A CRITICAL ANALYSIS OF THE GRADING SYSTEM AT PRESENT IN USE AT GROOTE SCHUUR HOSPITAL CYTOLOGY LABORATORY FOR ENDOCERVICAL GLANDULAR ATYPICAL CHANGES WITH RECOMMENDATIONS FOR IMPROVED CRITERIA AND TERMINOLOGY

BY

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DEDICATION

This dissertation is dedicated with thanks to my colleagues and mentors,

Drs.Richenda Fry, Anne Linder, Margaret Bull and

Joy Mc Murray

who inspired and taught me in the art of cytology.

I, Judith Whittaker, hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, or is being, or is to be submitted for a degree in this or any other University.

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22/3/93 .....

Date

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ABSTRACT.

The records of all patients on whom the cytological diagnosis of endocervical glandular atypia had been made by the Department of Anatomical Pathology, Cytopathology division, at Groote Schuur Hospital during 1987 were investigated.

The diagnosis was reviewed in all cases in which there was a complete five year follow-up. This included those cases in which the original smears, clinical records and patient details were available. A total of 407 (53.7% of the patients who had received the cytological diagnosis of endocervical glandular atypia) met these inclusion criteria.

The terminology applied to these cases was mild, moderate and severe endocervical glandular atypia (EGA) and graded for the purposes of this study into grades 1, 2, and 3 respectively. If the diagnosis of adenocarcinoma in situ (AIS) was suggested these were graded as 4.

It was found that no consistent diagnostic criteria had been applied in making the original diagnoses. Sixty seven percent of the grade 1 and grade 2 atypias regressed and the majority of the remainder did not progress. On review of the grade 1 cases, the majority showed anisonucleosis and regenerative changes only, although some of those which persisted were associated with low grades of squamous cervical intraepithelial neoplasia (CIN) and/or human papilloma virus infection (HPV) infection. There were similar findings in the grade 2 group, except that a higher proportion were associated with squamous CIN of all grades, and the majority of these persisted.

In the more severe lesions the association with squamous CIN3 was a prominent finding and this appeared to be related to a difficulty in differentiating CIN3 with gland involvment from AIS and pure endocervical glandular atypia. Histological correlation in these severe grades showed that only 15% of grade 3 were related to a glandular neoplastic or preneoplastic lesion and only 54% of grade 4 showed correlation with glandular neoplasia. Microglandular hyperplasia was misinterpreted as severe EGA/AIS in 10% of grades 3 and 4.

In view of these findings, it is recommended that in future endocervical glandular abnormalities be classified in terms of causation, if possible, and the diagnosis of mild and moderate atypia should not be made indiscriminantly. Where squamous abnormalities predominate, the appropriate diagnosis should be given, and the diagnosis of EGA or AIS should be reserved for those cases which show architectural abnormalities, as described in the literature.

ABBREVIATIONS

AIS	adenocarcinoma in situ
EGA	endocervical glandular atypia
CGA	cervical glandular atypia
EGD	endocervical glandular dysplasia
CIGN	cervical intraepithelial glandular neoplasia
CIN	cervical intraepithelial neoplasia
HPV	Human Papilloma virus
OC	oral contraceptive
depo	Depo provera (Medroxyprogesterone)
IUCD	intrauterine contraceptive device
GSH	Groote Schuur Hospital
Pap	Papanicolaou
TBS	The Bethesda System

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1. INTRODUCTION

Diagnostic cytology of the female genital tract was introduced by Papanicolaou (Papanicolaou 1928) in 1928 in New York and almost simultaneously by Babes (Babes 1928) in Bucharest. However, neither of these early publications made significant impact at that time and it was not until the 1950's when Papanicolaou published his atlas (Papanicolaou 1954) that the Pap smear became a meaningful diagnostic test. Today four decades after the publication of that atlas and several critical assessments of the value of the cytological test, it can be stated that the Pap smear must rate as one of the most effective public health tools that has ever been introduced.

Reduction of the mortality from squamous carcinoma of the cervix has been eloquently demonstrated (Boyes et al 1977, Fiddler et al 1968, Guzick 1978, Stenkvist et al 1984) and in the United States according to the American Cancer Association statistics, (American Society of Cytology 1988) death rate from cervical cancer has decreased by more than 70% in the last 40 years.

