, the number of high-grade CIN and carcinoma of the cervix, vulva and vagina is expected to decrease by 70%. Genital warts (caused by HPV types 6 and 11) are also expected to decrease by 90% subsequent to Gardasil vaccination. This vaccine also provides protection against genital warts and anal cancer in males.

HPV Testing

A few methods can be used to detect HPV infection in cytologic samples:

1. In situ hybridization is performed directly on cellular specimens and the infected cells can be visualized microscopically and correlated with morphologic abnormality. This assay is commercially available.

2. Polymerase chain reaction is the standard reference method for detecting and typing HPVs. It may also be used to assess the viral load. The assay is commercially available.

3. Hybrid Capture 2 (hc2), "Digene® test" (Qiagen, Gaithersberg, Maryland) is the most commonly used method for detecting HPV DNA. It is an in vitro nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of 18 types of HPV DNA in cervical specimens. The hc2 HPV DNA Test contains 18 RNA probes to various HPV types.

The test can differentiate between 2 HPV DNA groups: low-risk HPV types 6,11,42,43,44 and high-risk HPV types 16,18,31,33,35,39,45, 51,52,56,58,59 and 68, but it cannot determine the specific HPV type present in the specimen. It can be performed on the residual cell samples collected for the ThinPrep® Pap Test or with the Standard Transport Medium™ (Qiagen). It is the only test approved by the US Food and Drug Administration for detecting HPV for patient care. The test has a high sensitivity and low specificity, as not all patients with positive results have CIN or invasive cancer. For high-volume sample-throughput testing, the hc2 HPV DNA test can be performed using the Rapid Capture® system Instrument Application, but only the oncogenic high-risk HPV Probe was approved for high-volume testing. As HPV infection is selflimiting in young women, this test is not useful in women under 30 years of age.

Goal of HPV DNA testing

The test is not intended for use as a screening tool in the general population. It is designed to augment existing methods for the detection of cervical disease and should be used in conjunction with clinical information. The test results should not be used as the sole basis

for clinical assessment and treatment of patients. ***Its main utility is to screen patients with ASC-US Pap smear results to determine the need for referral to colposcopy***. However, results of the test are not intended to prevent women from proceeding to colposcopy.

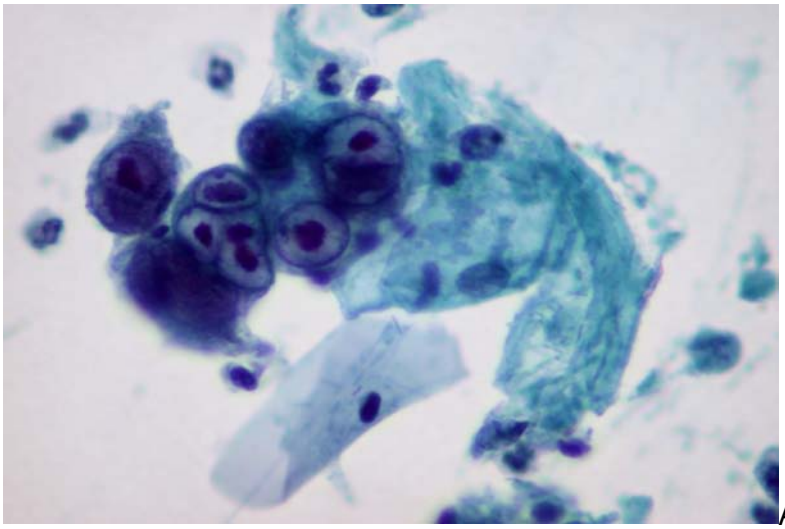
Interpretation

HPV DNA test results should be interpreted in conjunction with clinical findings and data derived from other diagnostic procedures.

- If the high-risk HPV probe is negative: there is a high probability that a high-grade CIN lesion will be not found at colposcopy.
- If the high-risk HPV probe is positive: there is a low but increased probability that a high- grade CIN lesion or a more severe lesion will be detected at colposcopy.

2. Herpes simplex virus.

HSV commonly infects cervix and vagina. The reported rate of genital herpes virus infection in North America range from 87 to 217 per 100,000, and it is caused by HSV-1 and HSV-2. The infection causes inflammatory epithelial ulcers. Multinucleated giant squamous cells with nuclear molding and intranuclear inclusions or chromatinic liquefaction with “ground-glass” appearance are seen at the ulcer borders and are characteristic for the infection. When the cytomorphologic changes are equivocal, an immunocytochemical staining of the infected cells with a commercial Herpes simplex antibody will be helpful for confirmation. (Fig. 3.6). This antibody is specific for HSV-2 but also cross-reacts with HSV-1. This immunocytochemical confirmatory test has 91% sensitivity and 95% specificity.



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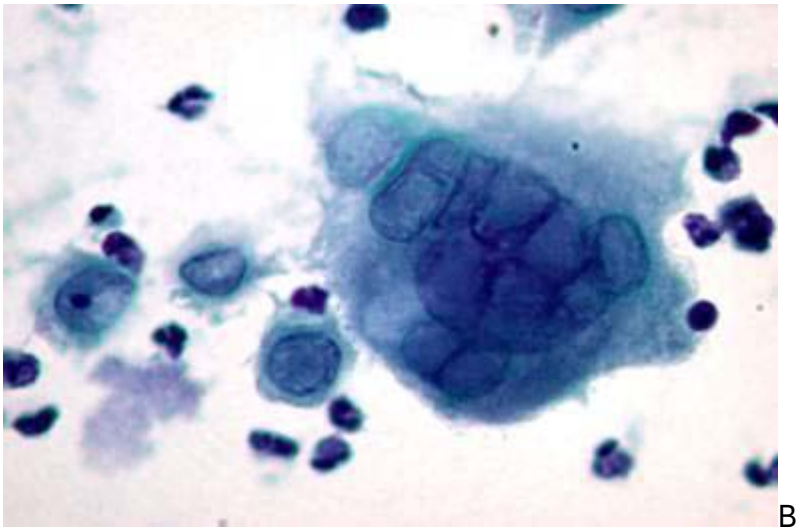
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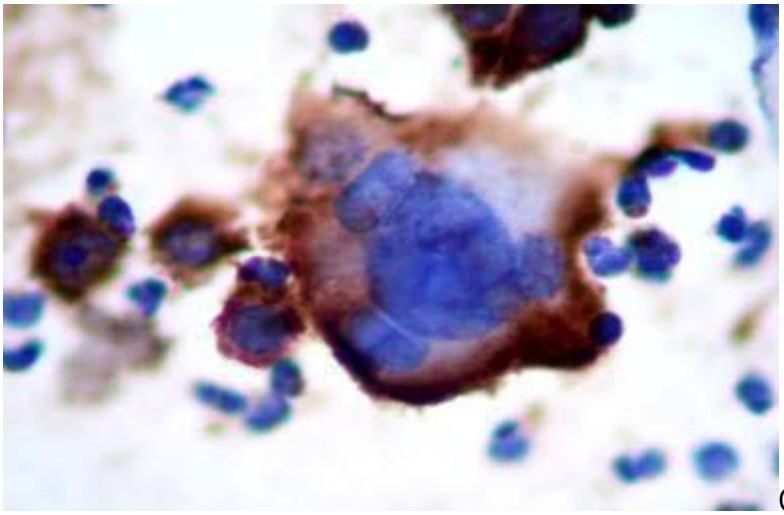
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Fig.3.6. Herpes simplex infection showing in a CP smear:
A. Infected epithelial cells with intranuclear inclusions.
B. Infected cells displaying multiple nuclei with “groundglass” change.
C. Infected cell showing positives cytoplasmic reaction to Herpes simplex antibody. (Immunoperoxidase).

4. Cytomegalovirus infection.

This infection is rarely detected by Pap smear. The infection affects endocervical and endometrial cells with production of characteristic large eosinophilic or amphophilic intranuclear inclusions.

FUNGAL INFECTION

Candidiasis. Elements of *Candida species* are normally found in the cervix and vagina and are present in 3% of all Pap smears. Its presence is not indicative of a fungal infection

requiring treatment. *Candida albicans* is the most common microorganism causing cervical vaginal candidiasis. Both yeasts and nonseptated pseudohyphae are seen. The budding yeasts are 3 to 7 µm in greatest dimension and the pseudohyphae are eosinophilic to gray-brown. The pseudohyphae are formed by elongated buddings that display constrictions along their length. They should be differentiated from elements of Trichophyton that may contaminate the Pap smears. (Fig.3.7).

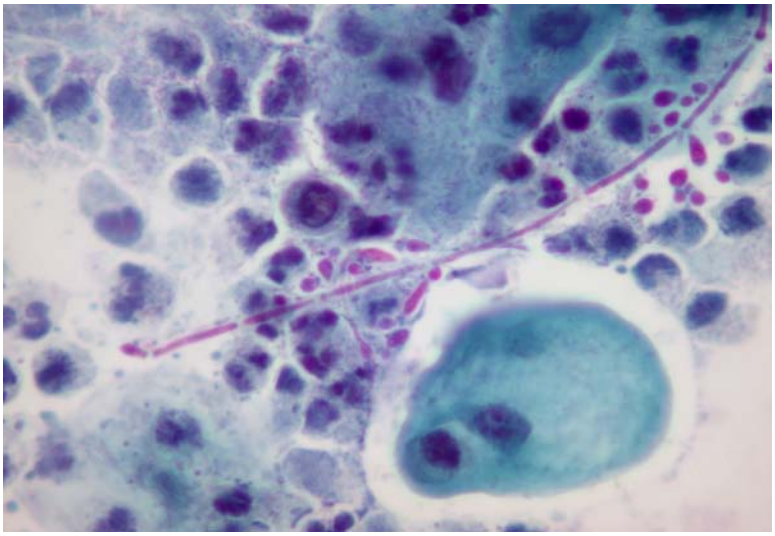
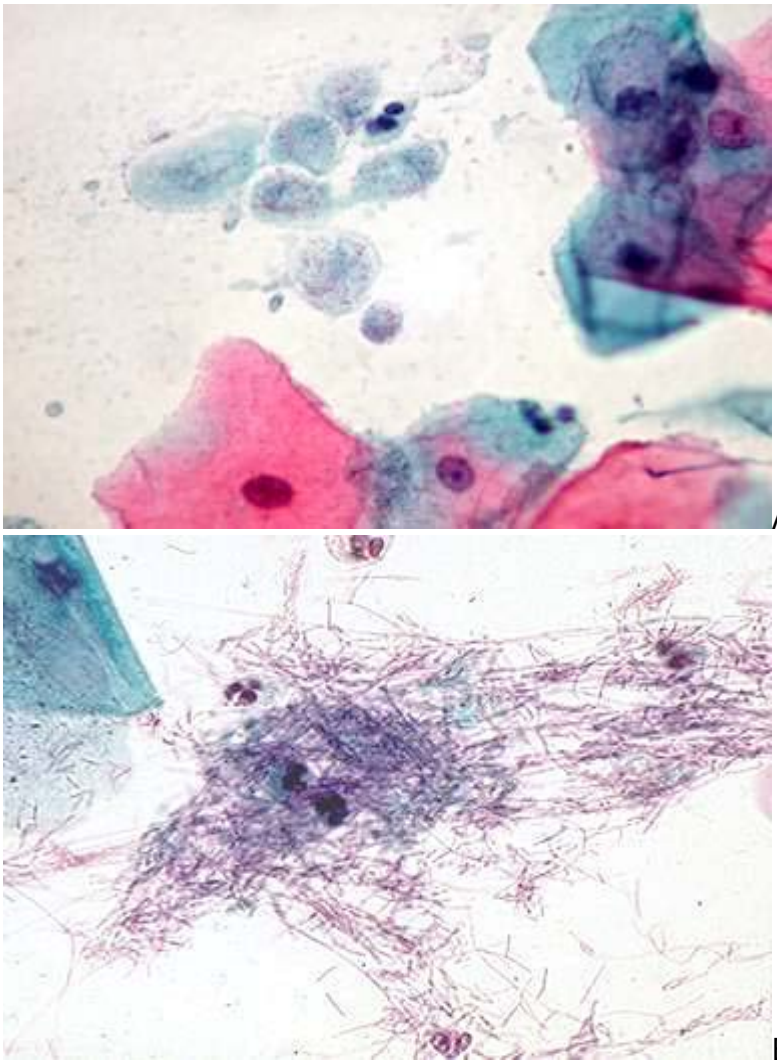


Fig.3.7. Vaginal candidiasis showing in a CP smear inflammatory exudate containing yeasts and nonseptated pseudohyphae of *Candida species*.

PARASITIC INFECTION

Trichomonas vaginalis is the most common STD of the lower female genital tract. *Trichomonas vaginalis* is a facultative anaerobic protozoan parasite without mitochondria or peroxisomes. The infection is characterized by a dense inflammatory exudate containing "pus balls" or large aggregates of polymorphonuclear leukocytes and *Trichomonas vaginalis* organisms. The organisms can be identified in routinely stained Pap smears. They are pear-shaped, oval or round cyanophilic organisms ranging in size from 15 to 30 µm. The nucleus is pale, vesicular and eccentrically located. Intracytoplasmic eosinophilic granules are often present and flagellae are usually not observed. Identification of Trichomonas vaginalis organisms in conventional Pap smears can be difficult, but its identification in liquid-based preparations is highly accurate and does not require a confirmatory test. The squamous cells present in the smear commonly show basophilic and eosinophilic cytoplasm and slightly enlarged hyperchromatic nuclei with perinuclear haloes, mimicking ASC-US or LSIL cells. Leptothrix infection is a commonly associated infection with Trichomonas vaginalis. Elements of *Leptothrix* have a "spaghetti and meat balls" configuration. (Fig.3.8). Trichomonas vaginalis must be treated even if it is asymptomatic.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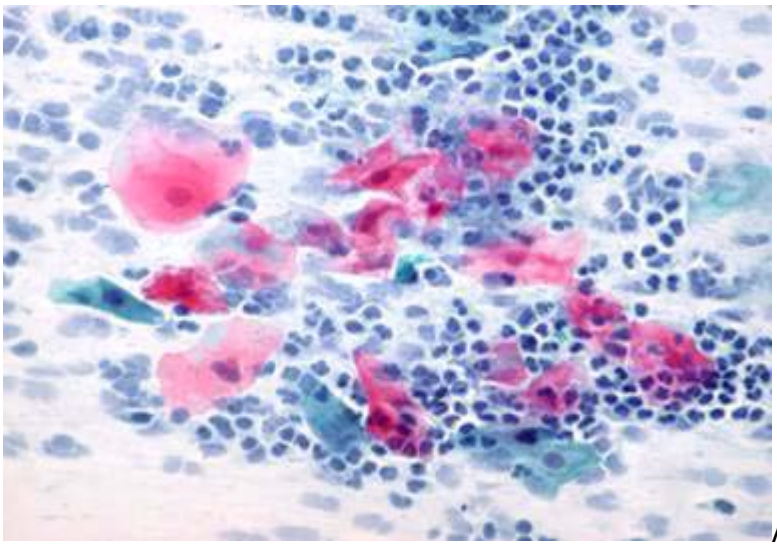
B

Fig. 3.8. A CP smear showing *Trichomonas vaginalis* organisms with intracellular eosinophilic granules (A) and elements of *Leptothrix* (B).

INFLAMMATION-ASSOCIATED CELLULAR CHANGES

Acute inflammation is often associated with changes in squamous cells. The affected cells are seen singly or in loose aggregates. They may show perinuclear halos or cytoplasmic vacuolization. Membrane rupture may be seen and the nuclei are enlarged, hyperchromatic with regular contours. (Fig. 3.9). The chromatin is clumped, fuzzy and may show karyorrhexis, karyolysis and karyopyknosis. The smear background contains numerous polymorphonuclear leukocytes. Marked cytolysis may be seen in cervicovaginal smears containing abundant Döderlein bacilli that produce enzymes causing destruction of intermediate squamous cells. Cellular debris and free lying rod-shaped bacilli are characteristic of the condition. This type of cytolysis is seen in conditions in which

intermediate squamous cells are the predominant cell present such as with pregnancy, the secretory phase of the menstrual cycle, steroid therapy and postmenopause.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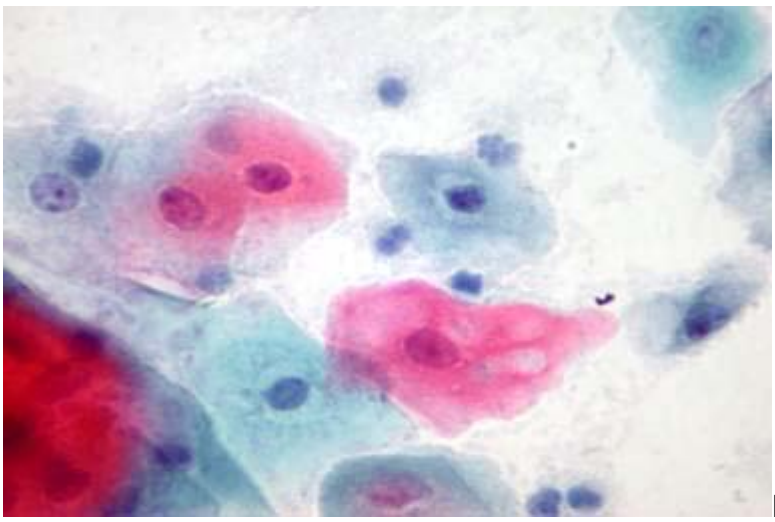
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Fig.3.9. Perinuclear halos and slight nuclear enlargement in intermediate squamous cells caused by an acute inflammation seen in a CP smear.

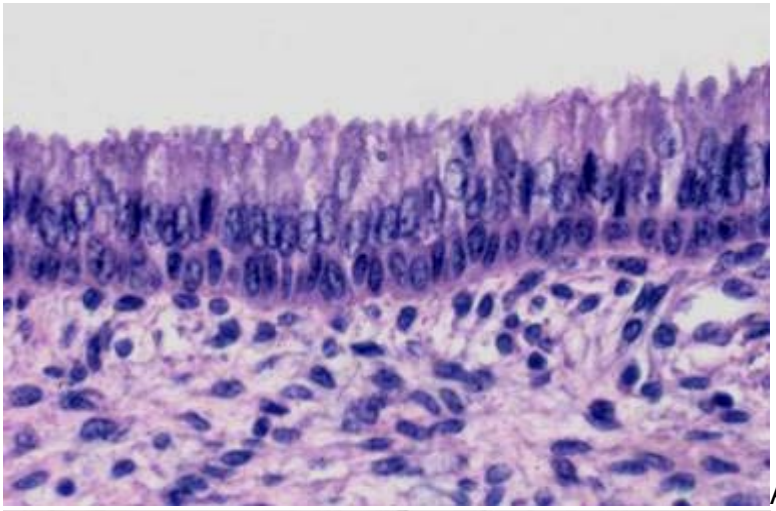
NONNEOPLASTIC EPITHELIAL PROLIFERATIONS

The cervical epithelium is under the effects of hormonal stimulation, inflammation or physical irritation. It may undergo hyperplasia, metaplasia and keratinization.

A. RESERVE CELL HYPERPLASIA

Reserve cells form a discontinuous layer between the endocervical columnar cells and

the basement membrane. These cells are capable of differentiating into either squamous cells or endocervical glandular cells and proliferate as a response to physical or chemical irritation. They are usually seen in clusters and show oval, bland nuclei with scant, ill-defined, vacuolated cytoplasm. Rarely, they are present singly and they may also be seen in tissue fragments. (Fig. 3.10).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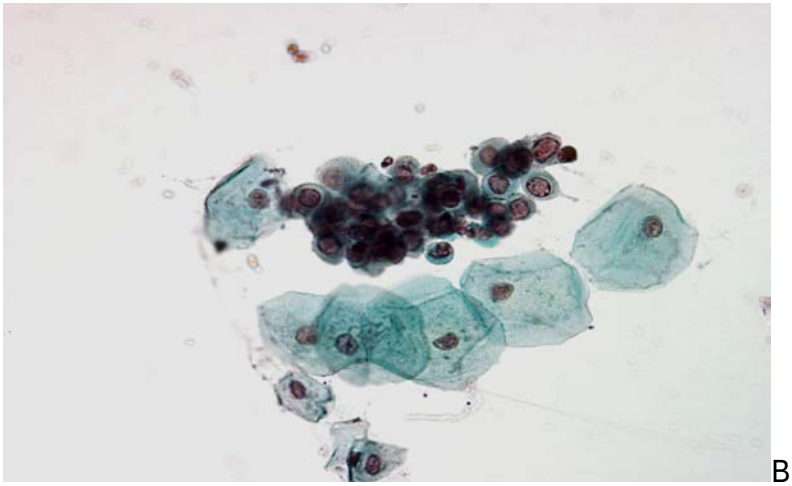

B

Fig.3.10. Reserve cell hyperplasia.
A. Histology of endocervical mucosa showing reserve cell hyperplasia.
B. A CP smear showing a cluster of hyperplastic reserve cells with oval, bland nuclei and scant cytoplasm.

B. SQUAMOUS METAPLASIA

Hyperplastic reserve cells gradually transform into immature squamous cells that exfoliate in sheets or singly. The immature squamous cells have pale, vacuolated cytoplasm and may show cytoplasmic extensions or tails (spider cells). Their oval nuclei have fine chromatin and are slightly hyperchromatic. With time, immature metaplastic squamous

cells change into mature squamous cells with more waxy, basophilic or eosinophilic cytoplasm. (Fig.3.11).

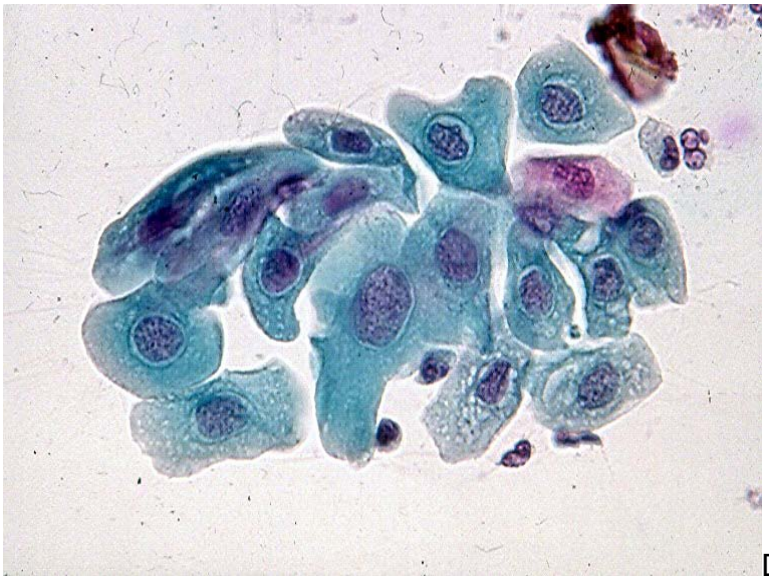

D

Fig.3.11. Endocervical epithelium with squamous metaplasia.
A. Histology of the lesion.
B. Metaplastic cells showing “spiderleg” cytoplasmic extensions,
C. Metaplastic cells with some displaying intracytoplasmic mucous vacuoles and
D. Mature metaplastic cells with mild nuclear enlargement.
(B - D: CP smears)

C. HYPERKERATOSIS AND PARAKERATOSIS

Hyperkeratosis is a protective process by which nonkeratinized squamous epithelium protects itself from injuries. The squamous epithelium becomes acanthotic and is covered by a thick layer of keratinous material that is characterized clinically as leukoplakia. It commonly occurs on the cervices of prolapsed uteri. Thick orangeophilic layers of anucleated squamous cells with keratohyaline granules are observed. (Fig.3.12).

Parakeratosis is a form of hyperkeratosis in which small round or spindle-shaped keratinized squamous cells retain their pyknotic nuclei. (Fig.3.13). These cells exfoliate singly or in loose clusters. It is important to differentiate these cells from *pseudokeratotic cells* that are also small squamous cells with eosinophilic or basophilic cytoplasm and nonpyknotic oval nuclei with fine chromatin. Both *parakeratotic* and *pseudoparakeratotic cells* are commonly seen in condylomatous lesions of the cervix and vagina.

A

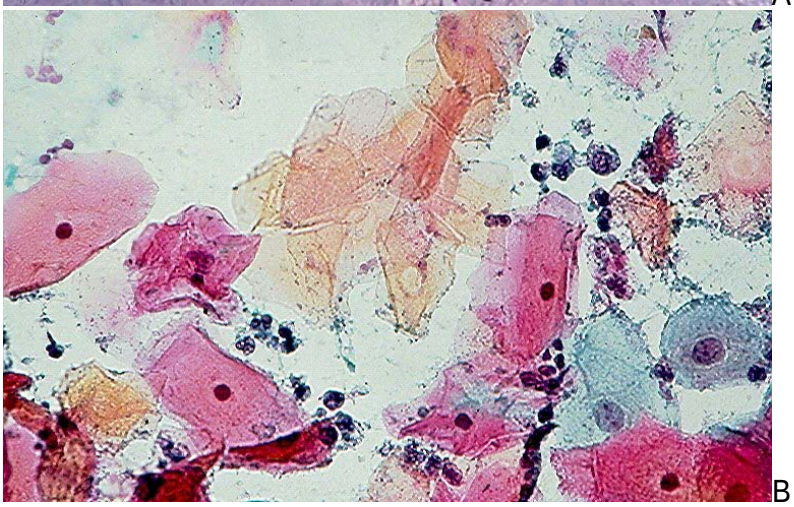

B

Fig. 3. 12. Hyperkeratotic cervical squamous epithelium.
A. Histology of the lesion.
B. An irregular aggregate of anucleated, orangeophilic squames in a CP smear.

A

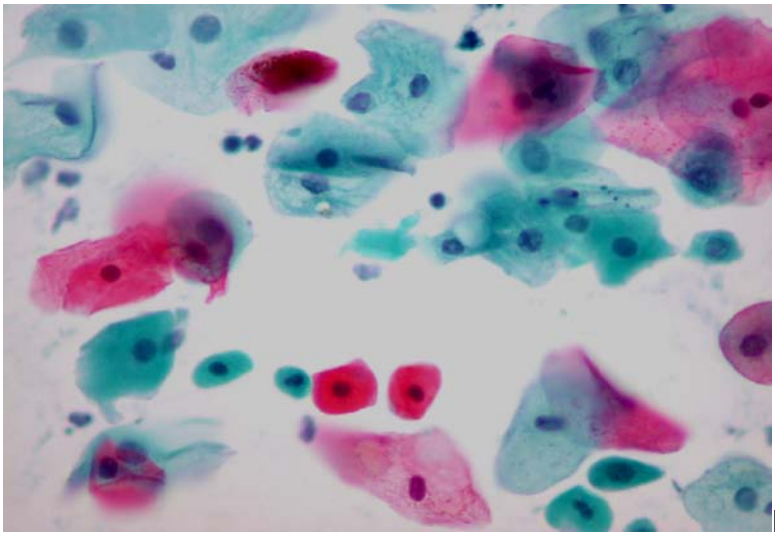

B

Fig.3.13. A , B. CP smear showing small parakeratotic cells with keratinized, orangeophilic or eosinophilic cytoplasm and pyknotic nuclei.

D. TUBULOENDOMETRIAL METAPLASIA

Tubuloendometrial metaplasia is common, affecting about 30% of women. The lesion is located in the upper portion of endocervical canal, often in deep clefts. It may represent a response to injury. Cytologic material from this lesion may reveal ciliated cells with clear cytoplasm and abundant apical ciliae, secretory cells and intercalated cells with scant cytoplasm and thin, long nuclei (peg cells).

E. UROTHELIAL METAPLASIA

Urothelial metaplasia may be seen on exocervical atrophic squamous epithelium in elderly patients. The exfoliated metaplastic cells display oval, bland nuclei with longitudinal grooves. (Fig.3.14)

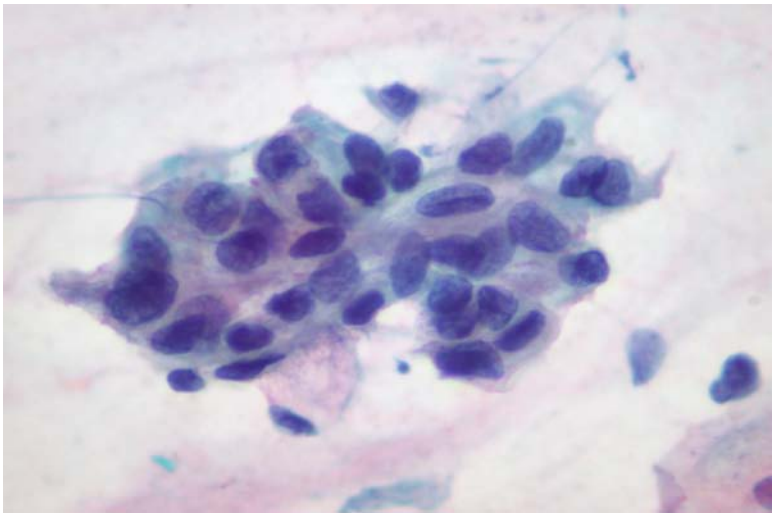
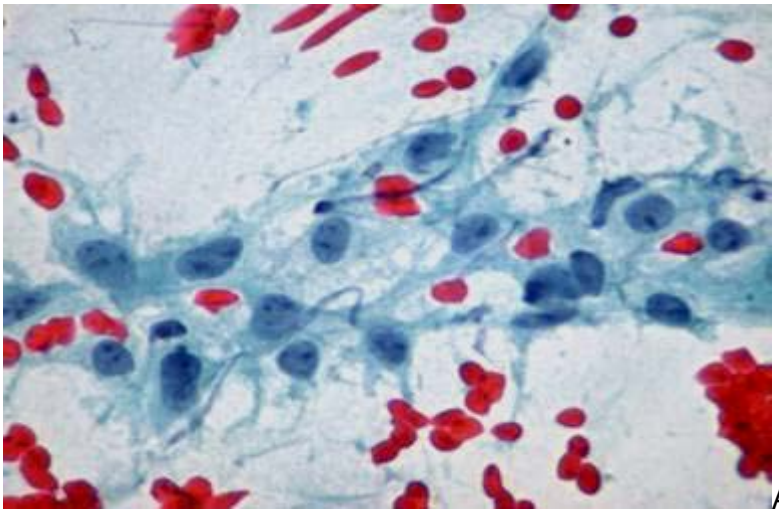


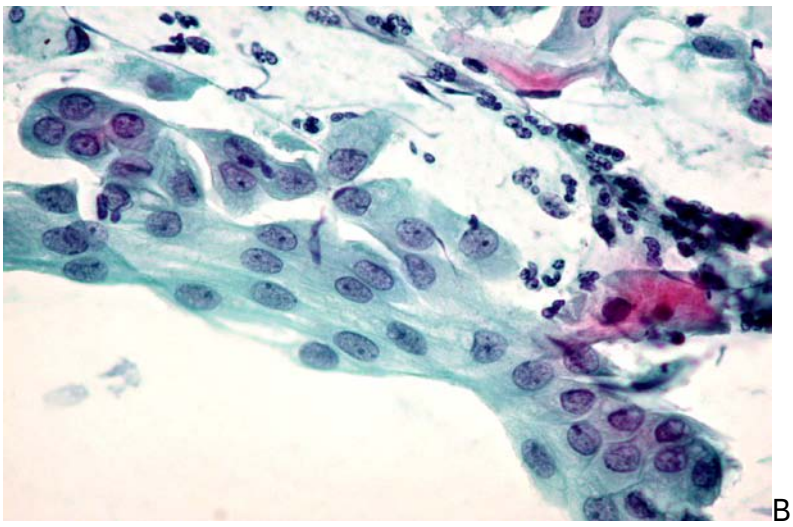
Fig.3.14. CP smear showing a cluster of metaplastic urothelial cells showing thin cytoplasm and oval nuclei with some displaying longitudinal grooves.

F. REACTIVE CELLULAR CHANGES DUE TO INFLAMMATION AND REPAIR CELLS

Reactive cellular changes are benign in nature. They are often associated with inflammation, radiation, IUD and other nonspecific causes. Criteria for reactive changes are not always well defined, and as the result, the interpretation may lack reproducibility. Repair cells are seen in Pap smears of patients with inflammatory epithelial ulcers, and with previous biopsy, cautery and cryosurgery of their uterine cervices. During the repair process the ulcer base is replaced by granulation tissue that is then covered by epithelial cells that proliferate from adjacent squamous or glandular epithelium. The repair cells present singly or in sheets, show cytoplasmic extensions and have single or double, regularly contoured nuclei with prominent nucleoli. They are spindle or columnar in configuration and may show intracytoplasmic vacuoles. Determination of the origin of repair cells (squamous versus glandular) is extremely difficult or impossible in the majority of cases.

Repair cells commonly show nuclear enlargement (1, 1.5 or 2 times the area of the nucleus of a normal intermediate squamous cell). Endocervical cells may show a greater nuclear enlargement. Nuclei may be double or multiple with smooth contours, and mildly hyperchromatic with fine chromatin. Prominent single or multiple nucleoli are observed. The cell cytoplasm may display polychromasia, vacuolization and perinuclear halos without thick cytoplasmic rims. Squamous metaplastic cells display similar nuclear and cytoplasmic changes; and cytoplasmic processes (spider cells) may be observed. (Figs.3.16 and 3.17).


A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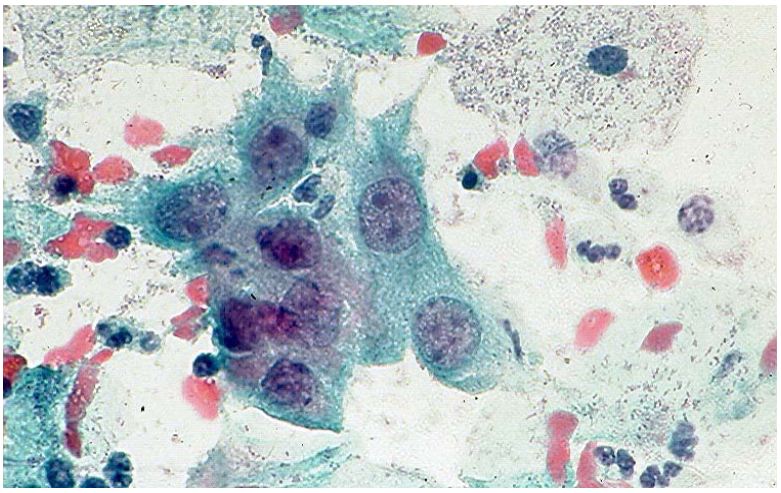
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Fig.3.16. Repair epithelial cells in CP smears:
A. Repair cells with cytoplasmic processes.
B. Repair cells with granular or vacuolated cytoplasm in a monolayered sheet.
C. Repair cells with prominent nucleoli and cytoplasmic processes.

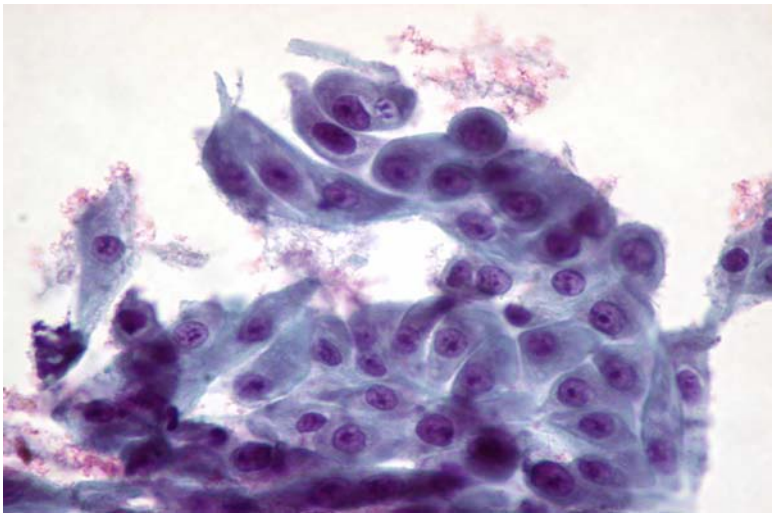


Fig.3.17. A monolayered sheet of repair squamous cells with conspicuous nucleoli in a LBP.

G. VITAMIN B12 AND FOLIC ACID DEFICIENCY-INDUCED CELLULAR CHANGES

These cellular changes are characterized by an enlargement of squamous cells and their nuclei. The nuclei are single or double and slightly hyperchromatic with fine chromatin. The presence of hypersegmented nuclei within polymorphonuclear leukocytes on the smear is an evidence supporting a diagnosis of pernicious anemia.

H. RADIATION AND CHEMOTHERAPY EFFECTS

Radiation and chemotherapy are routinely used in the treatment of patients with advanced solid cancers, lymphomas and leukemias. Their mechanisms of action on protein metabolism and mitosis on human cells differ, however they produce similar cellular changes.

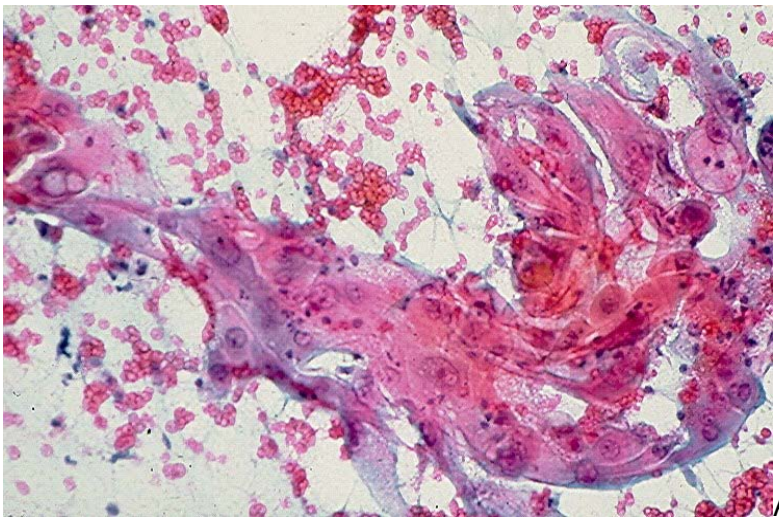
1. Radiation effects

Injury is caused by radiation-induced ionization of intracytoplasmic molecules from inhibition of DNA synthesis and destruction of cellular proteins and enzymes. The extent of cellular injury varies with the type of radiation, the duration of exposure and the radiosensitivity of the cells. Hematopoietic cells, germ cells, gastrointestinal cells and anaplastic tumors are highly susceptible to radiation injury because they have a high mitotic rate. Radiation changes may be classified as acute and chronic.

- **Acute post radiation changes** may appear a few days after the conclusion of treatment, persist for 6 to 8 weeks and then gradually subside. In the uterine cervix the changes affect mainly the squamous cells, however the endocervical glandular cells are

also affected albeit to a lesser degree. The smear shows inflammatory exudates with cellular debris, histiocytes and polymorphonuclear leukocytes forming “pus balls”.
The reactive epithelial cells are markedly increased in size without a substantial increase in the N/C ratio. Bizarre cell shapes may be seen and enlarged nuclei may show degenerative changes including nuclear vacuolization, pallor, wrinkling and smudging of chromatin. The nuclei vary in size, with some cell groups having both enlarged and normal-sized nuclei. Bi- or multinucleation and mild hyperchromasia are common and prominent single or multiple nucleoli are not uncommon. Cytoplasmic vacuolization, hyalinization or polychromasia may be present, as well as intracytoplasmic aggregates of leukocytes. Repair cells may also show radiation changes. (Figs.3.18 to 3.20).

- **Chronic radiation changes** appear about 6 months after cessation of the initial radiotherapy and may persist for years. The Pap smear displays an atrophic pattern with parabasal cells and intermediate squamous cells and pleomorphic giant cells. Cellular changes as seen in acute postradiation injuries may still be seen but they are less prominent.


A

B

Fig.3.18. CP smear showing squamous cells with radiation changes.
A. An aggregate of squamous cells with slightly pleomorphic, hyperchromatic nuclei and polychromatic cytoplasm.
B. A fragment of squamous epithelium showing large epithelial cells with mild nuclear enlargement and conspicuous nucleoli. Intracytoplasmic vacuoles are noted in some cells.

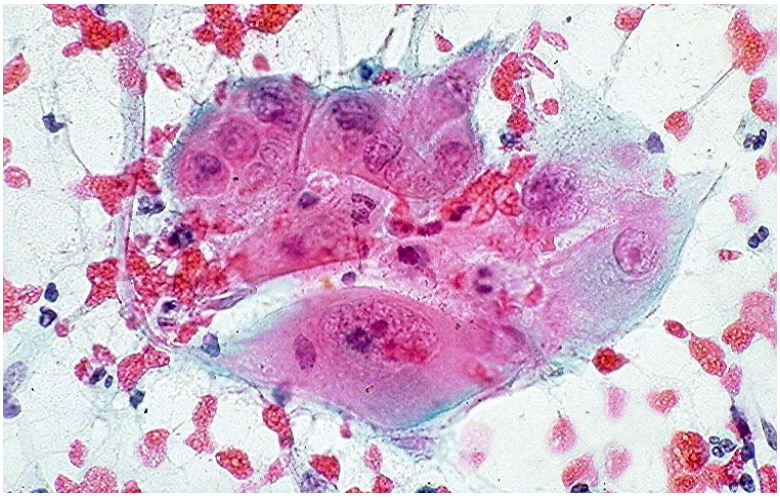


Fig.3.19. CP smear displaying a cluster of large epithelial cells with enlarged nuclei, prominent nucleoli showing thick, polychromatic cytoplasm with cytoplasmic extensions suggesting repair cells with radiation changes.

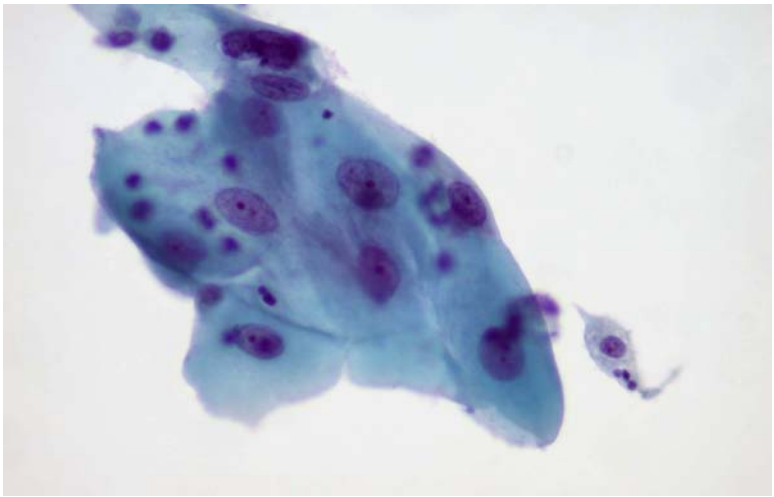


Fig.3.20. Squamous cells with radiation effects in a LBP.

2. Chemotherapy effects

Many drugs used to treat malignant diseases are alkylating agents, which are derivatives of nitrogen mustard. They alter the cellular DNA, RNA and proteins by different mechanisms. The cellular changes caused by alkylating agents are similar to those caused by radiation but they are systemic. (Fig.3.21). In Pap smears, the number of abnormal cells is much

smaller than in the case of radiation therapy. The epithelial cells from a patient receiving immunosuppressive agents, such as azathioprine and corticosteroids to prevent rejection of transplanted organs, may show cellular atypia simulating dyskaryotic or malignant cells. Therefore, an awareness of the patient's therapy is important to avoid a false-positive cytodiagnosis.

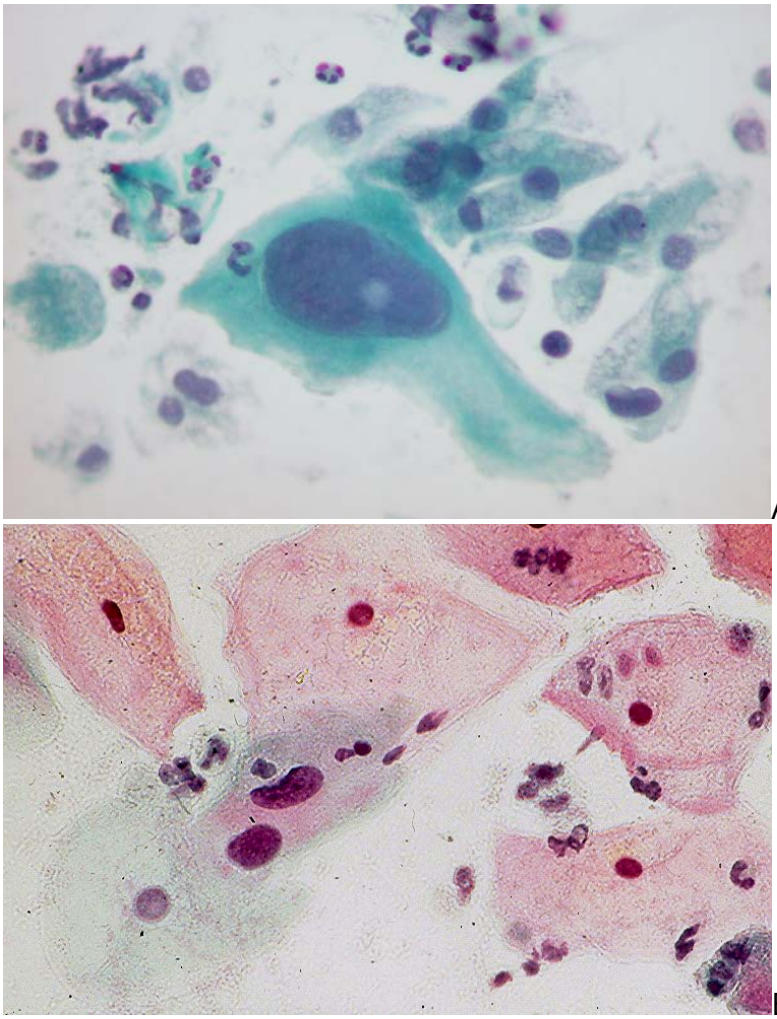


Fig.3.21. CP smear showing in A and B squamous cells with chemotherapeutic effects (Methotrexate) displaying enlarged, hyperchromatic nuclei.

It is important to note that if during radiotherapy and after its conclusion malignant cells are still present, a persistence of the original tumor should be suspected and a tissue biopsy should be obtained for histologic confirmation. In the event of interpretative uncertainty (suspicious report) the patient should be kept under observation with repeating cell samples every 3 to 6 months. In the majority of the cases, the cellular abnormalities regress. If tumor cells reappear after a tumor-free interval of several months or years a tumor recurrence is suspected and it should be confirmed by tissue biopsy. It should be

born in mind that patients with chemotherapy or immunosuppressive therapy have an increased risk of developing a second malignancy.

I. OTHER FINDINGS

- **"Cornflakes"** or brown artifact "cornflaking" is due to evaporation of xylene before coverslipping with deposition of air on superficial squamous cells. Cornflaking is more commonly seen in CP smears than in LBPs. (Fig.3.22).

Fig.3.22. CP smear showing superficial squamous cells with "cornflakes".

- **Blue blobs** represent condensed mucus, degenerated bare nuclei and precipitating hematoxylin. In postmenopausal women, they may represent parabasal/intermediate squamous cells with various degree of degeneration. Blue blobs appear as dark blue, round, oval, amorphous masses in Papanicolaou stained CP smear. (Fig.3.23).

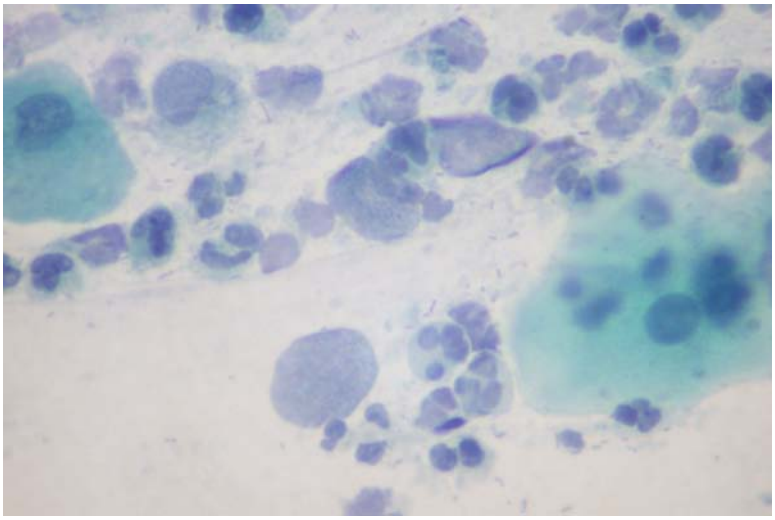


Fig.3.23. A few “blue blobs”, 2 intermediate squamous cells and several polymorphonuclear leukocytes in a CP smear.

- **Psammoma bodies** are laminated calcified round bodies. (Fig.3.24). They are rarely seen in Pap smears as they occur only about one in every 100,000 samples. In about 50% of patients a benign condition or no lesion is found. Psammoma bodies have been identified in Pap smears of patients taking oral contraceptive pills, using an IUD, with salpingiosis or pelvic inflammatory disease. In other cases, in particular postmenopausal women, an ovarian papillary serous carcinoma may be present. In those cases, the psammoma bodies are usually seen admixed with malignant epithelial cells that may wrap around some bodies. Therefore, further investigation is recommended in all patients showing psammoma bodies in their Pap smears to rule out the possibility of a clinically occult ovarian serous cancer.

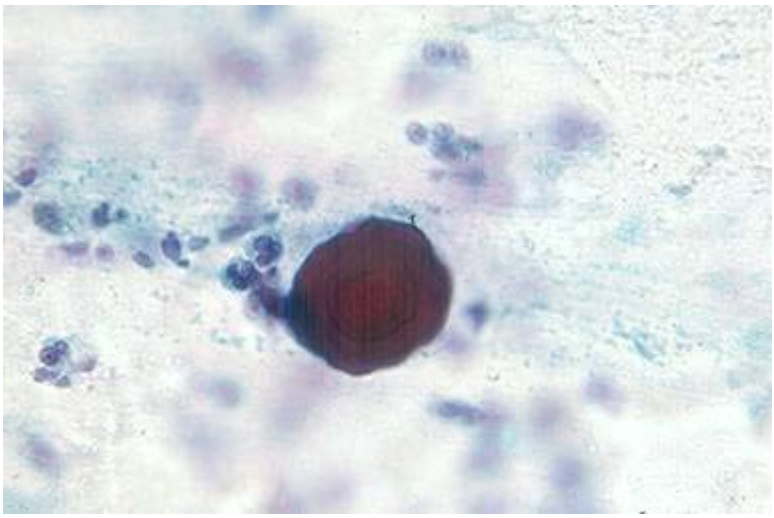


Fig. 3.24. A laminated round psammoma body in a CP smear.