Firm criteria have been established for the cytologic, colposcopic and histologic features of the precursor lesions of squamous carcinoma of the uterine cervix despite the fact that the terminology applied to these lesions remains under dispute. The identification of the precursor lesions of adenocarcinoma of the cervix is, comparatively, in its infancy. Initial histopathologic descriptions of precursor lesions are commonly based upon observations of cytological abnormalities in non-invasive lesions at the periphery of invasive carcinoma. Further evidence for the malignant potential of such peripheral lesions must rest upon documentation of progression to frank carcinoma in at least some of the

cases (Buscema & Woodruff 1984). This, however, has not been unequivocally shown.

1.1 PRECURSOR LESIONS OF ADENOCARCINOMA OF THE CERVIX

1.1.1.ADENOCARCINOMA IN SITU

Although alluded to by Hauser in 1894 (Hauser 1894) and Meyer in 1930 (Meyer 1930), the introduction of the term "AIS" is credited to Freidell and McKay who in 1953 described two examples (Friedell & McKay 1953). Because AIS is an uncommon lesion, pathologists and cytologists may not be familiar with its microscopic appearances and it is probably under-diagnosed. Various pathologists and cytopathologists have attempted to clarify the situation with the introduction of expanded criteria (Quizelbash 1975, Christopherson et al 1979, Bousfield et al 1980, Boon et al 1981, Wells & Brown 1986, Ayer et al 1987, Betsill & Clark 1986).

AIS has a varied histology, with three main histological subtypes: (a) endocervical type, (b) endometrial type, and (c) intestinal type. Endocervical AIS, either alone or in combination with other types was the most frequent variety. Miscellaneous types including adenosquamous, and clear cell, are rarely seen (Hasumi & Ehrmann 1987).

1.1.1.1 Endocervical AIS

This is characterized by nuclear stratification, moderate amounts of juxtaluminal cytoplasm (Ill.1), scattered mitoses and apoptotic bodies. Within Papanicalaou (Pap) smears, endocervical AIS is characterized by rosettes with prominent

nuclear pseudostratification, strips and crowded sheets of cells (Coleman & Evans 1988). The nuclei are enlarged, show hyperchromasia and generally show finely to moderately granular chromatin. Macronuclei may be encountered and apoptosis and mitotic activity is universally present. Abnormal mitoses may be identified. The architectural features are of primary diagnostic importance and are tabulated (Table I). (Ayer et al 1987).

TABLE I:

ARCHITECTURAL FEATURES OF 46 SMEARS WITH AIS
(Ayer et al 1987)

Exfoliation features.	no. with feature present
Tightly crowded sheets	46
Loosely crowded sheets	0
Strips off the sheets	38
Isolated strips	40
Rosettes	36
Gland openings	41
Feathering	35

1.1.1.2 Endometriod AIS

This is characterized by marked nuclear pseudo-stratification and absent intracytoplasmic vacuoles and with Pap smears thick "chunky" strips of cells are seen with exaggerated nuclear pseudo-stratification and scanty cellular cytoplasm. (Ill 2)

1.1.1.3 Intestinal AIS:

The existence of this type of AIS was alluded to by Qizilbasch (1975) and Gloor & Ruzicka (1982) however, it was Trowell (1985), who focussed major attention to the presence of argentaffin cells and goblet cells producing sialomucin. With Pap

smears, intestinal AIS is characterized by sheets of glandular cells with abundant opaque cytoplasm with large secretory vacuoles. The nuclei tend to be larger, rounded and more pleomorphic than those usually seen in endocervical AIS. (Ill 3 & 5)

The distribution of AIS in the cervix has important diagnostic and therapeutic implications and the usual pattern is that of transformation zone involvement and there is frequently overlying metaplastic and dysplastic squamous epithelium. Some investigators indicate that AIS is focal or localized while others report that it is usually extensive (Lauchlan & Penner 1967, Gloor & Ruzicka 1982).

The question of multifocality has only been addressed in a few reports and is thought to occur in approximately 15% of cases (Ostor et al 1984, Bertrand et al 1987).

The glands of AIS are usually surrounded by a compact stroma, but in the presence of concomitant cervicitis an oedematous stroma with an inflammatory infiltrate may be present. Cytologically an inflammatory background is commonly encountered occurring in almost two-thirds of cases. A blood stained background is present in half and does not necessarily signify invasion (Jaworski et al 1988).

1.1.2 ENDOCERVICAL GLANDULAR DYSPLASIA

Endocervical glandular dysplasia (EGD) may be defined as a lesion showing cytological and architectural atypia similar to adenocarcinoma in situ (AIS) but of lesser degree (Barter & Waters 1970, Bousfield et al 1980). Uniform histological criteria have not been widely accepted for EGD, and there is not yet solid

evidence of the progression of lesser degrees of EGD or atypias to carcinoma, either *in situ* or invasive. Three proposed classifications have been published in the last few years. Each of the classifications divides the pre-invasive changes into three categories: Van Roon et al (1983) use the terms mild/moderate atypia, severe atypia, and AIS; similarly, Brown & Wells (1986) and Wells & Brown (1986) use the terms low grade cervical glandular atypia (CGA), high grade CGA and AIS; and most recently, Gloor & Hurliman 1986, coined the term cervical intra epithelial glandular neoplasia (CIGN), divided into three grades. All four papers concentrate on the morphologic description of endocervical abnormalities without presenting follow up data to document the clinical utility of the classification.

Van Roon et al (1983) examined glandular epithelium in 208 cervical specimens, including 25 cases with mild/moderate atypia, 11 with severe atypia, and 20 with AIS. The diagnostic category as based on morphometric analysis of the degree of cytological change, ranging from mild to marked nuclear enlargement with variable distortion of the mean nuclear axis. Associated architectural changes included adenomatous changes, defined as glandular crowding and pseudostratification, and intraluminal shedding of atypical cells. The higher the degree of atypia, the more cases with adenomatous changes and intraluminal shedding there were. Reserve cell hyperplasia was frequently associated with cases classified as mild atypia. The mean patient age was 39.2 years.

Brown & Wells (1986) studied glandular atypias associated with CIN III lesions because of the known association between cervical intra-epithelial neoplasia (CIN) and AIS in the cervix. Seventeen cases of cervical glandular abnormalities were found: nine cases were considered to have low grade cervical glandular atypia, seven high grade cervical glandular atypia, and one AIS. The descriptions stressed

architectural as well as cytological abnormalities, low grade atypia having only minor deviations of glandular profile with intraluminal tufting and papillae and high grade atypia having more branching and budding of glands. The cytologic features of low grade atypia included nuclear enlargement and hyperchromatism, and stratification of nuclei limited to the basal two-thirds of the epithelium. High grade atypia was described as having pleomorphic, enlarged nuclei displaying hyperchromatism with occasional vesiculation, and stratification greater than two-thirds of the epithelial height. Less than one mitotic figure was seen per gland in high grade atypia. The single case designated AIS showed even more pronounced nuclear enlargement and numerous mitoses. Architecturally, glands were generally smaller than normal and crowded back to back. Transitional areas of lesser atypia were identified in the AIS case. All lesions were multifocal and often located high in the canal. The mean age of the 16 "atypia" patients was 36.9 years, and there was no consistent correlation with menstrual cycle or endometrial abnormalities. Because patients having atypia were slightly younger than the patients with AIS in some series and because of finding atypia at the border of AIS, the authors concluded that there was evidence of neoplastic progression.

Gloor & Hurliman (1986) described 23 cases of endocervical glandular lesions, all of which had foci equivalent to *in situ* adenocarcinoma. Foci with lesser degrees of atypia were variably present in all the lesions; these foci were divided into histological grades using the term CIGN, in analogy to the term CIN applied to squamous lesions. The criteria they used are similar to commonly applied criteria in the evaluation of gastrointestinal glandular dysplasia. CIGN grade I was characterized as having slightly hyper-chromatic nuclei, basally located, without significant stratification. CIGN grade II showed some pseudo-

stratification, crowding of nuclei, and more frequent mitoses. CIGN grade III was equivalent to classically described adenocarcinoma *in situ*, with distinct nuclear hyperchromasia, nuclear stratification, loss of basal location of nuclei, and decreased mucin. Architectural distortions were not commented upon. Further, Gloor & Hurliman (1986) also subdivided the cases into types A and B, type A containing the normal mucin pattern and type B resembling intestinal goblet cells. The significance of subdividing the lesions into these types is unknown. The average age of the reported patients was 34, similar to other series of AIS.