- **Carpet beetle part** is a contaminant from cotton applicator or tampon. It has a distinctive morphology permitting the correct identification when present. (Fig.3.25).

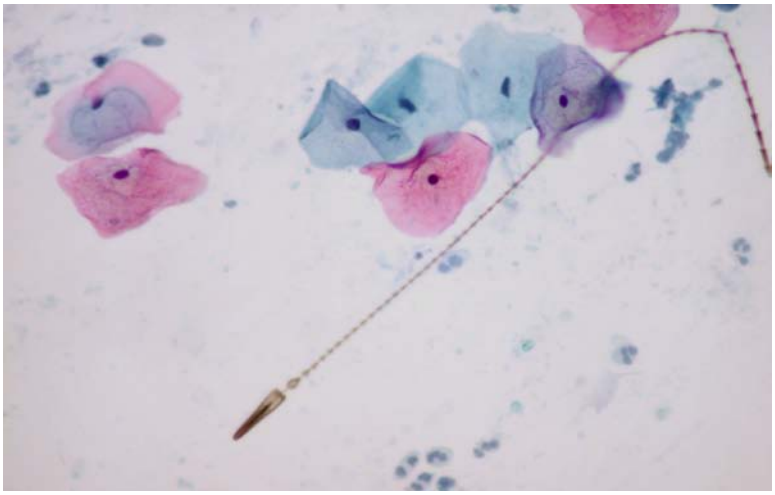


Fig. 3.25. Carpet larva part with distinctive morphology in a CP smear.

- **Curschmann spirals** are rarely found in Pap smears. They are inspissated mucous threads within cervical glands or clefts and have no diagnostic significance. (Fig.3.26).

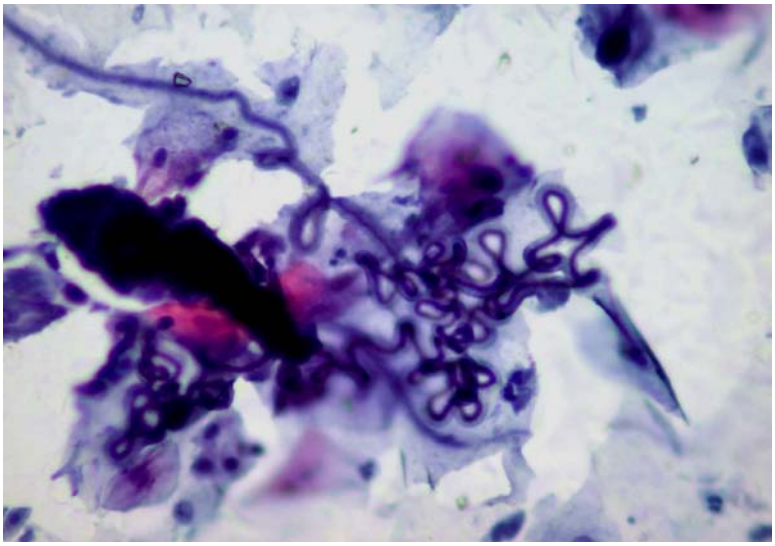


Fig 3.26. Curschmann spiral in a CP smear.

Common cellular features of abnormal and nonneoplastic squamous cells of the cervix and vagina are summarized in Table 3.1.

Table 3.1: Common Cellular Features of Abnormal and Nonneoplastic Squamous Cells*

CELLULAR FEATURES	INFLAMMATION ASSOCIATED CELLULAR CHANGES	VITAMIN B12 OR FOLIC ACID DEFICIENCY	RADIATION OR CHEMOTHERAPY	REPAIR CELLS
Architecture	Singly Clusters, loose	Singly	Clusters,cohesive Singly	Sheets, monolayered Singly
Cytoplasm - Border - Perinuclear halo with thin cyto-plasmic rim - Vacuolization - Enlargement - Extensions	 Distinct +, small +/- + -	 Distinct +, small +/- + -	 Distinct +/- + ++ +/-	 Distinct - - ++ ++
Nucleus - Irregular contours - Enlargement - Multinucleation - Chromatin	 - +/- - Clumped, fuzzy	 - + - Granular,fine	 - ++ + Granular,fine	 - + - Fine
Increased N/C ratio	-	+	-	-
Inflammatory background	+++	-	++	+
Other -Intracytoplasmic vacuoles -Macronucleolus	 - -	 - -	 + +/-	 +/- +

* Adapted with modifications from Nguyen GK, Kline TS: Essentials of Cytology. An Atlas. New York, Igaku-Shoin, 1993, p. 4 and 6.

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Chapter 4

Cervical Squamous Cell Lesions

Since the 1950s with the introduction of nationwide cytologic screening programs for cervical cancer in the United States, the incidence of squamous cell carcinoma (SCC) of the uterine cervix has decreased from 32.6 in the 1940s to 8.3 per 100,000 women in 1983 and 1984. However, the disease is far from being eradicated, as about 11,070 new cases of cervical cancer are expected to be diagnosed in 2008. Studies over the past 5 decades have demonstrated that cervical SCCs develop through a multistep process involving preinvasive lesions. In the majority of cases, the tumor occurs as the end result of a series of epithelial changes, ranging from mild to severe atypia, at the T-zone of the cervix. These lesions have been given various names: dysplasia and carcinoma in situ, cervical intraepithelial neoplasia (CIN) and squamous intraepithelial lesions (SIL).

In recent years, studies of cervical SILs and SCCs using molecular biology techniques have documented that the sexually transmitted human papillomaviruses (HPV) play an important role in the pathogenesis of these lesions. Of over 80 different types of HPV which have been identified, only about 40 are found in female anogenital tract lesions.

The HPV types 6 and 11 are mainly associated with cervical condylomas and CIN 1 but only rarely with HSILs (CIN 2 and 3) and almost never with cervical SCCs. Among the high-risk viruses HPV type 16 is most commonly detected in cervical SCCs. Of the SILs, LSILs are extremely heterogeneous with regard to their association with HPV types. In contrast to HSILs, LSILs may be caused by any single or combined HPV types of low- or high-risk HPV types, while HSILs, in about 88% of cases, are associated with HPV types 16, 18 or 31; and only 7% of them contain more than one HPV–DNA type.

SQUAMOUS INTRAEPITHELIAL LESIONS

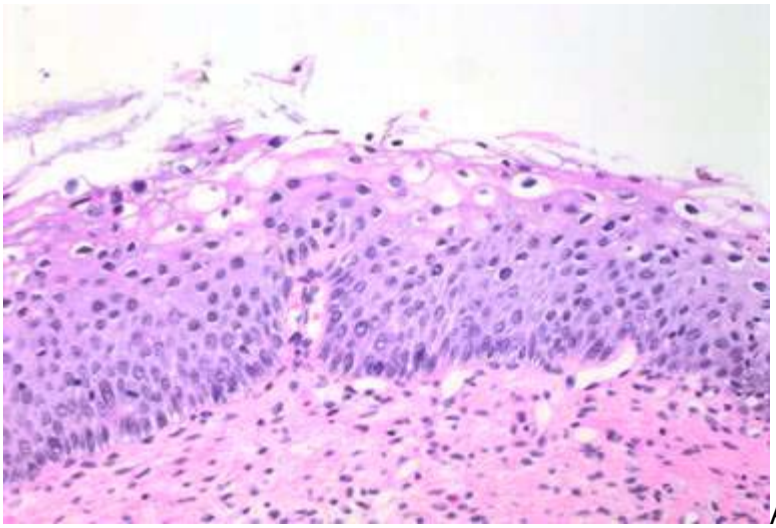
Cervical SILs have been generally regarded as precursors of cervical SCCs. SIL is predominantly a disease of women in their reproductive years. CIN lesions consist of three grades with grade 1 equivalent to mild dysplasia, grade 2 to moderate dysplasia, and grade 3 to severe dysplasia and carcinoma in situ (CIS). In TBS only two grades are recognized: LSIL that is equivalent to flat condyloma and CIN 1, and HSIL that is composed of CIN 2 and CIN 3 or CIS.

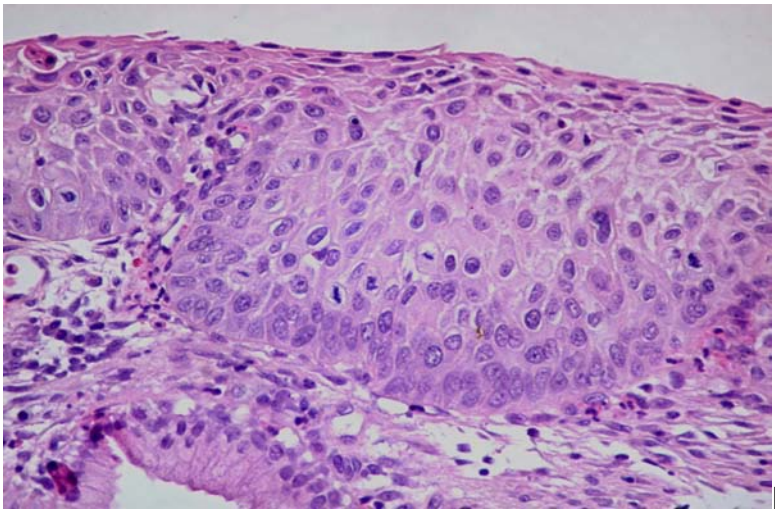
LSIL is defined as an intraepithelial lesion showing a preservation of differentiation, maturation and organization of squamous epithelium with mitoses confined to basal and

parabasal epithelial layers, koilocytosis, dyskeratosis, multinucleation, and enlarged hyperchromatic nuclei. HSIL, on the other hand, is characterized by a lack of squamous differentiation, epithelial disorganization, and severe cellular dyskaryosis with the presence of mitoses throughout the entire or lower 2/3 of the epithelium. (Figs.4.1 and 4.2).

Two biomarkers p16 and Ki-67 or MIB1 are useful for identification of an SIL, but they are not reliable for grading it (HSIL versus LSIL). Ki-67 antibody shows positive nuclear staining in over 30% of nuclei in the upper epithelial layers of a SIL, and p16 is strongly expressed by nuclei and cytoplasm of dyskaryotic cells in both LSIL and HSIL associated with high-risk HPV types. (Fig. 4.3). Normal, atrophic, reactive and metaplastic squamous epithelia, in contrast, are negative for p16; and Ki-67 is expressed only by parabasal cells.

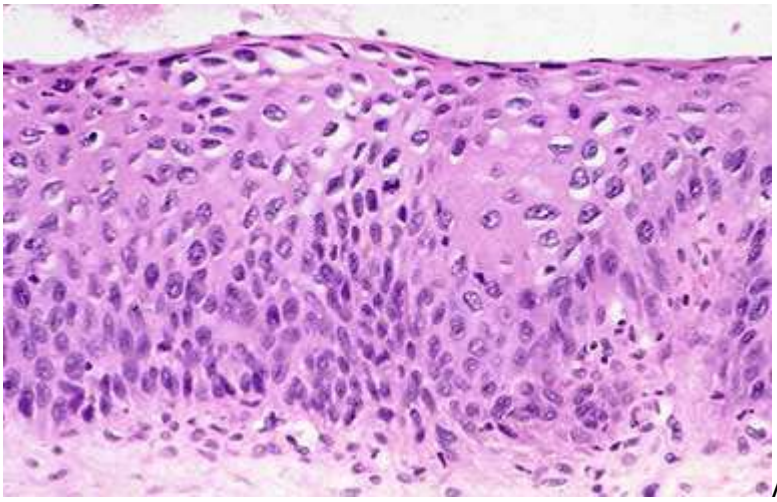
LSILs caused by low-risk HPV tend to regress and those caused by high-risk HPV tend to persist and progress to HSILs or SCCs. In a critical literature review of CIN lesions, Östor has found that the rates of regression, persistence and progression to CIN 3 and to SCC for CIN 1 lesions were about 60%, 30%, 10% and 1%, respectively. For CIN 2 lesions the corresponding approximations were 40%, 40%, 20% and 5%, respectively. The likelihood of CIN 3 regression, persistence and progression to SCC were about 33%, 56% and over 12%, respectively. He has also noted that the probability of an atypical squamous epithelium progressing to a SCC increased with the severity of atypia but this did not occur in every case. In a small number of cases foci of CIN 3 arose de novo, higher up in the cervical canal or adjacent to those of CIN 1, and did not develop from the progression of CIN 1 lesions. This observation challenges the Richart's popular concept of CIN. In a more recent meta-analysis by Melnikow et al. fairly similar rates of regression and progression of CIN/SIL lesions were found: about 47% of LSILs regress, 21% progress to HSIL and 1.5% progress to invasive cancer. For HSILs, 35% regress and 14% progress to invasive neoplasms.

 A



B

Fig. 4.1. Histology of LSIL:
A. Flat condyloma with koilocytes.
B. CIN 1/mild dysplasia.



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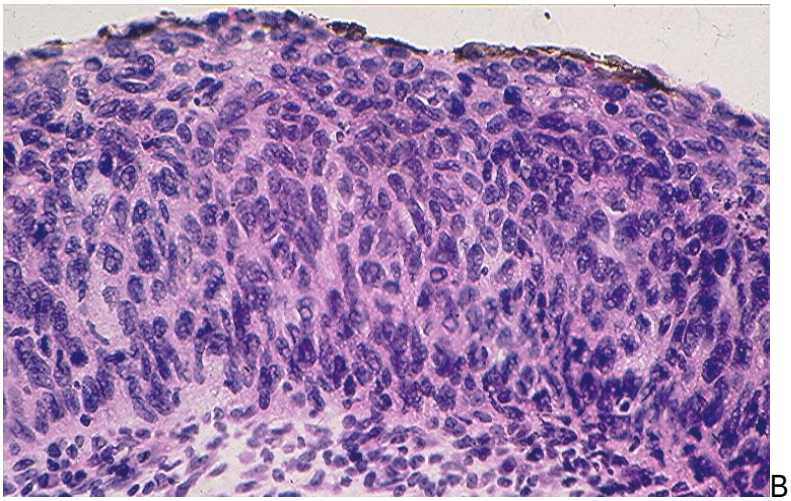

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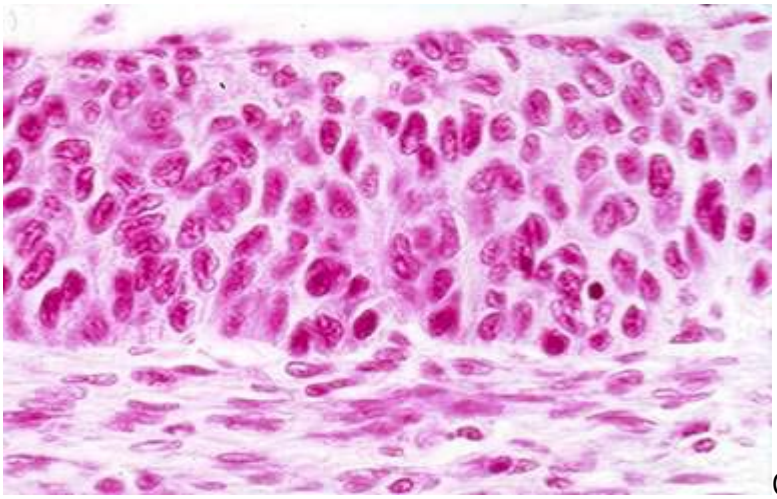
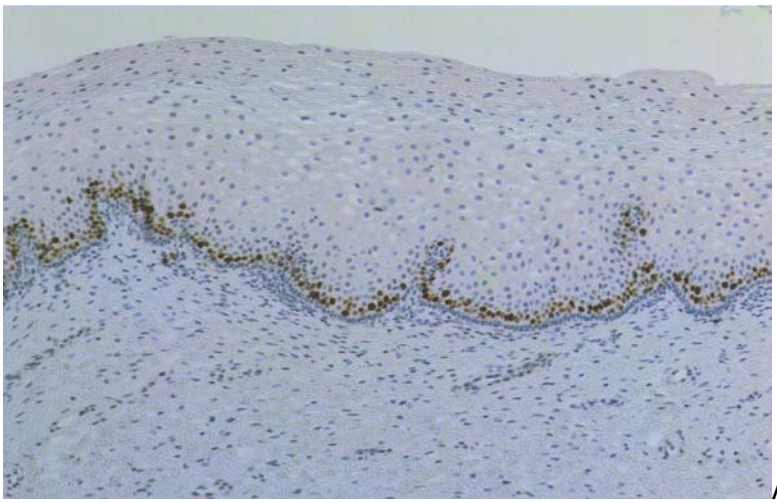


C

Fig.4.2. Histology of HSIL.
A. HSIL/CIN 2/Moderate squamous dysplasia showing focal squamous differentiation.
B. HSIL/CIN 3/Severe squamous dysplasia/CIS consisting of small cells.
C. HSIL/CIN 3/CIS consisting of large, pleomorphic cells.



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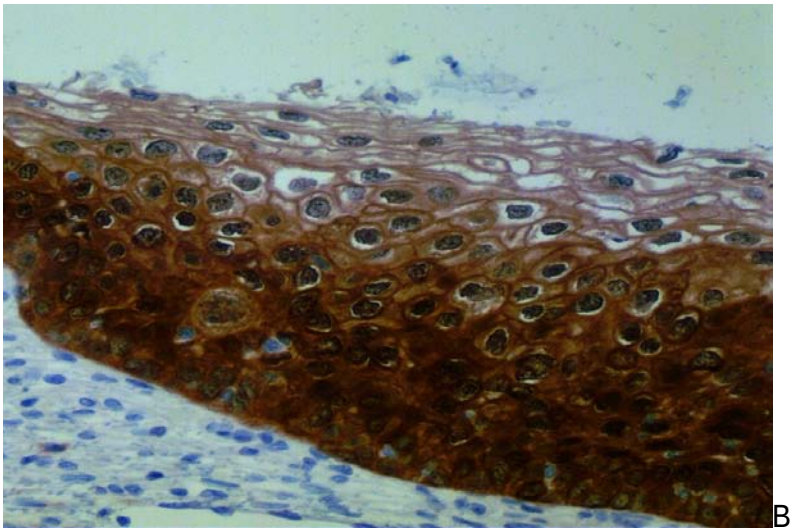

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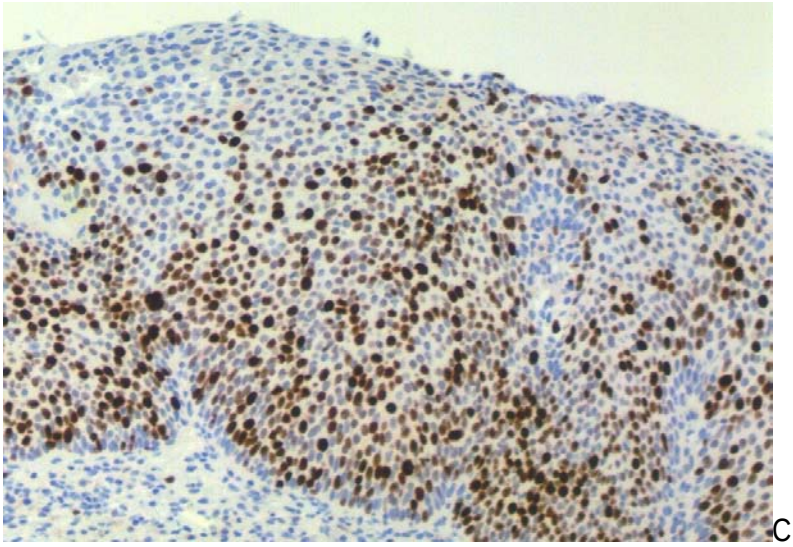
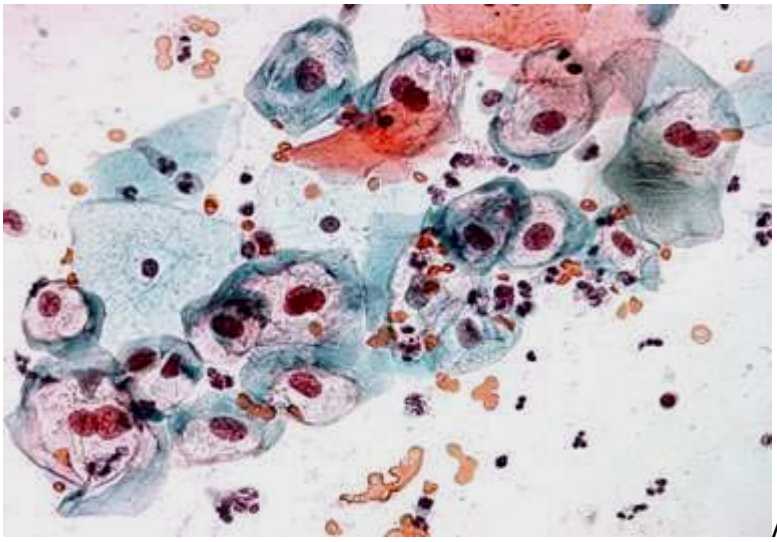
 C

Fig.4.3. Immunohistochemical features of normal squamous epithelium and HSIL:
A. Normal cervical squamous epithelium is negative for p16, and only its parabasal cell nuclei express Ki-67.
B, C. Dysplastic epithelium in this HSIL/CIN 2 showing positive nuclear and cytoplasmic reactions to p16 antibody (B), and over 30% of dyskaryotic nuclei express Ki-67 (C). (Avidin-biotin-complex).

A. LSIL

LSIL includes flat condyloma, mild dysplasia and CIN 1. LSIL is caused by a large number of different HPVs of low-risk and high-risk types. LSIL cells are found in about 2% of all Pap smears. The majority of women with LSIL Pap results have LSIL (CIN 1), but 18% of them are found to have HSIL (CIN 2 and 3) on cervical biopsy.

1. LSIL/Flat condyloma is characterized by the presence of koilocytes with dyskaryotic nuclei presenting singly and in sheets. These koilocytes are superficial and intermediate squamous cells displaying enlarged, hyperchromatic, single or multiple nuclei with granular or smudged chromatin pattern and irregular nuclear contours or membranes. The nuclei are surrounded by a perinuclear clear halo with a well–defined, thick cytoplasmic rim. The perinuclear halo is caused by degeneration of perinuclear cytoplasmic microorganelles caused by the HPV infection. Small or miniature parakeratotic and pseudoparakeratotic squamous cells are commonly seen, as well as dyskaryotic keratinizing squamous cells with atypical nuclei and thick, eosinophilic or orangeophilic cytoplasm. (Fig. 4.4).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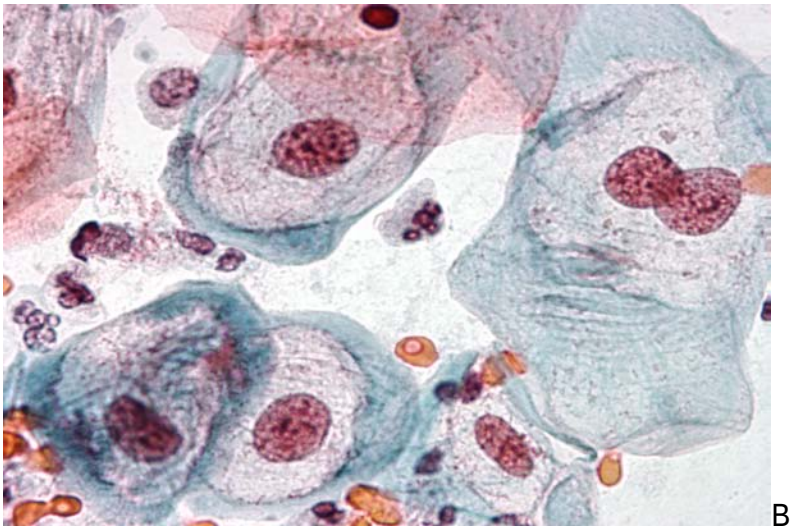
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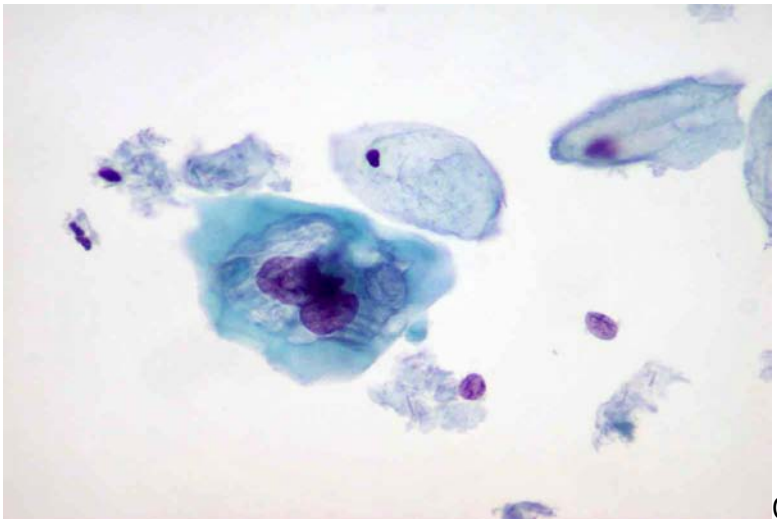
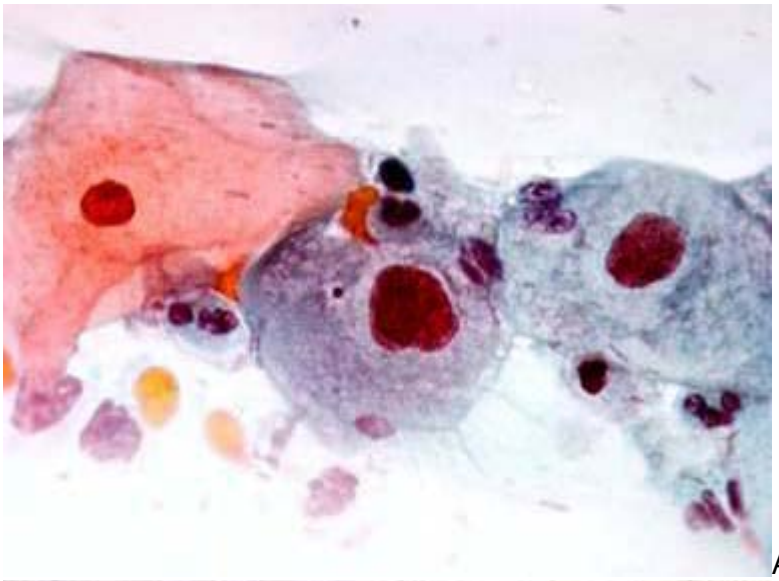

C

Fig. 4.4. LSIL/Flat condyloma:
A, B. Classic koilocytes with dyskaryotic nuclei in a CP smear.
C. A koilocyte with 2 nuclei in a LBP.

2. LSIL/Mild dysplasia/CIN 1 exfoliates superficial and intermediate squamous cells with enlarged, hyperchromatic and irregularly contoured nuclei. Nucleoli are absent and koilocytic changes are common. (Fig.4.5). LSIL cells should be distinguished from squamous cells with inflammatory changes, repair cells, squamous cells with radiation and chemotherapy effects and atypical squamous cells of undetermined significance. The reader is referred to Chapter 3 for discussion and illustrations of the three first cell types.



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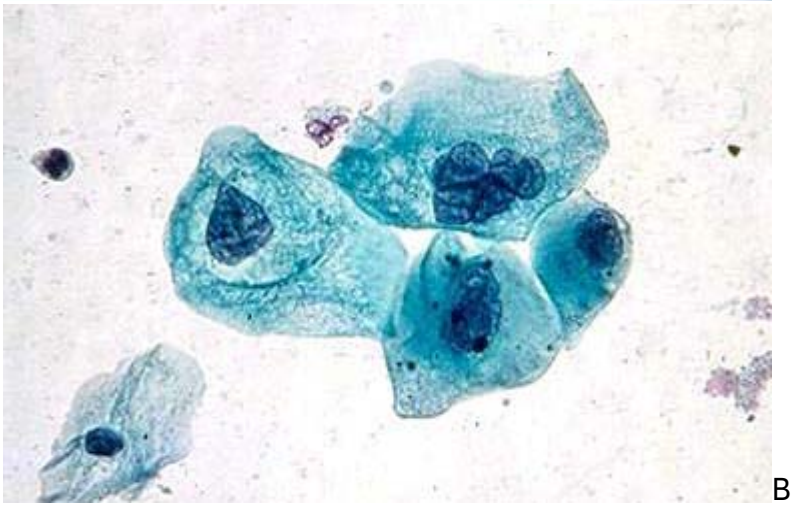

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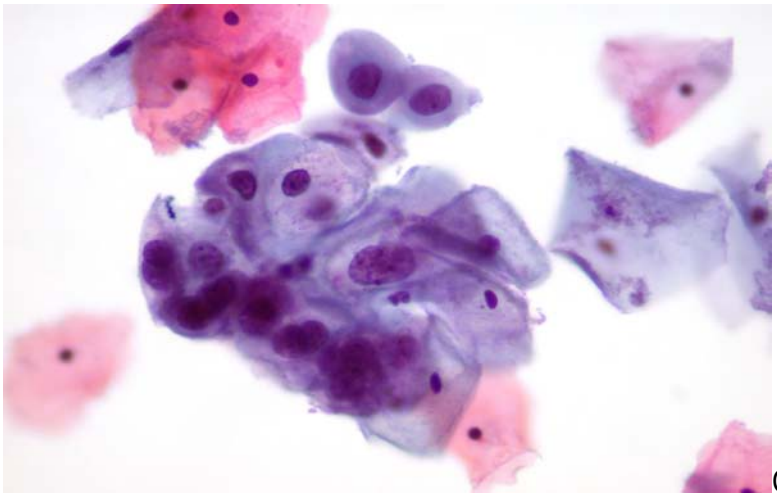
 C

Fig.4.5. LSIL:
A, B. CP smears showing mildly dyskaryotic superficial and intermediate squamous cells. One of the cells in (B) shows koilocytic change with a poorly formed perinuclear halo.
C. Mildly dyskaryotic squamous cells with some having perinuclear halos in a LBP.

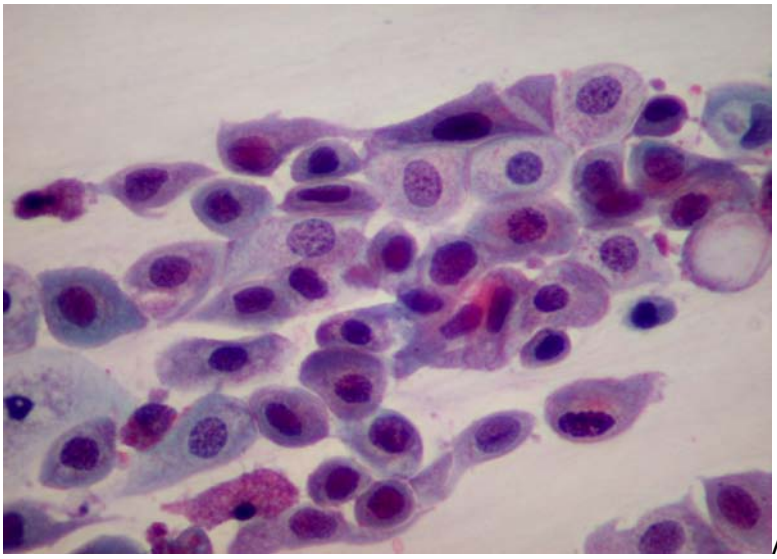
Management of LSIL

The ASCUS/LSIL triage study has found that high-risk HPV types were detected in 85% of LSIL cases and that HPV DNA testing was not a useful triage strategy. Colposcopy is generally recommended for initial management of LSIL patients. For pregnant women, a colposcopically directed biopsy may be performed, but an endocervical tissue sampling is contraindicated. Otherwise, the colposcopy evaluation may be deferred to 6 weeks postpartum.

B. HSIL

HSILs include moderate and severe dysplasias, CIS, CIN 2 and CIN 3. HSIL accounts in about 0.5% of all Pap smears, and 97% of women with HSIL Pap result are positive for high-risk HPV. If left untreated about 14% of them will develop cervical invasive squamous cell carcinoma.

1. CIN 2 lesions exfoliate parabasal-type cells singly or in sheets with thick, well–defined cytoplasm and enlarged hyperchromatic nuclei showing smooth or irregular nuclear contours. The chromatin is evenly distributed and may be finely or coarsely granular. Nucleoli are absent and koilocytic change may be present. (Fig.4.6).



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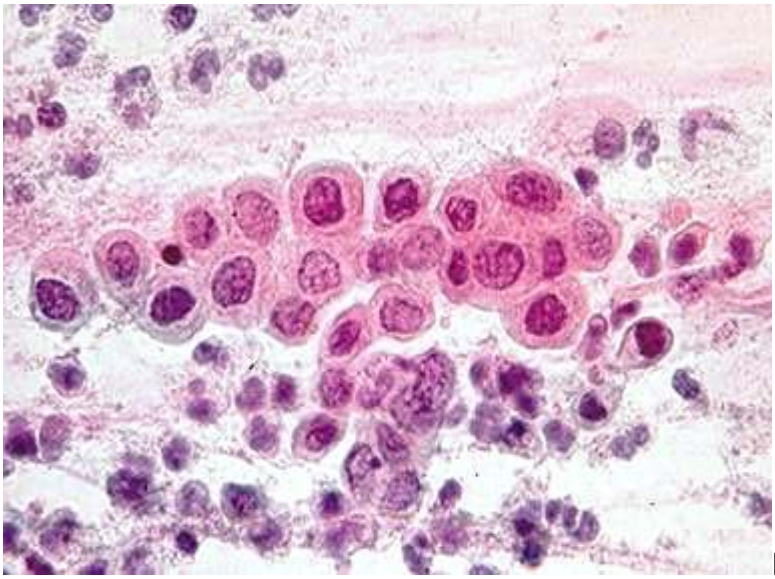

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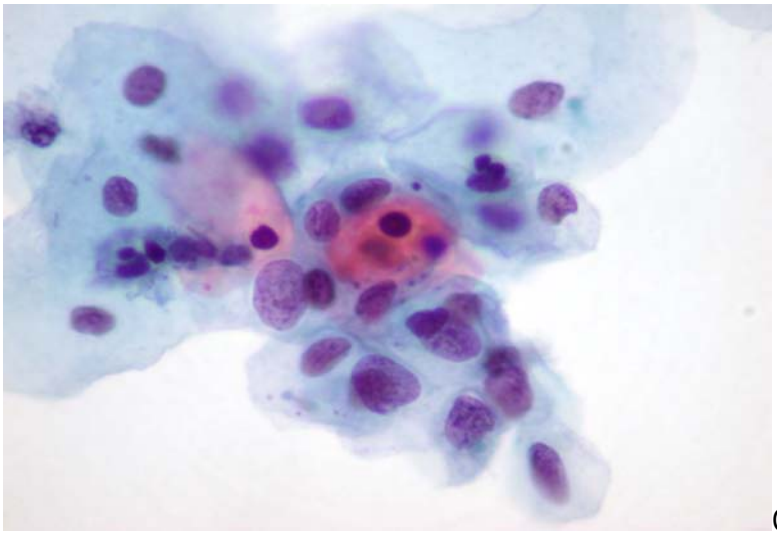
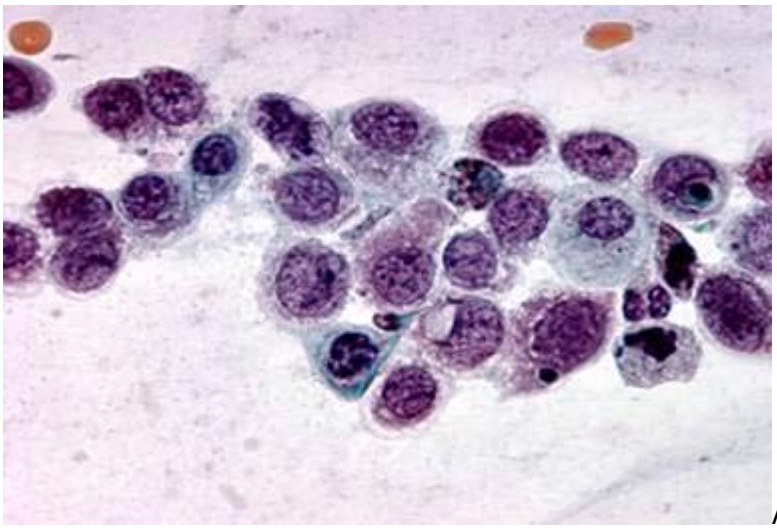


C

Fig.4.6. HSIL/CIN 2 showing in CP smears:
A, B. Moderately dyskeratotic squamous cells of low intermediate/parabasal cell type with enlarged, hyperchromatic nuclei with irregular nuclear contours and no nucleoli.
C. Similar moderately dyskaryotic squamous cells and normal intermediate cells in a LBP.

2. CIN 3 lesions consist of three main histologic patterns: large cell non-keratinizing, keratinizing and small cell types. A mixed cellular pattern is common.

Cells exfoliated from a **nonkeratinizing CIN 3** are large and pleomorphic. They show abundant, well– or ill–defined cytoplasm and enlarged, hyperchromatic nuclei displaying irregular nuclear contour. The nuclear chromatin is evenly distributed, and may be coarsely or finely granular. Cells in syncytial clusters are commonly encountered as well as epithelial fragments. (Figs.4.7 and 4.8). Occasional cells with keratinizing cytoplasm may be observed. Nucleoli are absent, and the smear background is free of necrotic debris. Rarely, a nonkeratinizing CIN 3 is composed of spindle-shaped cells. (Fig.4.9).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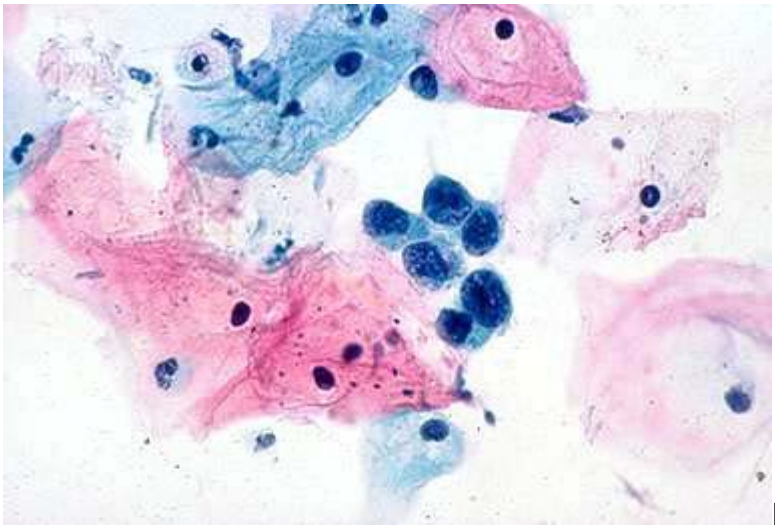
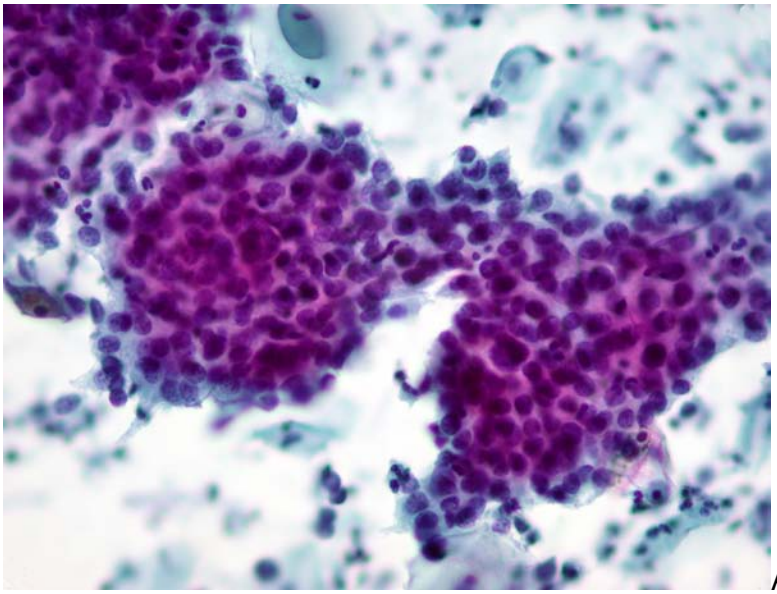

B

Fig.4.7. Cytology of HSIL/CIN 3, nonkeratinizing type in CP smears:
A. Markedly dyskaryotic medium-sized cells with hyperchromatic nuclei and irregular contours.
B. A cohesive cluster of markedly dyskaryotic small cells showing nuclei with similar changes with those of the cells in A.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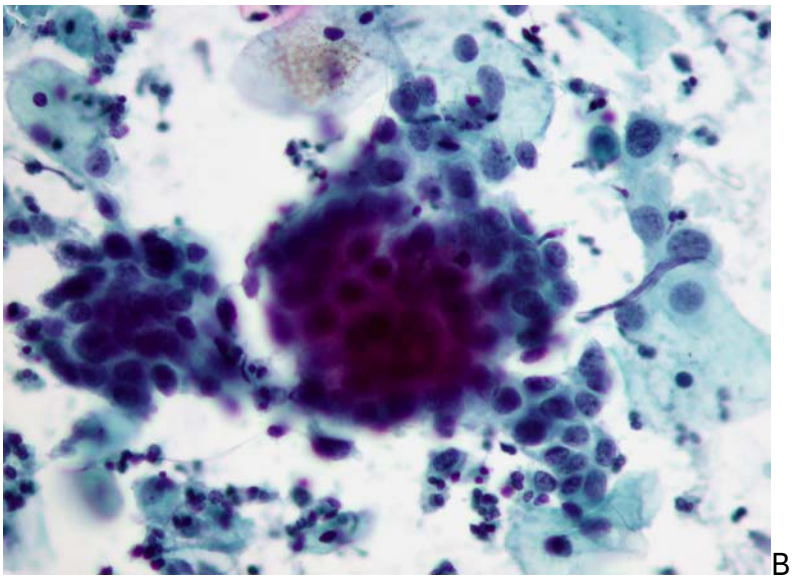

B

Fig. 4.8. A, B. Large tridimensional clusters of markedly kyskaryotic squamous cells removed by cytobrush from a HSIL/nonkeratinizing CIN 3 in CP smears.

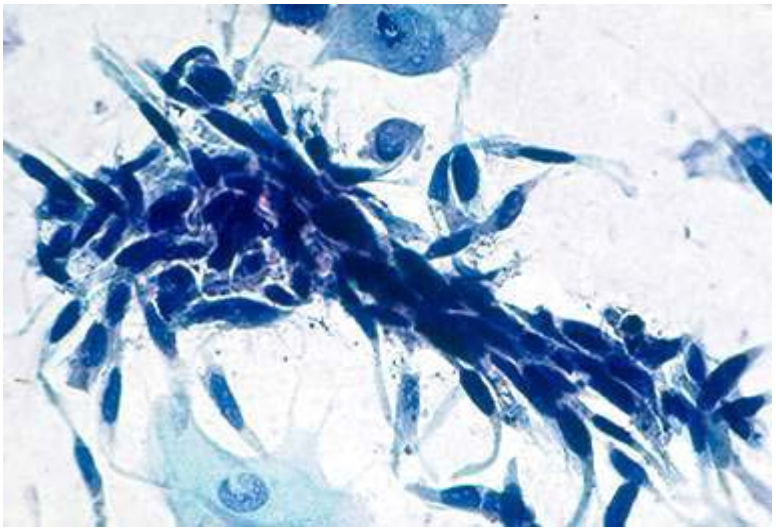
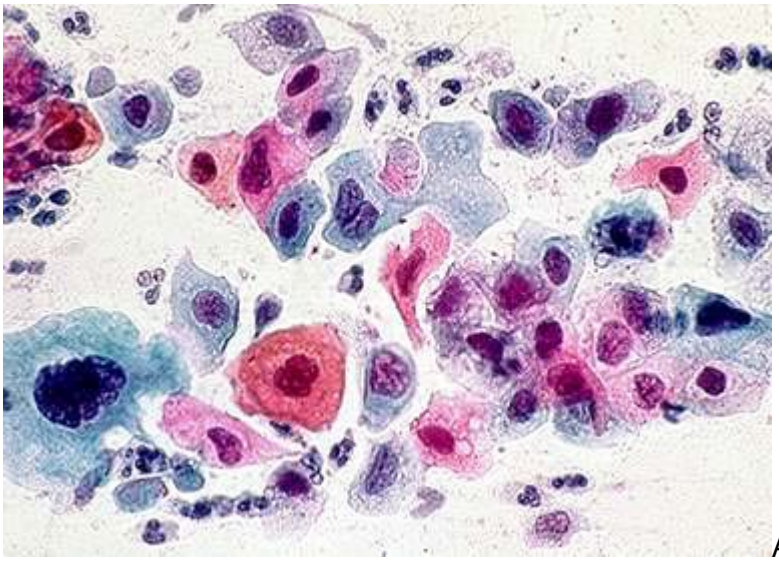


Fig. 4. 9. Nonkeratinizing HSIL/CIN 3 showing in a CP smear spindle-shaped cells with hyperchromatic, spindle nuclei.

Keratinizing CIN 3 yields spindled-shaped cells with orangeophilic, well-defined, thick cytoplasm and enlarged hyperchromatic nuclei. They are mainly seen as single cells and rarely in clusters. A **small-cell CIN 3** exfoliates cells with hyperchromatic nuclei and scant, ill-defined cytoplasm, singly or in loose aggregates, with or without nuclear molding. (Fig.4.10).



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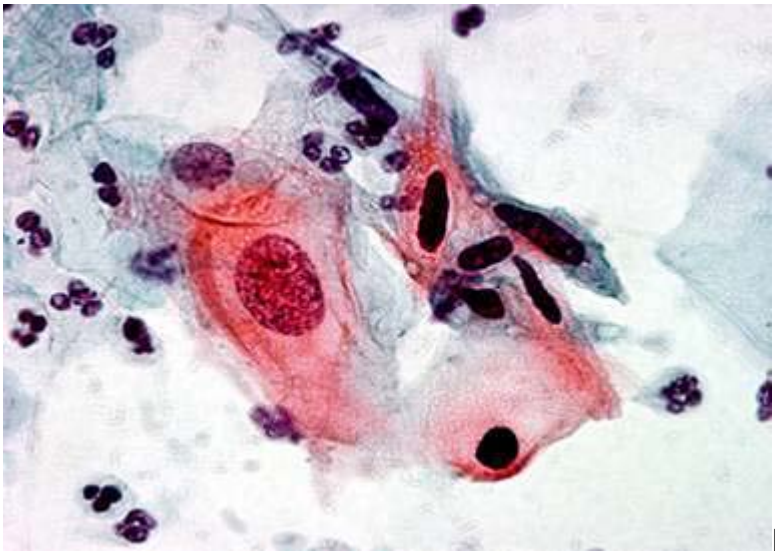

B

Fig.4.10. Keratinizing markedly dyskaryotic pleomorphic squamous cells in a CP smear from a HSIL/keratinizing CIN 3.

Management of HSIL

For patients with a HSIL Pap result, colposcopic evaluation is mandatory, as most patients will have confirmed biopsies of CIN 2 or 3. However, for pregnant women, the colposcopy may be deferred to 6 weeks postpartum. In pregnant women a colposcopically directed biopsy may be performed but an endocervical tissue sampling is contraindicated. If a histologically confirmed CIN is not identified at colposcopy, all cytologic and histologic materials of the patients should be reviewed. If the cytologic diagnosis of HSIL is correct, a diagnostic excisional procedure should be performed.

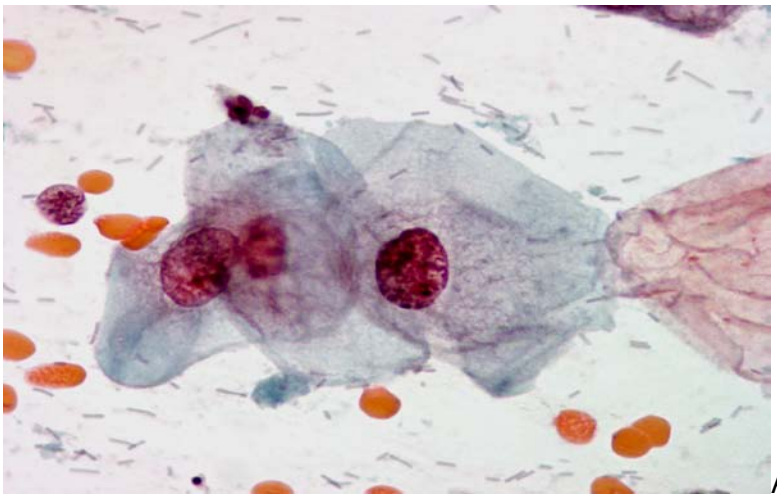
ATYPICAL SQUAMOUS CELLS

Atypical squamous cells (ASC) are seen in less than 5% of all Pap smears and the ASC/SIL ratio is about 3:1. Patients with ASC diagnosis are found to have a CIN lesion on colposcopically directed cervical biopsy in 10% to 20% of cases. In The Bethesda System-2001, ASCs are divided in 2 categories: ASC of undetermined significance (ASC-US) and ASC, cannot exclude a high-grade squamous intraepithelial lesion (ASC-H). An ASC diagnosis is made when an SIL is suspected cytologically. Cytologic criteria for identification of ASC-US and ASC-H cells are somewhat subjective, and the diagnoses suffer high inter-observer and intra-observer variation rates. ASC-US represents about 90% of all ASC cases.