CIN was a term which evolved through the need to provide more consistent pathological diagnoses and to couple the pathologic interpretation with clinical management decisions, whereas CIGN was created de novo, without clinico-pathologic correlation. At this time, proof that CIGN grade I progresses to CIGN grade II or III is absent. In addition, the lack of HPV in glandular atypias, which can be demonstrated in adjacent adenocarcinomas, has been taken as evidence that the development of adenocarcinoma may not evolve through a dysplastic sequence (Farnsworth et al 1989).

1.1.3 THE BETHESDA SYSTEM

Terminology is obviously a problem and there is no consensus as to what glandular atypias should be called.

The Bethesda System (Addendum I) attempts to provide uniform diagnostic terminology in cytology reporting (Vooijs 1990, Solomon 1992). Atypical glandular cells of undetermined clinical significance (AGUS) is the term TBS uses to encompass these atypical endocervical glandular lesions and this terminology is

commented on by Dianne Solomon in the Compendium of Diagnostic Cytology (Solomon 1992). These cells demonstrate changes beyond those encountered in benign reactive processes yet that are insufficient for a diagnosis of invasive adenocarcinoma. Therefore, lesions in this category include a morphologic spectrum from atypical-appearing reactive processes to adenocarcinoma *in situ* (AIS).

Atypical endocervical cells of undetermined significance may be further subclassified according to whether a reactive or a premalignant/malignant process (AIS) is favoured. Atypical endocervical cells of undetermined significance, probably reactive, demonstrate nuclear enlargement three to five times normal and slight hyperchromasia. A honeycombed pattern with distinct cell borders is often maintained. Atypical endocervical cells of undetermined significance, probably AIS, are characterized by cellular strips and rosettes demonstrating elongated, overlapping nuclei with moderately coarse chromatin and hyperchromasia. It may be difficult to differentiate SIL with gland involvement from AIS.

1.2 AETIOLOGY OF ENDOCERVICAL ADENOCARCINOMA

The risk factors for cervical adenocarcinomas seem more comparable to endometrial carcinoma than squamous carcinoma (Korhonen 1984). Estrogens, either endogenous or exogenously administered, are clearly implicated as etiologic factors in some cases of endometrial carcinoma. In parallel with the endometrium, the glandular epithelium of the endocervix is hormonally responsive. A number of epidemiologic studies have been addressed to the issue of the relationship between oral contraceptive (OC) use and cervical carcinoma; however,

most of them suffer from confounding factors such as sexual history, detection bias, and selection of contraceptive use (Taylor et al 1967, Stern 1977, Vessey et al 1983, Celentano et al 1987, Ebeling et al, 1987, Irwin et al 1988). Of the case control studies, the majority favor OC's being etiologically important, with relative risks increasing with increasing duration and early onset of use (Schwartz & Weiss 1986). Peters et al (1986) examined a population-based tumor registry for Los Angeles and found that among women under 35 years of age, there was an increase of about 8% per year of cervical adenocarcinoma between 1972 and 1982.

In the late 1960's a distinct type of endocervical glandular hyperplasia, designated by Kyriakos et al (1968) as "micro-glandular hyperplasia" was recognized as the result of oral contraceptive use. This lesion could be mistaken for well-differentiated adenocarcinoma (Jones & Silverberg 1989). Microglandular hyperplasia was seen in only a minority of oral contraceptive users, and a similar lesion had been previously described in pregnancy (Nichols & Fidler 1971). There has been no evidence that microglandular hyperplasia progresses to endocervical carcinoma.