1. ASC-US

ASC-US cells show cellular features that are more severe than those of squamous cells with reactive changes but less than those of a SIL. (Figs.4.11 and 4.12). Thus, the diagnosis of ASC-US is made by exclusion of cells with known cytologic features. Cytologic criteria of ASC-US cells include:

- ASC-US cells are of superficial or intermediate type with
- Enlarged mononucleus or binuclei that are 2.5 to 3 times larger than the nucleus of a normal intermediate squamous cell (~35 μm^2)
- Slightly increased N/C ratio
- Slightly hyperchromatic nuclei with irregular chromatin distribution.
- Regular nuclear contours, but it may show focal irregularity.
- Dense and eosinophilic or orangeophilic (keratinized) cytoplasm
- Perinclear halo may be present
- Nucleoli may be seen in ASC-US repair cells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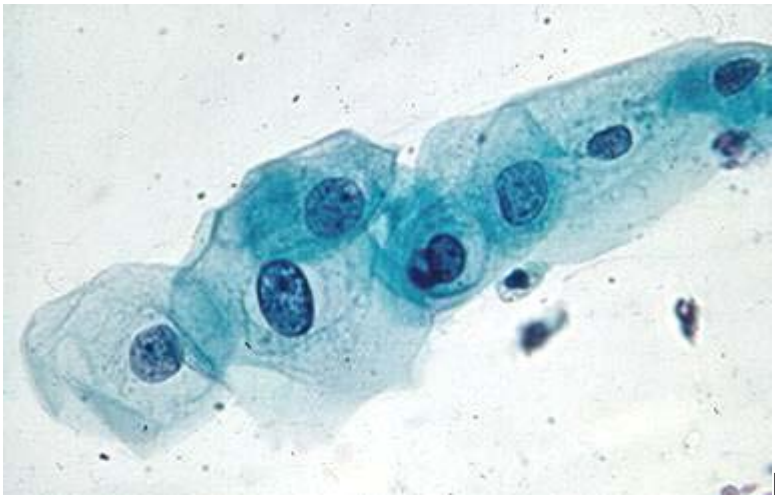
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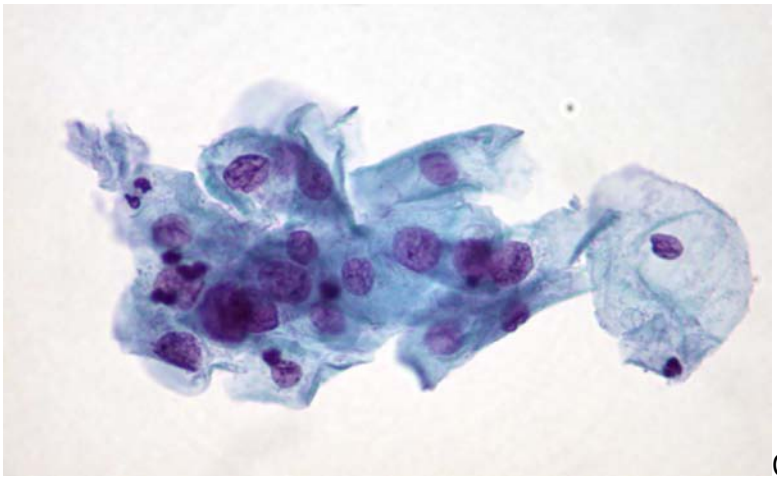
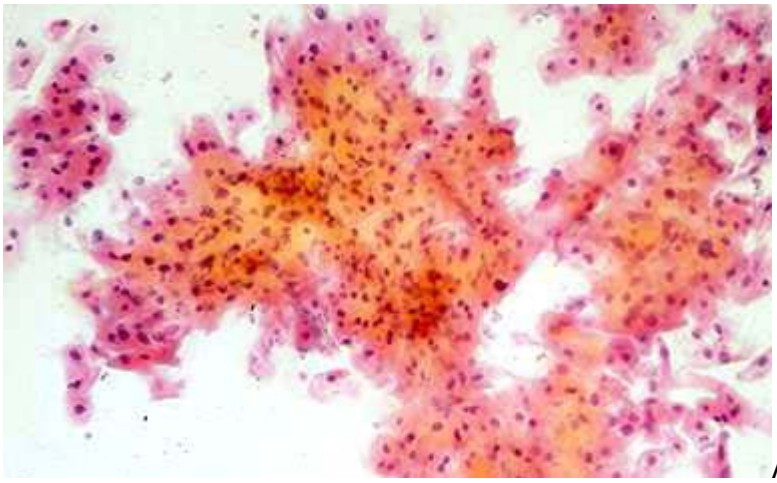


C

Fig.4.11. ASC-US cells:
A, B. Single and loosely clustered ASC-US cells in a CP smear showing smooth nuclear contours with minimally hyperchromatic nuclei and no nucleoli are seen.
C. Similar ASC-US cells are seen in a LBP.



A

B

Fig. 4.12. A, B. ASC-US cells with keratinized cytoplasm in CP smears.

Atrophic vaginitis may yield cells with features suggesting ASC-US changes. (Fig.4.13). In atrophic vaginitis a short course of intravaginal treatment with estrogen cream for 4 to 7 days will be helpful to solve this diagnostic dilemma. This treatment will induce a maturation of squamous cells, but dyskaryotic cells will remain unchanged.

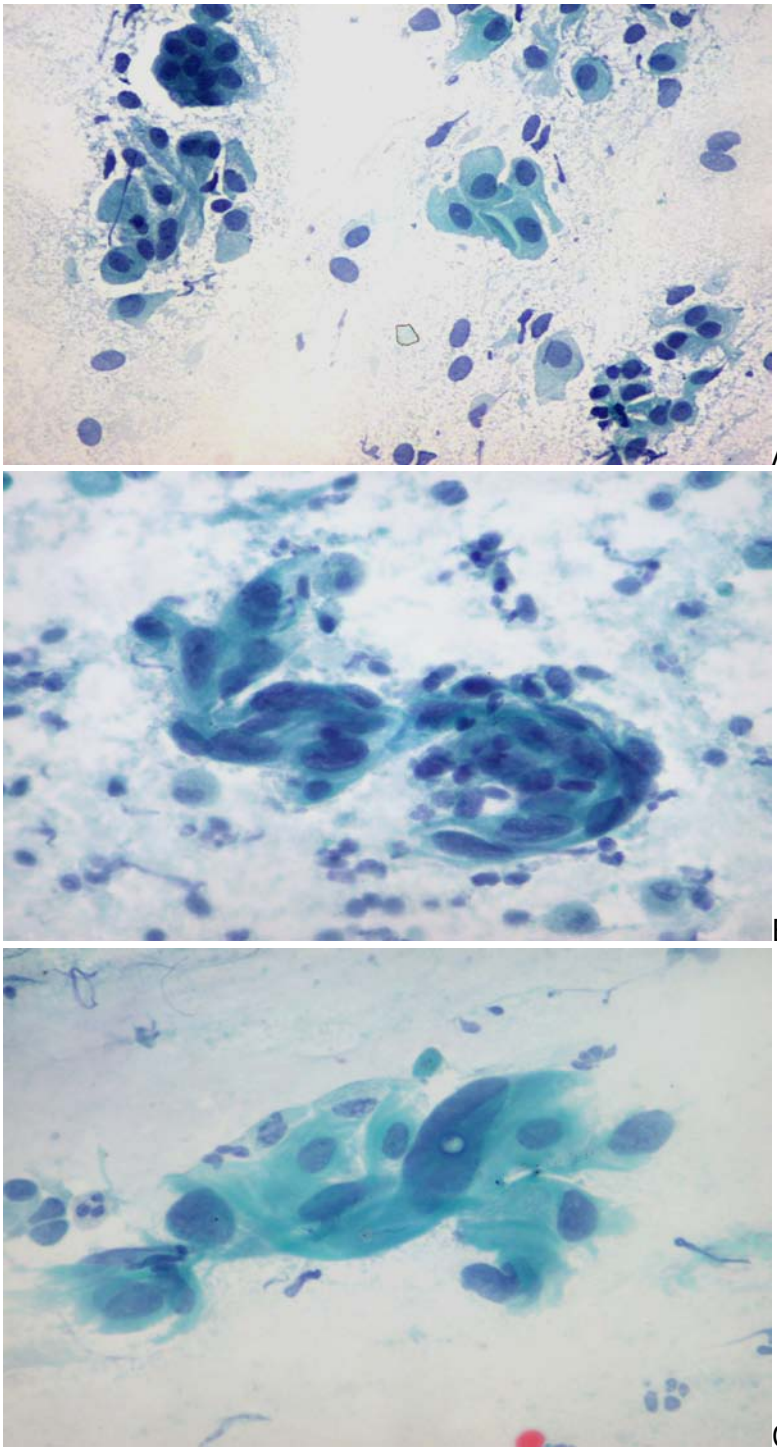


Fig. 4.13. A-C: Atrophic vaginitis showing in CP smears ASC-US cells.

Management

Oncogenic (high-risk) HPV DNA testing is the preferred management for patients with ASC- US Pap results, especially when it can be performed concurrently. The test should not be performed in women younger than 30 years of age because the HPV infection in these patients is often caused by a mixture of low- and high-risk virus types, making the interpretation of the test results difficult, if not impossible. Follow-up with repeat Pap tests at 6-month intervals or immediate colposcopy is also acceptable. In pregnant women, the colposcopy may be deferred to 6 weeks postpartum.

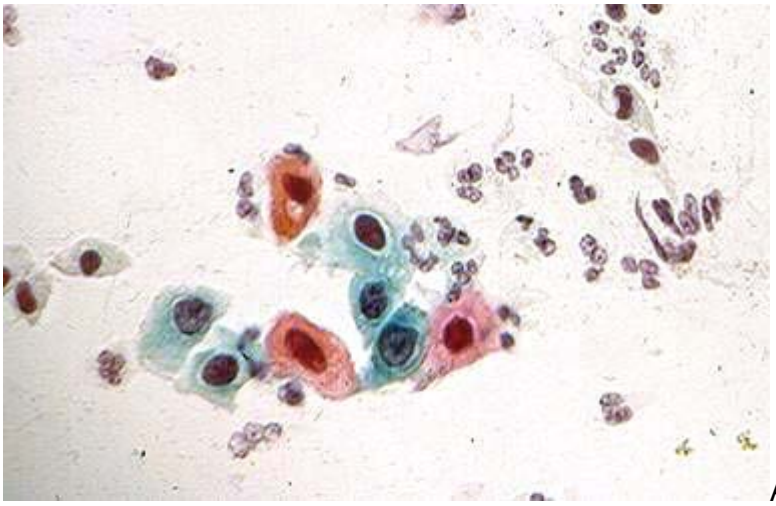
If the HPV DNA testing is positive for high-risk viruses, the patient should be referred to colposcopy. If the test is negative for high-risk viruses, she should be followed by a repeat Pap smear every 6 months for 2 years. If the cellular atypia is cleared within 2 years, she can return to routine annual screening.

If HPV DNA testing is unavailable and if the cellular atypia persists over 2 years, she should be referred to colposcopy for further evaluation.

2. ASC-H

ASC-H represents 5% to 10% of all ASC cases. ASC-H cells are metaplastic squamous cells with nuclear atypia that fall short of a definitive diagnosis of HSIL. (Fig. 4.14). Cytologic criteria of ASC-H cells include:

- ASC-H cells are usually small in number.
- The cells occur singly, in small groups or in epithelial fragment containing less than 10 cells.
- ASC-H cells are polygonal in shape and have the size of a squamous metaplastic cell and dense cytoplasm.
- Their hyperchromatic nuclei are 1.5 to 2.5 times larger than that of a normal metaplastic squamous cell, irregular chromatin and mildly irregular contours.
- No nucleoli.
- The N/C ratio is increased and is about that of an HSIL cell.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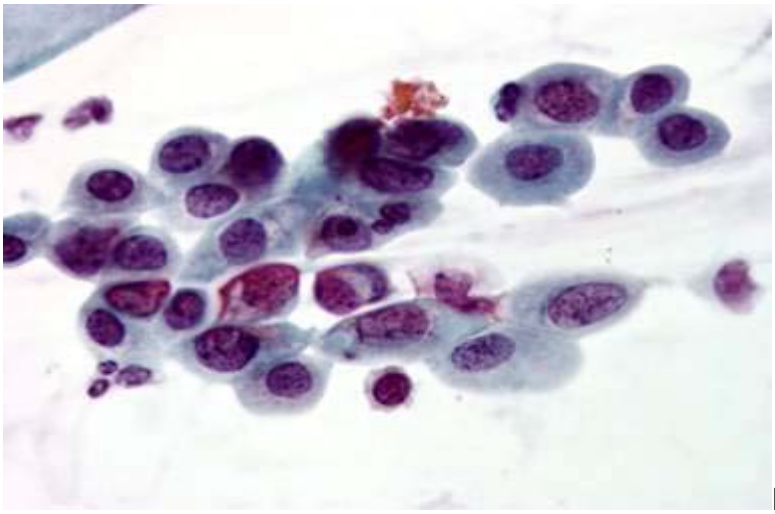
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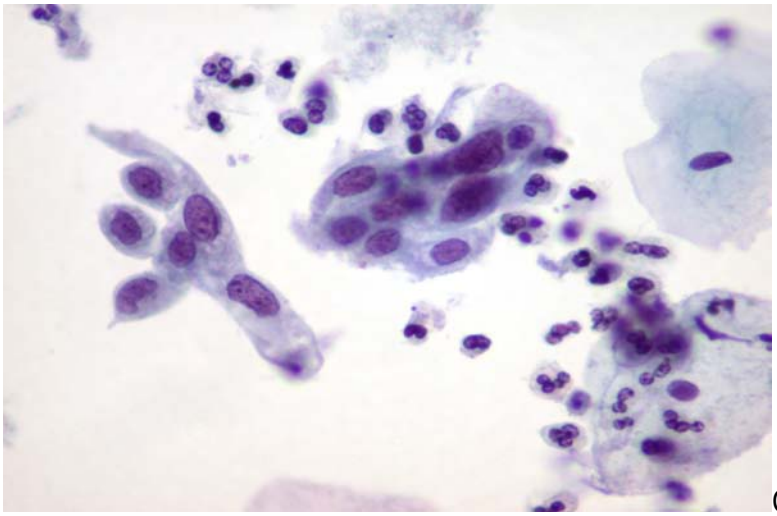

C

Fig. 4.14. ASC-H cells:
A, B. ASC-H cells present singly and in loose aggregates in CP smears.
C. Loosely clustered ASC-H cells in a LBP.

ASC-H cells in a crowded sheet or epithelial fragments may show a loss of nuclear polarity. The cell cytoplasm has squamoid features, however. On smears these thick tissue fragments may display peripheral cells in vague palisades mimicking those of a cervical adenocarcinoma in situ. Nucleoli are virtually absent. However, it should be borne in mind that an adenocarcinoma, either in situ or invasive, may coexist with a squamous lesion of the cervix. Cytobrush bristles may remove large fragments of CIN 3 epithelium lining cervical glandular crypts, and these tissue fragments are difficult to distinguish from a large epithelial fragment containing ASC-H cells.

Management

Patient with ASC-H diagnosis should be referred to colposcopy as ASC-H has a positive predictive value for histologic CIN 2 or 3 much higher than that of ASC-US (50% versus 17%). If a CIN 2 or 3 is not found she should have a repeat Pap test in 6 months or a HPV DNA test. If her repeat Pap test result is ASC-US or worse, or if her HPV DNA is positive for high-risk viruses, she should be referred to a second colposcopy. If the patient is pregnant, the colposcopy may be deferred until 6 weeks postpartum.

INVASIVE SQUAMOUS CELL CARCINOMA

Invasive SCCs are the most common type of cervical cancers, accounting for 60% to 80% of all malignant tumors of the cervix. They occur mainly in adults with a peak incidence in the 5th and 6th decades of life. Their common clinical manifestation is abnormal vaginal bleeding that may occur spontaneously or following a sexual intercourse. Cervical SCCs are histologically classified as well- and poorly differentiated (keratinizing and non-keratinizing SCCs). Cervical epithelium adjacent to SCC commonly shows foci of LSIL or HSIL.

CYTOLOGIC MANIFESTATIONS OF CERVICAL SCC

Cervical SCCs have distinctive cytologic manifestations. (Figs.4.15 to 4.18). Common cytologic features of cervical SCC include:

- Cancer cells with keratinized cytoplasm are seen predominantly singly.
- Nonkeratinized cancer cells predominantly seen in small aggregates and in clusters.
- Prominent nucleoli are present mainly in nonkeratinized tumor cells.
- Necrotic debris or tumor diathesis is almost always observed in CP smears, but it is subtle or minimal in LBP in which the necrotic debris is collected at the periphery of tumor cell groups (“clinging diathesis”).
- Cells characteristic of SILs may be present, as SILs may coexist with SCC
- Cervical adenocarcinoma in situ (AIS) cells may be seen if the AIS coexists with SCC.

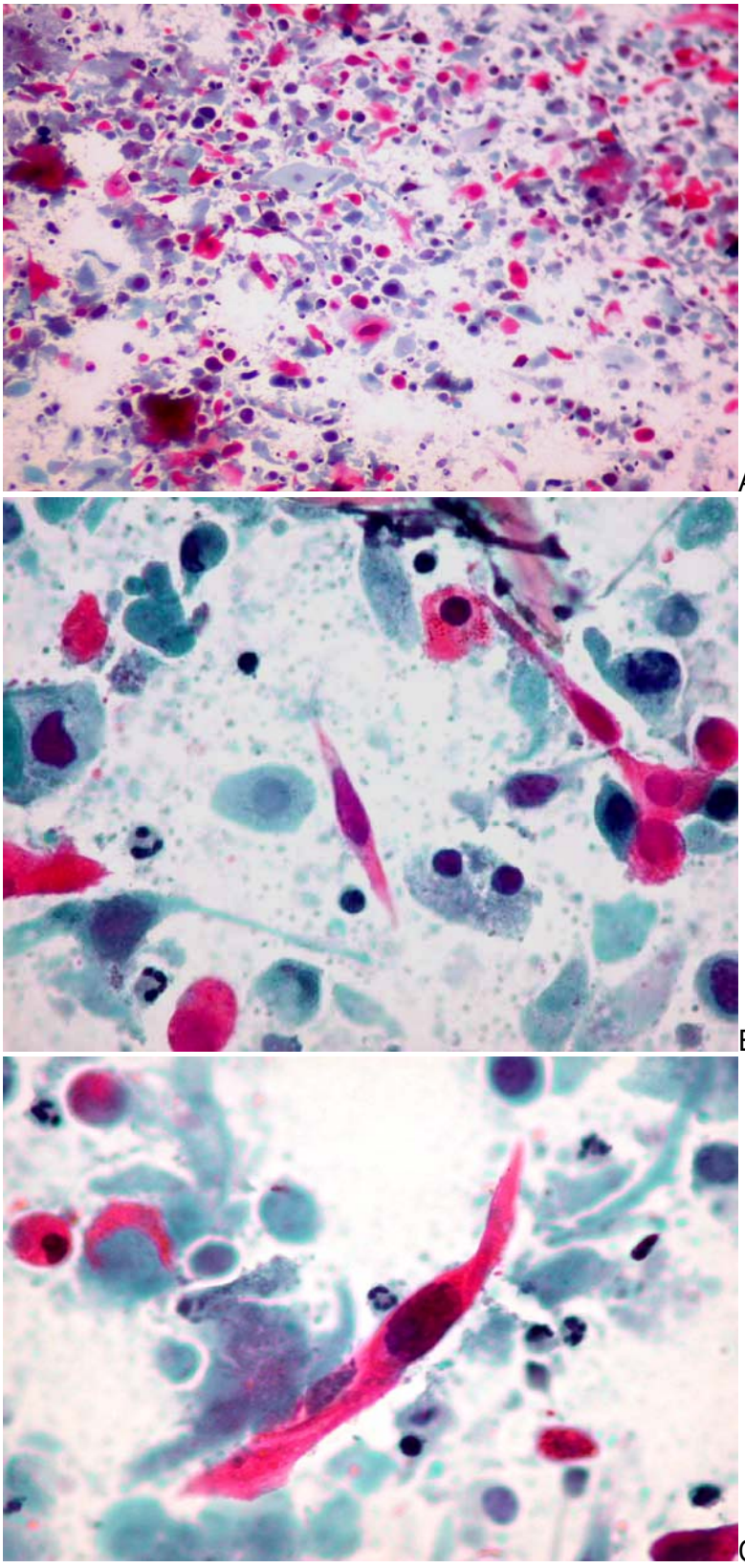


Fig. 4.15. Keratinizing SCC showing in a CP smear keratinizing, pleomorphic malignant squamous cells in a necrotic background (tumor diathesis).

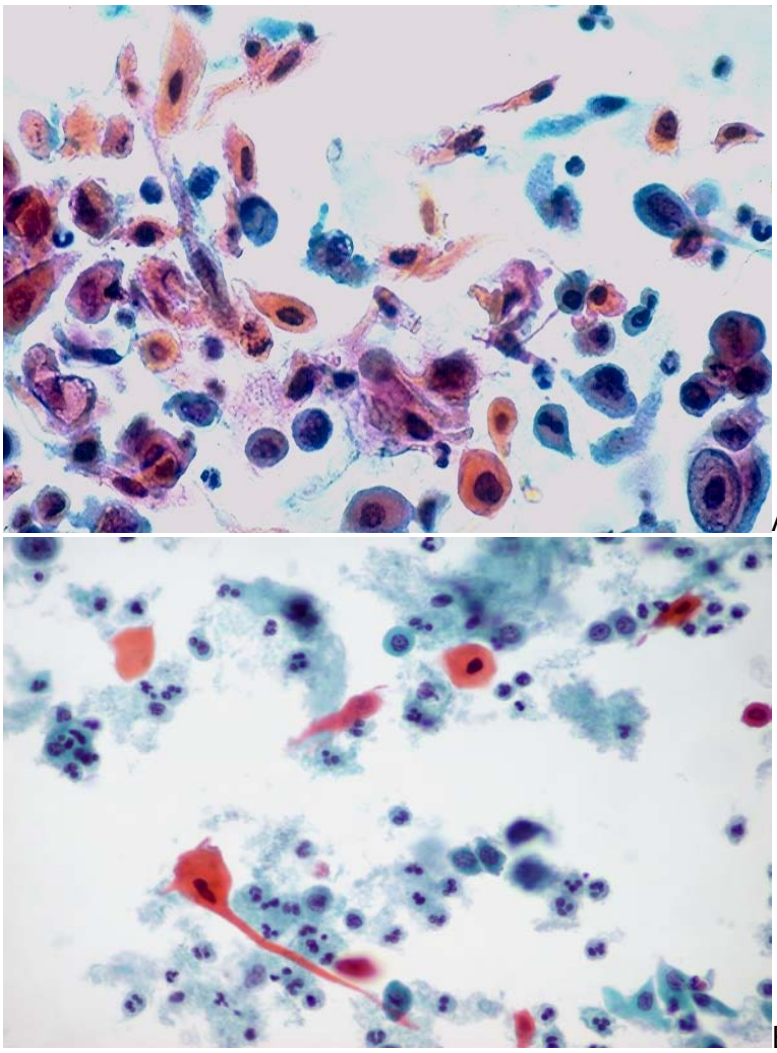
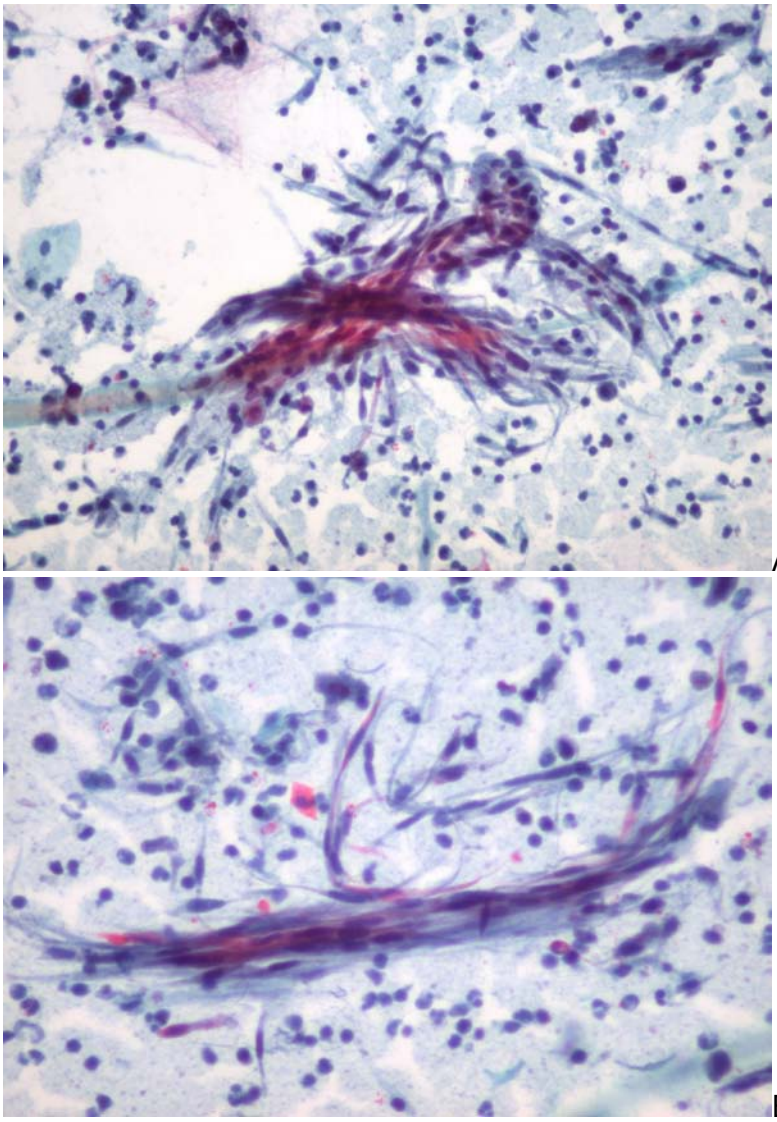


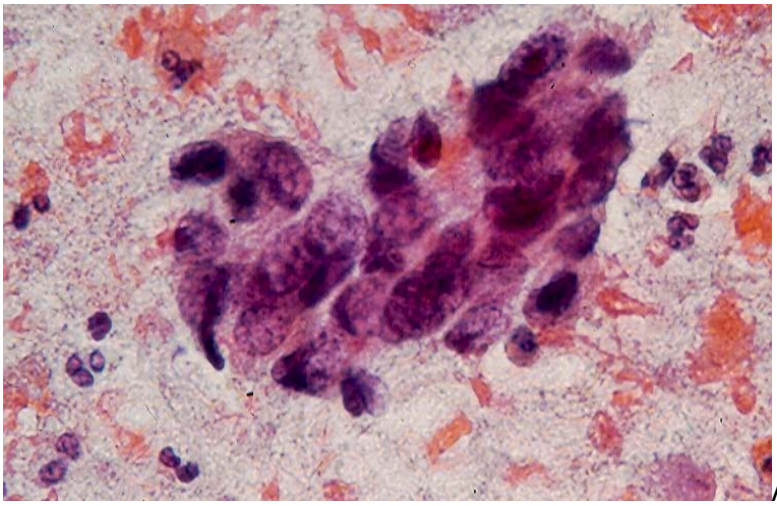
Fig.4.16. Keratinizing SCC showing:
A. In CP smear pleomorphic malignant cells with keratinized cytoplasm and necrotic debris. A few tumor cells with tadpole configuration are present.
B. Similar cancer cells in a LBP.



A

B

Fig. 4.17. A and B. Keratinizing SCC showing in CP smear spindle-shaped, “fiber” tumor cells, singly and in bundles.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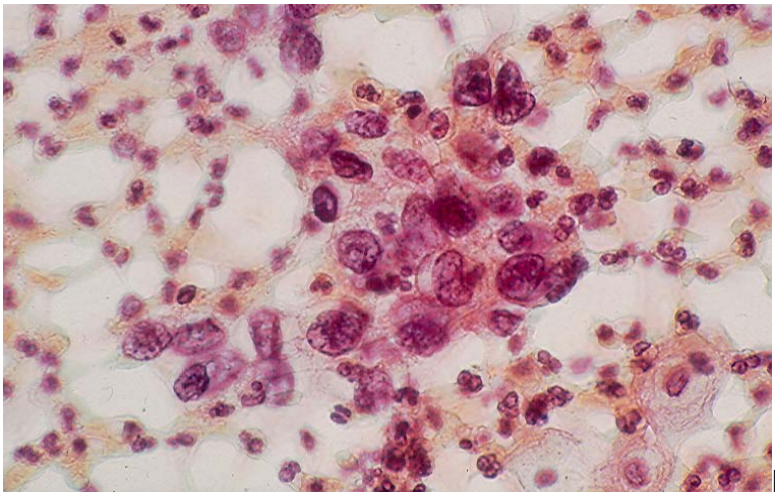

B

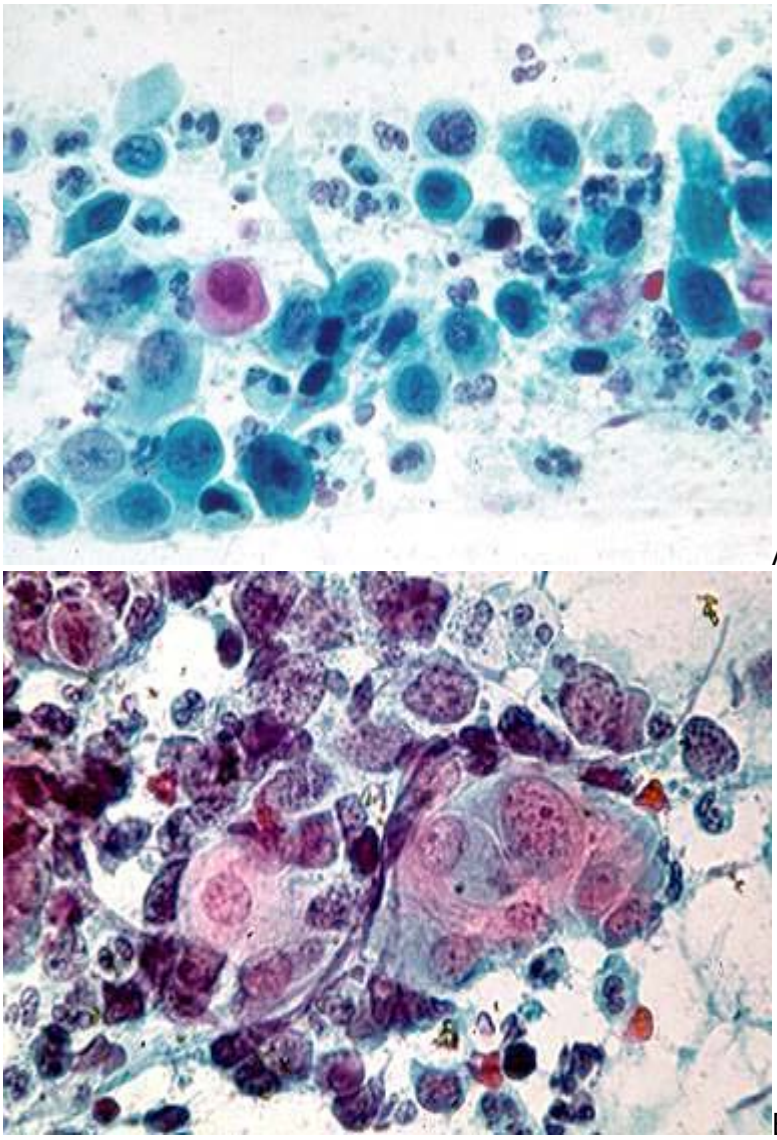
Fig.4.18. A, B. Two poorly differentiated SCC showing in CP smears syncytial clusters of tumor cells with ill-defined, nonkeratinized cytoplasm, pleomorphic nuclei and tumor diathesis.

MICROINVASIVE SQUAMOUS CELL CARCINOMA

Microinvasive squamous cell carcinoma (MICA) of the cervix is defined as an early invasive cancer up to 3 mm below the overlying basement membrane in which no vascular or lymphatic tumor invasion is identified. Cervical MICA, so-defined, has an incidence of pelvic lymph node metastasis lower than 1%. The cytologic manifestations of cervical MICA in CP smears include:

- Pleomorphic, single HSIL cells with or without keratinization.
- HSIL cells or malignant nonkeratinizing squamous cells in loose clusters, syncytia and hyperchromatic cell groups or tissue fragments. (Fig.4.19).
- Nuclei with irregular chromatin clumping and distribution.
- Micro– and macronucleoli.
- Small amount/focal tumor diathesis.

Of these criteria, the first three are the most important ones, however they are not present in every single case. In the case of keratinizing MICA, keratinized malignant squamous cells with thick, orangeophilic cytoplasm are seen and tumor diathesis may be present. MICA may be suspected cytologically, but its definitive diagnosis must be made by careful histologic examination of the excisional cervical cone biopsy.



A

B

Fig.4.19. Cervical microinvasive squamous cell carcinoma showing in CP smears:
A. Slightly pleomorphic HSIL cells and tumor diathesis.
B. Clustered malignant nonkeratinizing squamous cells with conspicuous nucleoli.

Cytologic manifestations of LSIL, HSIL and Invasive squamous cell carcinoma of the uterine cervix in CP smears are tabulated in Table 4.1 below.

Table 4.1. Cytology Features of Low- and High-grade SILs and Invasive Squamous Cell Carcinoma (SCC)*.

FEATURES	LSIL/CIN 1	HSIL/CIN 2	HSIL/CIN 3	INVASIVE SCC
Abnormal cell number	+	++	+++	++/variable
Architecture	Singly Clusters, loose	Singly Clusters, loose	Singly Clusters, loose Syncytia	Singly Clusters, loose Syncytia
Defined cytoplasm Perinuclear halo	++ ++	++ +/-	+/- -	+/- -
Increased N/C ratio Irregular nuclear contours	+ +	++ +	+++ +	+ to ++ ++
Chromatin - Granular - Regular Nucleoli	 Fine + -	 Fine +/- -	 Fine - -	 Coarse/Dense - +
Tumor diathesis	-	-	-	+++
Others - Cervical AIS cells**	-	-	+/-	+/-

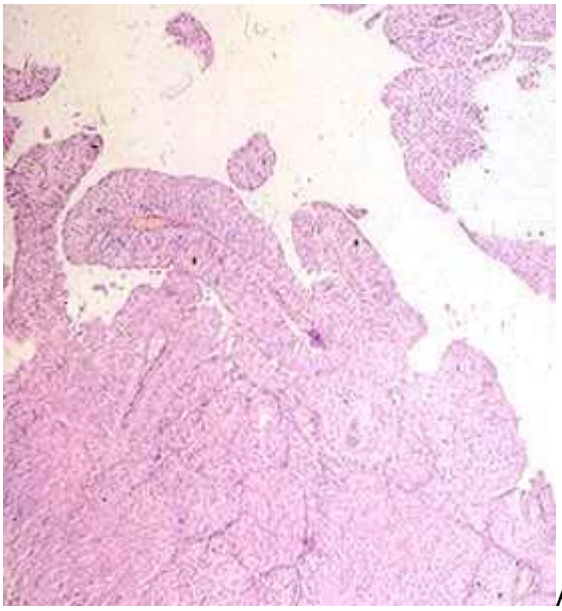
* Adopted with modifications from Nguyen GK, Kline TS. Essentials of Cytology. An Atlas. New York, Igaku-Shoin, 1993, p 3 and 4.
** Adenocarcinoma in situ (AIS) cells.

VARIANTS OF SQUAMOUS CELL CARCINOMA

1. Verrucous carcinoma is a very rare and distinctive type of well-differentiated squamous cell carcinoma of the cervix and vagina. The tumor tends to grow slowly and recur locally but it does not metastasize. HPV type 6 has been identified in verrucous carcinoma by molecular techniques. Macroscopically, the tumor has a warty and fungating appearance. It is characterized histologically by an undulating hyperkeratinized surface consisting of pointed papillary projections. The tumor cells usually show normal-appearing nuclei or minimal nuclear atypia. In CP smears, abundant benign-appearing squamous cells with keratinized cytoplasm admixed with abundant anucleated, keratinous squames are seen.

2. Papillary squamous (transitional) cell carcinoma is a rare variant of invasive squamous cell carcinoma of the cervix and vagina, with about 40 cases reported in the literature. Microscopically, it can be graded as high- or low-grade tumor. In CP smear a high-grade neoplasm is characterized by single and clustered small cancer cells with scant cytoplasm and hyperchromatic nuclei, similar to those of a HSIL (CIN 3), admixed with necrotic debris and inflammatory cells (tumor diathesis). (Fig.4.20).

A case of **cervical low-grade papillary transitional cell carcinoma** showing in CP smear a few single and benign-appearing transitional cells with oval nuclei and well-defined, granular cytoplasm and a few syncytial, 3-dimensional, well-defined, cohesive clusters of medium-sized epithelial cells with granular, eosinophilic cytoplasm and oval nuclei with a loss of nuclear polarity has been reported.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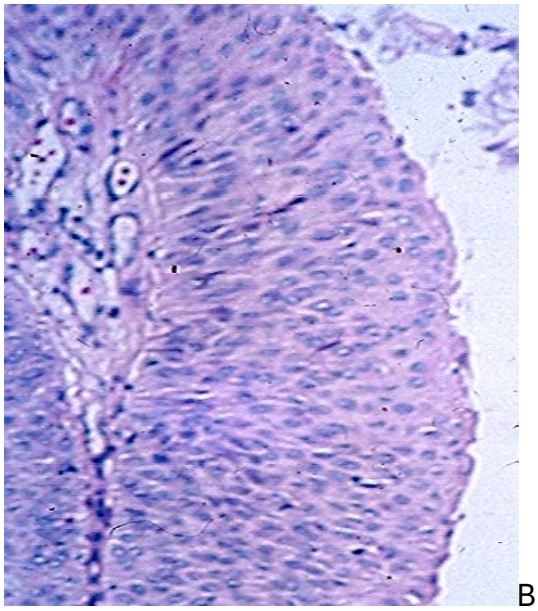
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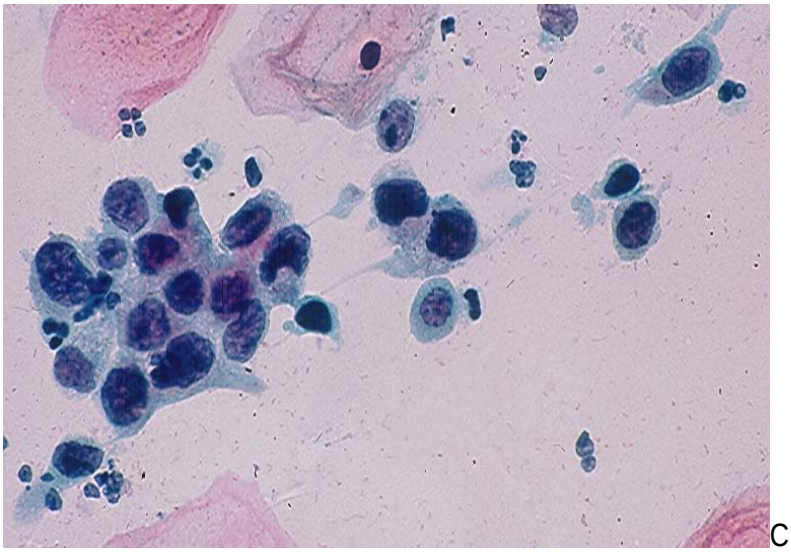
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Fig. 4.20. Papillary high-grade transitional cell carcinoma:
A, B. Histology of the tumor.
C: CP smear showing single and clustered tumor cells similar to those of a HSIL.

3. Lymphoepithelioma-like carcinoma of the cervix is also a rare tumor with lower rate of lymph node metastasis, potentially radiosensitive and better prognosis. Histologically, it is characterized by solid cords of poorly differentiated polygonal malignant cells with squamoid differentiation and a lymphocyte–rich stroma. It shows in CP smear numerous benign lymphoid cells and rare single and clustered malignant non–keratinizing, poorly differentiated or anaplastic epithelial cells with irregular nuclear contours, hyperchromatic nuclei, coarse chromatin and prominent nucleoli. These cells are obscured by heavy inflammation and blood, a background resembles that of a menstrual smear.

4. Sarcomatous squamous carcinoma of the cervix uteri is rarely encountered. The tumor yields in CP smear single and loosely clustered pleomorphic malignant cells that are difficult to differentiate from those of a soft tissue sarcoma. Immunohistochemical and electron microscopic studies of the tumor tissue obtained by biopsy are necessary for a correct diagnosis.

SENSITIVITY, ACCURACY AND ERRORS

In the screening of cervical cancer, about 85% to 90% of women in the general population have a normal Pap result, while about 10% of them show squamous cell atypia, and the remainder are diagnosed as having a SIL. Of these cervical SILs, 75% to 90% are low–grade lesions and the remainder are high–grade lesions.

The cytodiagnosis of SIL is subjective and suffers remarkable interobserver variations. On rare occasions, a SIL cannot be graded as low– or high–grade, and a diagnosis of ungraded SIL is made.

Squamous cells with perinuclear haloes and normal nuclei are not specific for LSILs. (Fig. 4.21). These changes are non-specific, and HPV–DNA is often not detected within the cell cytoplasm by in situ hybridization.

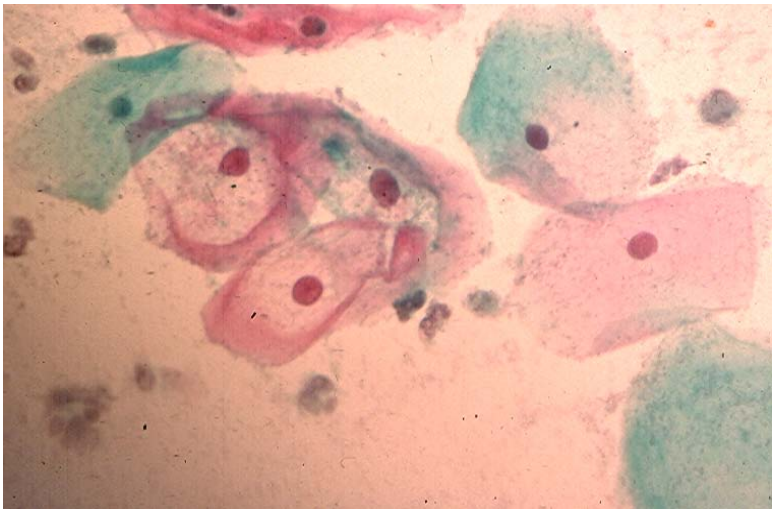

Fig. 4.21. CP smear showing intermediate squamous cells with perinuclear haloes and normal, nondyskaryotic nuclei.

The diagnostic accuracy rates of LSILs varied tremendously in different reported series. The sensitivity of the Pap test in detecting cervical SILs varied widely, ranging from 30% to 80%, with a mean of 47%. Its specificity ranged from 86% to 100%, with a mean of 95%.

For LSILs, a correct cyto–histologic correlation was obtained in about 38% to 56% of cases, and in one series, the cervical biopsy showed HSIL in 12% and was unremarkable in 50% of the cases. In another series, a poor correlation between cytologic and histologic diagnoses of various grades of CIN was observed: 50% of patients with a cytodiagnosis of CIN 1 showed a higher grade CIN in biopsied cervical tissues, and the overall false-negative rate of cervical smears for CIN 2 and 3 was 19%. In Koss' experience, about 20% of cases with a cytodiagnosis of LSIL show HSIL in biopsied tissues. For HSILs, a cytodiagnostic accuracy rate of 85% to 100% has been reported.

For invasive cervical cancers, the Pap test had a more variable sensitivity, ranging from 16% to 82%; and many patients had one or more negative test result. SCCs with keratinized surfaces are often underdiagnosed as scraping these tumors may yield only benign appearing keratinized squamous cells. It should be borne in mind that a false–negative cytodiagnosis is potentially dangerous as the cancer may be left untreated. A false–positive diagnosis is undesirable but it is less dangerous as the patient will be subsequently evaluated by colposcopy and cervical biopsy.

False-positive diagnoses of cervical cancer occurred in 10% to 15% of cases, and the 3 most common errors were:

- atrophic smear with benign atypia in a granular pseudonecrotic background, followed by
- reparative changes, and
- keratinizing HSIL.

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Chapter 5

Cervical Glandular Lesions

Cervical adenocarcinomas are believed to originate from the multipotential subcolumnar reserve cells of the endocervical canal. These tumors display complex growth patterns consisting of different cell types. Of these, adenocarcinoma of endocervical cell type is the most common one. It may occur in a pure form or coexist with a squamous cell carcinoma. The etiology of cervical adenocarcinoma has not been fully elucidated. Recently, HPV types 16 and/or 18 have been identified in cervical adenocarcinoma tissues suggesting a common etiology with squamous cell cancer. In contrast to cervical squamous cell carcinoma, the sequential changes of cervical glandular epithelium leading to the development of adenocarcinoma have not been well documented. Currently, adenocarcinoma in situ (AIS) is widely accepted as the immediate precursor to cervical adenocarcinoma.

ADENOCARCINOMA IN SITU

Cervical AIS tends to occur in women in their 3th and 4th decades of life. Histologically, it involves the transformation zone of the cervix in the majority of cases and consists of three main cell types: endocervical, endometrioid and intestinal; with endocervical-type lesions being the most common ones. Lesions containing more than one cell type are not uncommon. Cervical AIS has been found in association with squamous dysplasia and squamous carcinoma in about 50% of cases. On the other hand, AIS is found only in about 5% of cervical HSILs. AIS cells are ER, PR, CEA negative and p16 and MIB1 positive.

Depending on the degree of cellular differentiation, AIS may be classified as well and poorly differentiated. A well-differentiated AIS displays fairly distinctive cellular manifestations permitting its identification in a high percentage of cases, while a poorly differentiated tumor does not have any specific cytological pattern and may be readily mistaken for an invasive adenocarcinoma. (Fig.5.1).

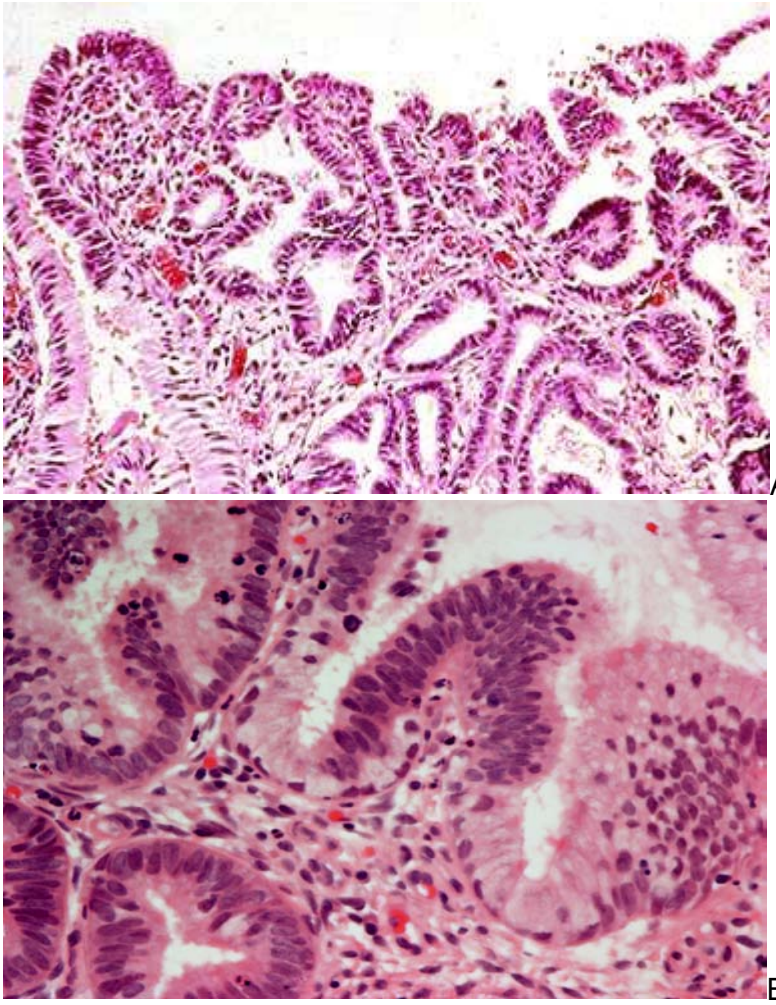
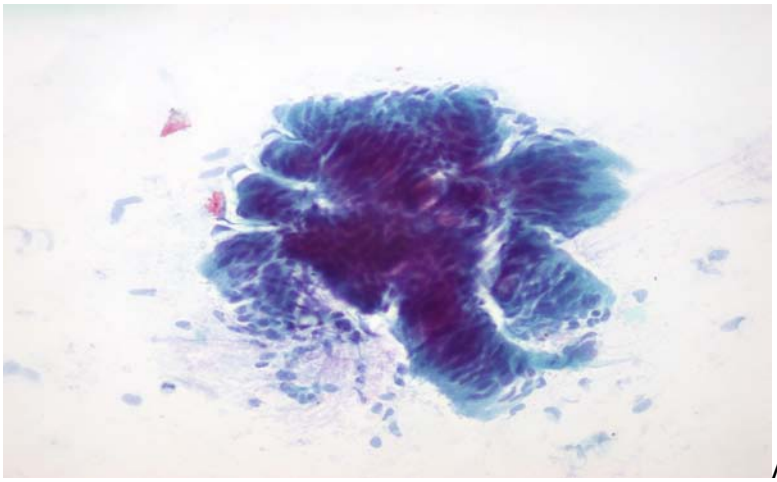


Fig. 5.1. A, B. Histology of cervical adenocarcinoma in situ.

Well-differentiated AIS, endocervical cell type displays the following cytologic features in a CP smear:

- Large sheets of malignant glandular epithelium with crowded columnar tumor cells showing nuclear stratification that is well visualized at the edges of the sheets.
- Short strips of tumor cells with cytoplasm extending off the edges of the tumor cell sheets (feathering). (Fig. 5.2).
- Short strips of tumor cells with palisading nuclei.
- Tumor cells forming rosettes.
- Isolated tumor cells and cells in papillary clusters may be seen.
- The smear background is free of necrotic debris or tumor diathesis.
- The individual tumor cells are two to three times larger than normal endocervical glandular cells and have enlarged hyperchromatic nuclei with finely or coarsely granular chromatin pattern.
- Nucleoli are absent in about 50% of cases.

Atypical or malignant squamous cells may be seen if there is a coexisting squamous cell lesion that is usually present in about 50% of cases. Each of these features provides a key to an accurate cytodiagnosis which can be made in about 90% of cases.