However, there have been several reports of endocervical adenocarcinomas occurring in conjunction with the use of oral contraceptives. The most convincing study was by Dallenbach-Hellweg (1984) who reported that of 28 patients having invasive adenocarcinoma, 23 had a history of oral contraceptive usage. Microglandular hyperplasia was diagnosed prior to or coincident with carcinoma in all the women using OC's. No other authors have demonstrated as strong an association.

From epidemiological studies, a sexually transmitted infectious agent has been suspected in the etiology of cervical carcinoma. During the past decade, clinical, pathological and molecular studies have all pointed to the human papilloma virus (HPV) family of viruses as the most likely group of agents. Earlier studies stressed the importance of keratinizing epithelium as the specific target cell of HPV; however, more recent studies have documented the presence of HPV in glandular epithelium of the endocervix (Tase et al 1988, Okagaki et al 1989, Griffin et al 1991). This evidence, together with the high percentage of adenocarcinomas with concurrent squamous cervical intra-epithelial neoplasia (CIN), lend support for a common etiology of both squamous and glandular neoplasms. The bulk of the evidence links HPV type 18 to endocervical adenocarcinoma- and adenosquamous carcinomas. HPV 16 has also been demonstrated in a minority of glandular carcinomas of the endocervix. DNA sequences of HPV have been documented in *in situ* adenocarcinomas of the endocervix, but not in "atypias" of the endocervix (Tase et al 1989). A disproportionately small percentage of adenocarcinomas *in situ* contain detectable HPV 18 compared to invasive carcinomas, and it has been proposed that HPV 18 may induce a more rapid malignant transformation than do some of the other HPV types (Kurman et al 1988).

Other infectious agents may act as co-carcinogens, including herpes virus. Pertinent studies (Wentz et al 1981, Zur Hausen 1982) are primarily of squamous cervical cancers; nevertheless, the conclusions may be applicable to adenocarcinomas as well. Numerous retrospective studies show that a higher percentage of women with cervical carcinoma have antibodies to herpes virus type 2 than do matched controls. However, one large prospective study

demonstrated no difference in the rate of cervical carcinoma in patients with herpes virus type 2 and controls (Vonka et al 1984).

1.3 PSEUDONEOPLASTIC BENIGN GLANDULAR LESIONS OF THE CERVIX

In Lee et al's 1991 article, benign endocervical conditions are grouped together when compared with AIS. All of these benign conditions have lead to problems in cytological interpretations and have lead to false positive diagnosis of AIS. These benign conditions included tubal metaplasia and focal endometriosis, but the majority showed only cervicitis with varying degrees of reactive glandular hyperplasia. Other authors (Pacey et al 1988, Novotny et al 1992) have also found tubal metaplasia, endometriosis and micoglandular hyperplasia as the only histological findings in cone biopsies performed as a result of the cytological diagnosis of AIS.

Young and Clement 1991, give an overview of pseudoneoplastic glandular lesions of the uterine cervix. It is their experience that certain benign glandular lesions pose diagnostic difficulties as they may have atypical architectural and/or cytological features. These conditions are reviewed in their article and include:

1. Papillary endocervicitis
2*. Tunnel clusters
3*. Deep glands and deep Nabothian follicles
4. Microglandular hyperplasia
5*. Mesonephric hyperplasia
6. Diffuse laminar endocervical glandular hyperplasia
7. Glandular hyperplasia, not otherwise specified

8. Metaplasia (tubal, endometrial, intestinal)
9. Endometriosis
10. Arias-Stella reaction
11*. Changes secondary to extravasation of mucin
12. Infective and reactive atypias and other miscellaneous lesions

Those conditions asterisked (*) occur deep to the superficial lining epithelium of the endocervical canal and do not constitute a cytological diagnostic problem. All of the other conditions may be problematic both cytologically and histologically.

1.3.1 PAPILLARY ENDOCERVICITIS

This is perhaps the most common of the discussed lesions and there is a complex micropapillary pattern associated with chronic endocervicitis. If there is in addition reactive atypia, this may be interpreted as a neoplastic glandular lesion.