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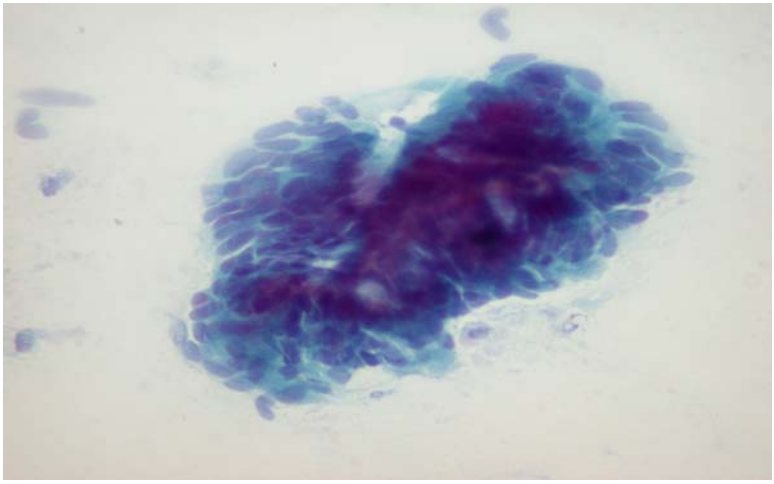

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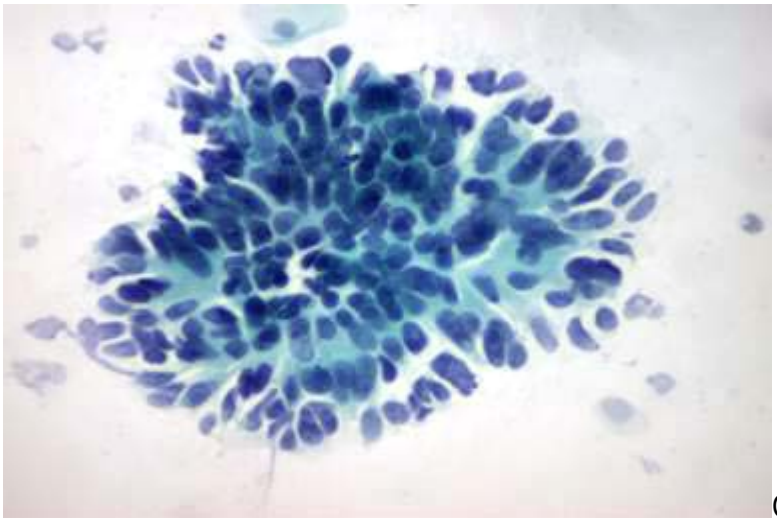

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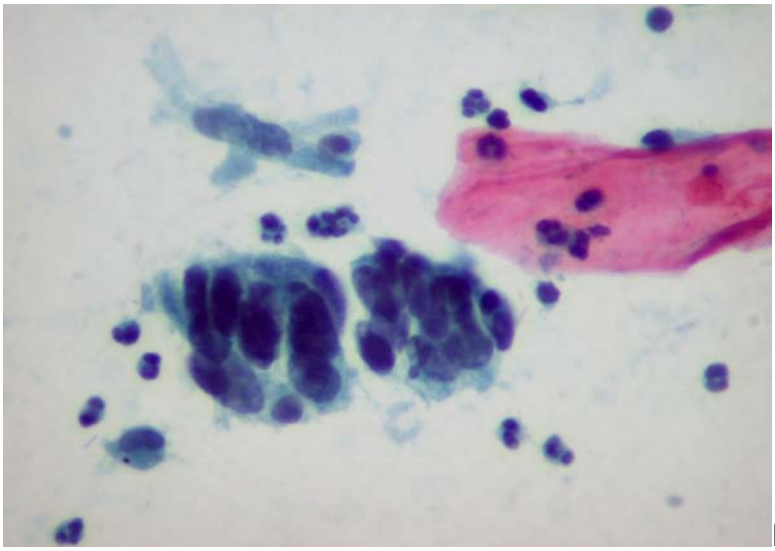

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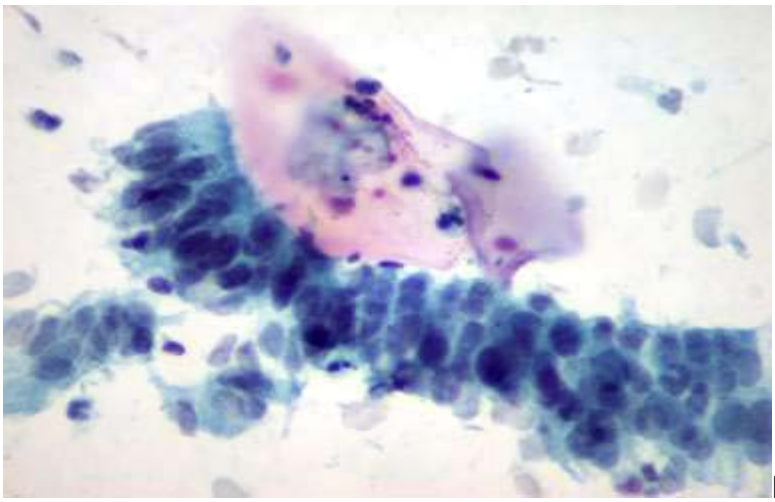

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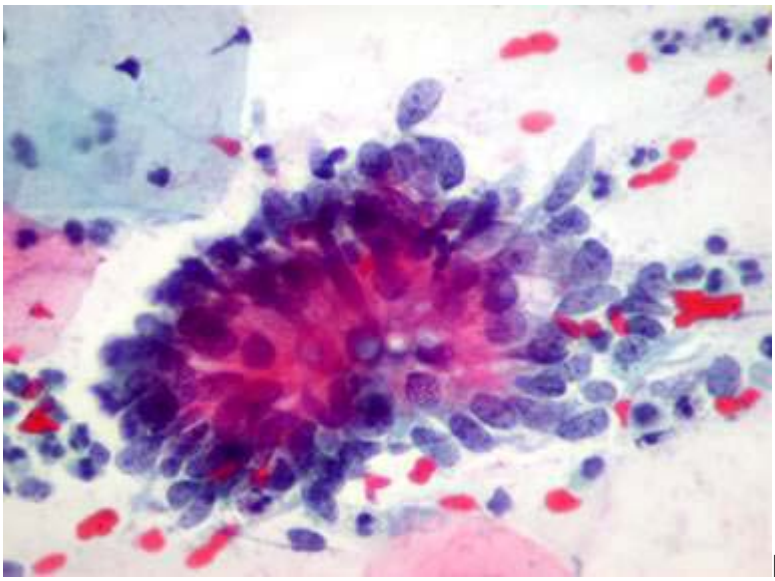

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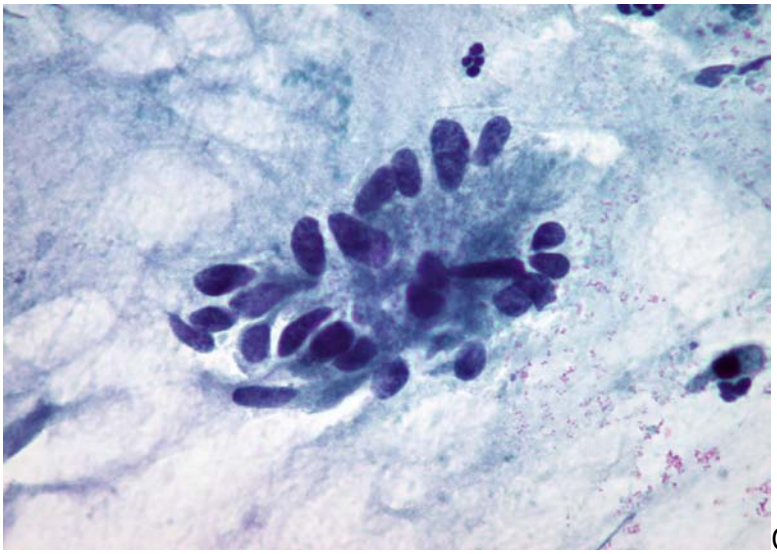
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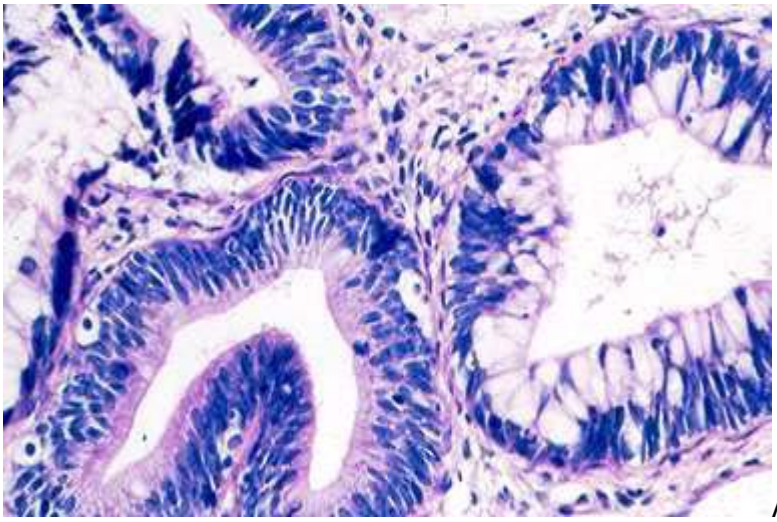
Fig. 5.2. Cytology of cervical AIS in CP smear:
A-C: Irregular monolayered sheet of tumor cells with cytoplasmic extensions or feathering.
D-E: Strips of tumor cells with pseudostratified nuclei.
F,G: Tumor cells forming rosettes.

AIS, intestinal variant almost always coexists with AIS, endocervical type. Cytologically, the tumor cells are large and occur singly, in clusters and in large epithelial fragments. They demonstrate single intracytoplasmic mucous vacuoles, resembling colonic epithelial sheets. (Fig. 5.3).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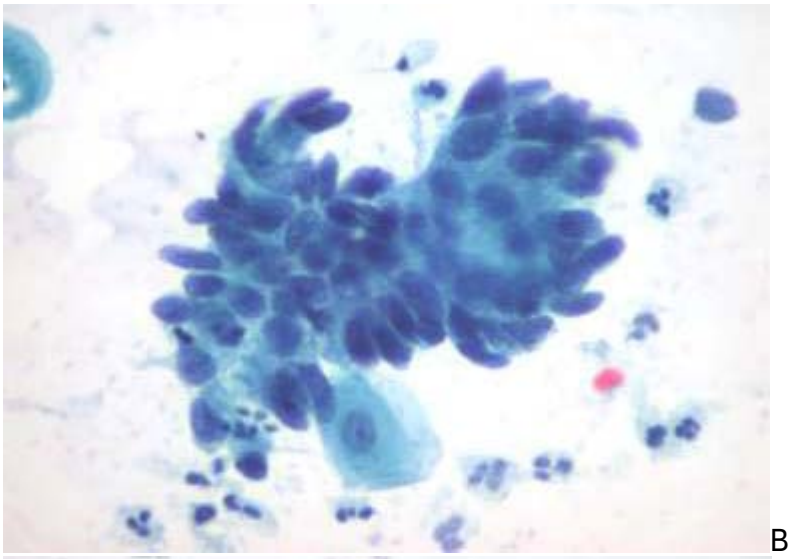
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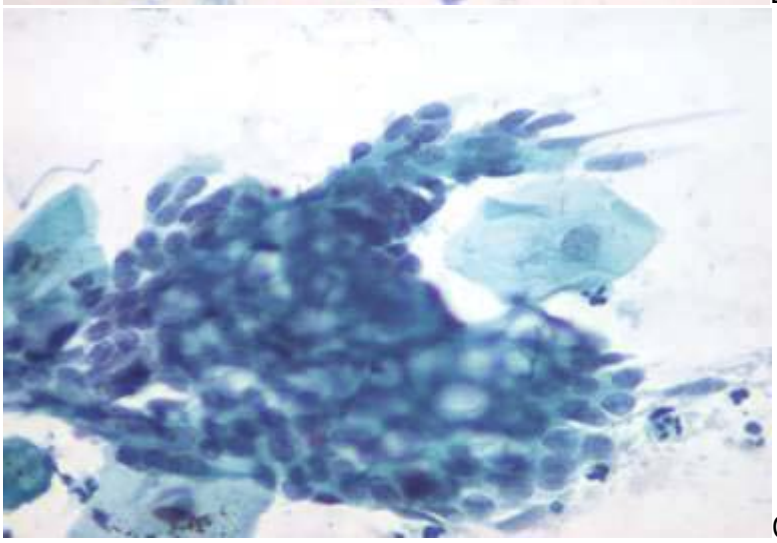
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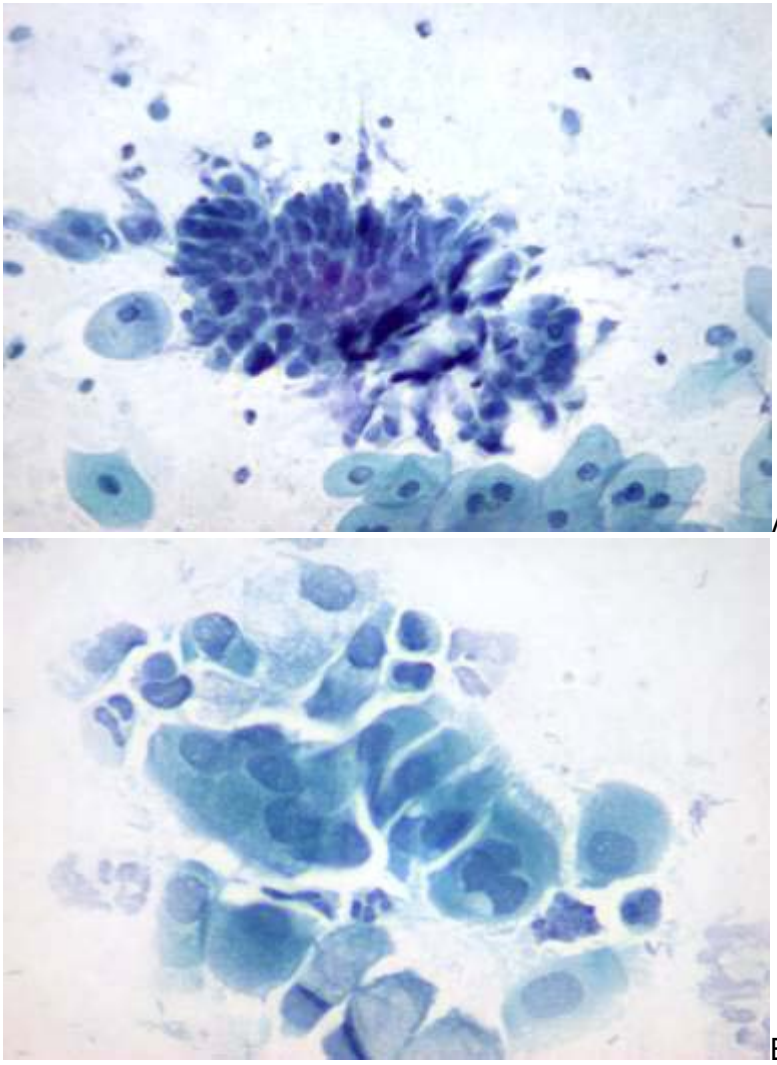
Fig. 5.3. Cervical AIS, intestinal variant.
A. Histology of the tumor
B, C: The tumor showing in CP smear:
B. Epithelial fragment with elongated nuclei in vague palisade.
C. Epithelial fragment with multiple round, clear spaces or vacuoles.

Differential diagnosis

A few conditions may exfoliate cells mimicking those of cervical AIS.

1. Cervical endometriosis exfoliates cells that may be mistaken for those of cervical AIS. Efforts should be made to look for endometrial glandular fragments and clusters of endometrial stromal cells to avoid a false-positive diagnosis.

2. Tubal metaplasia, a fairly common lesion of the cervix, may yield cell clusters that are readily mistaken for those of AIS. Ciliated cells can be recognized in well-preserved cellular strips. (Fig.5.4). However, loss of cilia due to degenerative changes is not uncommon. An awareness of tubal metaplasia and the potential for cytodiagnostic error is necessary to avoid an unnecessary cone biopsy.



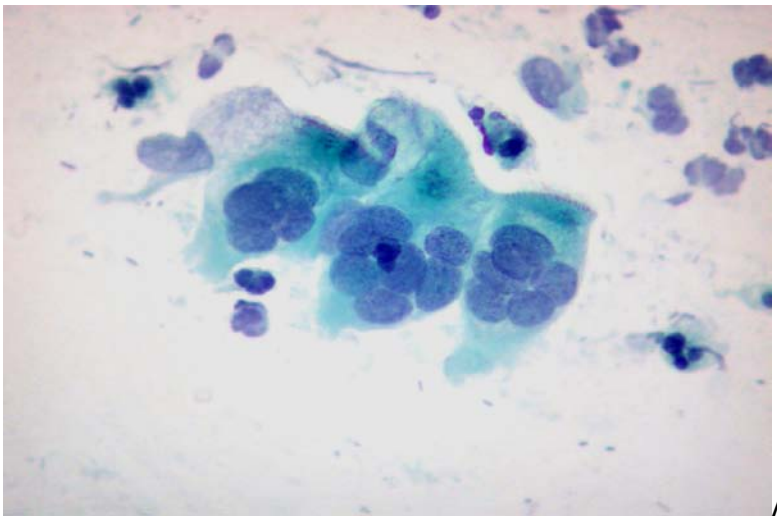
A B

Fig. 5.4. Tubal metaplasia showing in CP smear ciliated columnar cell singly and in row.

3. Postcone biopsy smears may contain fragments of endometrium from lower uterine segment with crowded, hyperchromatic, and pseudostratified nuclei simulating those of cervical AIS. These endometrial epithelial fragments are usually mixed or surrounded by endometrial stromal cells. Clinical data will be helpful in avoiding a false-positive diagnosis in this setting. The reader is referred to Chapter 2 for illustrations of lower uterine segment endometrium.

4. Reactive endocervical cells. Cytologic criteria of reactive endocervical glandular cells include:

- Reactive endocervical cells tend to occur in flat sheets with minimal nuclear crowding. (Fig. 5.5).
- Fairly abundant cytoplasm with well-defined cytoplasmic borders.
- Slightly enlarged nuclei with fine chromatin and prominent nucleoli.
- N/C ratio is within normal limits or only slightly increased.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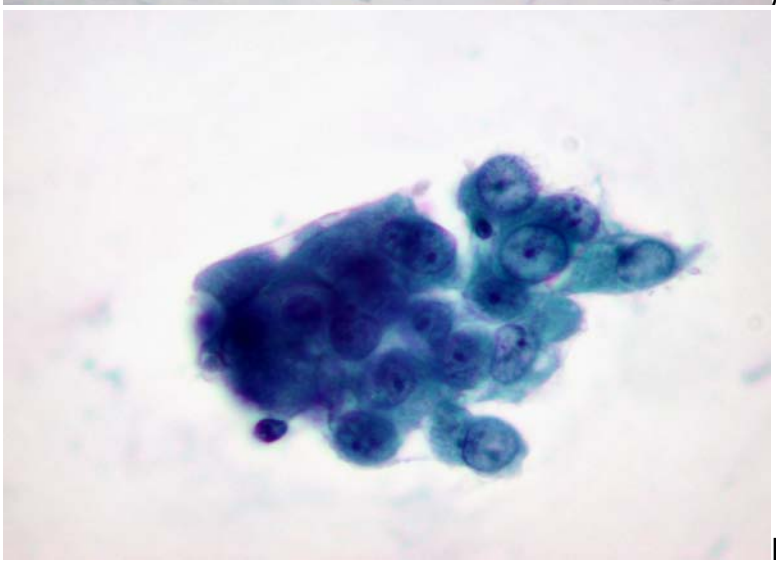
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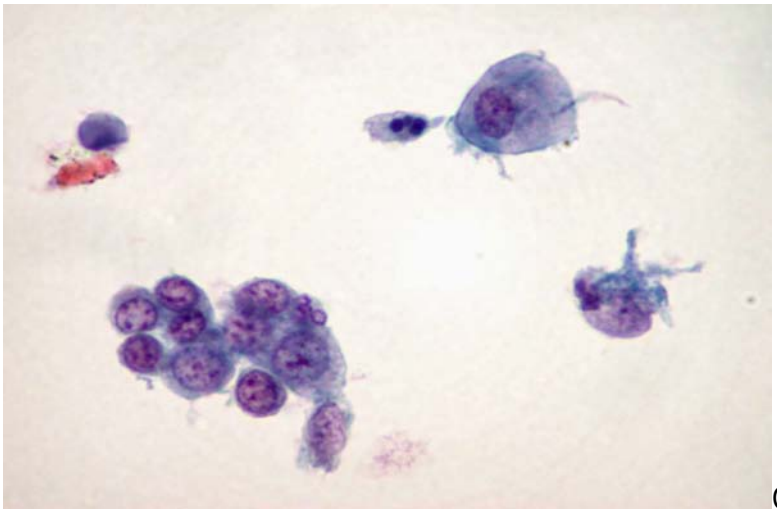
 C

Fig. 5.5. Reactive endocervical glandular cells:
A, B. In CP smear these cells have well-defined cytoplasm and multiple, enlarged nuclei with prominent nucleoli.
C. Similar cells seen in a LBP.

5. Atypical cervical glandular cells. The reader is referred to the section on Atypical glandular cells below for discussion and illustrations.

ATYPICAL GLANDULAR CELLS

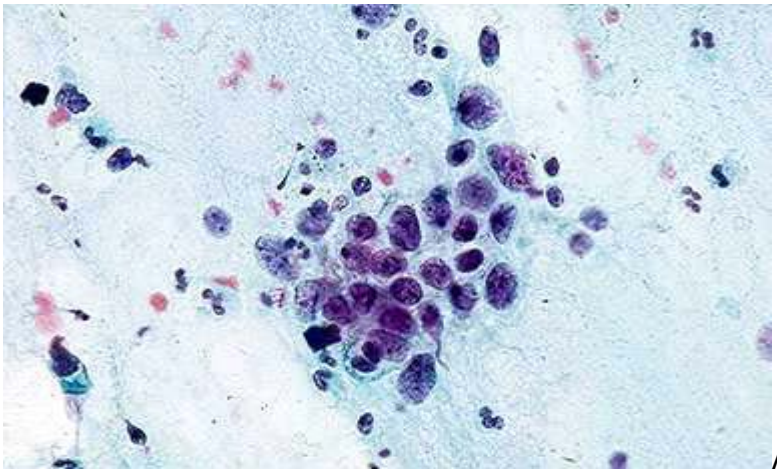
In The Bethesda System-2001 Atypical glandular cells (AGC) are defined as cells showing cellular changes that fall between those of definite benign reactive process and those of an unequivocal AIS or adenocarcinoma. AGCs are divided into 2 subtypes: AGC, NOS and AGC, favor neoplastic. AGCs are further divided into endocervical and endometrial types.

AGC accounts for about 0.2% of all Pap tests, with about 30% of patients having a significant cervical lesion: 5% being AIS and adenocarcinoma, and 20% being CINs.

1. Atypical endocervical cells, NOS

Cytologic criteria of AGCs, NOS. include:

- AGCs occur in sheets and strips with some cellular crowding and overlap. (Fig. 5.6).
- Nuclei are enlarged, up to 3 to 5 times the area of normal endocervical nucleus.
- Some variation in nuclear size and shape.
- Fairly abundant, distinct cytoplasm.
- Increased N/C ratio.
- Mild nuclear hyperchromasia.
- Nucleoli may be present.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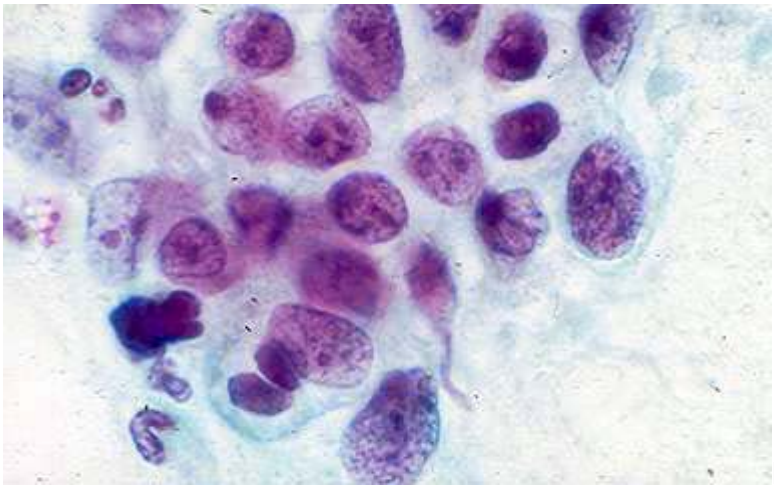
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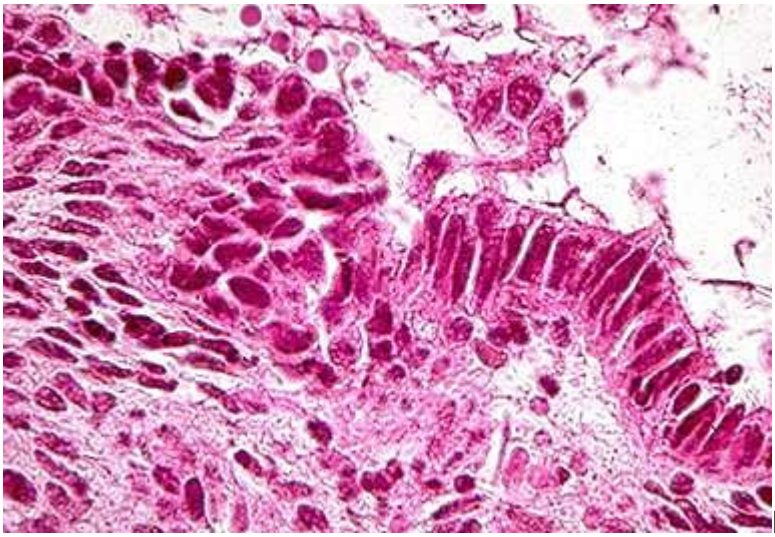
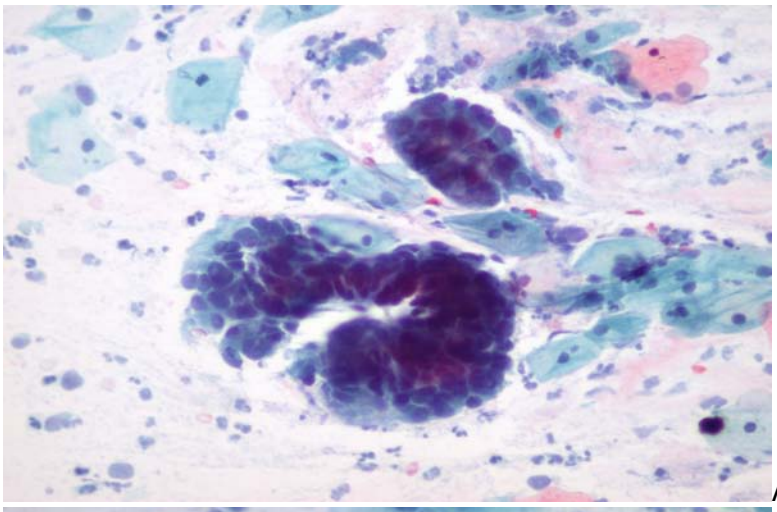

B

Fig. 5.6. A, B. CP smear showing a sheet of atypical endocervical glandular cells, NOS, displaying enlarged, slightly hyperchromatic nuclei and conspicuous nucleoli.
C. Cervical biopsy in this case revealed atypical endocervical glandular epithelium with no definitive histologic features of an AIS.

2. Atypical endocervical cells, favor neoplastic

By definition, atypical endocervical cells, favor neoplastic are AGCs with morphologic changes that qualitatively fall short of the cells derived from a cervical invasive or in situ adenocarcinoma. (Figs. 5.7 and 5.8). Their cytologic criteria include:

- Cells exfoliate in sheets, clusters and strips with nuclear crowding and overlap.
- Rare cell groups with rosette or acinar formation with feathering.
- Hyperchromatic, enlarged nuclei.
- Increased N/C ratio.
- Relatively scant cytoplasm with ill-defined cell borders.
- Nucleoli rarely observed.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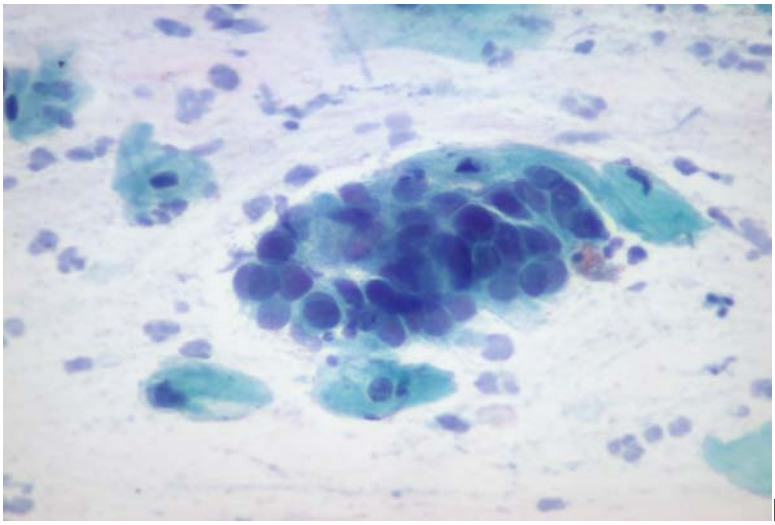
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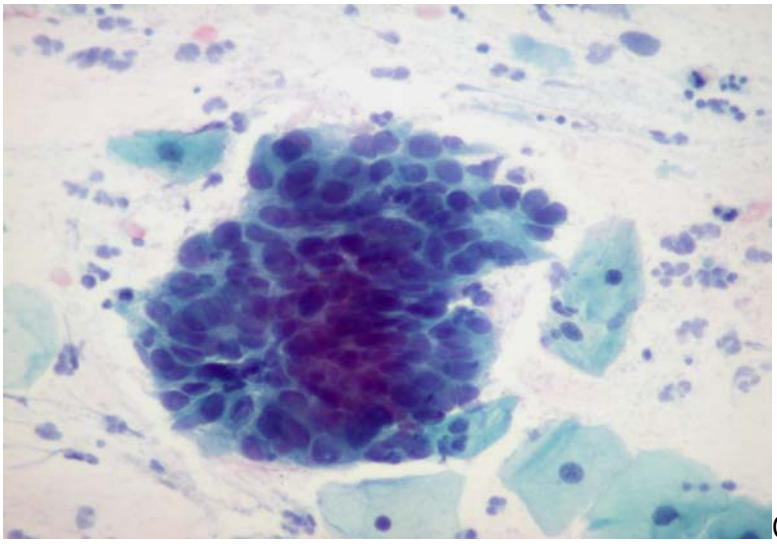

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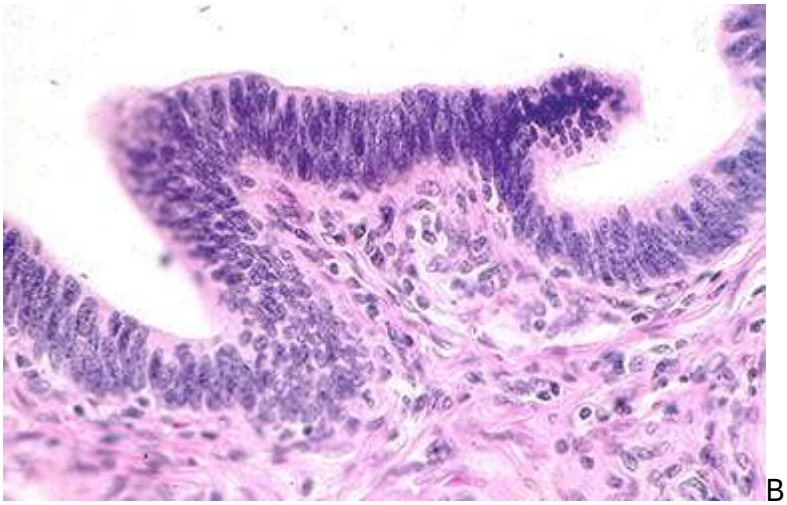
Fig. 5.7. Clusters of atypical cervical glandular cells, favor neoplastic displaying nuclear crowding and overlapping. Nuclei in palisade are seen in A and B.

A



B

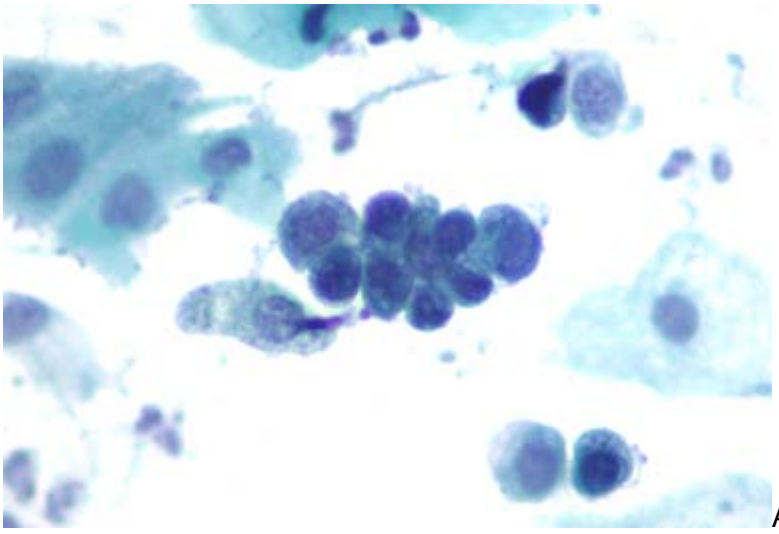
Fig. 5.8. A. CP smear showing atypical endocervical glandular cells, favor neoplastic, in a dyshesive cluster. The atypical cells show enlarged, hyperchromatic nuclei and ill-defined cytoplasm. The patient was subsequently found to have AIS on cervical biopsy.

3. Atypical endometrial glandular cells

Diagnostic criteria of endometrial AGCs include:

- Small glandular cells present singly or in rounded clusters.
- Scant or moderately abundant or vacuolated cytoplasm. (Fig. 5.9).
- Enlarged, hyperchromatic nuclei with 1 of the 2 additional nuclear changes below:
- Irregular nuclear contours, or
- Prominent nucleoli

These cellular changes may also be seen in association with endometrial polyp, chronic endometritis, endometrial hyperplasia and IUDs. The endometrial cell atypias can be difficult to identify because of cellular degeneration. On the other hand, normal endometrial cells exfoliated in menses may display reactive changes with slight nuclear enlargement and pleomorphism or degeneration that could be misinterpreted as abnormal. As about 50% of postmenopausal women shedding atypical endometrial cells have a significant endometrial pathology including carcinoma. Therefore, it is more practical to lump all degrees of endometrial cell atypia into one category of “endometrial cell atypia” and rely on histologic examination of endometrial tissue samples for grading of endometrial cell atypias. The presence of a high maturation index of squamous cells or a tumor diathesis also represents a risk for malignancy.



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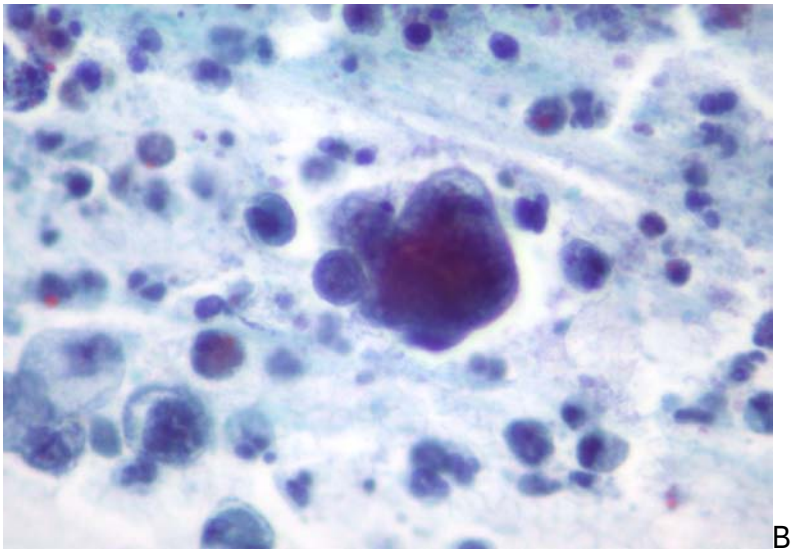

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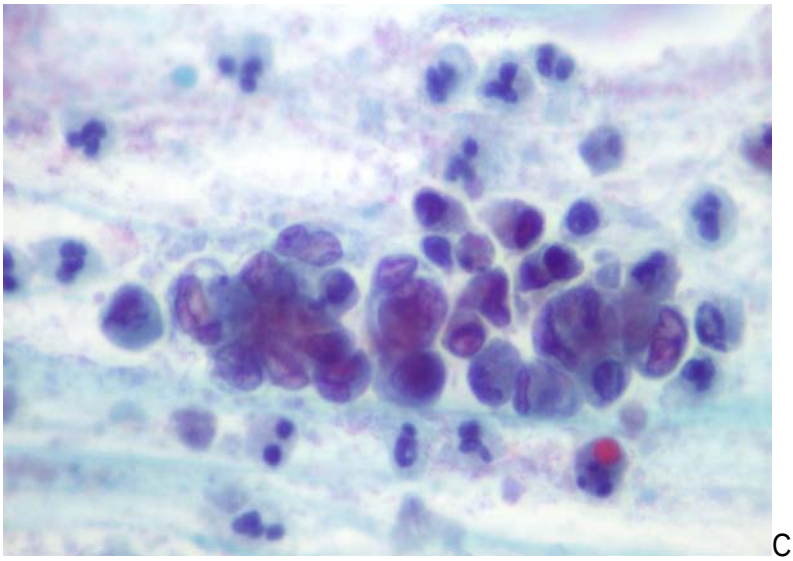


C

Fig. 5.9. A-C. CP smear showing 3-dimensional clusters of atypical endometrial cells.

Diagnostic difficulties

Cervical AGCs, favor neoplastic show changes that are qualitatively slightly more severe than those of AGCs, NOS. On several occasions AGCs are difficult to distinguish from atypical squamous cells, and in many patients with AGCs on CP smears, the cervical biopsy revealed a squamous cell lesion or no significant epithelial abnormality. However, according to DeMay, AGCs constitute a high-risk finding that predicts adenocarcinoma in 5% to 10% of cases. Therefore, patients with Pap smears showing persistent AGCs, NOS or AGCs, favor neoplastic should undergo colposcopic examination with biopsy and fractional uterine curettages to rule out a squamous cell lesion of the cervix and glandular neoplasm of the uterus. Testing for oncogenic HPV-DNA may be of diagnostic help, as cervical adenocarcinoma is strongly associated with HPV types 16 and 18.

INVASIVE ADENOCARCINOMA

Invasive adenocarcinoma of the cervix occurs more frequently in the 5th decade of life and accounts for up to 25% of all cervical cancers. AIS is found at the edge of invasive cervical adenocarcinomas in 43% to 100% of patients. The tumor may be associated with an ovarian mucinous or endometrioid adenocarcinoma. It is p16, HPV16, HPV18, ER and PR positive in almost all cases. It may be well- to poorly differentiated, and a mixed pattern consisting of areas of well and poor differentiation is not uncommon. Endocervical mucinous carcinomas are the most common type, accounting for 70% to 90% of cases followed by carcinoma of endometrioid type. Intestinal and signet-ring adenocarcinomas are rarely encountered. The neoplasm spreads first to pelvic structures then pelvic lymph nodes, ovaries, upper abdomen and distant organs. The 5-year survival rates of patients with cervical adenocarcinoma vary with the tumor stage: 79% for stage I, 37% for stage II and less than 9% for stage III and IV tumors. Poor prognostic factors include: high stage, depth of invasion >5 mm, angiolymphatic invasion, over-expression of HER2 and elevated level of serum CA125. The tumor is usually graded as follows:

- Well-differentiated or grade I: if it shows a glandular and papillary pattern and consists of columnar cells with uniform, oval nuclei; and 10% or less of the tumor has a solid growth pattern. (Fig.5.10).
- Moderately differentiated or grade II: if 11% to 15% of the tumor displays a solid, cribriform, complex glandular growth pattern and is composed of tumor cells with round or irregular nuclei and prominent nucleoli.
- Poorly differentiated or grade III: if the tumor shows pleomorphic cells.

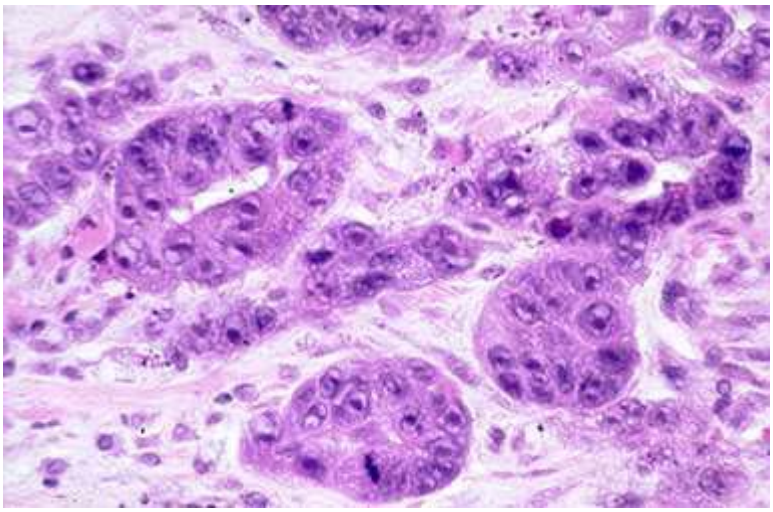
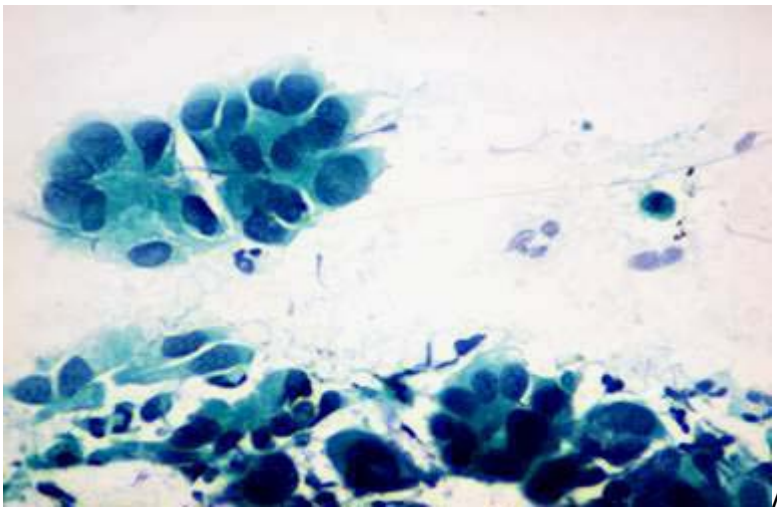


Fig. 5.10. Histology of a moderately differentiated cervical adenocarcinoma.

A. Well-differentiated cervical adenocarcinoma has cytologic manifestations similar to those of AIS, endocervical type, previously described.

B. Moderately differentiated cervical invasive adenocarcinoma usually has cytologic manifestations different from those of a well-differentiated tumor. Cytologic criteria include:

- Abundant malignant glandular cells commonly forming acini, balls, sheets, papillary clusters, strips, rosettes or syncytia. (Fig. 5.11).
- Dyshesive tumor cells are more commonly found (than AIS).
- Oval or pleomorphic nuclei with normo- or hyperchromasia.
- Finely or coarsely granular chromatin.
- Single or multiple micro- or macronucleoli.
- Cytoplasm is variable, ill defined, and rarely vacuolated.
- Tumor diathesis is present in about 30% of cases.
- Dyskaryotic or malignant squamous cells are found in about 20% of cases.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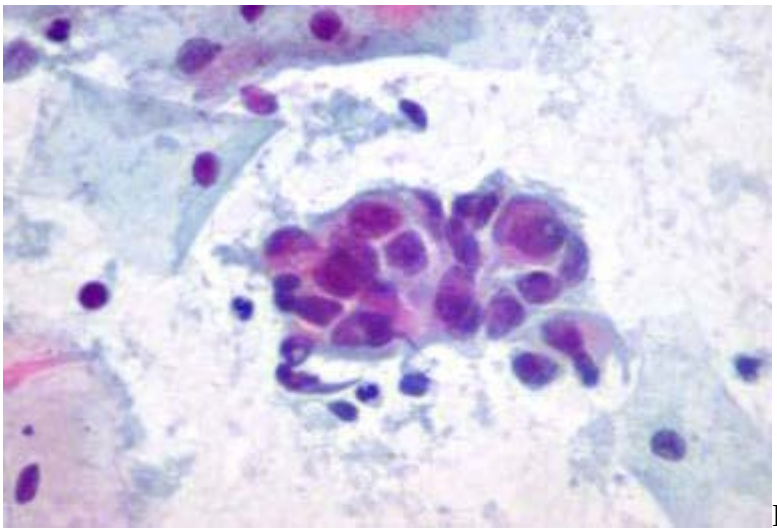
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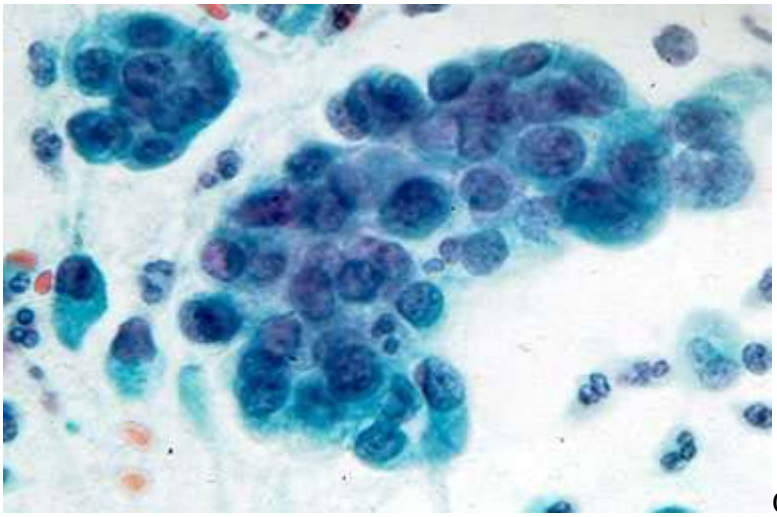


C

Fig. 5.11. Clusters of fairly polygonal malignant glandular cells with ill-defined cytoplasm, hyperchromatic nuclei and small or inconspicuous nucleoli in CP smears from 3 cases of moderately differentiated endocervical adenocarcinoma.

C. Poorly differentiated cervical adenocarcinoma exfoliates pleomorphic malignant glandular cells singly and in clusters. The tumor cells display pleomorphic nuclei, ill-defined cytoplasm and prominent nucleoli. (Fig. 5.12).

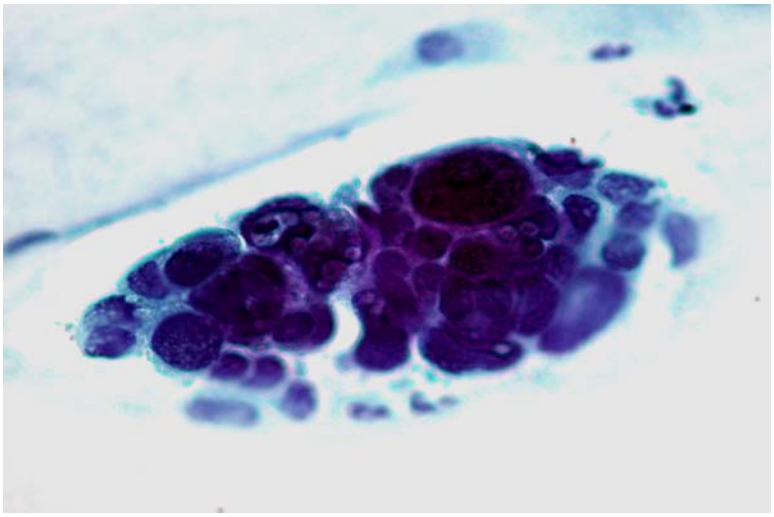


Fig. 5.12. Clustered pleomorphic malignant glandular cells in a CP smear from a poorly differentiated cervical adenocarcinoma.

Differential diagnosis

Cells from a cervical adenocarcinoma should be differentiated from cells derived from a reparative epithelium, endometrial adenocarcinoma and extrauterine cancer. The reader is referred to Chapter 2 in this book for discussion and illustration of repair epithelial cells.

- **Endometrial adenocarcinoma cells** can be difficult to distinguish from those of a cervical glandular malignant tumor. In general, cells from an endometrial tumor are smaller than those of an endocervical cancer and they are usually associated with a large amount of necrotic debris. In difficult cases, a fractional uterine curettage should be done to distinguish these two lesions histologically. A well-differentiated endometrial adenocarcinoma invading the cervix may exfoliate tumor cells in sheets with nuclei in picket-fence at periphery, in strips with pseudostratified nuclei and in rosettes, as seen in cell samples from a cervical AIS. The reader is referred to Chapter 7 for discussion and illustrations of malignant endometrial cells.

- **Extrauterine adenocarcinoma cells** may reach the cervix by traveling through the fallopian tubes and uterine cavity. These malignant cells are, in most cases, found in a smear background that is free of necrotic debris and inflammatory exudate. In doubtful cases, clinical data and fractional curettages of the uterus would be of diagnostic help. The reader is referred to Chapter 6 for a more detailed discussion on extrauterine cancers.

DIAGNOSTIC ACCURACY

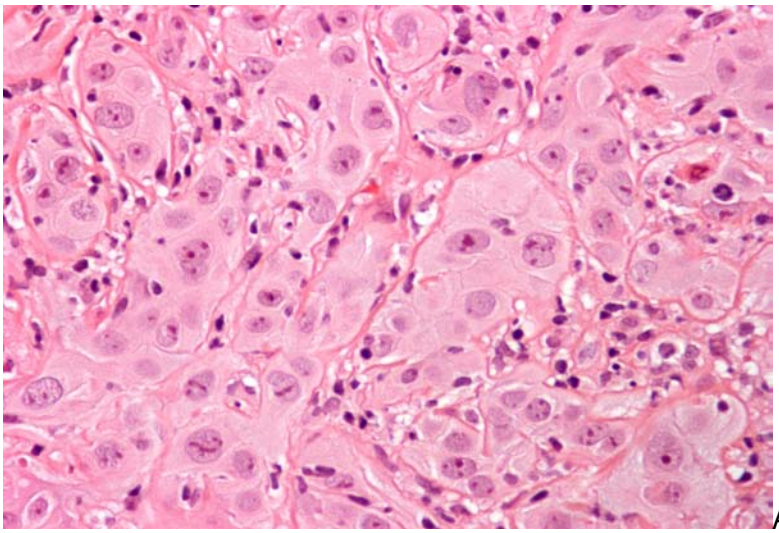
Cytodiagnosis of cervical AIS is challenging. In one series consisting of 94 patients with cervical AIS, 65 (69%) cases showed a glandular lesion and 29 (31%) displayed a squamous or unspecified lesion. For pure cervical AISs a diagnostic sensitivity of 40% to 69% has been reported. When a combined lesion consisting of AIS and HSIL the reported rate of detection of glandular cell abnormality was 16% to 23% only, depending on the types of cell preparation (CP smear or LBP). However, if the cell samples were diagnosed as having either an AIS plus HSIL or a HSIL, the sensitivity rates were about 63% and 74%, respectively.

The diagnostic accuracy rate of invasive cervical adenocarcinomas in different reported series ranged from 86% to 97.4%. The cytologic detection of cervical adenosquamous carcinomas appears to be more difficult leading to a false-negative rate up to 55%. It is important to note that cervical biopsy is adequate for confirming an invasive adenocarcinoma but it is not adequate for diagnosing a cervical AIS which requires a deep cone biopsy for histologic confirmation.

VARIANTS OF CERVICAL ADENOCARCINOMA

Relatively common variants of cervical adenocarcinoma are mixed adenosquamous carcinoma, serous papillary carcinoma, and clear cell carcinoma. Adenoma malignum and adenoid cystic carcinoma are rare occurrences in this location.

1. Glassy cell carcinoma is a poorly differentiated adenosquamous cell carcinoma and accounts for 1% to 2% of all cervical cancers. It more commonly occurs in relatively young patients, with a mean age of 41 and is HPV types 16 and 18 positive. The tumor most often appears as a bulky exophytic mass consisting of nests and masses of polygonal cells with granular “glassy” cytoplasm and oval nuclei with prominent nucleoli. Patients with this type of tumor may show blood eosinophilia. In typical cases, the tumor yields in Pap smears single and clustered large malignant epithelial cells with oval nuclei and prominent nucleoli, similar to those of a nonkeratinizing squamous cell carcinoma. (Fig. 5.13). Tumor cells with ground-glass cytoplasm may be observed. In other cases exfoliated tumor cells do not display a ground-glass cytoplasm and a firm diagnosis of glassy cell carcinoma can be only made by tissue biopsy.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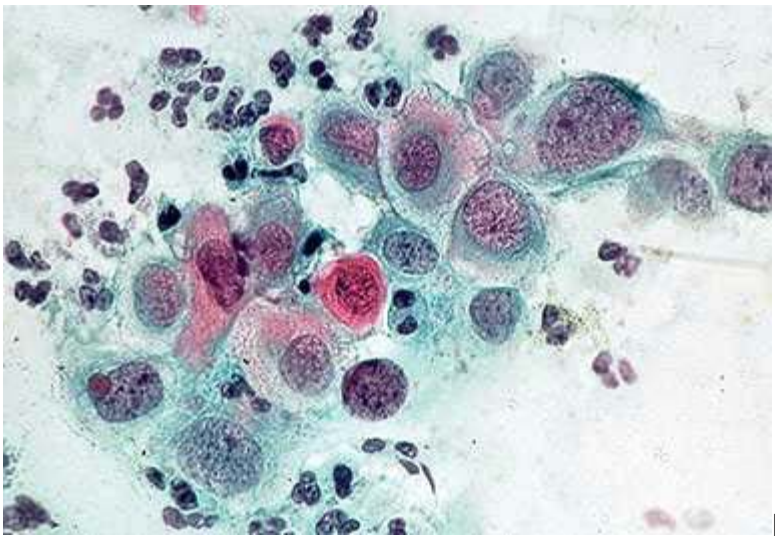
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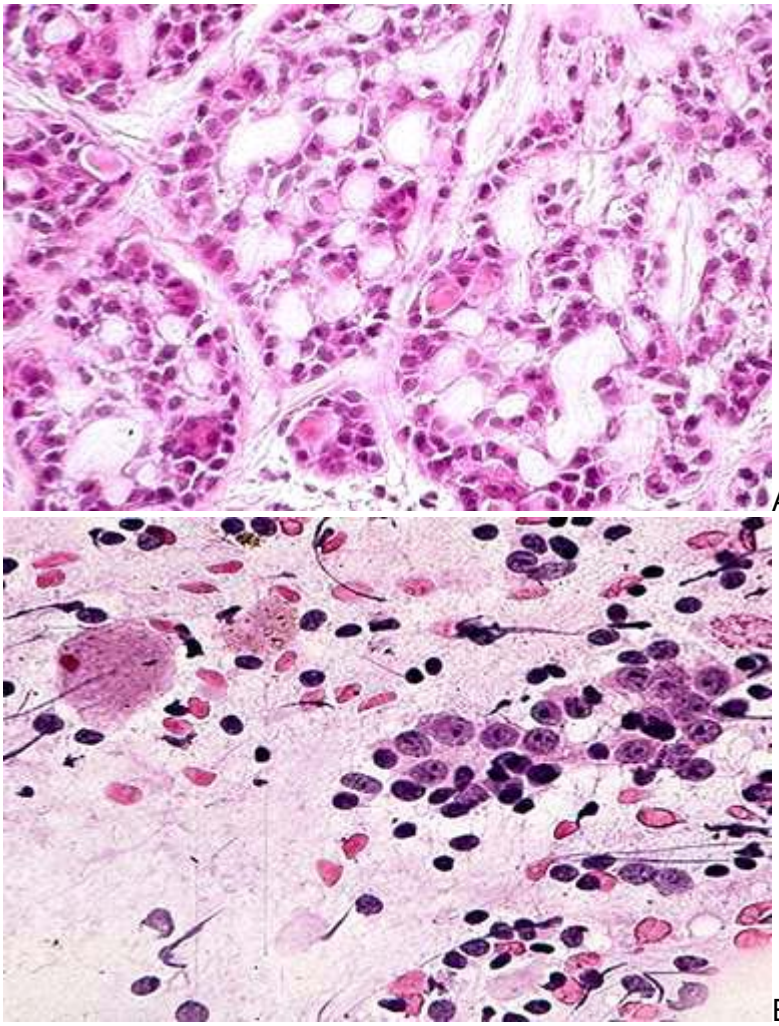
Fig. 5.13. Glassy cell carcinoma:
A. Tumor histology showing cells with abundant, homogenous, eosinophilic, “glassy” cytoplasm, oval nuclei and prominent nucleoli.
B. CP smear showing clusters of malignant epithelial cells with abundant, “glassy” or granular cytoplasm and large and oval nuclei. Small nucleoli are noted in some cells.

2. Adenoid cystic carcinoma accounts for about 1% of all cervical adenocarcinomas. The tumor occurs mainly in elderly patients, but it may occur in patients under 50 years of age. The neoplasm commonly forms a polypoid friable mass and consists of small cancer cells forming clusters, cords and trabeculae with lumens containing hyaline eosinophilic material. It shows in Pap smears clusters of small cells with scant cytoplasm and small, oval, hyperchromatic nuclei. Globules of basophilic material may be observed. (Fig.5.14). The tumor is HPV 16 positive and has a poor prognosis.



A

B

Fig. 5.14. Adenoid cystic carcinoma:
A. Tumor histology showing small tumor cells forming round spaces containing dense, hyaline eosinophilic material.
B. A round hyaline body and smaller clusters or sheets of tumor cells with scant, ill-defined cytoplasm and round nuclei with conspicuous nucleoli seen in a CP smear.

3. Minimal deviation adenocarcinoma is a rare tumor accounting for about 1% of all primary cervical adenocarcinomas. Histologically, it may be divided into 3 types: cervical, endometrioid and non-specific.

- *Cervical minimal deviation adenocarcinoma (MDA), mucinous type* is the most common type, occurring in young women between 32 to 42 years of age. It is usually sporadic but it may rarely occur synchronously or precede an ovarian tumor that is commonly mucinous in nature. The tumor is usually HPV negative and often missed by small cervical biopsy. Due to diagnostic delay, this neoplasm is usually diagnosed at a high stage and therefore will have a poor prognosis. In about 50% of cases, foci of moderately or poorly differentiated adenocarcinoma are present. A cervical MDA, mucinous type, yields in CP smears sheets and clusters of glandular cells with monomorphic nuclei, small nucleoli and clear cytoplasm that may show wispy cytoplasmic extensions or tails. Higher grade tumor cells are not uncommonly found. (Figs. 5.15 to 5.17).

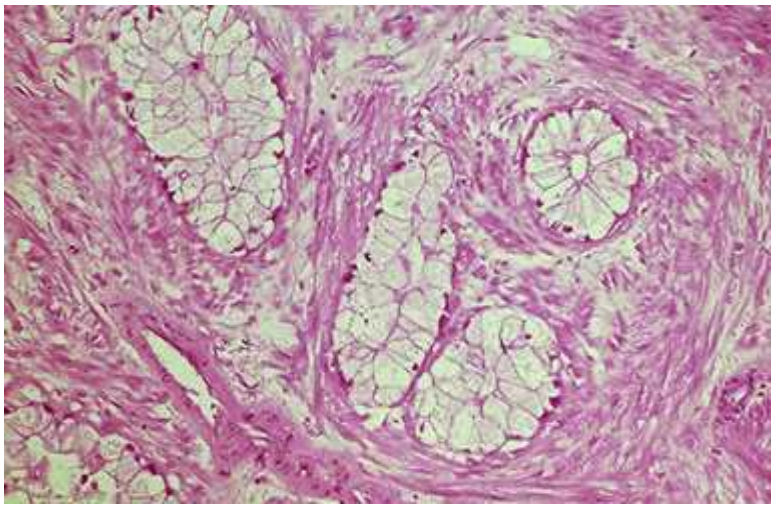
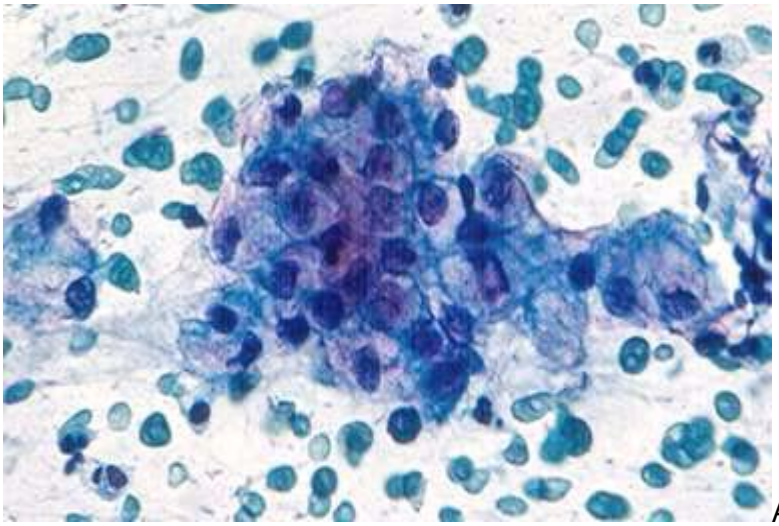


Fig. 5.15. Histology of cervical MDA, mucinous type showing mucous glands with small, bland nuclei invading the cervical fibromuscular wall.



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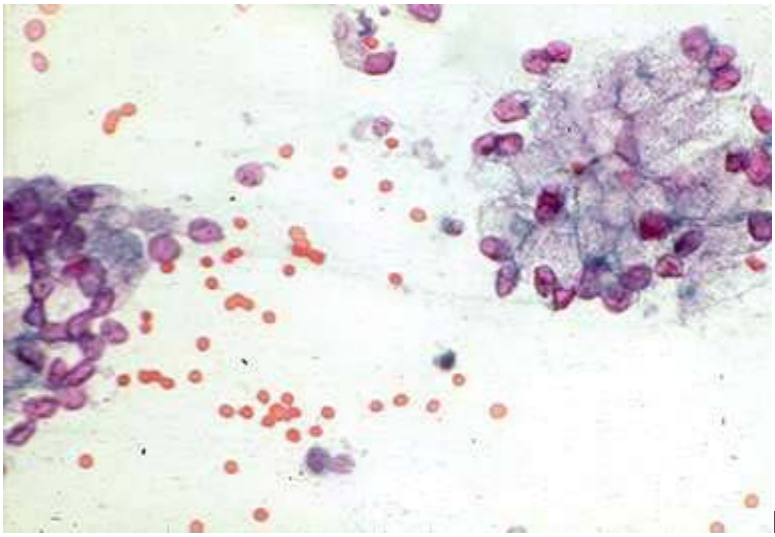
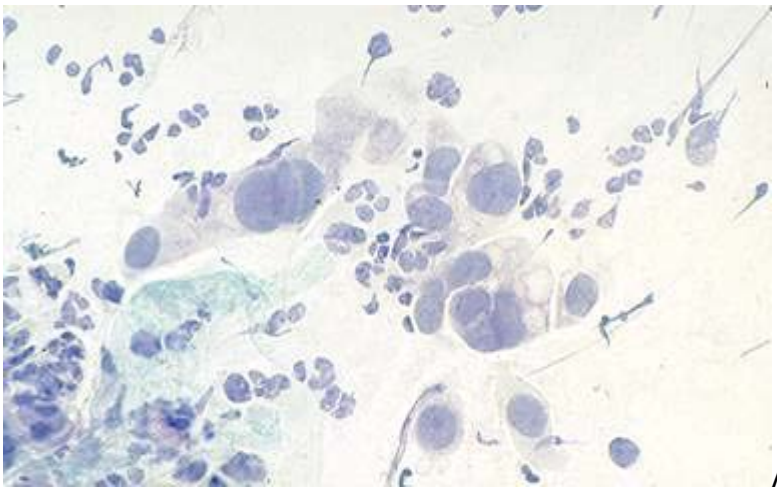


B

Fig. 5.16. A, B. Cervical MDA, mucinous type, showing in CP smear:
Irregular sheets and clusters of benign-appearing mucus secreting cells in honeycomb pattern.



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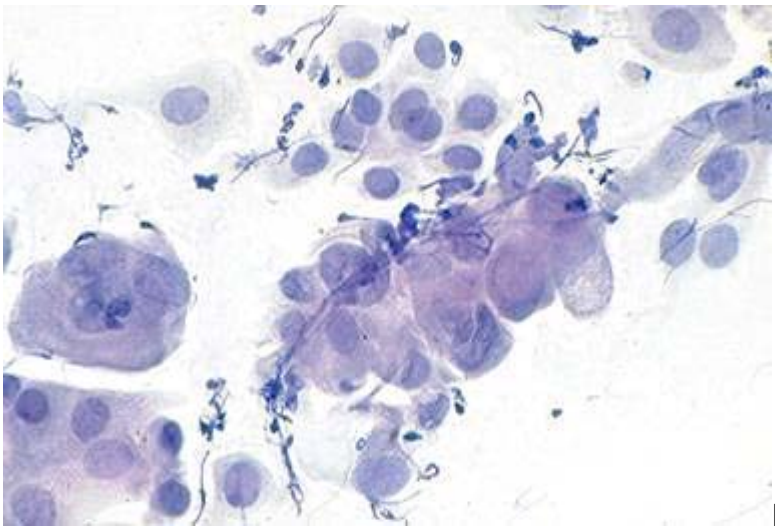

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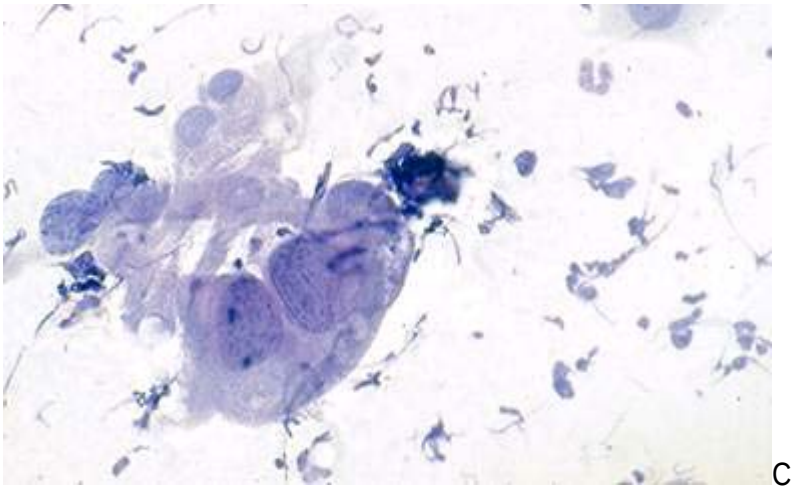
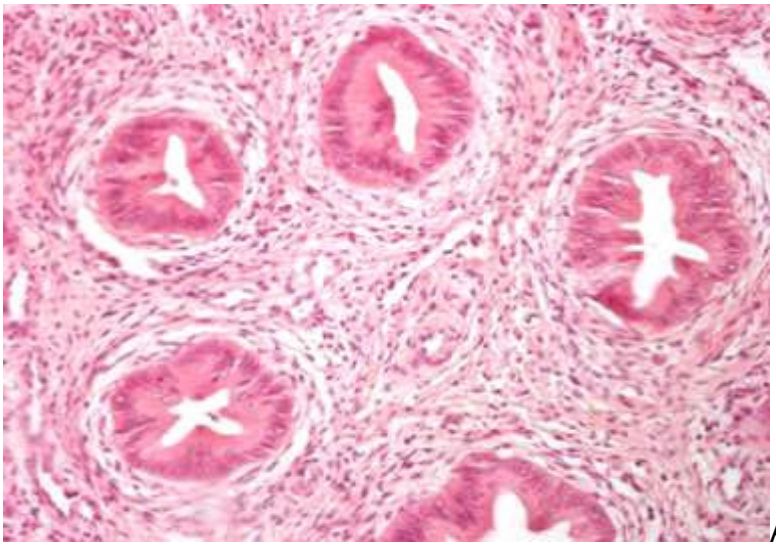

C

Fig. 5.17. In another case of cervical MDA, mucinous type, abundant tumor cells with relatively bland nuclei are seen (A and B). But a few more pleomorphic tumor cells with large, irregular nuclei and conspicuous nucleoli are present (C). The poor preservation of glandular cells is evident in this air-dried CP smear.

- ***MDA, endometrioid type.*** The cytologic manifestations of this neoplasm have recently been reported. This type of tumor exfoliates sheets of columnar glandular cells with low-grade oval nuclei in palisade at free borders. Similar tumor cells forming cell stripes with vague pseudostratified nuclei and rosettes, as seen in cervical AIS, are present. Tumor cells with higher nuclear grade may also be found. (Fig.5.18). Cytologic manifestations of a cervical ***MDA, non-specific*** **type** have not been reported so far.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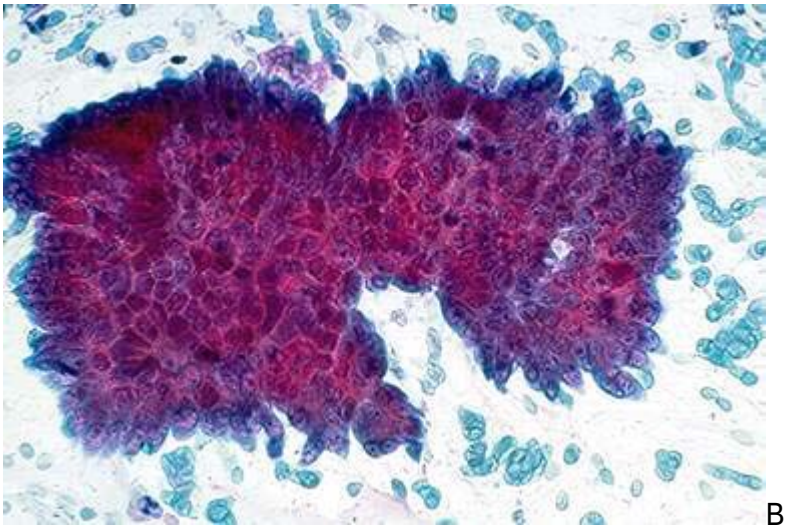

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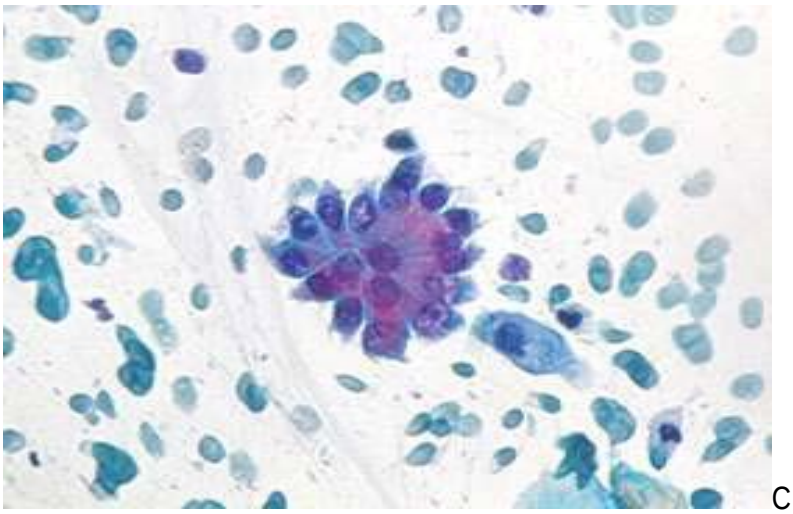

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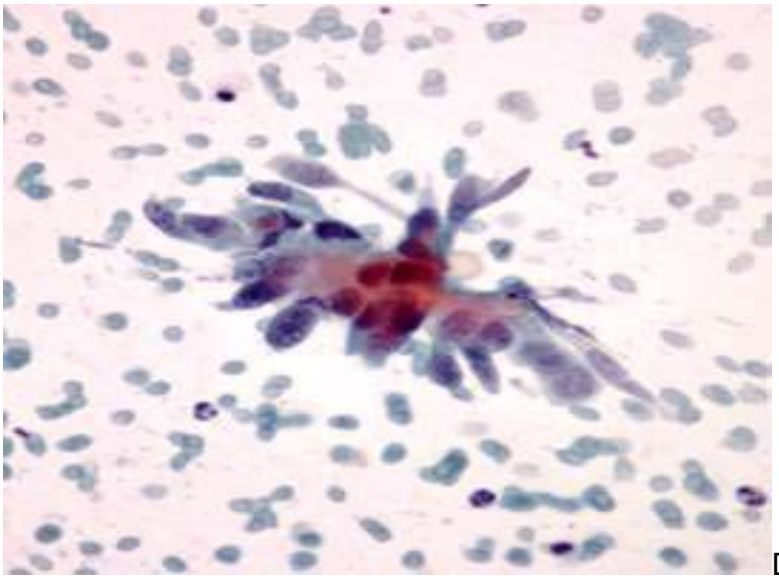

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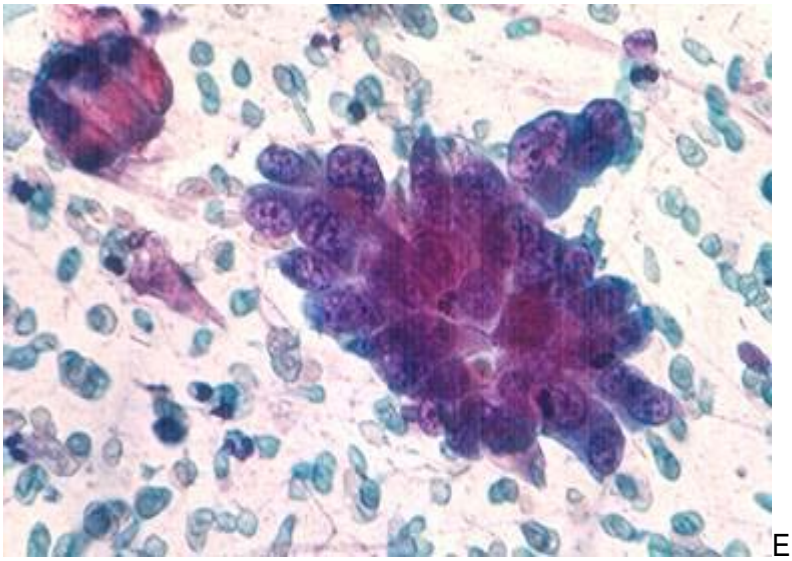
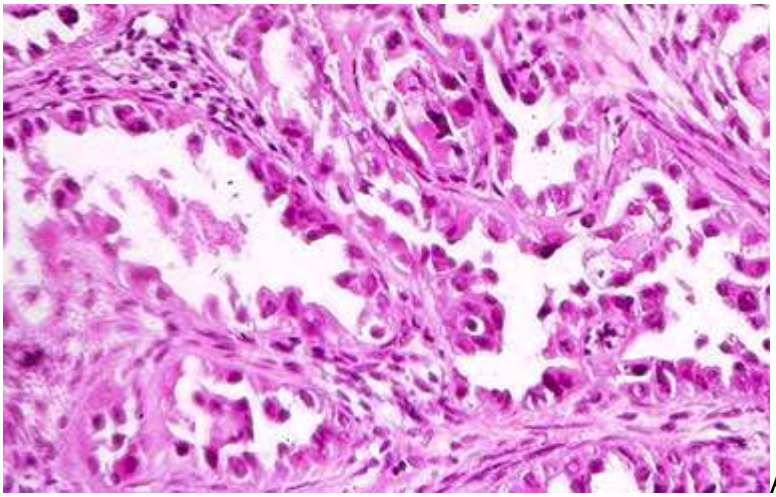

E

Fig. 5.18. Minimal deviation adenocarcinoma, endometrioid type:
A. Histology of the tumor.
B-E. CP smear showing tumor cells in a large sheet with nuclei feathering and in palisade formation at the periphery (B), tumor cells with low-grade nuclei and cytoplasmic “tails” in a rosette and cell strip (C,D), and more pleomorphic malignant glandular cells with conspicuous nucleoli forming a rosette (E).

4. Clear cell carcinoma accounts for about 4% of all cervical adenocarcinomas. About 2/3 of cases occur in young women who had an in utero exposure to diethylstilbestrol (DES) but it may occur in older women without DES exposure. In about 50% of cervical clear cell carcinoma related to DES exposure a vaginal adenosis is present. Grossly, the tumor appears as a nodular or an ulcerated lesion. The 5- and 10-year survival rates of patients with clear cell carcinoma are 55% and 40%, respectively. Histologically, it is characterized by a papillary, microcystic, tubular or solid pattern. The tumor cells show a clear or eosinophilic, granular cytoplasm and often have a “hobnail” configuration. The nuclei are oval and show prominent nucleoli. Cervical clear cell carcinoma shows in CP smears irregular sheets and clusters of epithelial cells with clear, granular or vacuolated cytoplasm and oval nuclei with prominent nucleoli. (Figs.5.19 and 5.20).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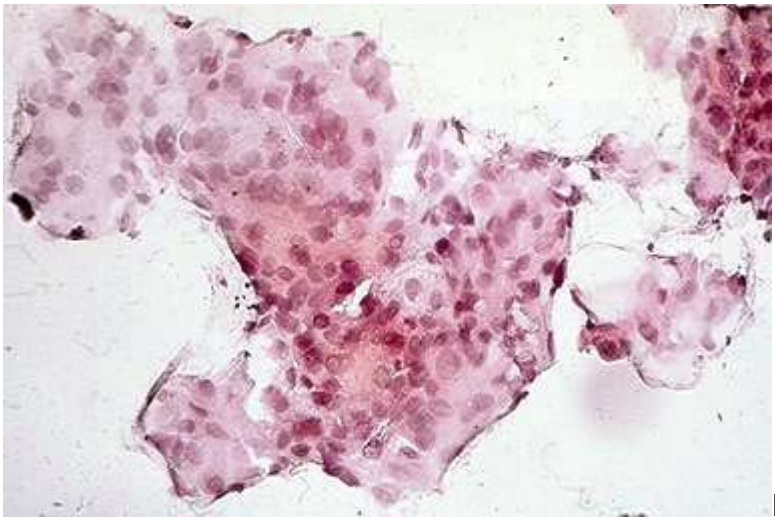
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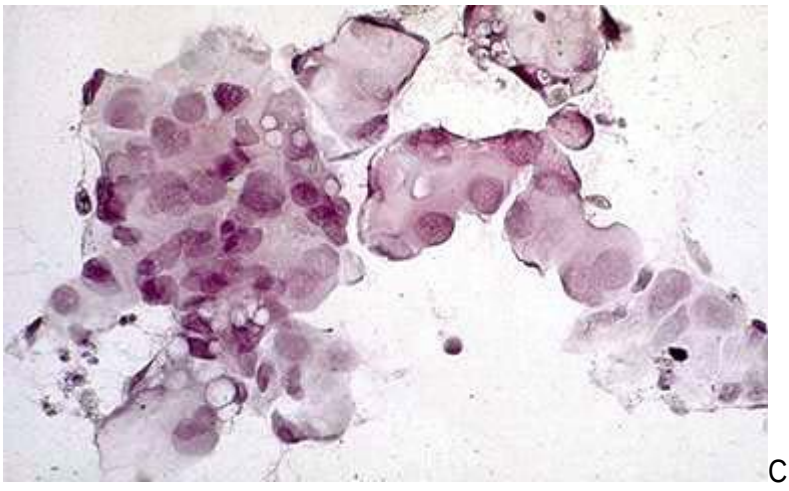

C

Fig. 5.19. Clear cell carcinoma:
A. Histology of the tumor showing cells in “hobnail” pattern.
B, C. CP smear showing sheets of tumor cells with round nuclei, granular or opaque, well defined cytoplasm and focal nuclear crowding.

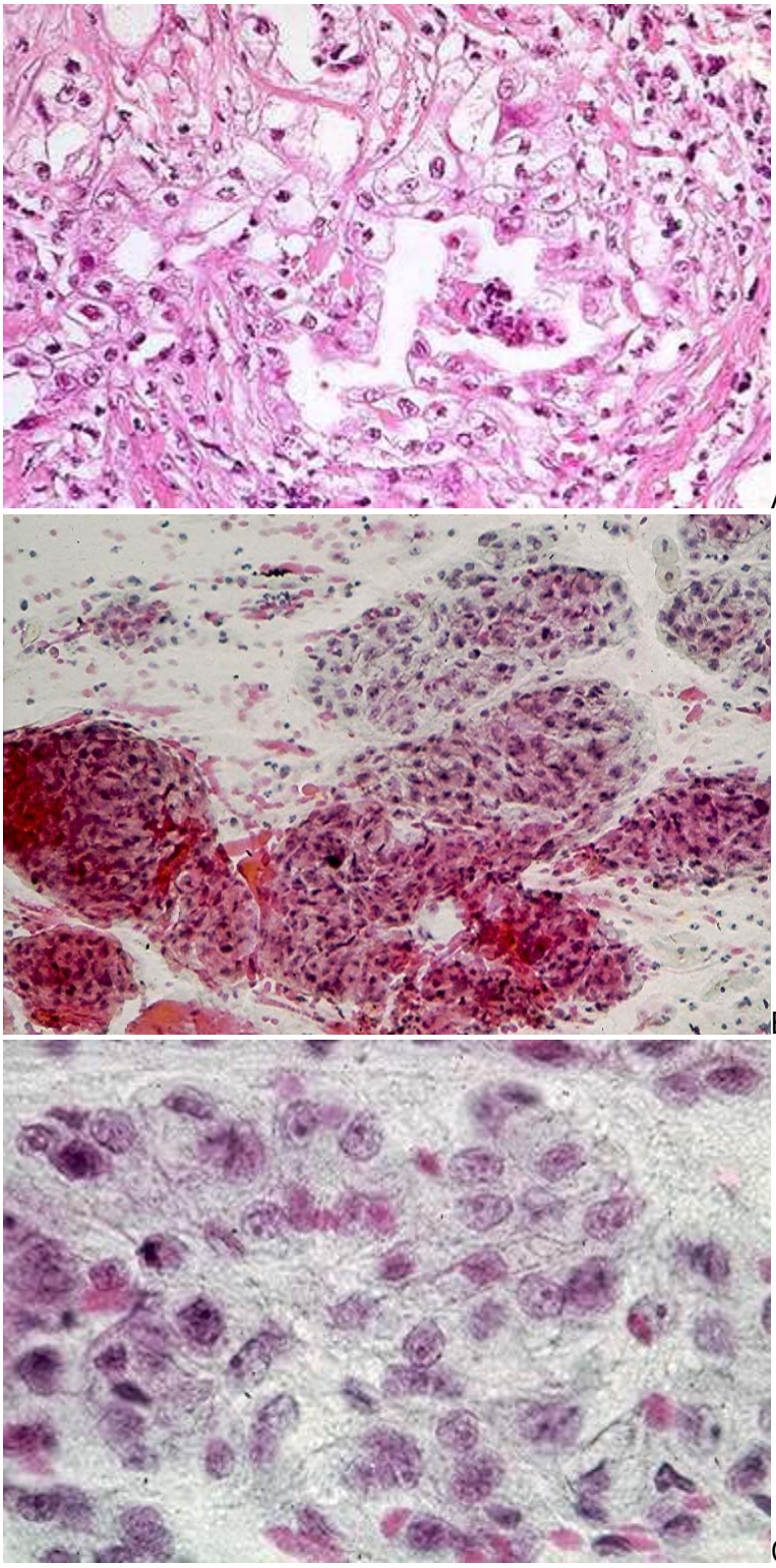
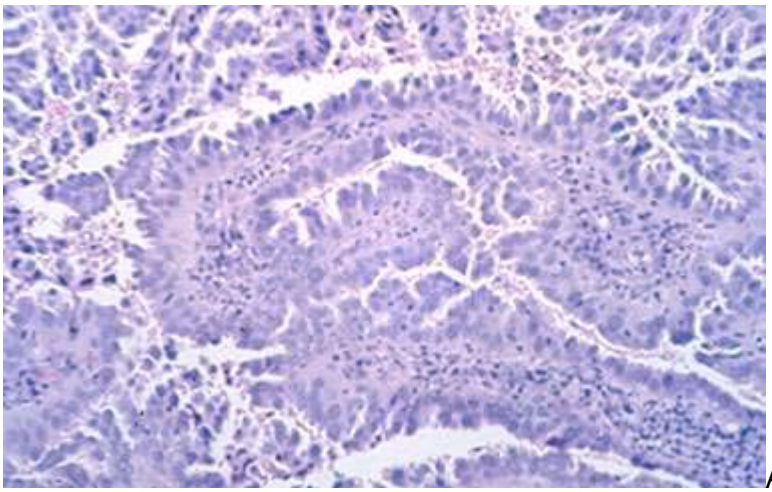


Fig. 5.20. Clear cell carcinoma:
A. Histology of the tumor.
B, C: Large sheets of tumor cells with clear, vacuolated cytoplasm, oval nuclei and conspicuous nucleoli seen in a CP smear.

5. Villoglandular carcinoma is a rare cervical cancer with low-grade nuclei and an excellent prognosis. It is composed of epithelial papillae with thick fibrovascular cores and shows in Pap smears monolayered sheets of malignant epithelial cells with folding and nuclear crowding. The tumor cell nuclei are oval, hyperchromatic and show inconspicuous nucleoli. (Fig. 5.21).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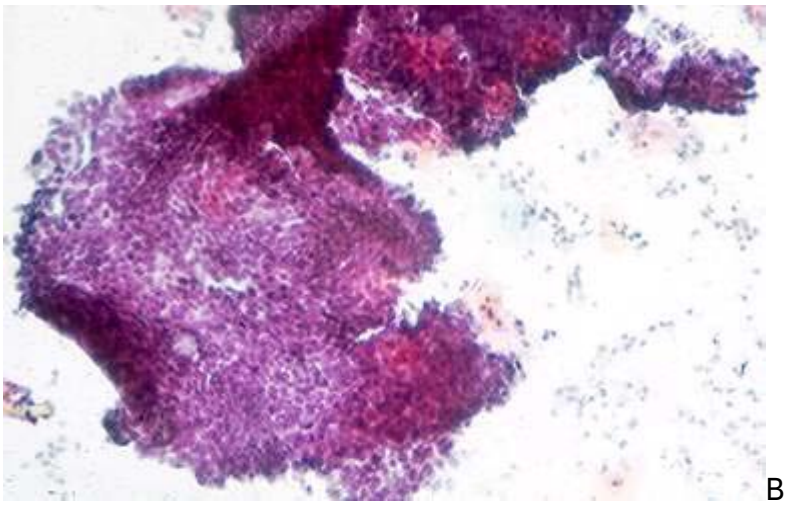
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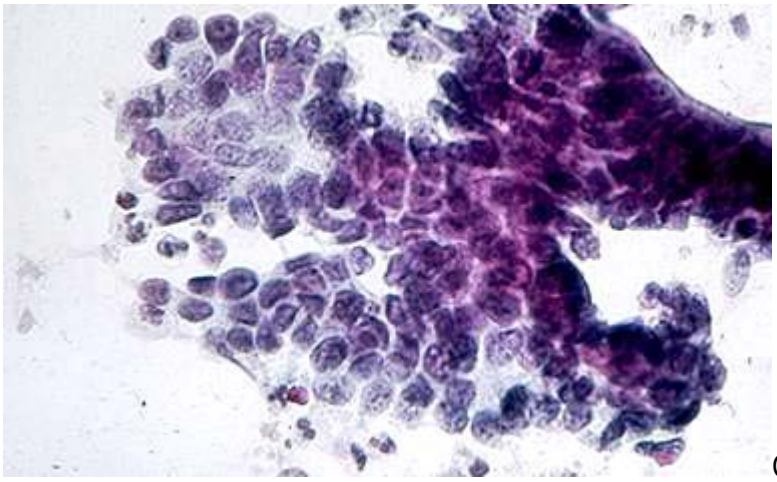
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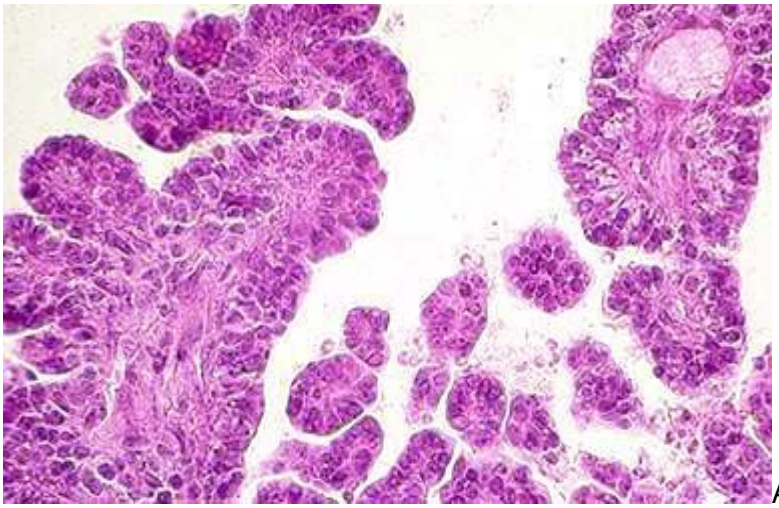
Fig.5.21. Villoglandular carcinoma:
A. Histology of the tumor.
B-D. CP smear showing a large monolayered sheet of tumor cells with folding(B) and thick tumor cell clusters showing round, hyperchromatic monomorphic nuclei with inconspicuous nucleoli (C,D).

6. Papillary serous carcinoma accounts for about 1% of all cervical carcinomas. The tumor is aggressive and has early pelvic and periaortic lymph node metastases. In some studies, tumors in young patients are HPV positive and those in older patients are HPV negative. Histologically the tumor is characterized by the presence of thin fibrovascular cores and covered by pleomorphic malignant glandular cells with prominent nucleoli. It displays in CP smear malignant glandular cells in tri-dimensional papillary clusters and in irregular, monolayered sheets. (Fig.5.22).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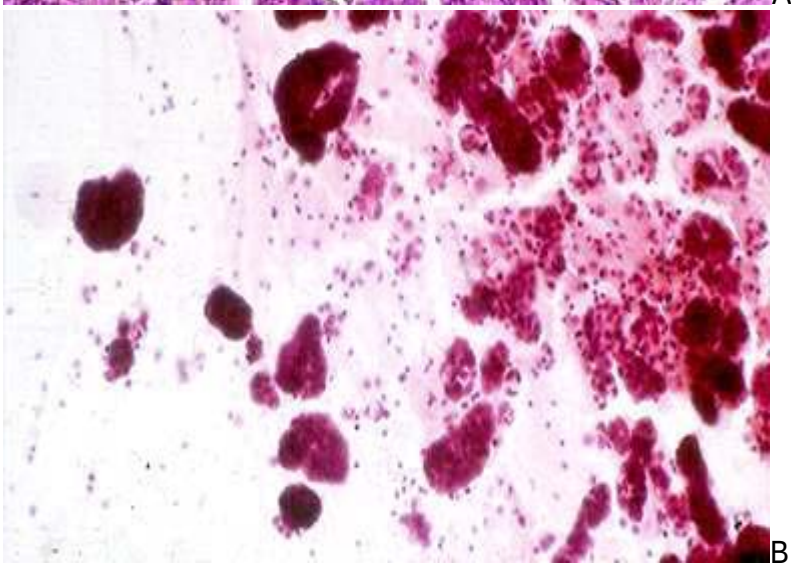
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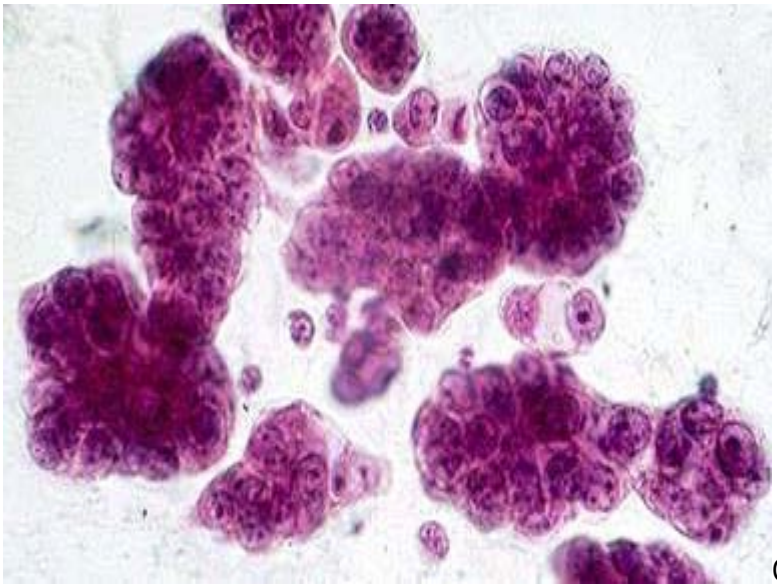
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Fig. 5.22. Papillary serous carcinoma:
A. Histology of the tumor.
B, C. Tri-dimensional papillary clusters of tumor cells with prominent nucleoli in CP smear.

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Chapter 6

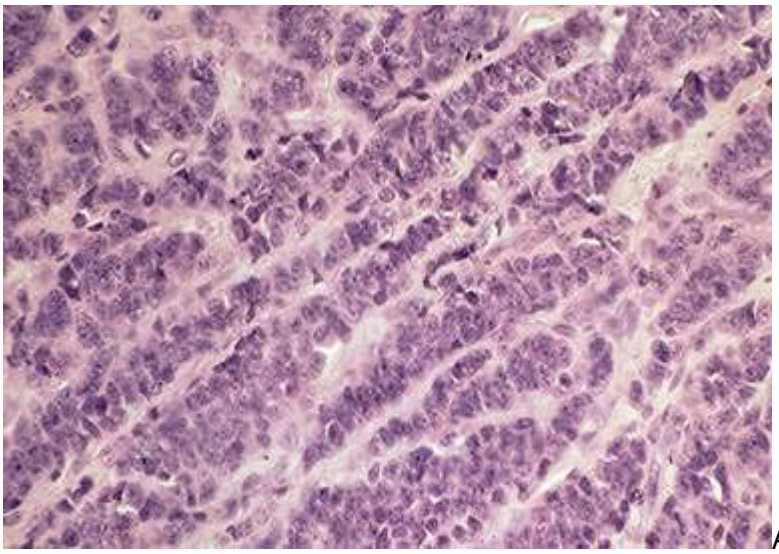
Other Cervical Cancers and Extrauterine Cancers

PRIMARY CERVICAL CANCERS

A. NEUROENDOCRINE CARCINOMAS

These are rare tumors that may occur alone or in association with a cervical adenocarcinoma of usual type. Histologically, these tumors are classified into 4 subtypes: typical and atypical carcinoid tumors, small cell carcinoma and large cell carcinoma with neuroendocrine differentiation. Carcinoid tumors are highly aggressive with a 3-year survival rate of 12% to 33%.

1. Typical carcinoid tumor yields, in a CP smear, single and loosely clustered oval cells with plasmacytoid configuration and oval nuclei with chromatin clumping. (Fig. 6.1).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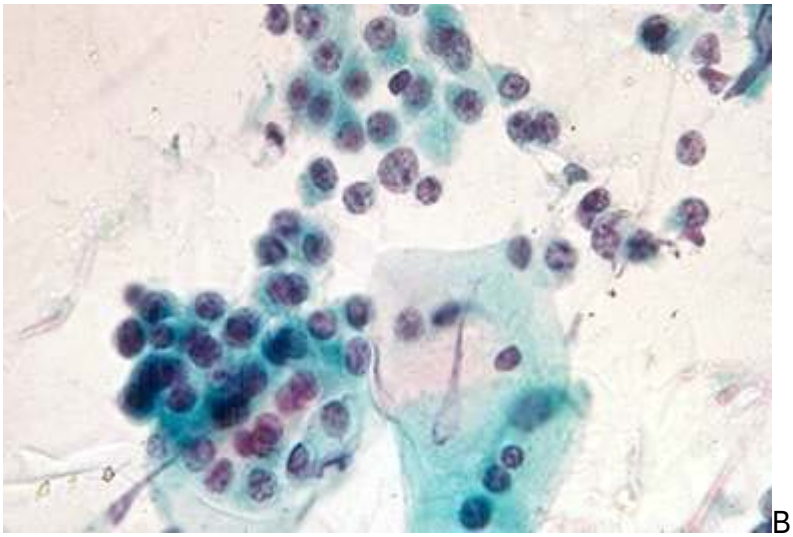

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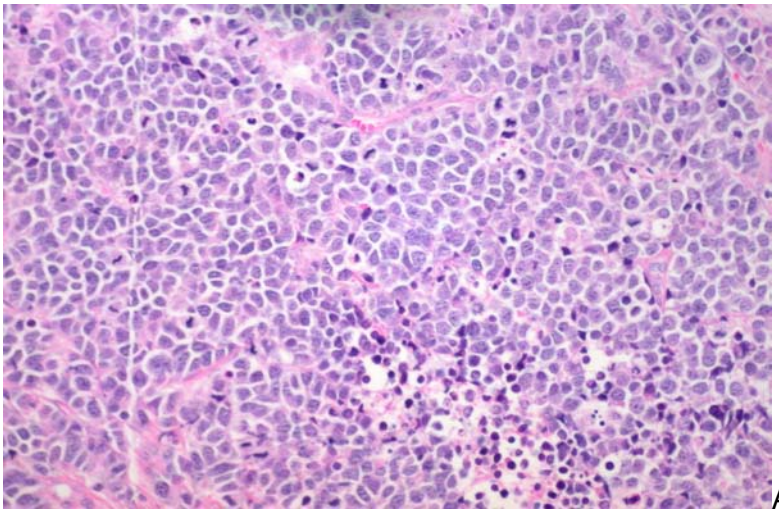
Fig. 6.1. Typical carcinoid tumor:
A. Histology of the tumor.
B. CP smear showing single and loosely clustered oval or polygonal tumor cells with eccentrically located nuclei.

2. Small-cell carcinoma (oat cell carcinoma) is an aggressive neoplasm, accounts for 2% to 5% of all cervical cancers and is strongly associated with HPV type 18. It is occasionally associated with Cushing syndrome or symptoms of other peptide hormones. It rarely coexists with SIL and its 5-year survival rate is 30% to 40%. It is histologically similar to small cell lung cancer and yields in CP smear small cancer cells with hyperchromatic nuclei with “salt and pepper” chromatin, nuclear molding and linear basophilic nuclear debris. (Fig. 6.2).



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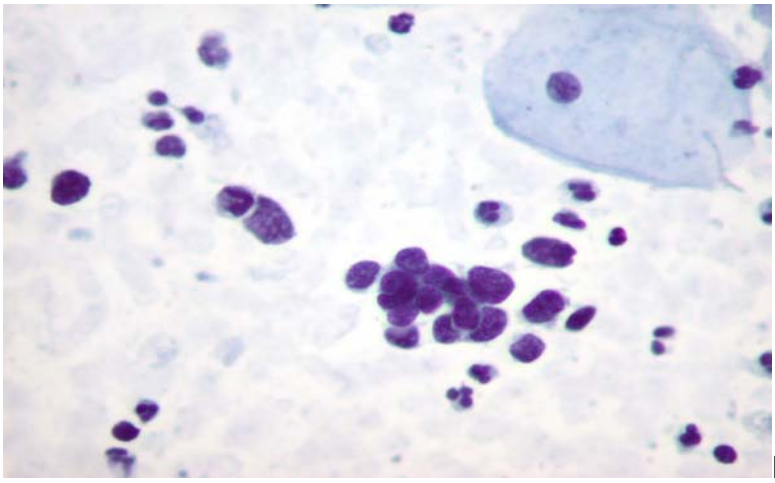

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Fig. 6.2. Small cell carcinoma:
A. Histology of the tumor.
B: CP smear showing single and clustered tumor cells showing scant cytoplasm, round hyperchomatic nuclei with molding.

B. MALIGNANT MIXED MÜLLERIAN TUMOR

MMMT is a very rare cervical cancer occurring in adult or elderly women (mean age, 50 to 65 years), often with a history of radiation therapy for cervical squamous cell carcinoma. Most MMMTs found in uterine cervix are actual endometrial MMMTs extending to the cervix. The tumors are classified as homologous and heterologous depending on the nature of its stromal neoplastic cells. The reader is referred to Chapter 7 for a more detailed discussion on MMMTs. These neoplasms commonly exfoliate malignant glandular cells in Pap smears, and stromal tumor cells are usually not observed. (Fig. 6.3).

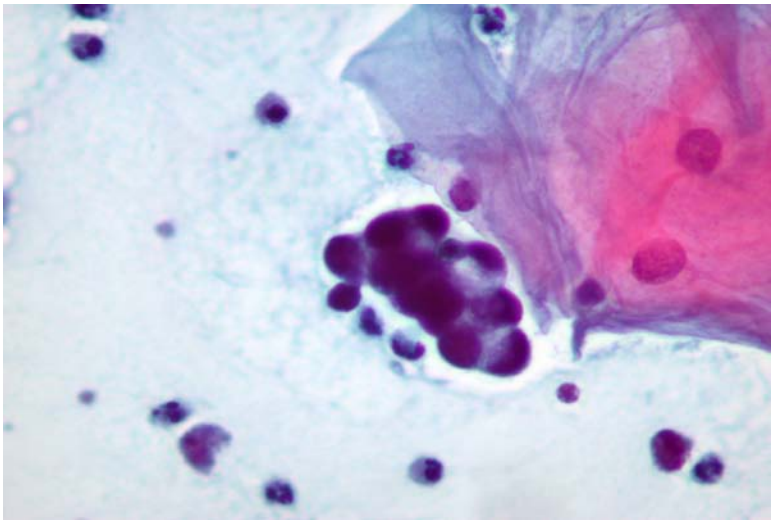


Fig. 6.3. Homologous MMMT showing in Pap smears a cohesive cluster of fairly monomorphic malignant glandular cells.

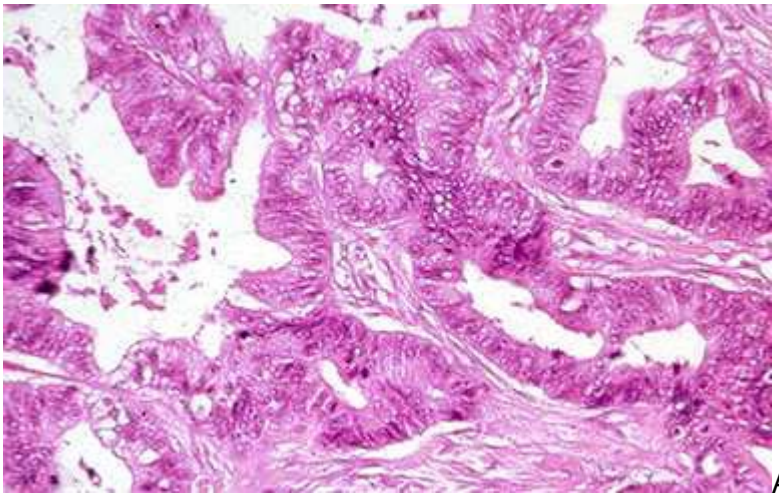
C. Stromal cell sarcoma of the cervix is a very rare tumor that tends to occur in postmenopausal women (mean age, 54 years). Histologically it is characterized by malignant, uniform cells or slightly pleomorphic cells with scant cytoplasm. It shows in Pap smear single and loosely clustered round or slightly pleomorphic malignant cells with scant cytoplasm, similar to the malignant stromal cells of a MMMT. The reader is referred to Chapter 7 for illustrations.

D. Other cervical nonepithelial cancers such as **rhabdomyosarcoma, leiomyosarcoma, non-Hodgkin lymphoma, adenosarcoma, alveolar soft part sarcoma, granulocytic sarcoma** are exceedingly rare tumors. And the cytologic manifestations of some of these tumors in CP smears have been reported.

METASTATIC CANCERS

Almost all malignant glandular neoplasms arising from extragenital organs can metastasize to the uterine cervix. Of these tumors, carcinomas of the breast, stomach and colon are the most common primaries while those arising from other anatomic sites such as the lung, pancreas, bladder, liver, kidney and gallbladder are rare. In one report consisting of 208 consecutive cases of primary and metastatic cervical adenocarcinomas, metastatic adenocarcinoma to the cervix (MAC) accounted for about 2 % of all cervical adenocarcinomas. Regardless of the primary site, nearly 90% of women with metastasis to the cervix have evidence of a disseminated cancer, and the most common symptom is vaginal bleeding, occurring in 75% of patients.

The cytologic manifestations of MAC in Pap smears are rather distinctive and different from those of a usual primary endocervical adenocarcinoma. A metastatic moderately differentiated colonic adenocarcinoma yields abundant necrotic debris and malignant glandular cells with elongated nuclei in syncytial clusters and in irregular sheets with tumor cells in palisade at free borders. (Fig. 6.4). Metastatic mammary duct carcinoma to the cervix yields malignant glandular cells in clusters and in Indian file arrangement. (Fig. 6.5). Metastatic carcinomas to the cervix are best confirmed by immunohistochemical studies of biopsied tissues: colonic adenocarcinoma cells are CK7 negative; and CK20, villin and CDX2 positive; breast cancer cells are ER, PR and gross cystic disease fluid protein fraction 15 positive; and endometrial carcinoma cells are ER and vimentin positive.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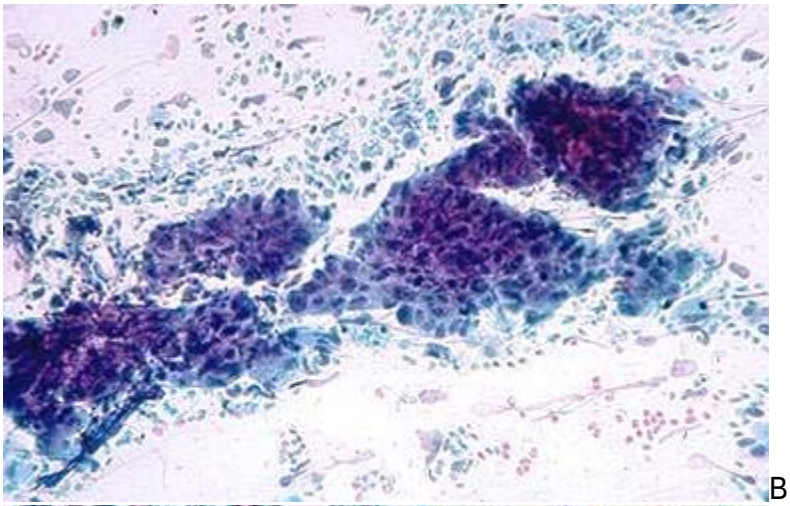
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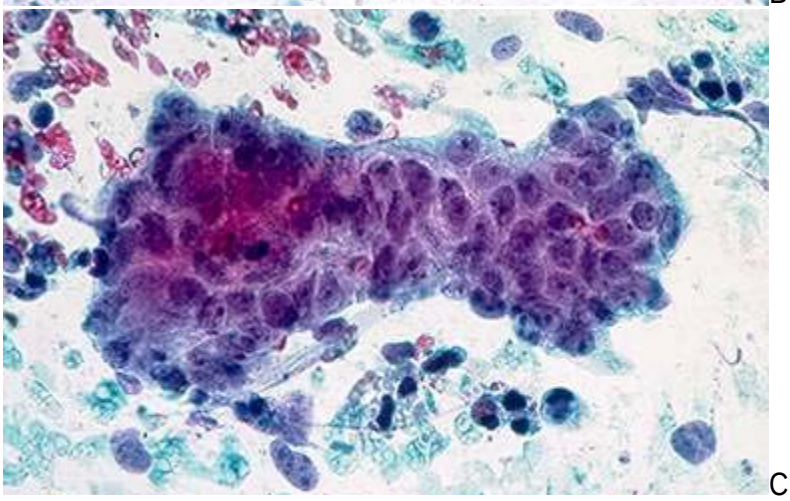
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Fig. 6.4. Metastatic well-differentiated colonic adenocarcinoma to the cervix:
A. Histology of the tumor.
B, C. CP smear showing irregular large sheets of tumor cells with cells at the periphery arranged in picket-fence. A large amount of necrotic debris is present.

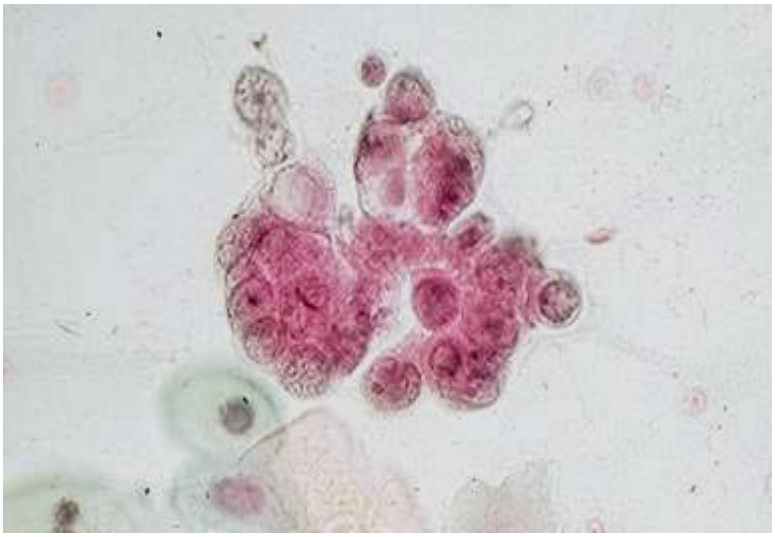
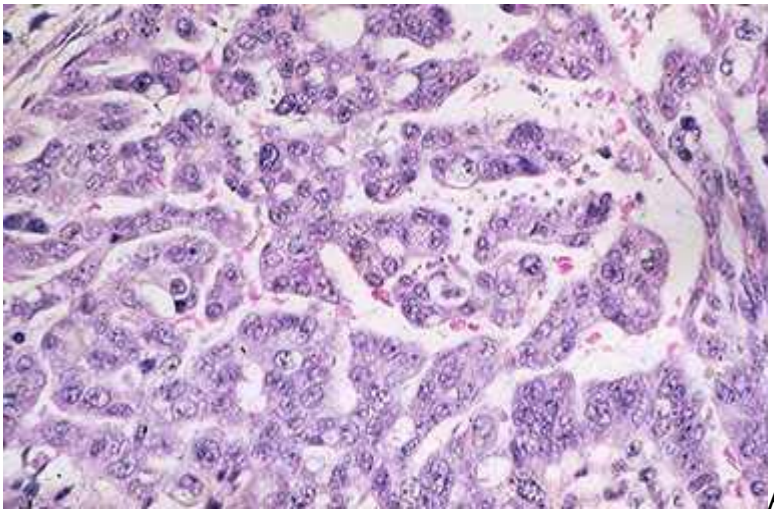


Fig. 6.5. Metastatic breast carcinoma showing in CP smear an irregular cluster of malignant glandular cells with conspicuous nucleoli.

EXTRAUTERINE CANCERS

Extrauterine cancers involving the abdominal peritoneum may traverse through the fallopian tubes and uterine cavity to accumulate in the posterior vaginal fornix. A vaginal smear in this case may reveal tumor cells in irregular, tight tri-dimensional clusters. The smear background is more commonly free of tumor diathesis. The presence of psammoma bodies should alert the observer to the possibility of a papillary serous ovarian carcinoma, and effort should be made to identify psammoma bodies surrounded by malignant epithelial cells to confirm the diagnosis. However, it should be born in mind that psammoma bodies may be seen in cervico-vaginal cell sample in patients without intra-abdominal cancer and in women with IUD. (Figs. 6.6 and 6.7).



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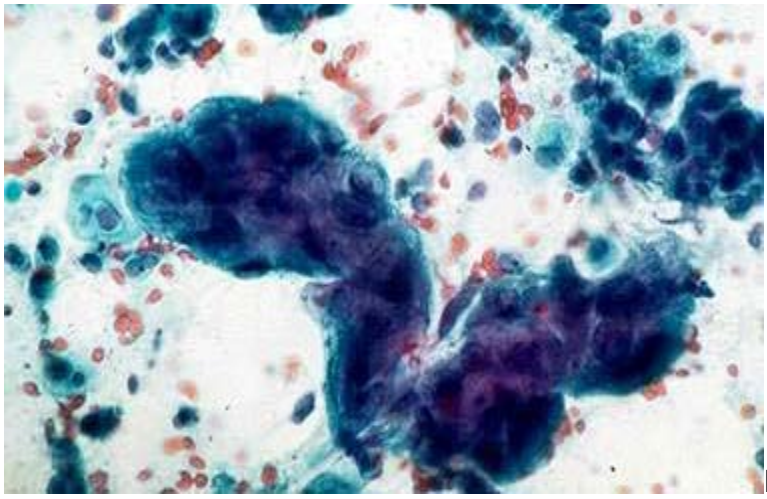
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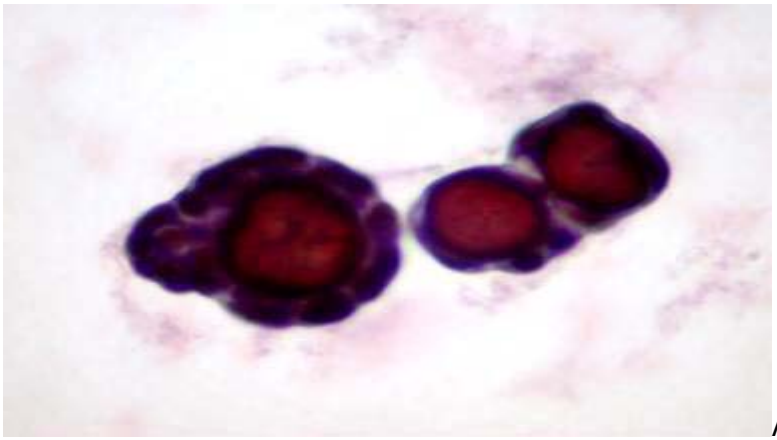
Fig. 6.6. Papillary serous ovarian carcinoma:
A. Histology of the tumor.
B. CP smear showing thick tridimensional papillary clusters of malignant glandular cells in a "clean" smear background.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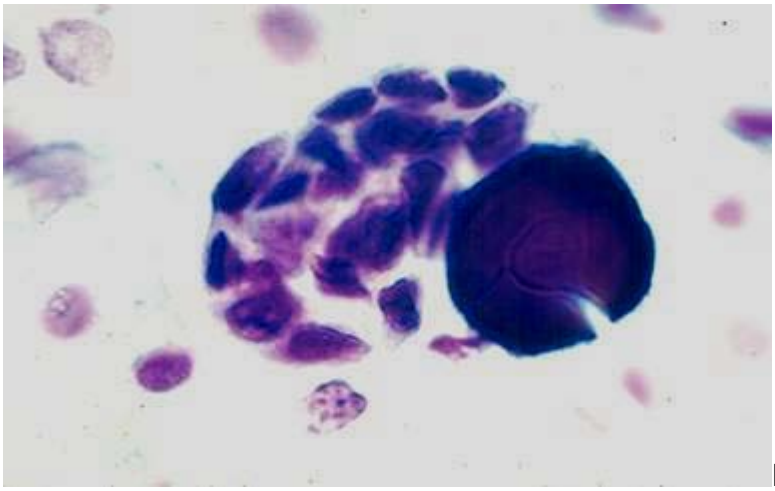
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Fig. 6.7. Ovarian carcinoma:
A. Borderline ovarian carcinoma showing in CP smear 3 psammoma bodies surrounded by

low-grade epithelial tumor cells in a clean background.

B. High-grade ovarian serous carcinoma showing in CP smear fairly pleomorphic malignant cells partially surrounding a psammoma body.

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Chapter 7

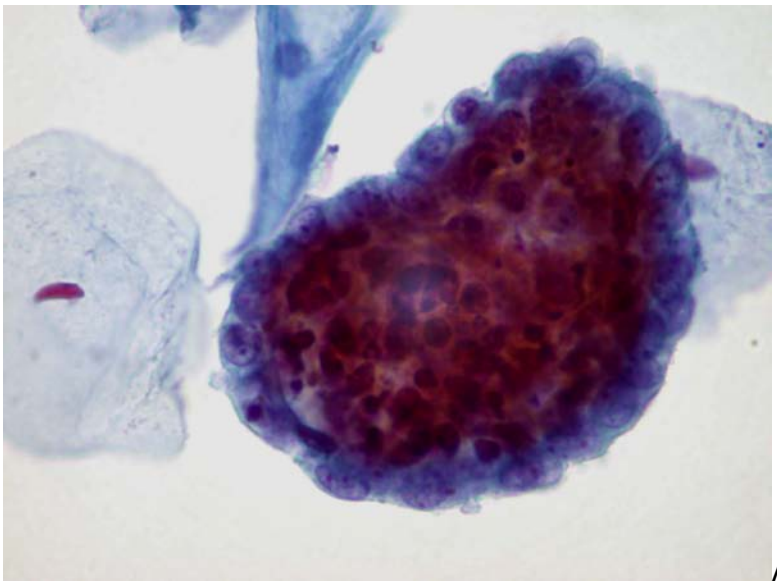
Endometrial Lesions

The endometrium is an important target for hormonal stimulation and may be affected by a wide variety of disease processes. The resulting lesions are often manifested clinically by abnormal uterine bleeding, and exfoliate endometrial cells which, after a variable period of retention within the uterine cavity, pass through the cervical canal and tend to accumulate in the posterior vaginal fornix. They often show a variable degree of degenerative change rendering their morphologic evaluation difficult. However, when these cells are well–preserved a correct cytodiagnosis may be made. In the past fifty years efforts have been made to design a simple–to–use device for use in the physician's office to obtain cells directly from the endometrium for cytologic examination. Those devices included cannula for washing of uterine cavity, Mi-Mak plastic helix, Endopap sampler and Endocyte sampler. Of these, Endocyte samplers have been the most popular ones. They are simple to use and cause little or no discomfort to patients, and the cell samples obtained have a low rate of cellular inadequacy. A recent study with direct samplers has demonstrated that if the endometrial cytology is properly used, it would be cost–effective by substantially reducing the number of unnecessary endometrial curettages. However, to date, there is no known cytology screening program for endometrial cancer using direct endometrial samplers.

BENIGN-APPEARING ENDOMETRIAL CELLS IN WOMEN OVER 40 YEARS OF AGE

The presence of spontaneously exfoliated benign-appearing endometrial cells in Pap smears of women over 40 years of age is not regarded as an epithelial abnormality in The Bethesda System-2001. These cells are seen in small tridimensional, round clusters and they are present in less than 1% of all Pap smears. (Figs.7.1 and 7.2). In the majority of cases, these benign endometrial cells are from a normal and cycling endometrium, and in other cases their exfoliation is secondary to a benign endometrial polyp, an IUD and a hormonal replacement therapy. Only in about 1% of these women an endometrial hyperplasia or carcinoma is found. Therefore, the presence of spontaneously exfoliated benign-appearing endometrial cells in an asymptomatic woman does not constitute an indication for endometrial biopsy for histologic evaluation.

Lower uterine segment (LUS) endometrial fragments scraped by a cervical cytology sampler do not belong to endometrial cells in this category. Clusters of atrophic endocervical cells, naked squamous cell nuclei or clustered histiocytes may be mistaken for benign endometrial cells.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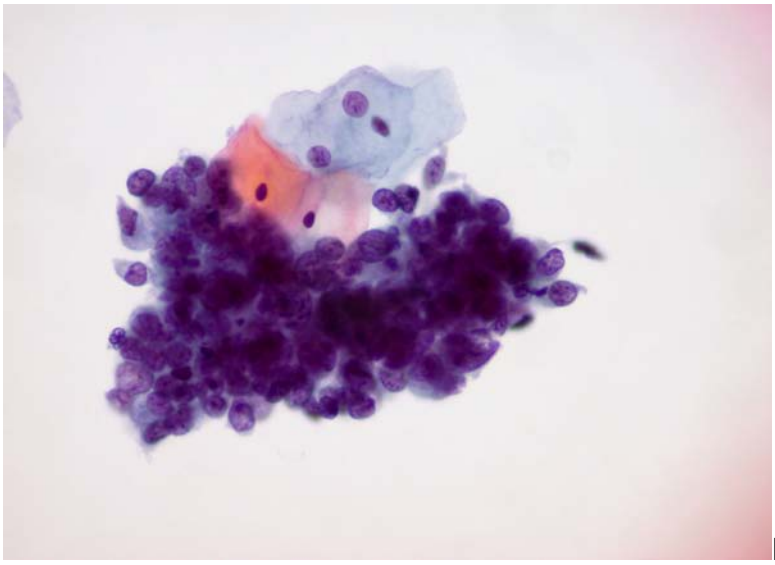
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Fig. 7.1. Benign endometrial cells in LBP from a woman over 40 years of age with cycling endometrium.

A. A tridimensional, ball-like cluster of endometrial stromal cells wrapped by endometrial glandular cells.

B. A tridimensional cluster of endometrial glandular cells with scant cytoplasm.

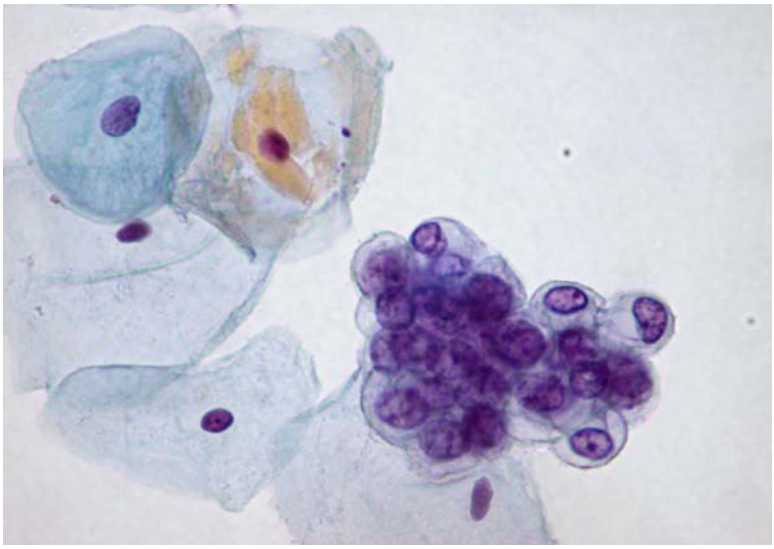
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Fig. 7.2. LBP showing clustered histiocytes mimicking endometrial cells. Note the bean-shaped nuclei and more defined and more abundant cytoplasm.

ABNORMAL SHEDDING OF NORMAL-APPEARING ENDOMETRIAL CELLS

Depending on a woman's menstrual status, abnormal shedding of normal-appearing endometrial cells may have different endometrial pathologies, according to several studies. Normal appearing endometrial epithelial cells have scant cytoplasm and a small bland, round or oval nucleus that is of the same size as the nucleus of a normal intermediate squamous cell. These cells usually occur in small groups or clusters and have no conspicuous nucleoli. (Fig. 7.3).

In a premenopausal woman, shedding of normal-appearing endometrial cells, epithelial and/or stromal types, beyond day 10 to 12 of the menstrual cycle is an abnormal finding that should be interpreted with caution in the light of clinical information. It can be secondary to an IUD, endometritis, anovulatory cycle, prior endometrial curettage or uterine endoscopy, endometrial polyp, hormonal therapy, submucosal myometrial leiomyoma, endometrial hyperplasia and rarely endometrial cancer.

In a postmenopausal woman, hormonal therapy, endometrial polyp, endometrial hyperplasia and endometrial cancer are the main causes of abnormal shedding of normal-appearing endometrial cells. Of these etiologies, hormonal replacement therapy is the most common one; a benign endometrial polyp is found in 23% of patients, endometrial hyperplasia and endometrial carcinoma are found in 5% and 5% of cases, respectively. In another series, about 6% of postmenopausal women with endometrial carcinoma shed only normal-appearing endometrial cells. Thus, the presence of unexplained normal-appearing endometrial cells in Pap smears in an asymptomatic woman needs further investigation to

rule out a significant endometrial pathology, especially when clinical risk factors for endometrial carcinoma are present (hypertension, obesity, nulliparity and hormonal replacement therapy). It should be born in mind that a large percentage of patients with abnormal shedding of normal-appearing endometrial cells show no pathology in biopsied endometrial tissues.

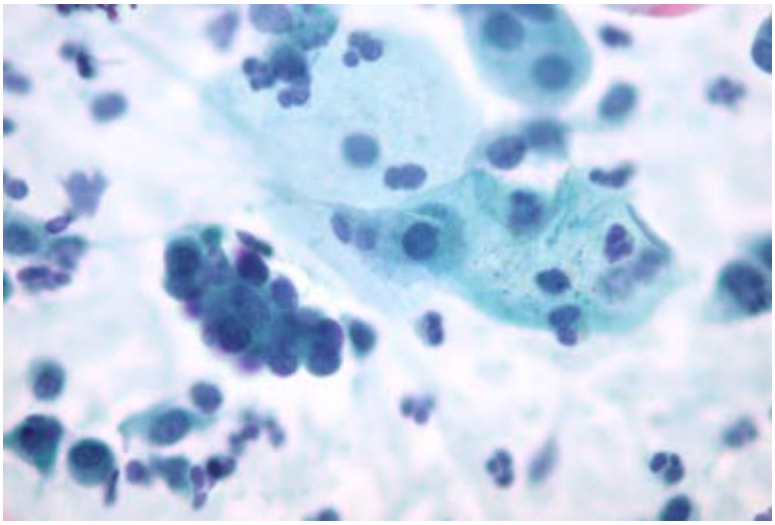


Fig. 7.3. A group of normal-appearing endometrial cells in a CP smear. The endometrial cell nuclei are of the same size with those of intermediate squamous cells.

IUD-INDUCED CELLULAR CHANGES

Women bearing IUDs usually show cellular atypias affecting endometrial glandular cells and cervical metaplastic squamous cells. These individuals may shed endometrial cells at any time of the menstrual cycle. By mechanical effects, reactive and regenerative endometrial cells are formed. These cells occur in groups or clusters and show cytoplasmic enlargement with intracytoplasmic vacuoles, conspicuous or prominent nucleoli thus mimicking malignant glandular cells. The cervical metaplastic squamous cells may show prominent nucleoli. Single endometrial cells with high N/C ratios and hyperchromatic nuclei with irregular nuclear membrane or contours mimicking HSIL/CIN 3 cells may be observed. (Fig. 7.4). It is important to note that these CIN 3-like cells are few in number and do not form hyperchromatic crowed groups as seen in cervical with HSIL/CIN 3, and there is an absence of other squamous cells with less dyskaryotic change, as usually observed in SIL cases. The differential diagnosis is more challenging when these CIN 3-like cells are present in abundance. (Fig. 7.5).

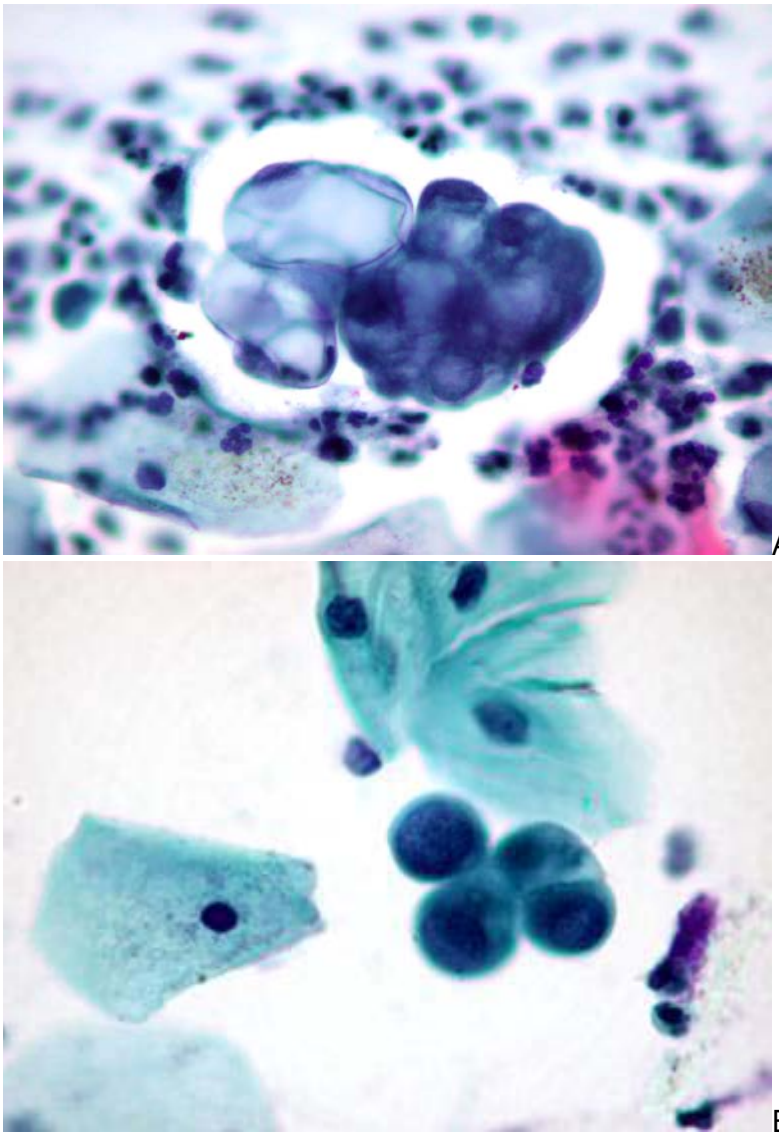
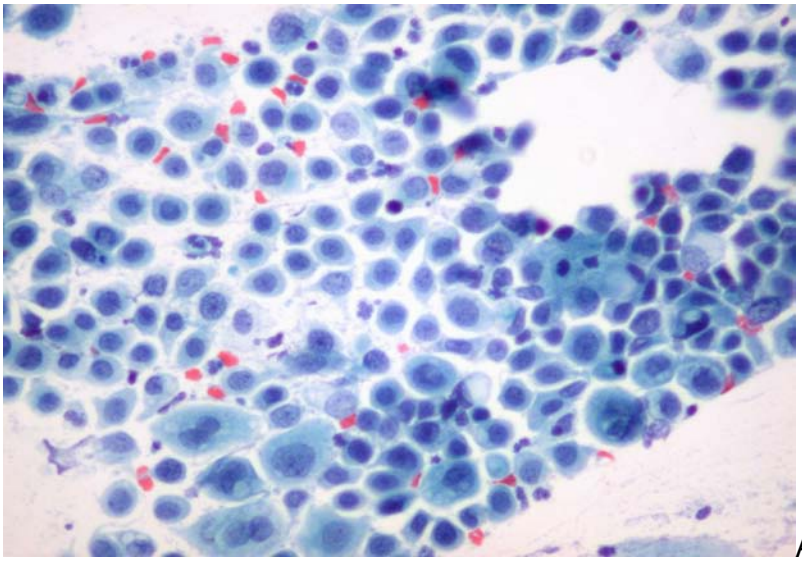


Fig. 7.4. CP smear showing IUD-induced cellular changes.
A. A cluster of glandular cells with vacuolated cytoplasm.
B. Four small HSIL-like cells.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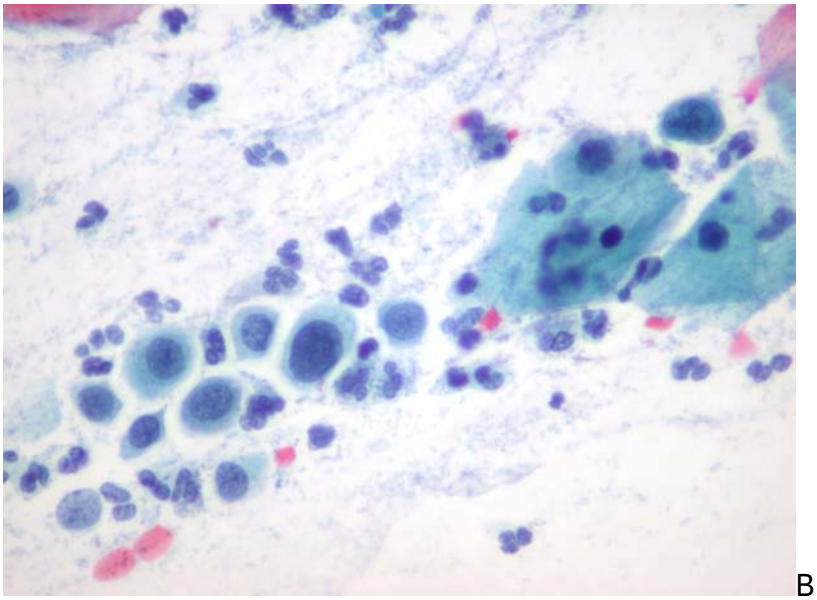

B

Fig. 7.5. A, B. CP smear from a 25-year-old woman with IUD showing a large number of epithelial cells with IUD-induced changes, mimicking HSIL cells. Small cytoplasmic vacuoles are seen in a few cells. Colposcopy, endocervical curettage and long-term follow-up revealed no cervical SIL or any significant endometrial lesion.

ENDOMETRIAL HYPERPLASIA

Endometrial hyperplasia is the result of an increased or uninterrupted estrogenic stimulation and it is an important factor in endometrial carcinogenesis. However, not all cases of endometrial cancer are estrogen-related, as the tumor can also arise from an atrophic endometrium.

Endometrial hyperplasias are classified into 3 main types: simple hyperplasia (cystic glandular hyperplasia), complex hyperplasia without atypia (adenomatous hyperplasia) and complex hyperplasia with atypia (atypical hyperplasia showing remarkable nuclear abnormality). About 40% of patients with atypical endometrial hyperplasia will eventually develop endometrial adenocarcinoma.

1. Endometrial hyperplasia without atypia yields in CP smears clustered normal-appearing endometrial cells in a well-estrogenized smear background.

Simple and complex endometrial hyperplasias without atypia display in direct endometrial samples similar cellular manifestations. Large and irregularly dilated branching endometrial glandular fragments and cell clusters or clumps with no remarkable nuclear atypia are seen. Nuclei in palisade, nuclear crowding and overlapping and inconspicuous nucleoli may be observed. (Fig.7.6).

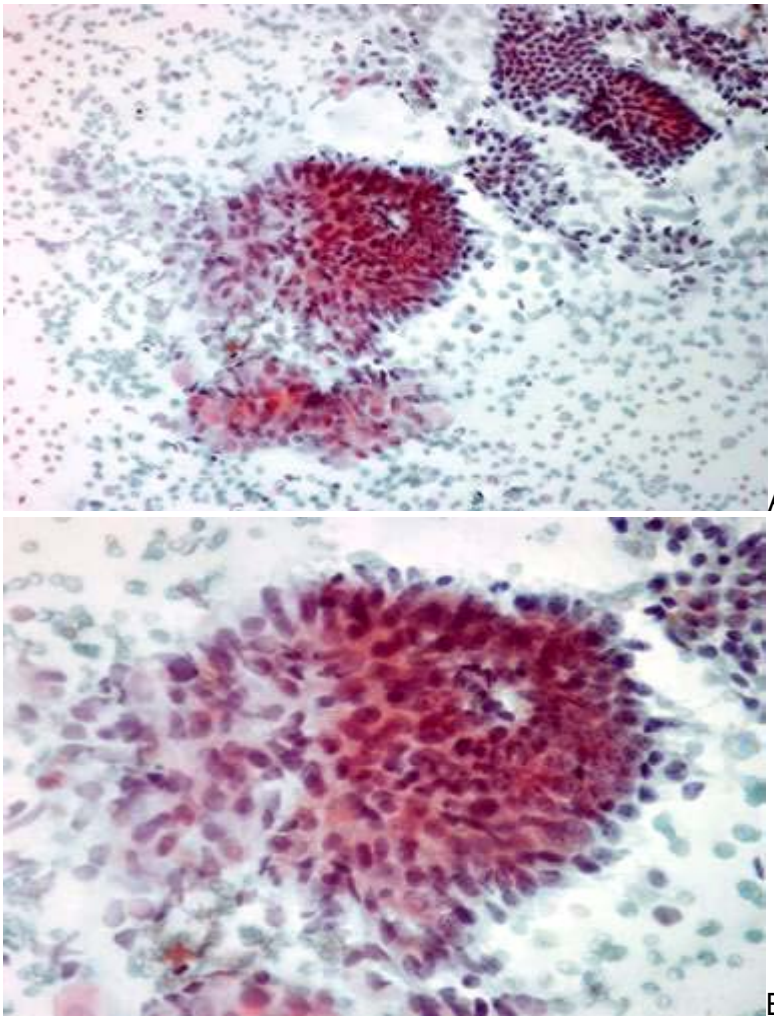


Fig. 7.6. Endometrial adenomatous/complex hyperplasia without atypia showing in direct endometrial sample an endometrial fragment displaying monomorphic oval or elongated, pseudostratified nuclei.

2. Endometrial hyperplasia with atypia is characterized by clustered atypical endometrial cells with enlarged nuclei, irregular nuclear contours, chromatin clumping, parachromatin clearing and nucleoli. However, all of the above-mentioned cellular changes are not present in a given case. The reader is referred to Chapter 5 for illustration of atypical endometrial glandular cells.

Endometrial hyperplasia with atypia yields in direct samples irregularly dilated or branched endometrial glands and clusters or clumps of endometrial cells. The cell clumps display a loss of nuclear polarity, nuclear pleomorphism, hyperchromasia, chromatin clumping and clearing and prominent nucleoli. (Fig. 7.7). These cell clusters or clumps are indistinguishable from those of a well-differentiated or grade 1 endometrial carcinoma. Large clusters of benign and slightly pleomorphic stromal cells are also commonly present.

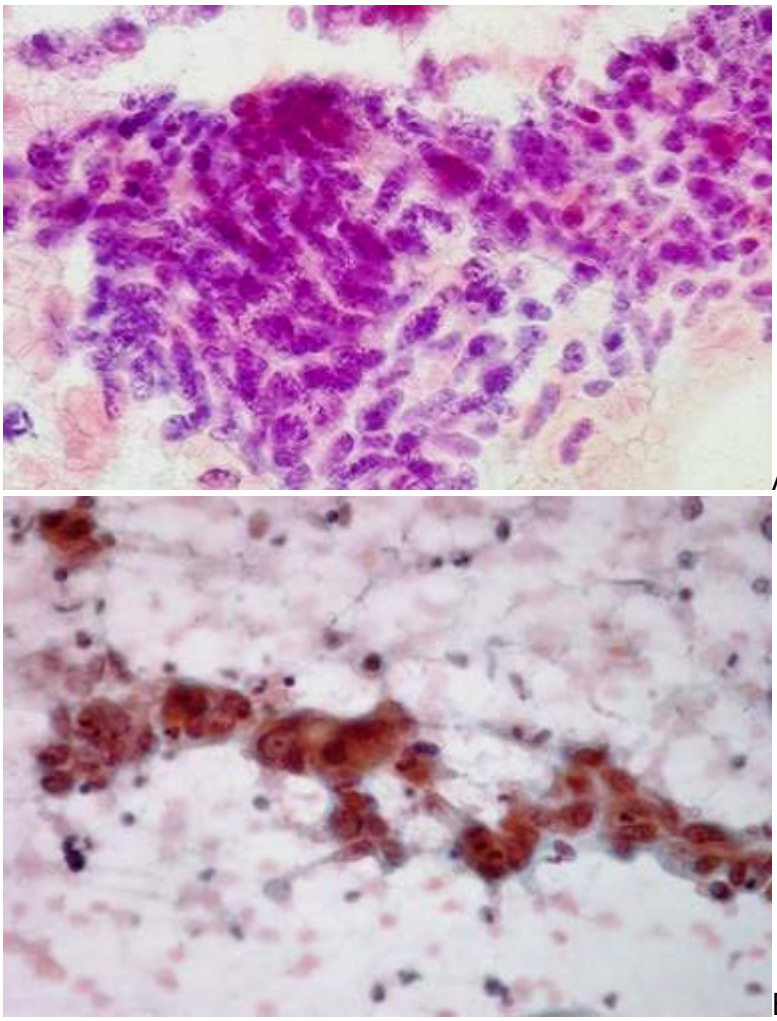


Fig. 7.7. Endometrial hyperplasia with atypia showing in direct sampling:
A. A large cluster of epithelial cells with loss of nuclear polarity, slight nuclear pleomorphism, focal nuclear crowding and conspicuous nucleoli.
B. Small clusters of atypical glandular cells with pleomorphic nuclei and conspicuous nucleoli.

ENDOMETRIAL ADENOCARCINOMA

Endometrial adenocarcinoma is the most common malignancy of the female genital tract in North America. It occurs mainly in postmenopausal women in their 6th and 7th decades of life, with an average age of 60 years at the time of diagnosis. About 75% of the cases are seen in patients over 50 years in age. Patients younger than 40 years constitute about 5% of all cases. The tumor may arise from a hyperplastic endometrium (Type I tumor) or from a normal or atrophic endometrium showing carcinoma in situ (Type II tumor). Type I tumors are common (80%), and low-grade tumors have a favorable prognosis and tend to occur in obese, younger and perimenopausal women. Type II tumors tend to arise in thin, older, multiparous and postmenopausal women. They show a high histologic grade and have an aggressive behavior and poor prognosis.

Epidemiological studies have identified a number of risk factors in patients with type I endometrial adenocarcinoma. The most important ones are atypical endometrial hyperplasia, obesity, unopposed estrogen effects, nulliparity and diabetes mellitus. Type II tumors usually lack those classic risk factors.

In recent years efforts have been made to screen asymptomatic endometrial carcinoma cytologically by direct endometrial sampling. In one large study consisting of 2,586 asymptomatic perimenopausal and postmenopausal patients with high–risk factors revealed a prevalence of endometrial carcinoma of 6.9/1000. In another study of 747 asymptomatic women over 45 years of age, a prevalence of endometrial cancer of 4/1000 was found. However, there are no controlled data to justify a mass screening of endometrial carcinoma in low-risk patients.

The most common clinical manifestation of endometrial carcinoma is an abnormal uterine bleeding. However, about 10% of the patients with early disease present with leukorrhea only.

From the cancer screening point of view, routine Pap smears are not efficient in detecting endometrial carcinomas as the tests fail to detect cancer cells in about 50% of cases. Depending on the degree of differentiation of endometrial adenocarcinomas, their cytologic manifestations in Pap smears and in direct endometrial samples are similar.

A well–differentiated tumor shows irregular clusters and groups of malignant epithelial cells displaying nuclear hyperchromasia, nuclear crowding and overlap, small or conspicuous nucleoli, as seen in an atypical endometrial hyperplasia. (Fig. 7.8).

A poorly differentiated neoplasm yields pleomorphic malignant glandular cells singly and in clusters that are readily identifiable. (Figs. 7.9 and 7.10). The smear background always contains a large amount of necrotic debris. Fragments of malignant glandular epithelium

are evident in endometrial aspiration. (Fig.7.11). Cytologic features of endometrial adenocarcinomas in Pap smears include:

- Tumor cells present singly and in small, tight clusters.
- Scant, basophilic and often vacuolated cytoplasm.
- Variation in nuclear size and loss of nuclear polarity.
- Nuclei with moderate hyperchromasia and irregular chromatin distribution.
- Prominent nucleoli with parachromatin clearing.
- Increased nuclear and nucleolar sizes are observed with higher tumor grade.
- Tumor diathesis variably present.

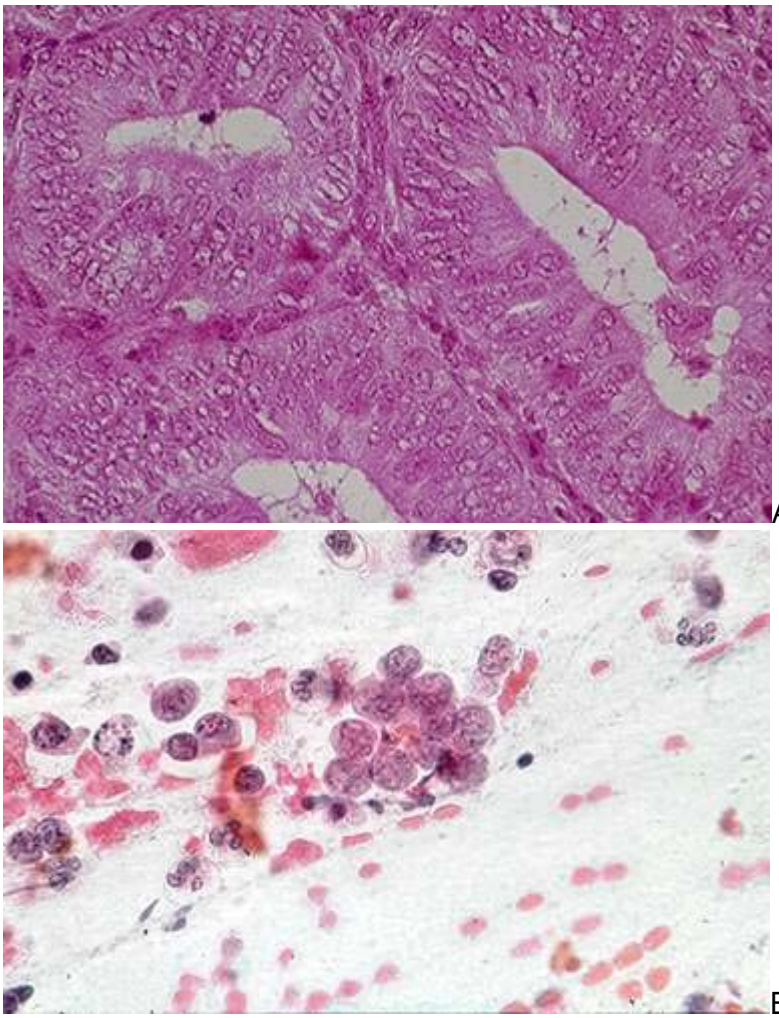
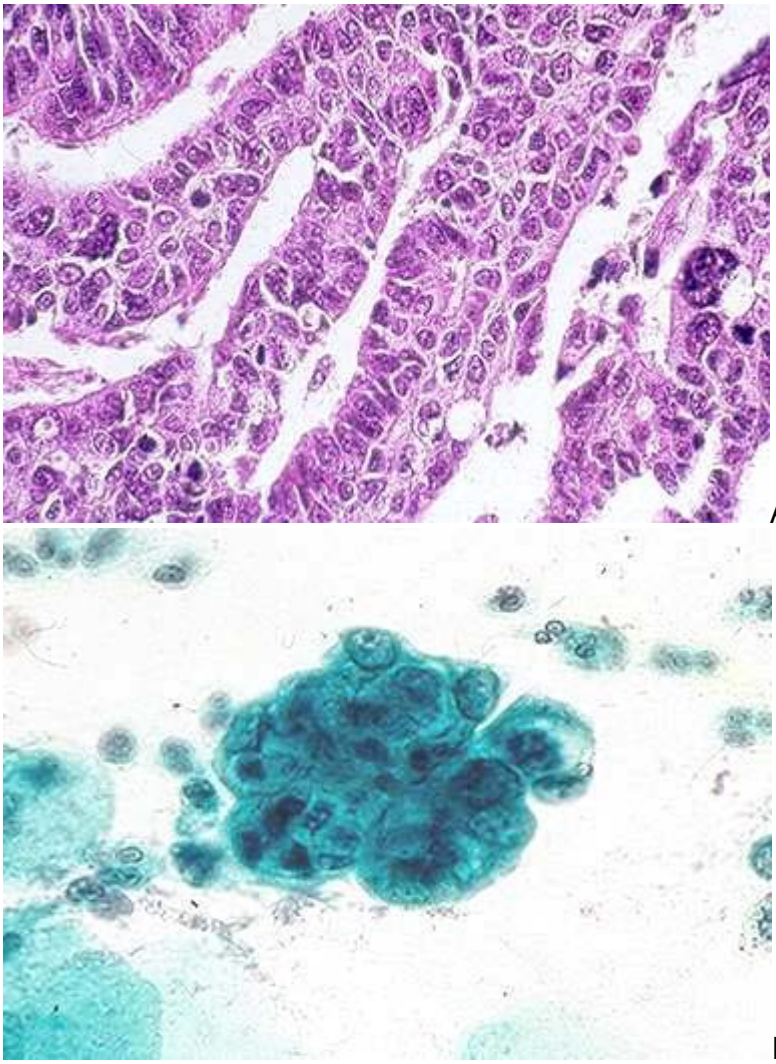

A
B

Fig. 7. 8. Low-grade endometrial adenocarcinoma:
A. Histology of the tumor.
B. CP smear showing clustered monomorphic tumor cells with enlarged, hyperchromatic nuclei and small nucleoli.



A

B

Fig. 7.9. Poorly differentiated endometrial adenocarcinoma.
A. Histology of the tumor.
B. CP smear showing a large, cohesive cluster of tumor cells with pleomorphic nuclei, irregular chromatin clumping, parachromatin clearing and prominent nucleoli.

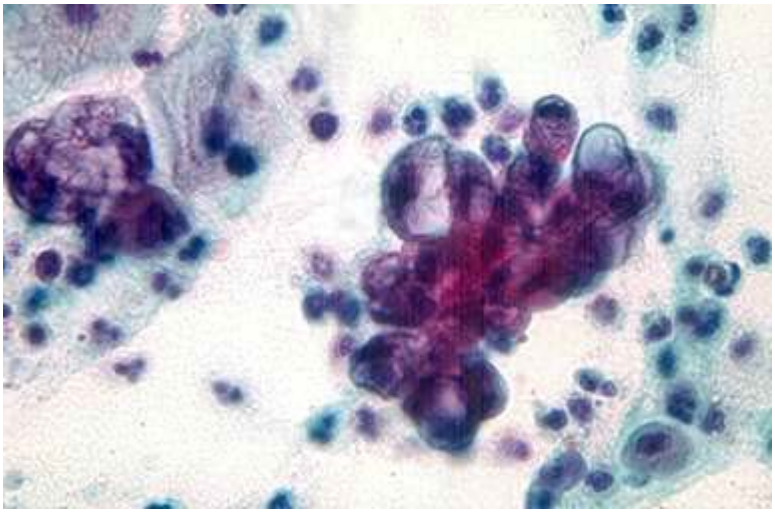


Fig. 7.10. Pleomorphic tumor cells with vacuolated cytoplasm in the CP smear of a patient with poorly differentiated endometrial adenocarcinoma.

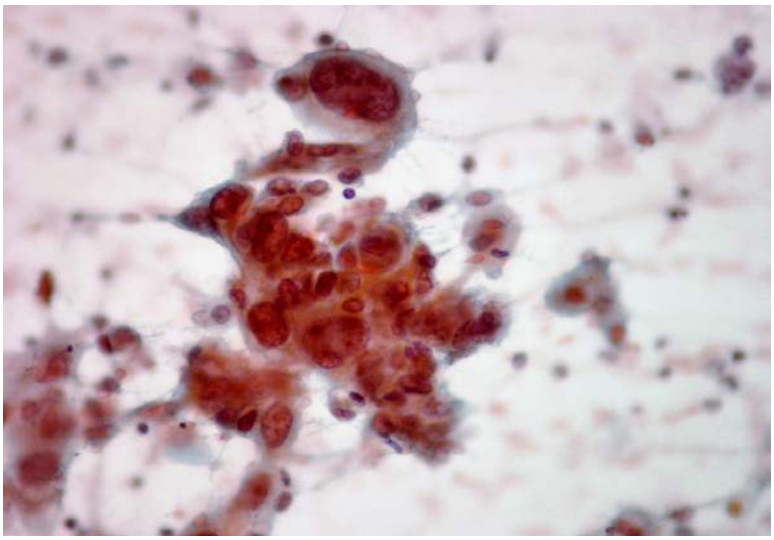
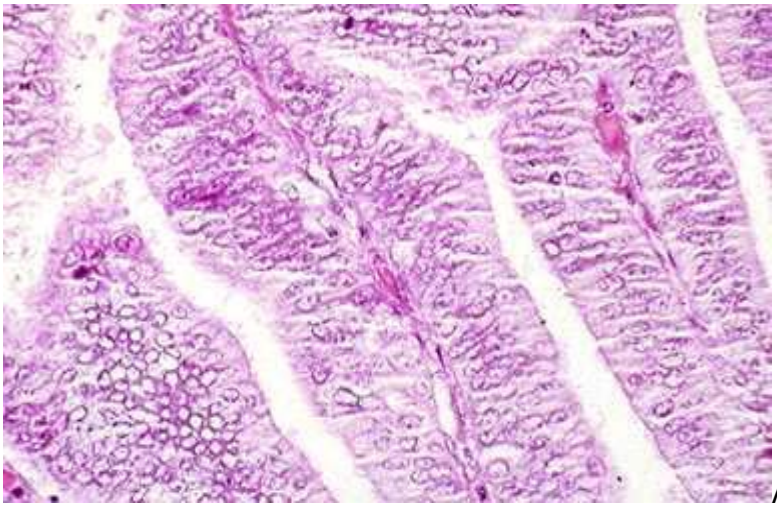


Fig. 7.11. Poorly differentiated endometrial adenocarcinoma showing in direct endometrial sample pleomorphic malignant epithelial cells with marked nuclear pleomorphism and prominent nucleoli.

OTHER ENDOMETRIAL CANCERS

1. Endometrial papillary serous carcinoma is a rare neoplasm. It yields in direct endometrial sampling large monolayered sheets of tumor cells with focal nuclear crowding and overlapping, and conspicuous nucleoli are present. (Figs.7.12 and 7.13).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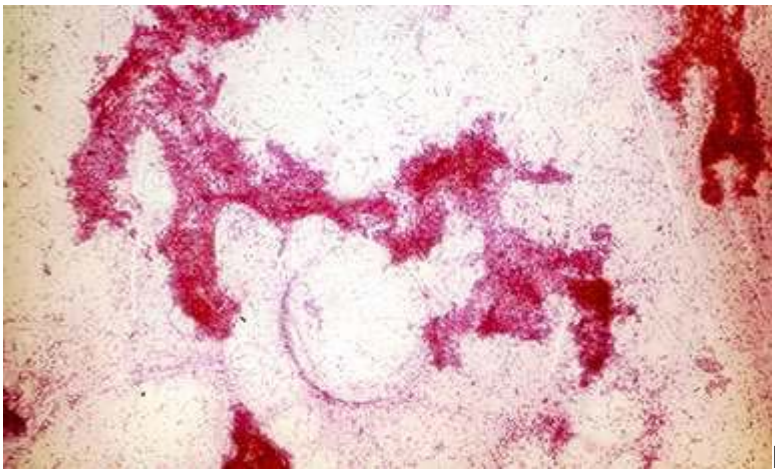
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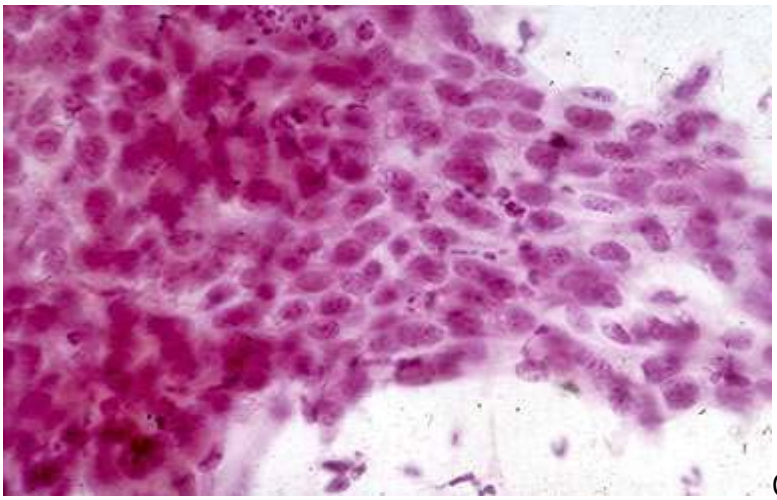
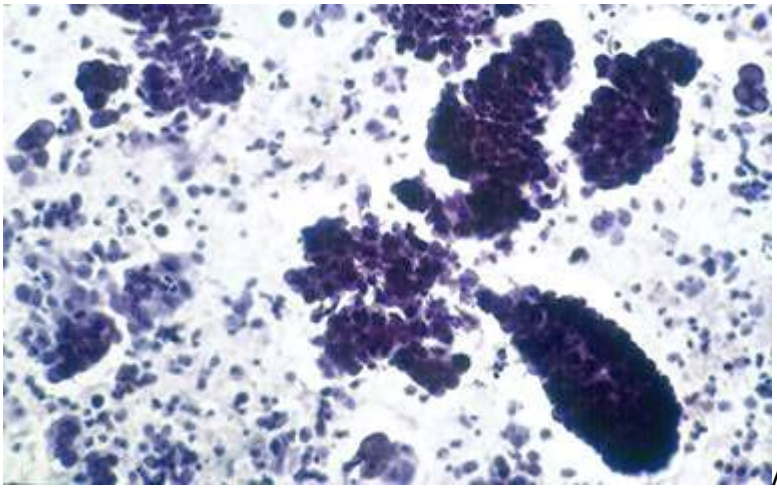

C

Fig. 7.12. Papillary serous carcinoma:
A. Histology of the tumor.
B. Direct endometrial sample showing irregular large, thick sheets of tumor cells and
C. A sheet of tumor cell displaying focal nuclear crowding and overlapping and inconspicuous nucleoli.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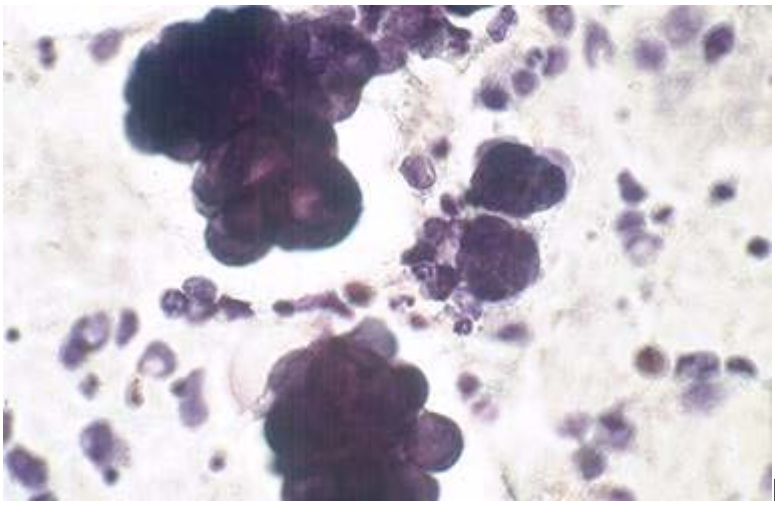
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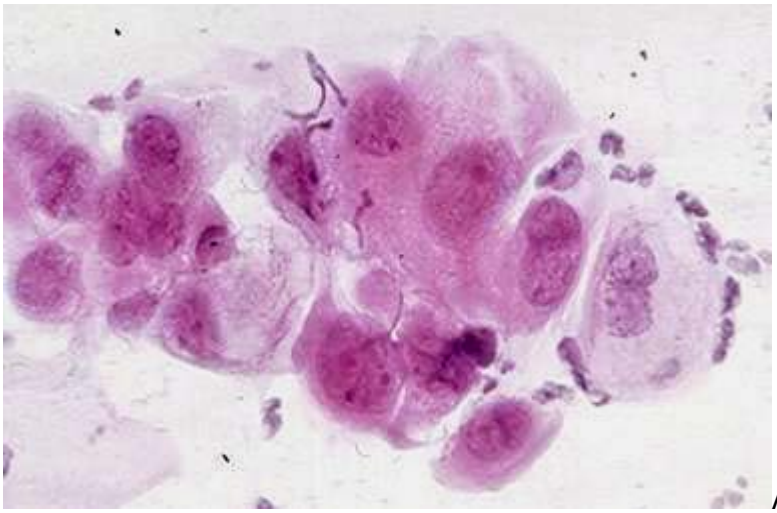
Fig. 7.13. A, B. In another case of papillary serous endometrial carcinoma the CP smear shows abundant 3-dimensional papillary clusters of tumor cells.

2. Squamous cell carcinoma of the endometrium is rare and consists of nonkeratinizing and keratinizing types. These two tumor types yield in direct samples single and clustered malignant squamous cells with keratinizing and nonkeratinizing malignant squamous cells, respectively. (Figs. 7.14 and 7.15).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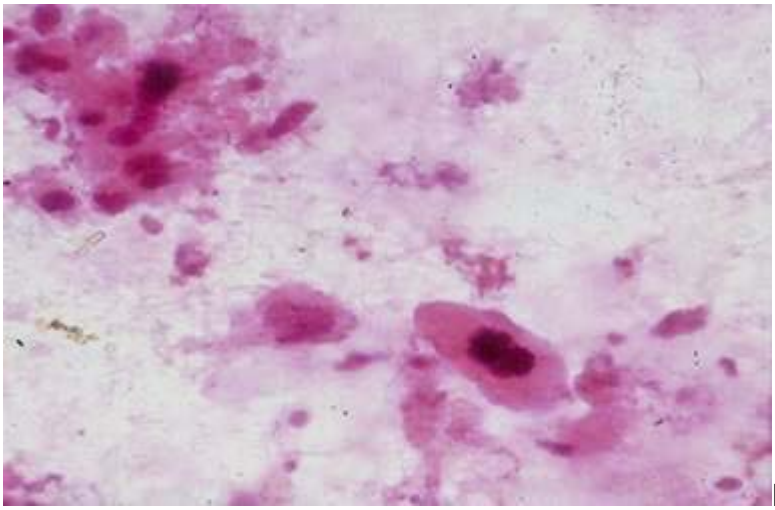
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Fig. 7.14. Keratinizing squamous cell carcinoma showing:
A and B. Clustered and single malignant squamous cells with eosinophilic and keratinizing cytoplasm in a direct endometrial sample.

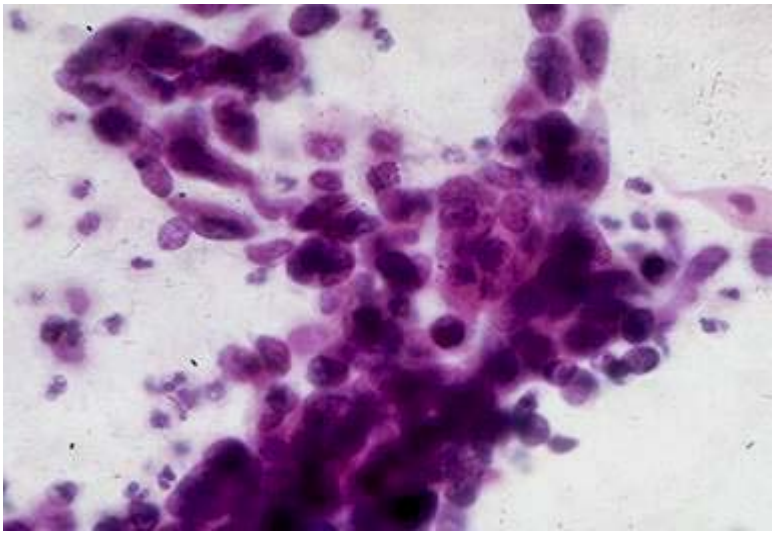


Fig. 7.15. Nonkeratinizing squamous cell carcinoma showing in a direct endometrial sample a cohesive cluster of cancer cells with scant, slightly eosinophilic and defined cytoplasm and hyperchomatic, vesicular nuclei.

3. Small cell carcinoma is a very rare endometrial cancer. It shows single and clustered pleomorphic small cancer cells with scant cytoplasm, hyperchromatic nuclei without nucleoli and nuclear molding. (Fig. 7.16).

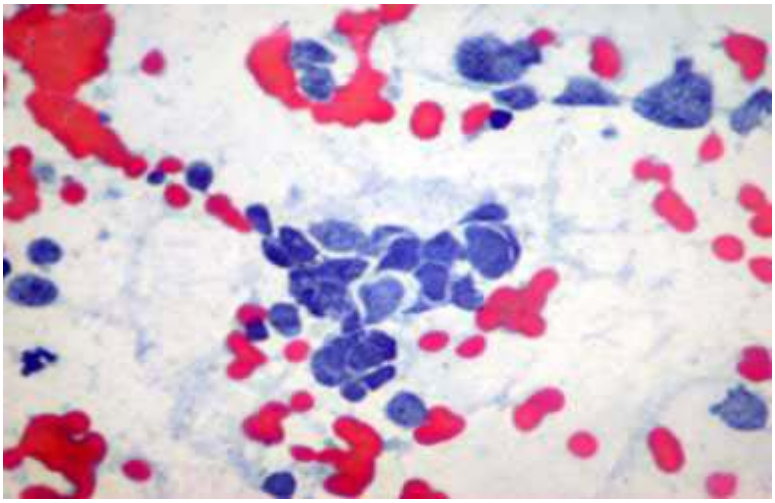
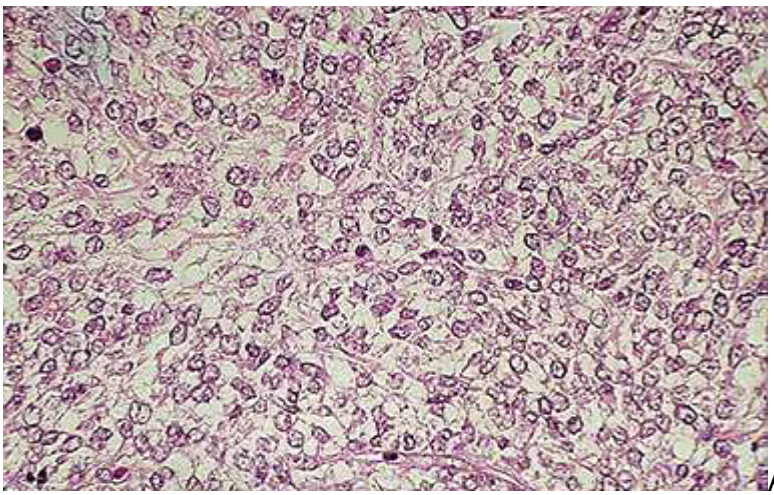


Fig. 7.16. Small cell carcinoma showing in direct endometrial sample tumor cells with nuclear molding.

4. Endometrial stromal sarcoma is a rare neoplasm. It occurs mainly in postmenopausal women. It may be classified as low– or high–grade, depending on the degree of cellular atypia and the number of mitotic figures. The cytologic manifestations of the tumor in vaginal pool smears and direct endometrial samples are similar and consist of numerous single or loosely clustered malignant round cells with oval or pleomorphic, hyperchromatic nuclei and scant, ill–defined cytoplasm. (Fig. 7.17).



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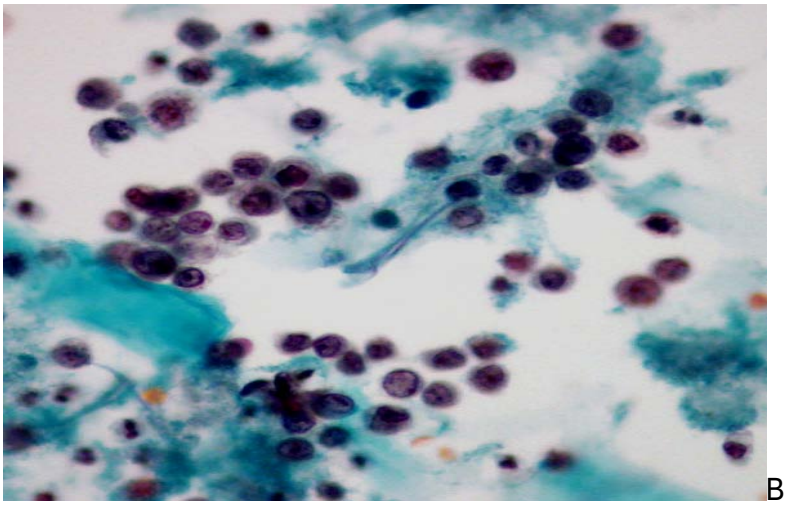
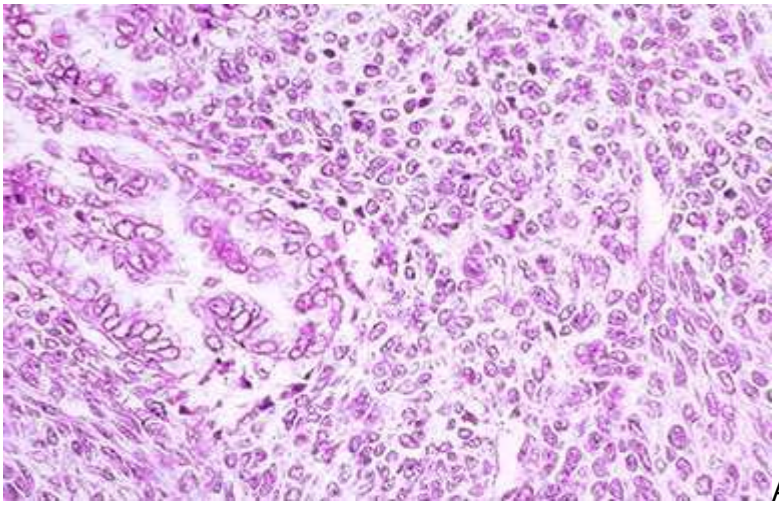

B

Fig. 7.17. Low-grade endometrial stromal sarcoma:
A. Tumor histology.
B. Single and loosely clustered round tumor cells in a direct endometrial sample.

5. Malignant mixed müllerian tumor (MMMT). This rare tumor occurs predominantly in postmenopausal women in the 6th or 7th decade of life, with up to 30% of patients having a history of exposure to radiation. Histologically, the tumor is classified as homologous or heterologous. A homologous tumor may yield in direct endometrial samples malignant epithelial and stromal cells and other malignant mesenchymal cells derived from the cells that are normally present in the uterus. (Fig. 7.18). A heterologous tumor may show in direct endometrial samples malignant epithelial and stromal cells and other malignant mesenchymal cells derived from cells that are not normally found in the uterus such as bone, cartilaginous, fat and striated muscle cells. It is important to note that in Pap smears only malignant glandular cells are identified in the majority of cases of endometrial MMMT.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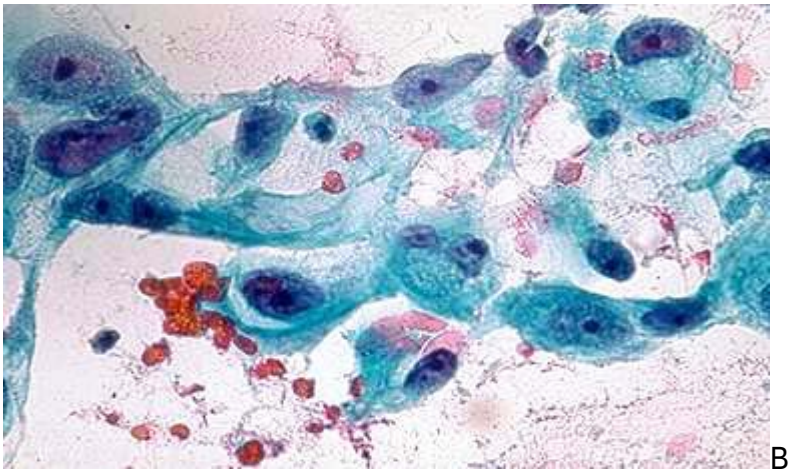
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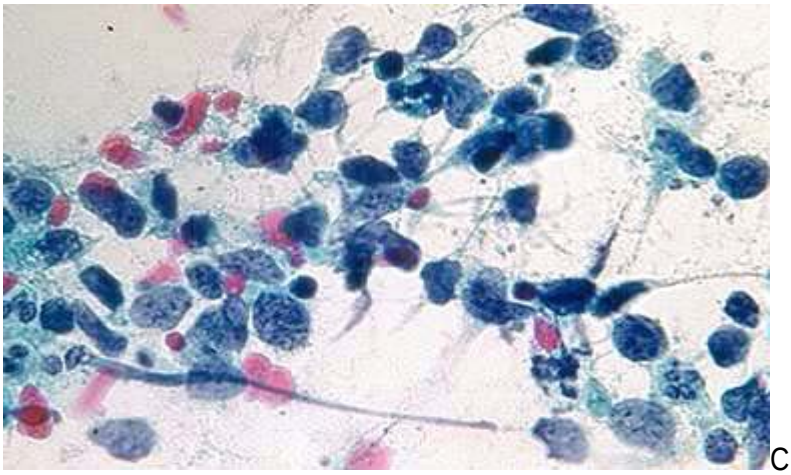
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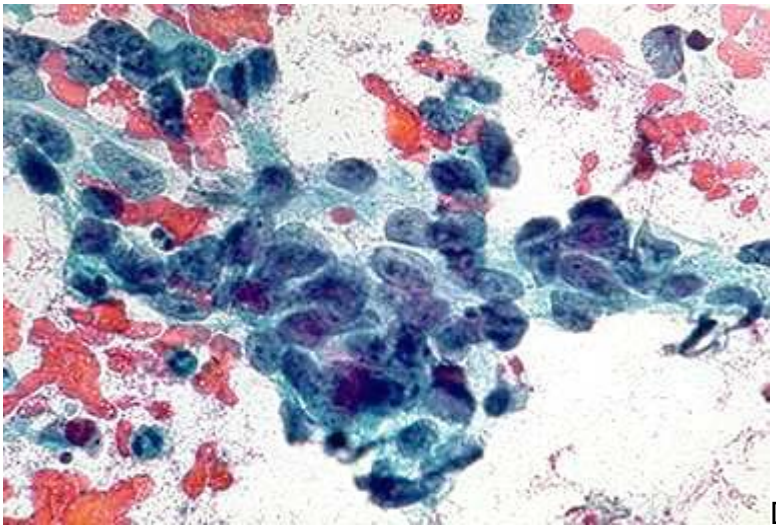
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Fig. 7.18. Homologous MMMT:
A: Histology of the tumor.
B-D. Malignant glandular/epithelial cells with prominent nuclei (B), malignant stromal cells present singly (C) and a syncytial cluster of malignant stromal cells with ill-defined cytoplasm and micronucleoli (D).

6. Leiomyosarcoma is a rare uterine tumor occurring mainly in adult women. The neoplasm peaks at ages 40 to 69, mean 54 years. Lung, bone and brain are the most common sites of metastatic deposits. When the tumor has invaded through the endometrium, malignant smooth muscle cells may be detected in cell samples collected from posterior vaginal fornix or in direct endometrial samples.

7. Metastatic cancers to the uterus are rare, with breast, gastrointestinal and kidney being the most common primary carcinomas followed by cutaneous melanoma. It may involve the myometrium but it may first appear in endometrial curettings, particularly in the case of lobular carcinoma of the breast. These neoplasms may be seen in cell samples collected from the posterior vaginal fornix or in direct endometrial samples.

DIAGNOSTIC ACCURACY

Pap smear is not efficient in screening endometrial cancer as it will miss about 50% of cases. Criteria for assessing the cellular adequacy of direct endometrial samples vary among investigators. With Endocyte samplers, 10 to 15 endometrial fragments are required to consider a sample as adequate or representative for cytologic evaluation of a nonneoplastic lesion. However, for endometrial cancer, the presence of 5 or 6 groups of well-preserved cancer cells with 5 to 10 cells in each group is adequate for a confident diagnosis. Usually, about 10% of endometrial samples procured by Endocyte or Endopap samplers show inadequate cells for cytologic evaluation. The specificity of this technique is high, ranging from 81% to 100%, according to literature reviewed by Nguyen and Redburn. In the current practice of medicine, endometrial dating by direct endometrial cytology is not requested by gynecologists. The cytodiagnostic accuracy of endometrial carcinoma by direct endometrial sampling is high, ranging from 75% to 100%, according to several reports. Endometrial hyperplasia is difficult to identify cytologically and a broad diagnostic accuracy rate ranging from 31% to 97% have been reported. Finally, it should be borne in mind that patients with abnormal uterine bleeding, especially postmenopausal women, should have an endometrial biopsy or curettage to rule out an important endometrial pathology.

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Chapter 8

Vaginal Lesions

PROCUREMENT OF VAGINAL CELL SAMPLES

Vaginal cell samples should be collected prior to all digital pelvic examinations as lubricant may obscure vaginal cell morphology. If an excessive mucous secretion is present, it should be removed by a cotton ball moistened with normal saline solution. The material is usually obtained from the posterior vaginal fornix of the patient in supine position. If a lesion is present, direct scraping of the lesion should be made. For detecting vaginal adenosis, circumvaginal scraping of the upper vagina or a four-quadrant downward scraping of the vaginal mucosa should be performed. The smears obtained are fixed with a commercial cytospray fixative for staining by the routine Papanicolaou method.

CYTOLOGIC FINDINGS

A. INFLAMMATORY LESIONS

1. Infections of the vagina are caused by the same microorganisms affecting the cervix. The reader is referred to Chapter 3 for discussion and illustrations of some of these disorders.

2. Noninfectious inflammatory lesions of the vagina include: atrophic vaginitis, malakoplakia and acute inflammation with granulation tissue formation of the vaginal vault following a total hysterectomy.

Cytology of **atrophic vaginitis** is described in Chapter 2. **Vaginal malakoplakia** may be uni- or multifocal. Scraping of the lesion may reveal histiocytes containing round, basophilic, concentrically laminated, calcified Michaelis-Gutmann bodies that are diagnostic of the lesion.

Granulation tissue in the vaginal vault following a total hysterectomy may show foreign body granuloma (suture material). A cell sample for the lesion may reveal inflammatory cells, repair squamous cells and suture material laden macrophages. (Fig.8.1).

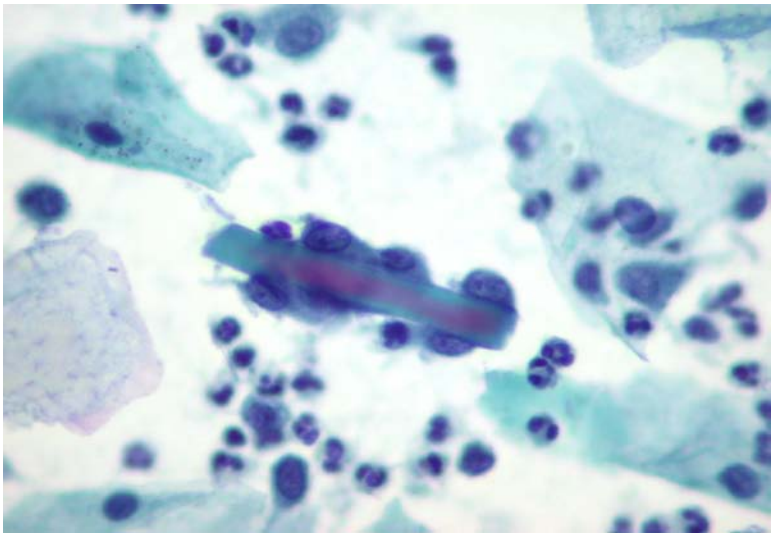

Fig. 8.1. A minute fragment of catgut surround by macrophages seen in a CP smear.

B. NONNEOPLASTIC LESIONS

1. Vaginal endometriosis may be formed by implantation of viable endometrial cells discharged during the menstrual period. The lesion may appear as a bluish submucosal cyst with chocolate colored liquid contents. In typical cases, a scraping smear or fine needle aspiration may yield fragments of endometrial epithelium, clusters of endometrial stromal cells, degenerated erythrocytes and a few hemosiderin laden macrophages. (Fig. 8.2).

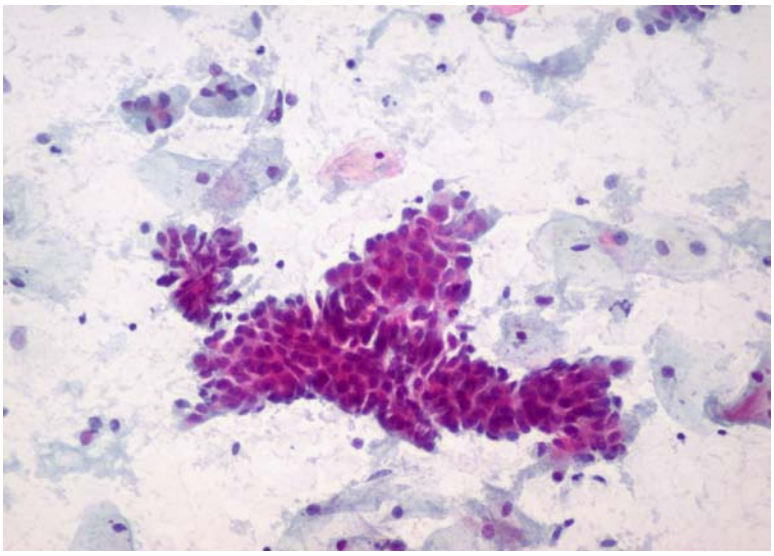

Fig. 8.2. Vaginal endometriosis showing in a scraping smear a fragment of benign endometrial epithelium.

2. Rectovaginal fistula may develop following a perforation of the rectal wall during a complicated total hysterectomy. Cell sample prepared from the vaginal secretion may reveal thick mucus containing colonic columnar epithelial cells, as well as digested food particles. (Fig.8.3).

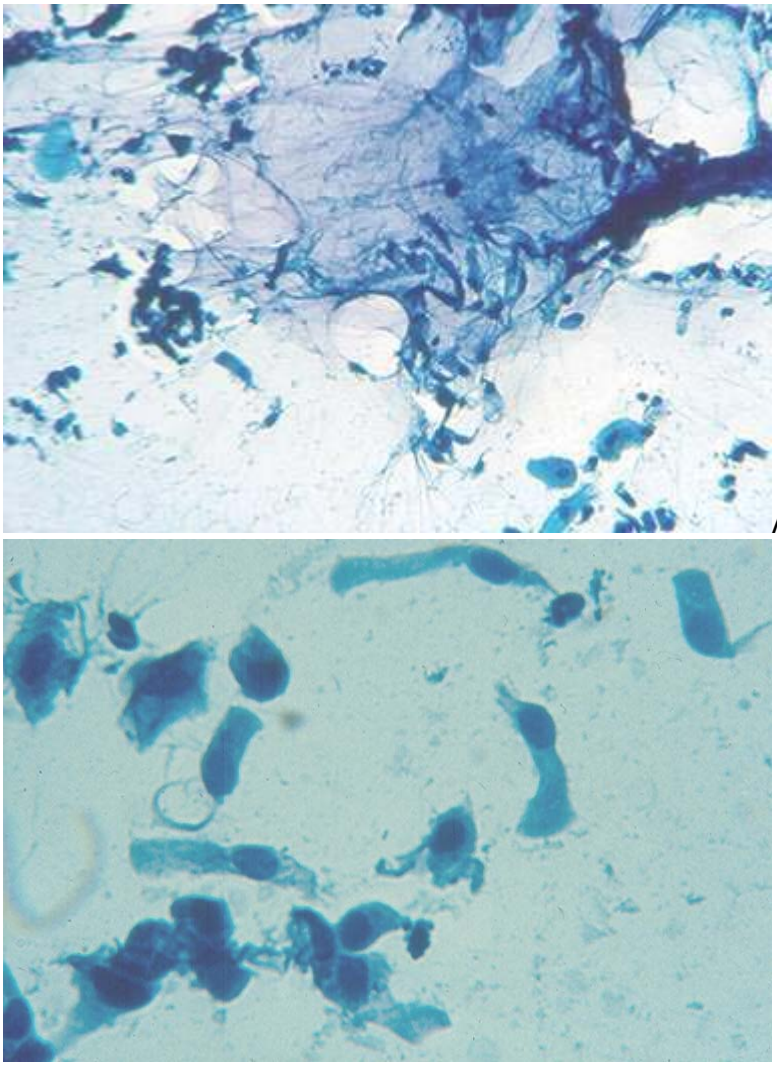
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Fig. 8.3. Vaginal smear showing thick mucus containing columnar epithelial cells.

3. Vaginal adenosis is defined by the presence of either endocervical glandular epithelium or tuboendometrial epithelium in the vagina. It most commonly affects the upper third of the anterior vaginal wall and it is diagnosed histologically by tissue biopsy. Approximately 35% to 90% of female offsprings with in utero exposure to diethylstilbestrol (DES), a synthetic estrogen that was commonly administered to women in early pregnancy to prevent spontaneous abortion in the late 1940s to the late 1950s, develop this lesion. However, vaginal adenosis is also found in women without in utero exposure to DES, and an incidence up to 41% has been reported. Vaginal adenosis containing endocervical-type epithelium is most commonly encountered and it may display squamous metaplasia. In a

scraping smear, the lesion yields endocervical glandular cells singly, in loose clusters and in monolayered sheets. Metaplastic squamous cells may be observed. (Fig. 8.4).

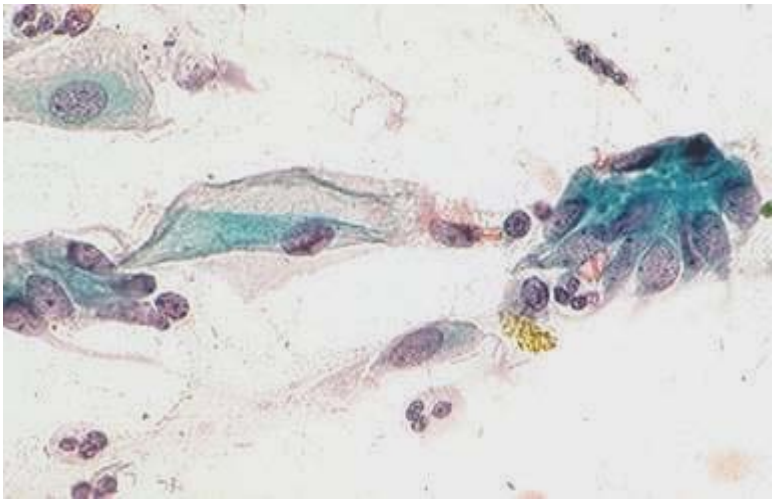


Fig. 8.4. Vaginal smear showing single and clustered columnar glandular cells exfoliated from a vaginal adenosis.

4. Glandular cells in posthysterectomy Pap smears. Benign glandular cells similar to normal endocervical cells may be occasionally seen in vaginal smears of patients with total hysterectomy. They are formed as the result of mucinous or glandular metaplasia of vaginal squamous epithelium, and they are more commonly seen in women with postoperative radiotherapy. (Fig. 8.5).

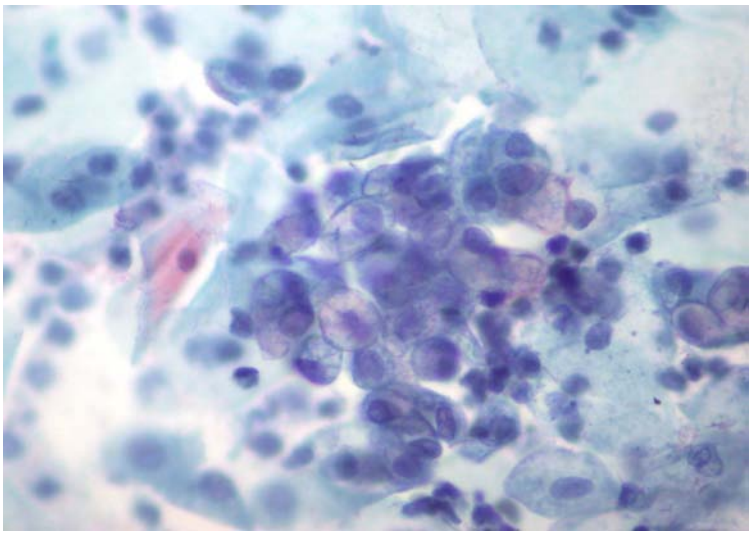


Fig. 8.5. A cluster of benign glandular cells with mucus-filled clear cytoplasm in a vagnal smear from a woman with prior total radical hysterectomy and radiotherapy for endometrial carcinoma.

C. PREMALIGNANT AND MALIGNANT LESIONS

1. Squamous intraepithelial lesions of the vagina or **Vaginal intraepithelial neoplasia** (VIN) are less common that those of the cervix. VIN is caused by HPV infection and is commonly associated with SIL or squamous cell carcinoma of the cervix or vulva. VIN lesions or vaginal SILs, are histologically similar to those of the cervix and are also graded as VIN grade 1, 2 and 3 according to the criteria used for CIN or for LSIL and HSIL of the cervix. Vaginal SILs display cytologic changes similar to those of cervical SILs previously described in Chapter 3.

2. Postradiation dysplasia of vaginal mucosa more commonly develops following radiotherapy to the lower genital tract. The vaginal cells show radiation changes as described in Chapter 2. Postradiation dysplasia may develop after a latency period ranging from months to years. It exfoliates dyskaryotic squamous cells as seen in a cervical SIL. Diagnosis of a vaginal posradiation dysplasia should be confirmed by tissue biopsy. (Fig. 8.6).

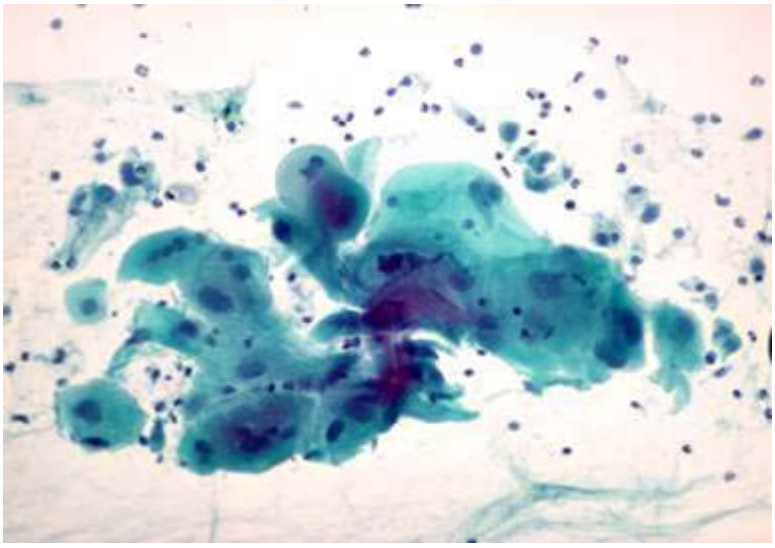


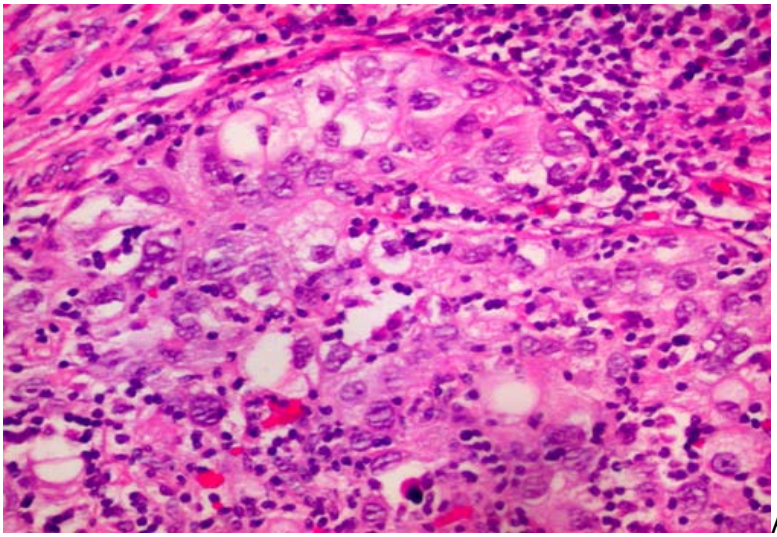
Fig. 8.6. Vaginal smear showing mildly dyskaryotic squamous cells from a postradiation dysplastic lesion of the vagina.

3. Primary carcinomas of the vagina are rare and account for 1% to 2% of all female genital tract cancers. Of these, squamous cell carcinoma is the most common neoplasm. However, most vaginal squamous cancers represent an invasion of either a cervical or vulvar squamous carcinoma.

i. ***Vaginal squamous cell carcinoma*** is most commonly of nonkeratinizing type, and its exfoliated cells are indistinguishable from those of the cervix of the same histologic type.

ii. ***Vaginal adenocarcinoma*** of nonspecific type is extremely rare and yields in a scraping smear nonspecific malignant glandular cells.

iii. ***Clear cell adenocarcinoma*** (CCA) of the vagina accounts for about 1% of all invasive carcinomas of the female genital tract. CCA developed as the result of intrauterine exposure to DES affects about 0.1% of patients up to 34 years of age. The mean age is 19.5 years with a range of 7 to 29 years. Vaginal CCA is often seen in association with adenosis but progression of adenosis to CCA has not been documented. CCA has 3 main histologic patterns: cystic, solid and papillary. Bulging of tumor cells in glandular lumens is referred to as a "hobnail pattern", one of the characteristic histologic features of the tumor. In cytologic material, exfoliated tumor cells are present singly, in sheets and in aggregates with "bulging" cells. (Fig. 8.7). The cell cytoplasm is fragile, poorly stained, vacuolated and rich in glycogen. (Fig. 8.8).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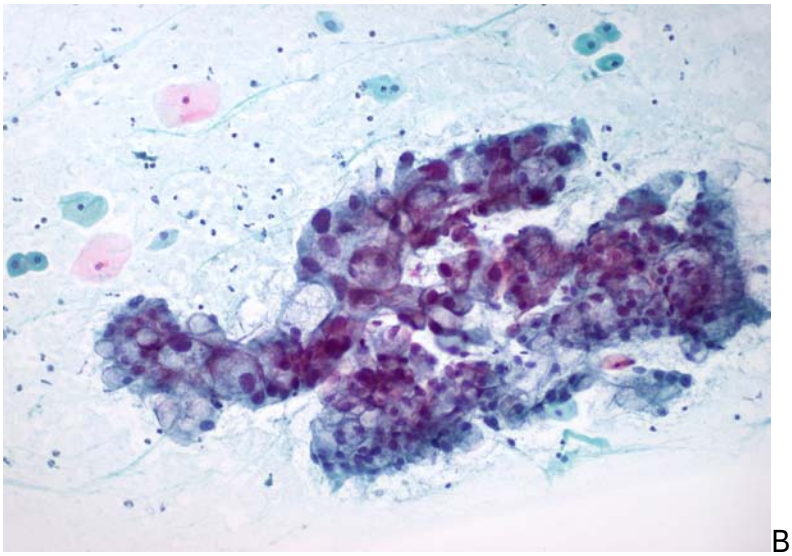
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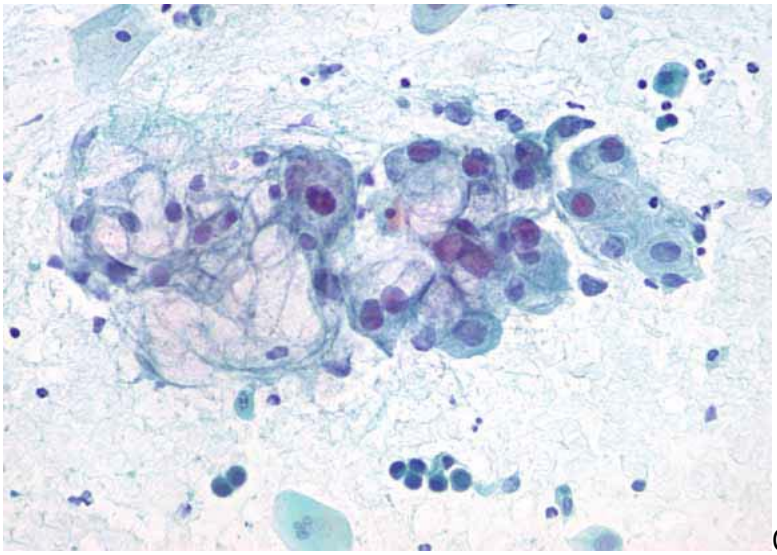

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Fig. 8.7. Vaginal clear cell carcinoma:
A. Histology of the tumor showing a mixed glandular and solid pattern.
B, C. Vaginal smear showing sheets of tumor cells with clear, vacuolated or granular cytoplasm.

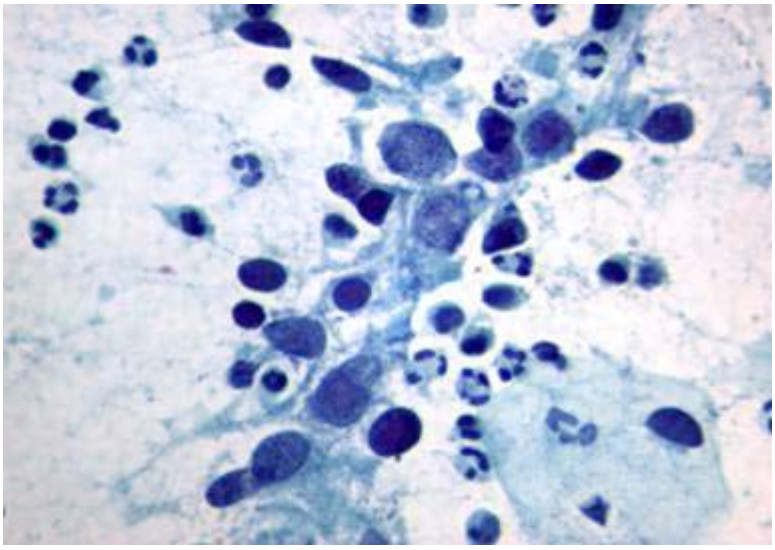

Fig. 8.8. Vaginal CCA showing in a scrapping smear an aggregate of tumor cells with prominent, large, hyperchromatic nuclei and ill-defined, granular cytoplasm displaying a vague ”bulging” pattern.

4. Primary nonepithelial malignant tumors of the vagina are very rare. Of these ***melanoma*** is the most common one in adult patients. Melanoma shows in Pap smears single and loosely clustered pleomorphic malignant cells with variable cytoplasm that may contain intracytoplasmic melanin pigment granules. (Fig. 8.9). When the tumor is amelanotic, a positive cytoplasmic reaction to S-100 protein, HMB-45 and MART-1 antibodies will confirm the diagnosis of melanoma.

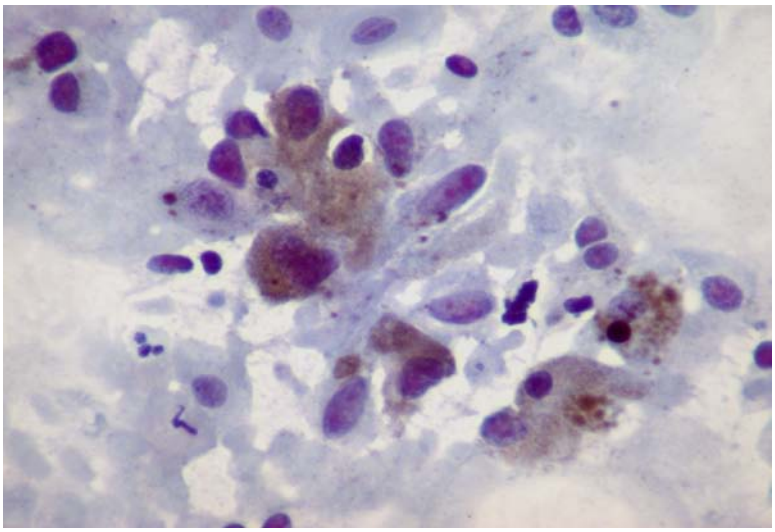

Fig. 8.9. Vaginal melanotic melanoma showing in a scrapping smear loosely clustered malignant cells with intracytoplasmic brownish melanin pigment granules.

5. Secondary tumors are the most common neoplasms of the vagina. They may occur by direct extension or via hematogenous or lymphatic spread. The most common primary cancer sites are cervix, endometrium, ovary, colon and urinary bladder. These neoplasms are usually submucosal and may be diagnosed cytologically by vaginal fine needle aspiration.

6. Benign tumors and Tumorlike lesions of the vagina include benign inclusion cyst (traumatic etiology), fibroepithelial polyp, fibroma, leiomyoma and hemangioma. These lesions are usually diagnosed by tissue biopsy and not by cytologic methods.

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Chapter 9

Uterine Annexal Mass Lesions

Uterine annexae include fallopian tubes and ovaries. Mass lesions of the uterine annexae are usually evaluated cytologically by fine needle aspiration. Depending on clinical settings, a transabdominal, transvaginal or laparoscopic FNA is used. Cancer arising from the fallopian tube is rarely encountered in medical practice. It may exfoliate its cells into the uterine cavity and can be diagnosed by cytologic examination of material collected from the posterior vaginal fornix.

INDICATIONS AND GOALS OF FNA OF UTERINE ANNEXAL MASS LESIONS

1. Confirming the benign nature of an ovarian cyst incidentally found during pregnancy and infertility evaluation.
2. Confirming an inoperable malignant ovarian tumor.
3. Documenting recurrence of a previously treated ovarian cancer.
4. Diagnosis of hydrosalpinx and fallopian tumors.
5. Diagnosis of a tubo-ovarian abscess

CYTOLOGIC FINDINGS

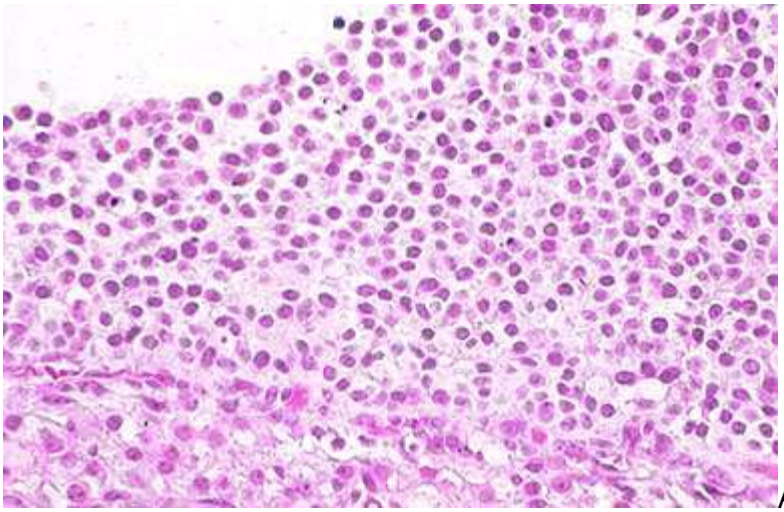
Fine needle aspirates are commonly obtained during laparoscopy. They may also be obtained by transvaginal, transrectal or transabdominal FNA under manual and/or ultrasound guidance. The cytologic samples obtained are prepared by direct smearing or by cytospin technique and stained by the Papanicolaou and/or Diff-Quik methods. Excessive material is used for cellblock (CB) preparation for histologic evaluation.

A. NONNEOPLASTIC LESIONS

These lesions include follicular cyst, corpus luteum cyst, endometrioid cyst and simple cysts.

1. Follicular cysts can be solitary or multiple and are benign macroscopically and ultrasonographically. They are <3 to 8 cm in greatest dimension and lined by an inner layer of stratified granulosa cells and an outer layer of theca cells. In FNAs, a follicular cyst

usually yields abundant isolated or clustered small, cuboidal benign cells with round nuclei, coarse chromatin and scant or vacuolated cytoplasm. Mitotic figures and cells with pyknotic nuclei may be seen. (Fig. 9.1). Similar cells are also seen in an FNA of a granulosa cell tumor. Follicular cyst constitutes a potential diagnostic pitfall as it may yield bizarre appearing follicular cells with abundant mitotic figures.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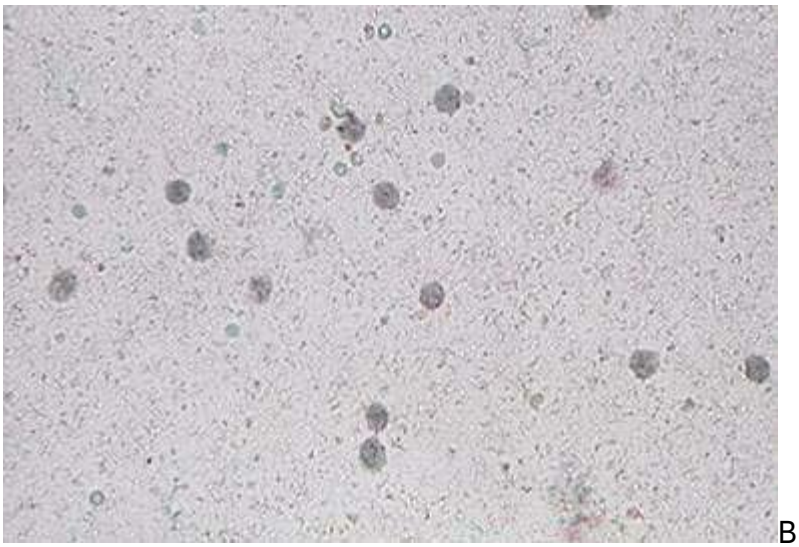
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Fig. 9.1: Follicular cyst:
A. Histology of a follicular cyst wall.
B. FNA showing isolated, monomorphic, granulosa cells with bland, round nuclei and scant cytoplasm.

2. Corpus luteum cyst is unilocular and consists of large luteinized granulosa and theca interna cells. These cells are seen singly and in clusters in FNA. (Fig. 9.2).

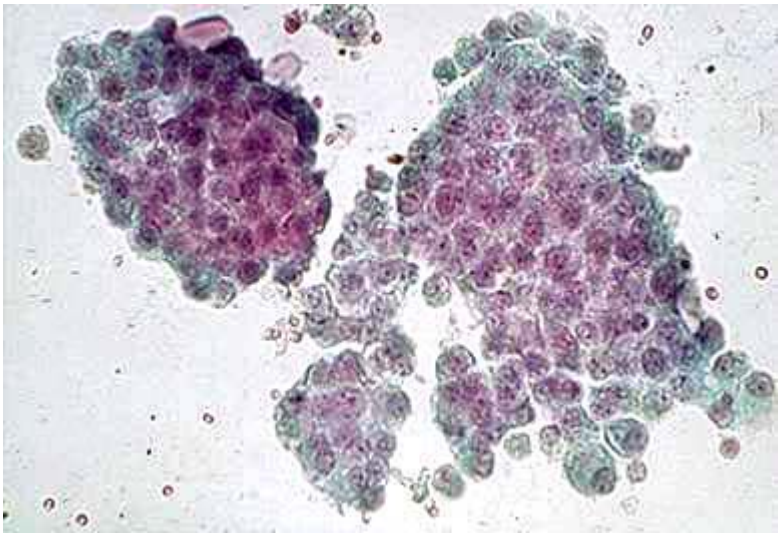
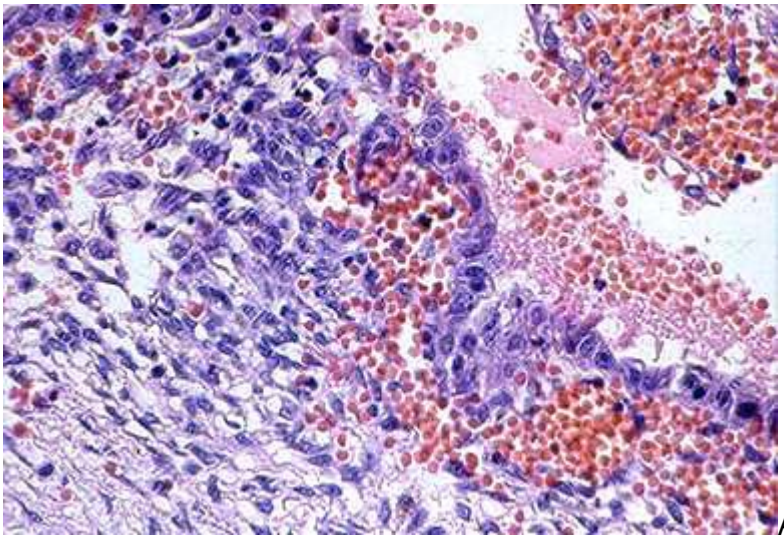


Fig. 9.2: Cohesive clusters of luteinized granulosa cells showing bland, round nuclei and a moderate amount of granular cytoplasm in an FNA.

3. Endometriotic cyst is commonly called endometrioma which is a misnomer. This lesion is commonly discovered during laparoscopy for infertility work-up. Ovarian endometriotic cysts are bilateral in about 50% of cases and contain hemolysed blood (chocolate cyst). In FNA, numerous hemosiderin-laden macrophages and hemolysed blood are found. (Fig. 9.3). Endometrial glandular and stromal cells are rarely seen in needle aspirates but minute fragments of endometrial tissue may be found in CB sections.



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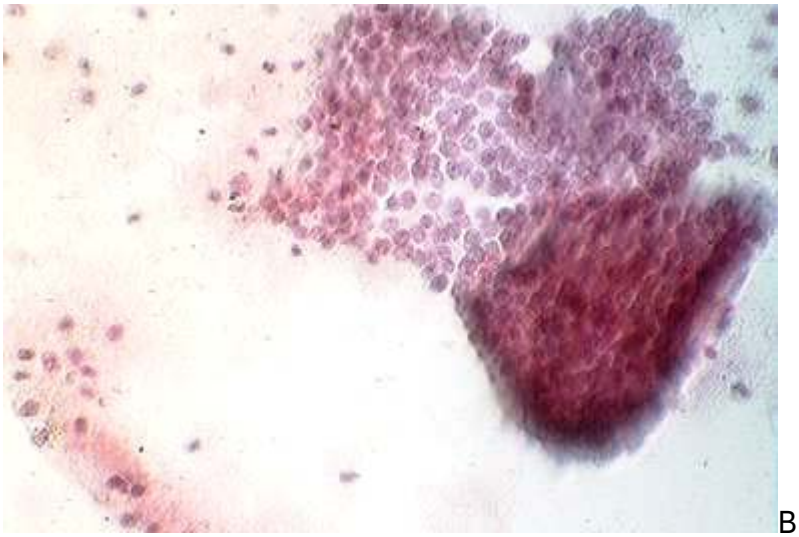
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Fig. 9.3: Endometrioid cyst:
A. Histology of the endometrioid cyst.
B, C. Fragments of benign epithelium admixed with hemolysed erythrocytes seen in FNA.

4. Simple cysts develop from invagination of mesothelium or surface epithelium of the ovary. They are usually small and most commonly seen in postmenopausal women. These cysts can also be seen in paraovarian or paratubal tissues. FNA of those cystic lesions reveals clusters and large monolayered sheets of cuboidal epithelial cells or mesothelial cells with a honeycomb pattern. (Fig. 9.4).

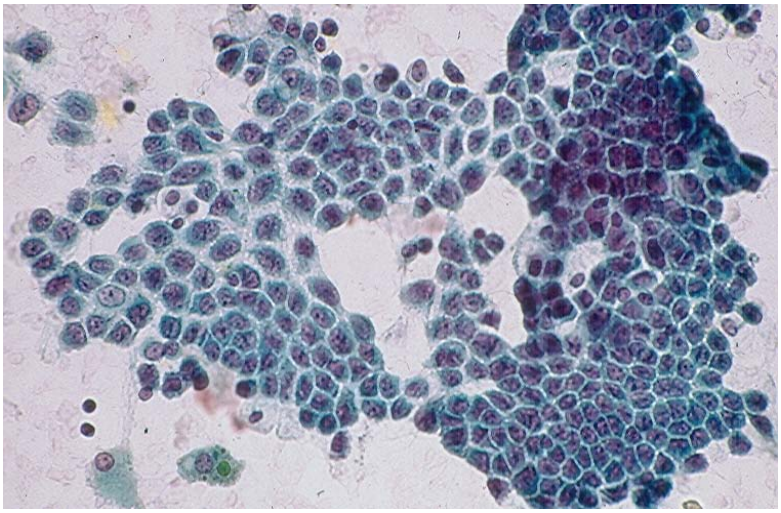

Fig. 9.4. FNA showing monolayered benign epithelial sheets from a simple cyst.

5. Hydrosalpinx is caused by salpingitis and appears as a large cystic adnexal lesion. It yields in FNA a scanty cellular fluid containing a few benign ciliated cuboidal or columnar epithelial cells. (Fig. 9.5).

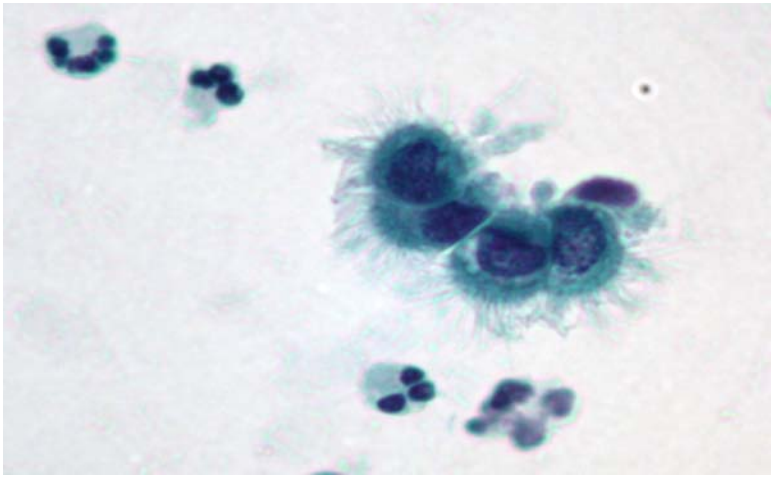

Fig. 9.5: Ciliated epithelial cells in an FNA from a hydrosalpinx. (oil immersion).

6. Tubo-ovarian abscess is the most severe result of pelvic inflammatory disease that is commonly caused by a Neisseria gonorrhoeae or Chlamydia trachomatitis ascending infection of the lower female genital tract. It yields in FNA purulent material consisting of numerous polymorphonuclear leukocytes and necrotic debris.

B. EPITHELIAL OVARIAN TUMORS

These tumors can be benign or malignant, arise from the ovarian surface epithelium and account for 60% of all ovarian neoplasms. Ovarian carcinomas are the commonest and account for 80% to 90% of all primary ovarian cancers.

Benign ovarian epithelial tumors are serous cystadenoma, cystadenofibroma and mucinous cystadenoma.

1. Serous cystadenoma and cystadenofibroma account for about 20% of benign ovarian tumors. They are usually cystic and unilocular with clear fluid contents and can be bilateral in up to 20% of cases. The lesions are lined by a single layer of benign cuboidal epithelial cells with basally located nuclei. Columnar, clear or ciliated cells may be seen in FNAs of the lesions. Psammoma bodies may be rarely observed. (Fig. 9.6).

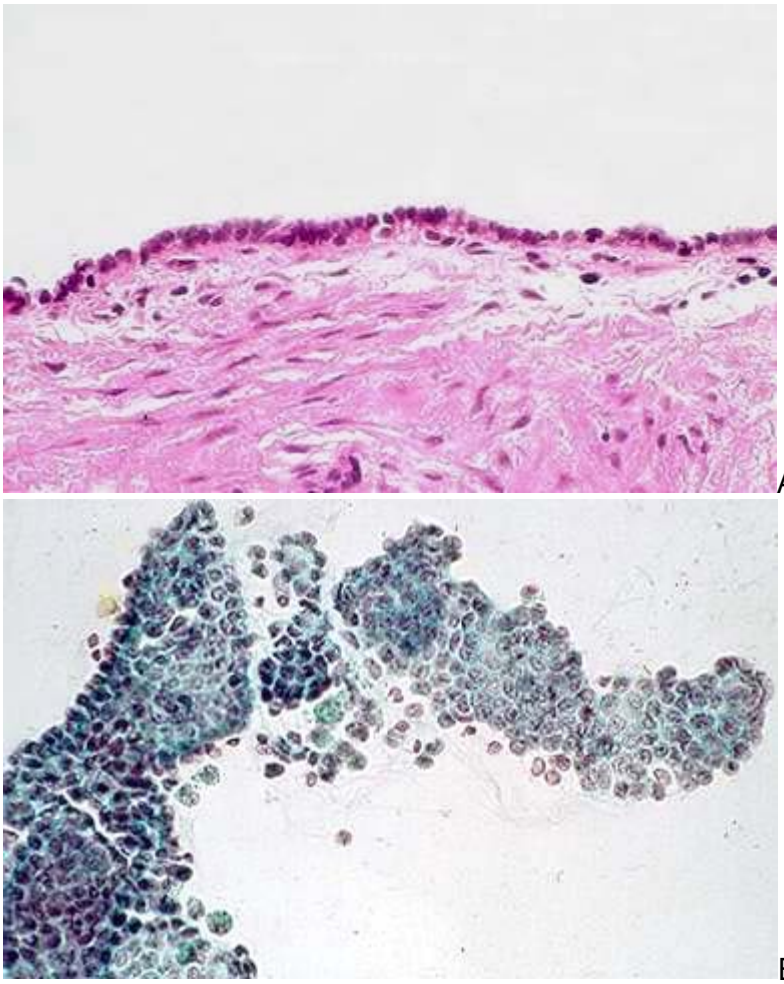
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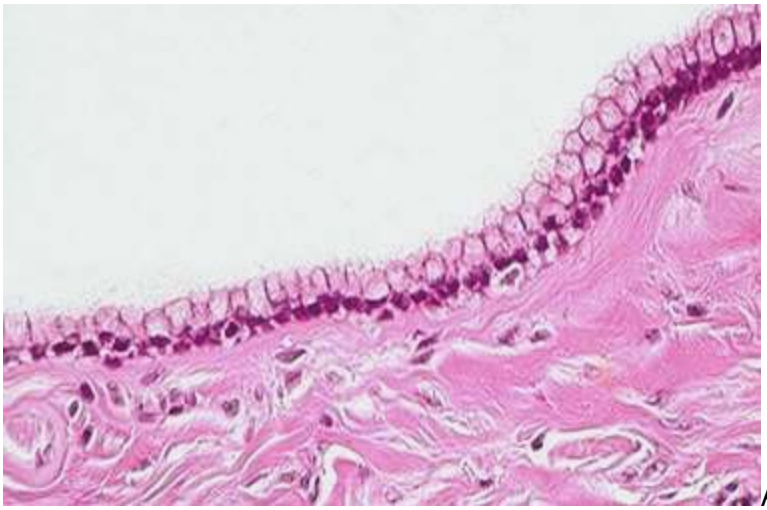
Fig. 9.6: Serous cystadenoma:
A. Histology of the tumor.
B. FNA showing fragments of benign neoplastic epithelium.

2. Mucinous cystadenoma accounts for about 20% of benign ovarian tumors. It is usually large and multiloculated and is rarely bilateral (2% to 3% of cases). Its epithelial lining is smooth and of either endocervical or intestinal type. It shows in FNA a large amount of mucinous material containing benign vacuolated mucus-secreting glandular cells that are seen singly, in sheets or ribbons. (Fig. 9.7).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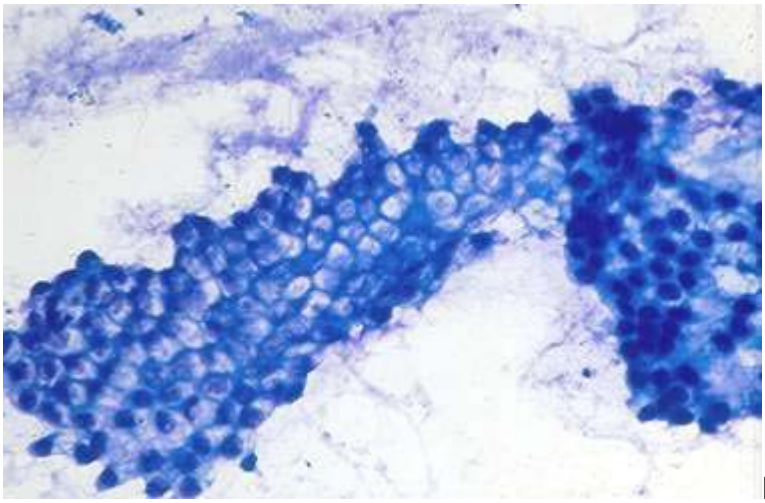
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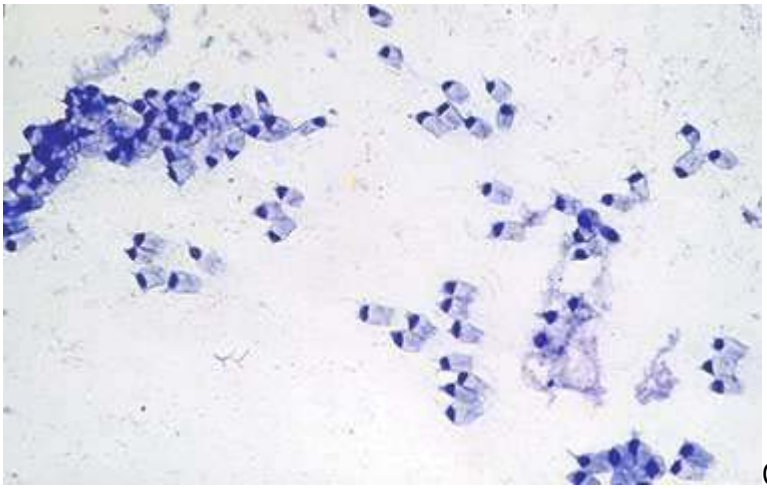
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Fig. 9.7: Mucinous cystadenoma:
A. Histology of the lesion.
B, C: FNA showing epithelial fragments and single and clustered mucus-secreting benign epithelial cells. (Diff-Quik).

3. Brenner tumor accounts for about 2% of all ovarian tumors. The tumor is bilateral in 10% of cases and consists of nests of transitional or urothelial epithelium surrounded by fibrous tissue. Most Brenner tumors are benign and less than 2% of them are borderline or malignant. In FNA a benign Brenner tumor is characterized by single and clustered benign urothelial cells with defined, granular cytoplasm and oval nuclei. (Fig. 9.8). Nuclear grooves may be observed.

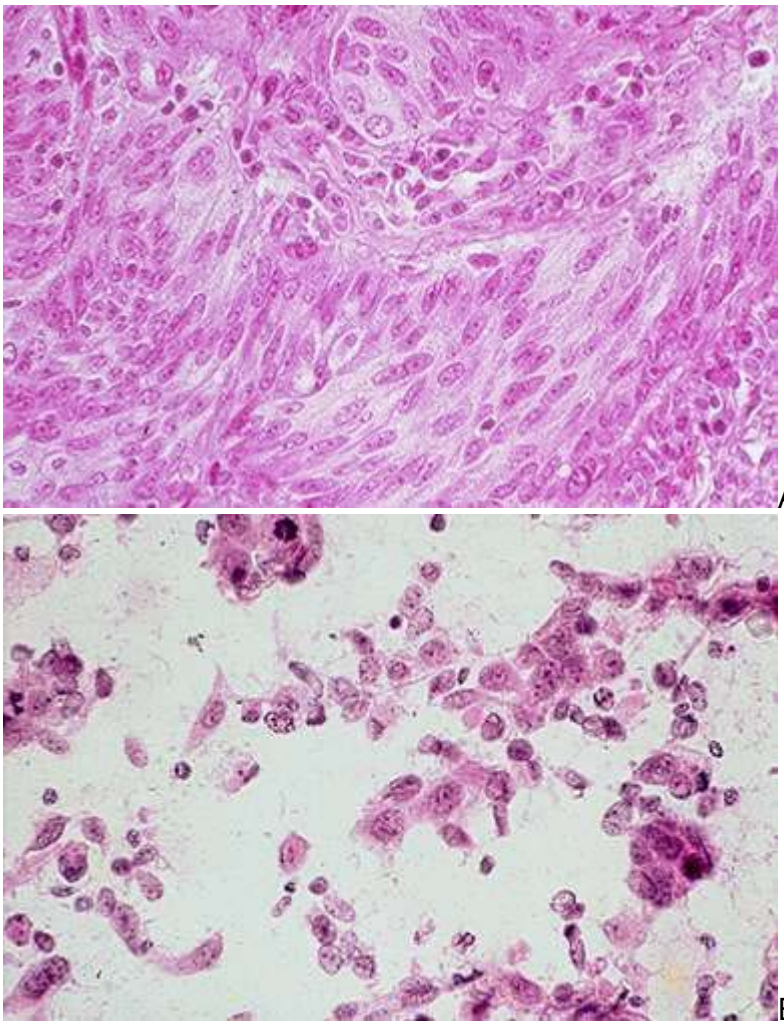

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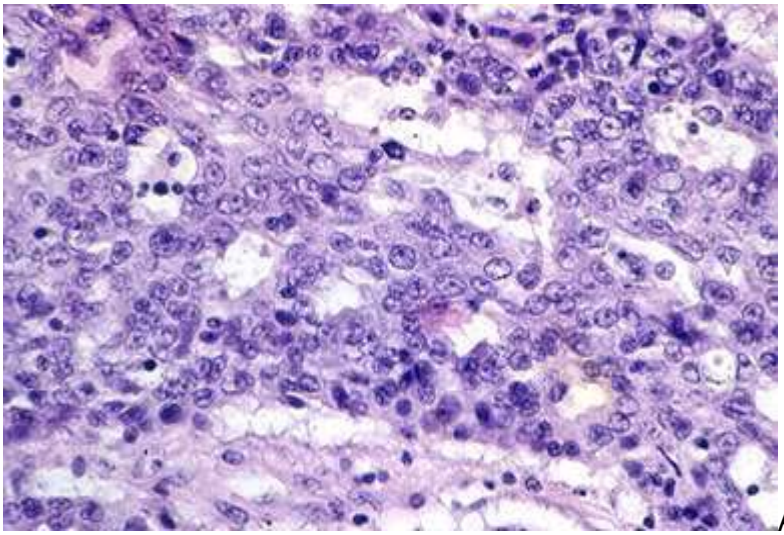
Fig. 9.8: Benign Brenner tumor.
A. Histology of the tumor.
B. Tumor FNA showing single and clustered benign, spindle-shaped epithelial cells with bland, oval nuclei and ill-defined, granular cytoplasm.

Ovarian carcinomas consist of several types:

- **Serous cystadenocarcinomas** are papillary and can be divided into borderline, low-grade and high-grade tumors. These tumors are the most common ovarian cancers and usually occur in patients 40 to 60 years of ages. They are often bilateral, solid and cystic. Histologically, serous cystadenocarcinomas are composed of malignant non-mucus secreting cells forming papillary projections. Depending on the cellular atypia the tumor is graded as low- or high-grade. Stromal invasion is present and psammoma bodies are present in about one third of the cases.

Ovarian cystadenocarcinomas are rarely sampled by transabdominal FNA for initial diagnosis but this diagnostic method commonly used to confirm a recurrent or inoperable tumor. Cytologically, a borderline serous tumor and low-grade serous cystadenocarcinoma are similar and consist of crowded sheets and irregular tridimensional clusters of epithelial cells with mild or moderate nuclear atypia, granular cytoplasm and psammoma bodies. (Fig. 9.9).

A high-grade serous cystadenocarcinoma yields hypercellular material containing single and clustered large pleomorphic malignant epithelial cells with round nuclei and prominent nucleoli. Psammoma bodies may be observed. (Fig. 9.10).



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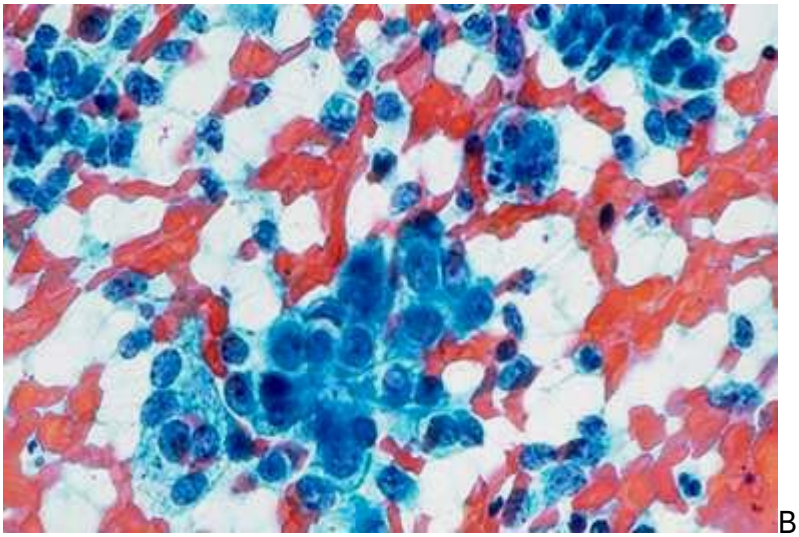
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Fig. 9.9: Low-grade serous carcinoma:
A. Tumor histology.
B. Tumor FNA showing cohesive groups and clusters of monomorphic tumor cells with small nucleoli.

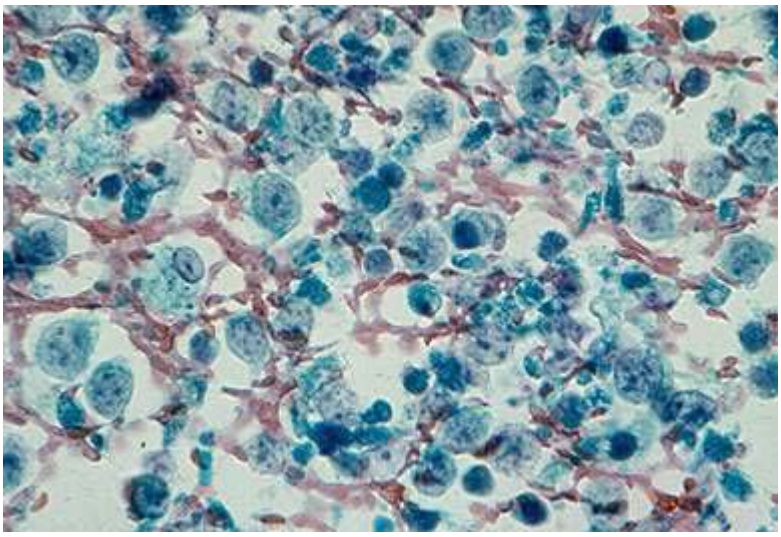


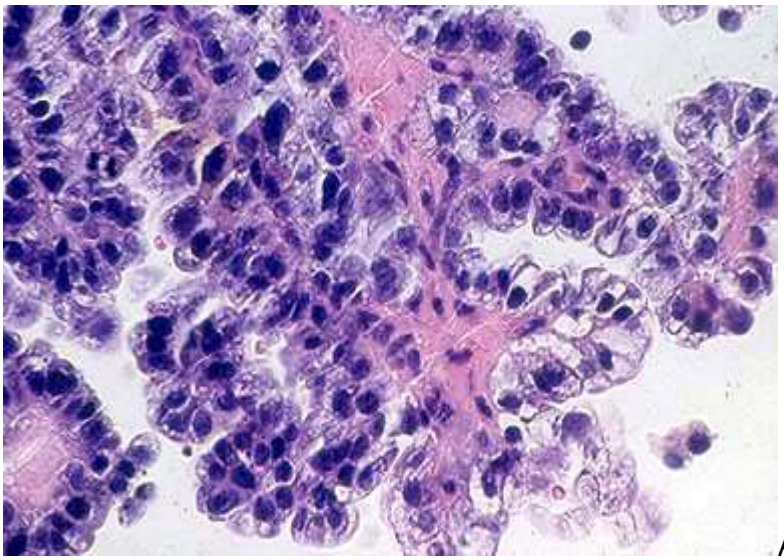
Fig. 9.10: High-grade serous carcinoma showing in FNA pleomorphic malignant cells with scant cytoplasm and prominent nucleoli admixed with a few macrophages.

- **Mucinous ovarian cystadenocarcinoma** is less common than serous carcinoma and accounts for 5% to 10% of all ovarian cancers. The tumor can occur at any age but is most commonly seen in patients 40 to 60 years of age, and about 75% of them are bilateral. It is large and mutiloculated and consists of solid areas of mucus-secreting malignant cells and areas with papillary formation. As in serous carcinomas, mucinous carcinomas are graded as low- and high-graded tumors. The borderline variant is cytologically similar to a low-grade tumor but it shows no stromal invasion histologically.

A borderline or low-grade mucinous cystadenocarcinoma shows in FNA clustered tumor cells with nuclear crowding and mild nuclear atypia. A high-grade tumor yields single and clustered pleomorphic malignant epithelial cells. Mucin production is not evident in routinely stained smears but can be demonstrated with mucicarmine and periodic acid-Shiff stain with prior diastase digestion.

- **Endometrioid carcinoma** accounts for about 20% of ovarian cancers and is bilateral in about 30% of cases. About 30% of patients with this neoplasm have endometriosis and 25% of them have a coexisting endometrioid uterine carcinoma. It yields in FNA numerous malignant cells singly and in crowded clusters, similar to those seen in an FNA of an ovarian serous cystadenocarcinoma.

- **Clear cell carcinoma** is a rare neoplasm and may arise from a focus of endometriosis. It yields in FNA sheets of malignant epithelial cells with prominent nucleoli and clear cytoplasm. (Fig. 9.11).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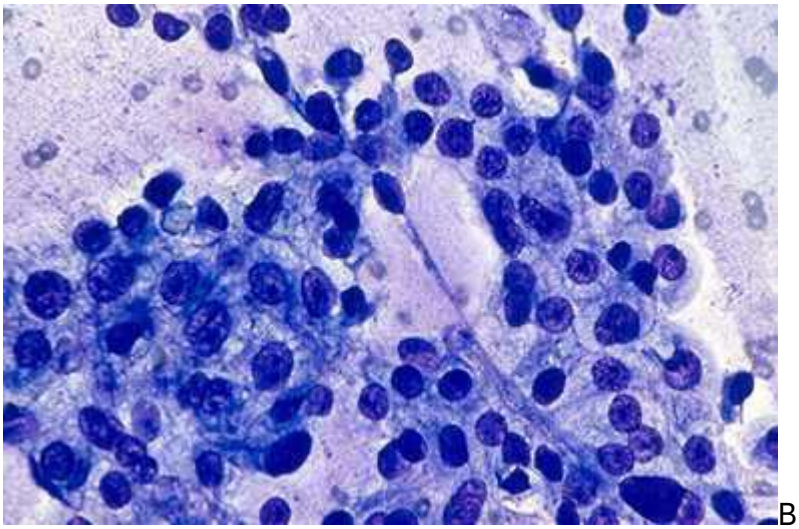
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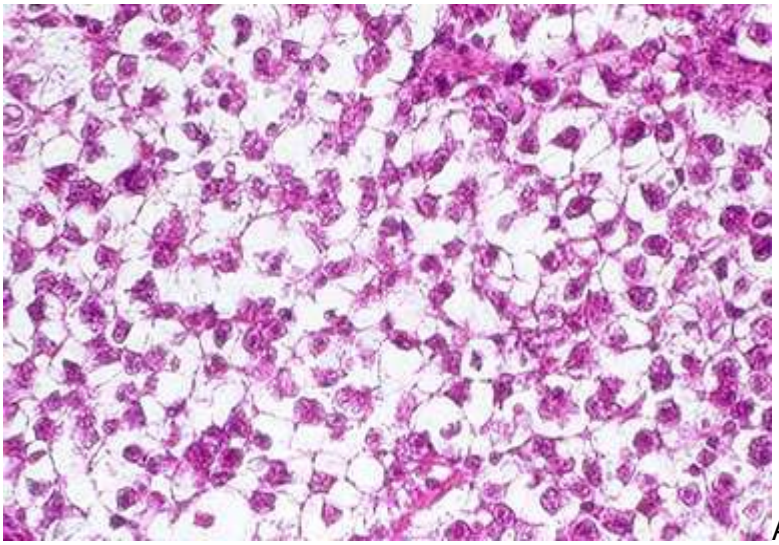
Fig. 9.11: Clear cell carcinoma:
A. Histology of the tumor.
B. Tumor FNA showing a sheet of malignant epithelial cells with clear or vacuolated, ill-defined cytoplasm, large, oval nuclei and prominent nucleoli. (Diff-Quik).

C. OTHER PRIMARY OVARIAN TUMORS

- **Germ cell tumors** account for 20% of all ovarian tumors. They consist of several histologic types: teratoma (mature and immature), dysgerminoma, embryonal carcinoma, endodermal sinus tumor and choriocarcinoma.

Mature teratoma is the most common germ cell neoplasm. It shows in FNA abundant anucleated squamous cells admixed with benign glandular cells. Immature teratoma is rare, malignant and usually solid. It is characterized by immature or embryonal tissue (usually neuroectodermal derivatives) admixed with benign elements as seen in its mature counterpart.

Dysgerminoma accounts for 1% to 5% of all ovarian cancers and about 40% of all malignant ovarian germ cell tumors. It occurs most frequently in young women under the age of 30 years, and yields in FNA single and loose aggregates of large tumor cells with ill-defined, variable cytoplasm and prominent nucleoli admixed with benign lymphoid cells. (Fig. 9.12). A "tiger-strip" background is only observed in air-dried smears with Romanowsky-type staining.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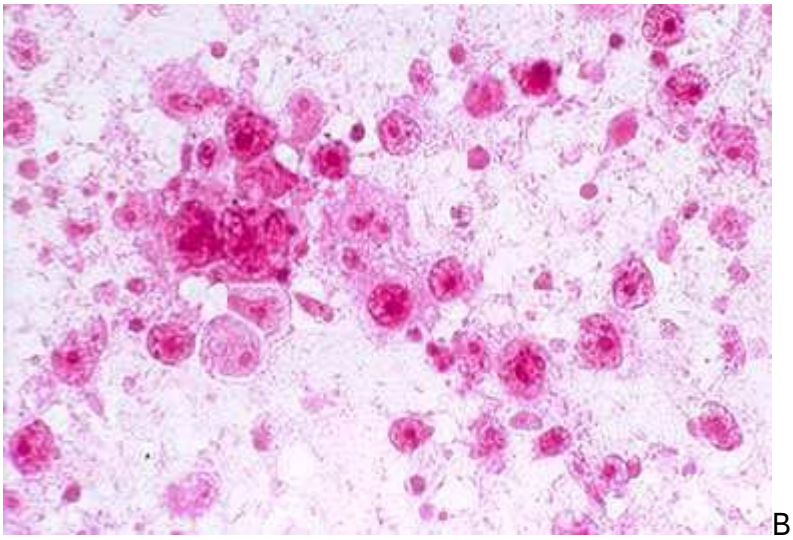


B

Fig. 9:12: Dysgerminoma:
A. Histology of the tumor.
B. Pleomorphic malignant cells with ill-defined, granular cytoplasm and prominent nucleoli in FNA of the above-illustrated tumor. (HE).

Embryonal carcinoma is a rare ovarian germ cell tumor. It shows in FNA cohesive clusters of pleomorphic malignant cells with ill-defined cytoplasm and large nuclei with prominent nucleoli. (Fig. 9.13).

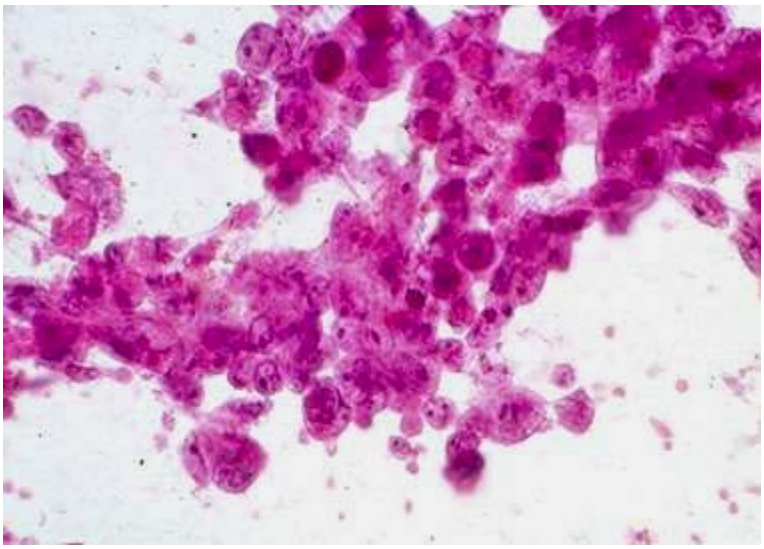


Fig. 9.13: Cohesive cluster of pleomorphic malignant cells with prominent nucleoli in FNA of an ovarian embryonal carcinoma. (HE).

Endodermal sinus or Yolk-sac tumor is an uncommon ovarian germ cell tumor. It shows in FNA cells resembling those of a poorly differentiated adenocarcinoma. Dense eosinophilic intracytoplasmic inclusions (alpha-fetoprotein) may be seen in some cells.

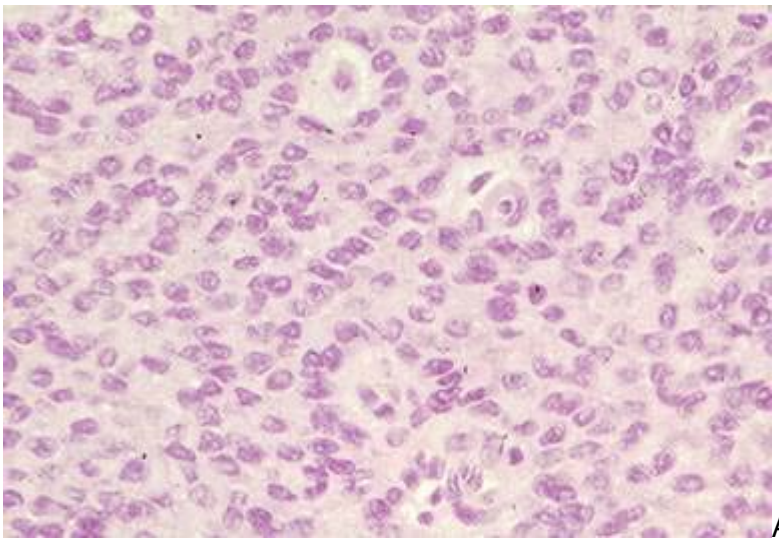
A rare ovarian choriocarcinoma shows in FNA bizarre malignant cells that stain positively with beta human chorionic gonadotropin antibody.

- **Sex Cord-Stromal tumors** account for about 8% of all ovarian tumors and include neoplasms arising from granulosa cells, Sertoli-Leydig cells and fibroblastic cells.

Granulosa cell tumor of adult *type* accounts for almost all granulosa cell tumors in adults. It occurs more commonly in postmenopausal women and is a slow growing tumor with metastatic potential. It secretes estrogen causing endometrial hyperplasia. Some tumors have an associated ascitis that usually shows no tumor cells. In FNA several clusters and sheets of small or medium-sized tumor cells with ill-defined cytoplasm, round nuclei and conspicuous nucleoli are seen, and tumor cells arranged in acini may be observed. (Fig. 9.14). Nuclear grooves may be seen but tumor cells forming Call-Exner bodies are rarely noted. These tumor cells are morphologically similar to those of a follicular ovarian cyst.

Sertoli-Leydig cell tumor and annular sex cord tumor yield cells that are cytologically similar to those of a granulosa cell tumor.

Thecoma and Fibroma are uncommon neoplasms that are usually non-functional and their needle aspirates are usually acellular.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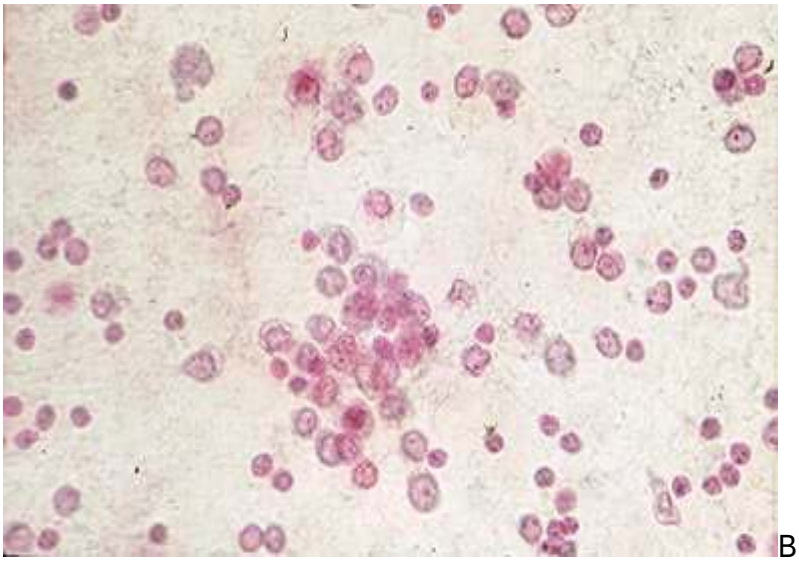
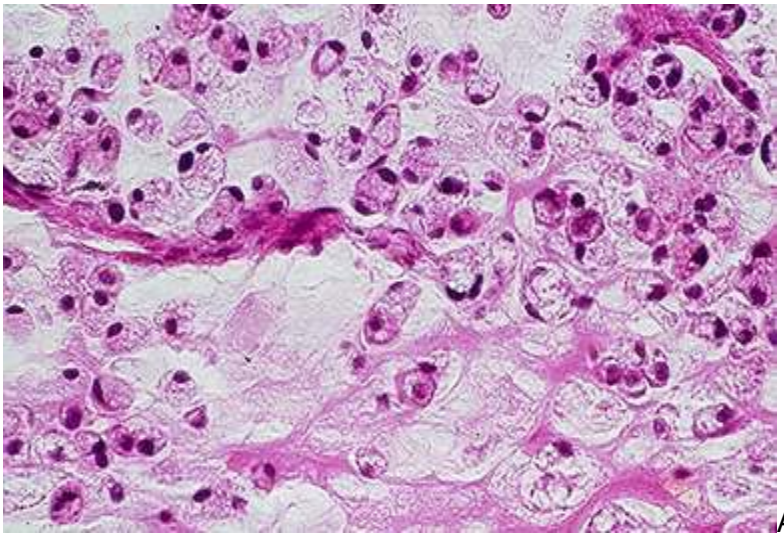


B

Fig. 9.14: Granulosa cell tumor:
A. Histology of the tumor.
B. Single and clustered tumor cells with scant cytoplasm and oval nuclei showing inconspicuous nucleoli and occasional nuclear grooves.

D. METASTATIC CANCERS TO THE OVARY

Tumors that most commonly metastasize to the ovary arise from the urogenital tract, colon, stomach and breast. About 15% to 20% of bilateral ovarian tumors are metastatic cancers. The well-known **Krukenberg tumor** is characterized by mucus-secreting signet-ring cells. Most of Krukenberg tumors have a gastric primary. (Fig. 9.15).



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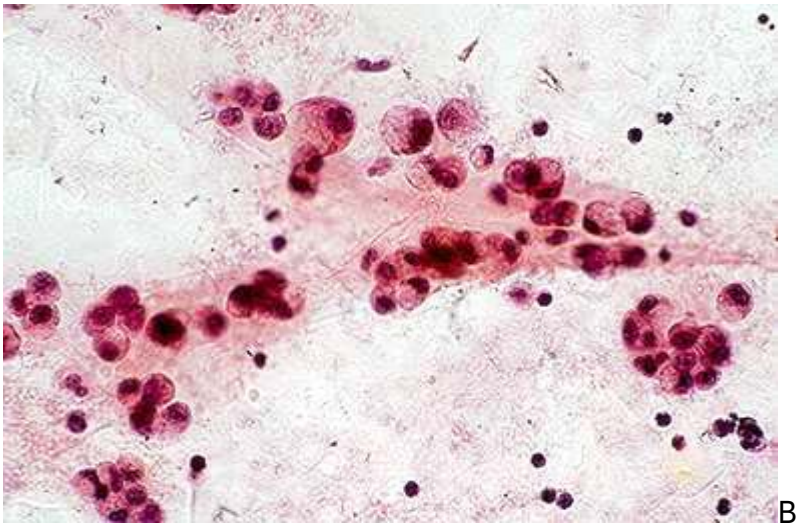
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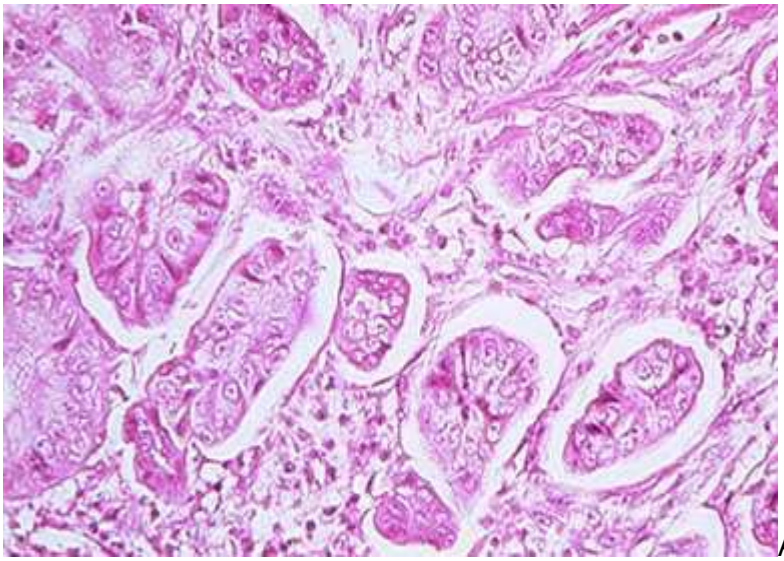
Fig. 9.15: Metastatic gastric signet-ring cell carcinoma:
A. Histology of the tumor.
B. FNA showing thick mucus containing single and clustered malignant tumor cells with large intracytoplasmic mucinous vacuoles pushing the tumor cell nuclei to the cell periphery.

A metastatic colonic adenocarcinoma yields in FNA sheets of malignant glandular cells in a “dirty” necrotic background. Tumor cell nuclei arranged in vague palisade may be visualized at the periphery of the aspirated tumor epithelial fragments. (Fig. 9.16).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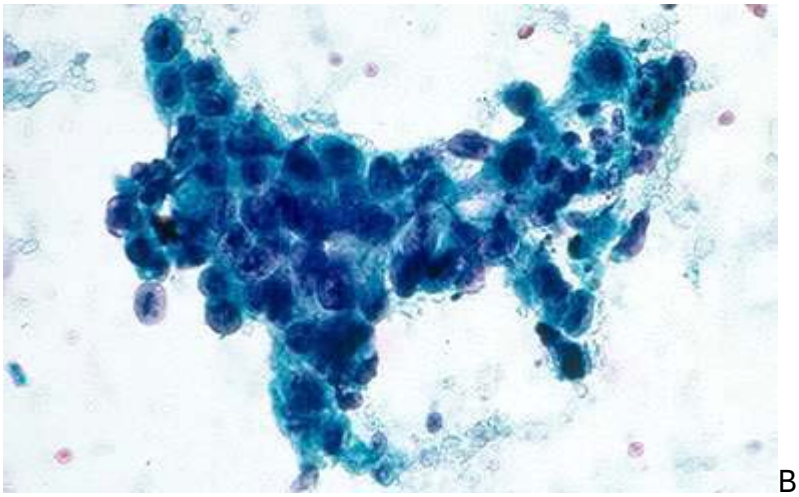
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Fig.9.16. Metastatic colonic adenocarcinoma to the ovary.
A. Histology of the tumor.
B. A cohesive malignant epithelial fragment in the tumor FNA.

DIAGNOSTIC ACCURACY OF OVARIAN CANCERS

An inadequate cell sample varying from 13% to 73% had been reported by some studies. According an extensive review by Cibas, the diagnostic sensitivity of malignancy in different reported series varied widely. It is low, at 26% to 40% if borderline ovarian tumors with a suspicious diagnosis were included with malignant tumors, and it ranges from 84% to 93% if those tumors are excluded from the malignant category. Both false-negative and false-positive diagnoses have been reported. A false-positive diagnosis may occur when a cellular sample from a follicular cyst shows abundant mitotic figures, and a false-negative diagnosis happens mainly in borderline ovarian neoplasms.

E. FALLOPIAN TUBE CARCINOMA

Primary fallopian tube carcinoma is a very rare neoplasm. Most patients are postmenopausal and nulliparous. Abnormal vaginal discharge or bleeding is a common clinical manifestation. Histologically, the cancer is of papillary serous type. It may shed cancer cells into the uterine cavity and these cells may be accumulated in the posterior vaginal fornix. The tumor cells commonly display features of a serous carcinoma and often have a vacuolated cytoplasm. (Fig. 9.17). Psammoma bodies may be observed.
A fallopian tube cancer should be suspected in an adult woman who shows malignant glandular cells in her Pap test and who also has a negative cone biopsy and endometrial curettage and who has no known primary cancer. Fallopian tube carcinoma may also be diagnosed cytologically by FNA.

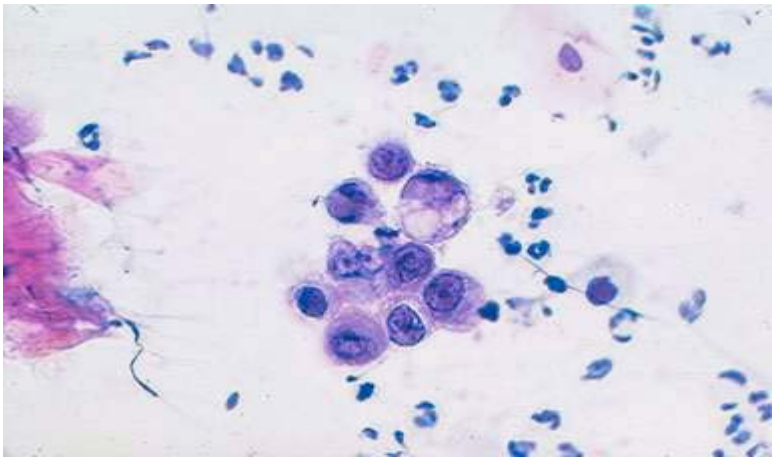

Fig. 9.17. A fallopian adenocarcinoma showing in posterior vaginal fornix smear a loose cluster of malignant epithelial cells with a moderate amount of granular cytoplasm and prominent nucleoli. A tumor cell with vacuolated cytoplasm is present.

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