1.3.2 MICROGLANDULAR HYPERPLASIA (MGH)

This lesion was often in the past confused with adenocarcinoma until its benign nature was appreciated in 1960 (Taylor et al 1967, Kyriakos et al 1968). MGH is usually related to progesterone stimulation and occurs typically in young women (mean age 33,5 years).

Microscopically MGH is characterized by closely packed glands that usually contain mucin secretion with or without inflammatory cells. The cells lining the glands are usually low columnar and there is often subnuclear vacuolation. The nuclei are almost always round and regular but rarely may have mild or moderate

atypical features. This combination of glandular hyperplasia with on occasion nuclear atypia can give rise to problems in cytological interpretation (Young & Scully 1989).

1.3.3 DIFFUSE LAMINAR ENDOCERVICAL GLANDULAR HYPERPLASIA

This term has recently been proposed for a subset of cases of endocervical hyperplasia characterized by a diffuse distribution of closely packed endocervical glands appearing as a superficial discrete layer. The mean age was 37 with no hormonal history of note and all lesions were incidental findings. Reactive cytological atypia was seen in some cases, but significant atypia was absent. Again this combination of hyperplasia with reactive atypia may be problematic in cytological interpretation (Jones et al, in press).

1.3.4 GLANDULAR HYPERPLASIA NOT OTHERWISE SPECIFIED

It is not widely appreciated that the endocervical epithelium may be hyperplastic, sometimes floridly so, without exhibiting any of the specific aforementioned patterns.

1.3.5 METAPLASIAS

1.3.5.1 Tubal metaplasia

Tubal metaplasia of the endocervix may be misinterpreted as EGD or AIS histologically or cytologically. It is characterized by normal endocervical glands lined by ciliated cells, non-ciliated cells and peg cells as seen in the normal tubal mucosa. Tubal metaplasia occurs in as many as 31% of cervices (Suh &

Silverberg 1990). Its frequency was, as expected, related to the number of sections examined. The problem was addressed by Novotny et al (1992) who reviewed cervical smears in which a diagnosis of EGD was suggested. Retrospective review of the cytology smears and histological slides from these patients revealed tubal metaplasia in 66% of cases. Terminal bars and cilia were the most helpful features in the cytological recognition of tubal metaplasia. Generally irregular crowded sheets with frayed or feathered edges with rosettes which are characteristic of AIS are lacking. Cilia unfortunately are particularly susceptible to drying artifacts and pH changes and may be difficult to discern in improperly prepared cervical smears.

1.3.5.2 Endometrial metaplasia

Pure endometrial metaplasia is rare and is usually a component of tubal metaplasia (Ismail 1991) and the above mentioned features are noteworthy.

1.3.5.3 Intestinal metaplasia

The rarest form of benign glandular metaplasia is intestinal in type and is characterized by goblet cells and argentaffin cells. Intestinal metaplasia is seen more commonly as a type of AIS and is discussed in that section.

1.3.6 ENDOMETRIOSIS

Cervical endometriosis may be superficial (also called primary) or deep (also termed secondary) (Clement 1990). Superficial endometriosis which is of relevance cytologically, is found in approximately 1% to 2% of the reproductive age group. It is usually found in a patient with a prior procedure, such as cone biopsy, which has involved the cervix (Ismail 1991). This entity is more problematic

cytologically rather than histologically and needs to be considered when contemplating the diagnosis of AIS or EGD in a previously conized patient. The cells are, however, smaller and more regular than endocervical dysplastic cells, and a stromal component may be present.

In post cone biopsy smears lower segment endometrium may be sampled; particularly when an endocervical brush is used. The unwarey may interpret the thick sheets of glandular cells as atypical endocervical cells. The nuclei are however all completely normal.

1.3.7 ARIAS-STELLA REACTION

The Arias-Stella reaction has been documented in endocervical glands in 9% of gravid hysterectomy specimens (Schneider 1981). The cytological features are similar to those of Arias-Stella within endometrial glands. The pleomorphic hob-nailed cells may lead to problems in interpretation, but the marked pleomorphism in an otherwise normal appearing cervix without a tumour diathesis on Pap-smear, should alert the cytologist to the diagnosis, particularly if a clinical history of pregnancy is given.

1.3.8 INFECTIONS AND OTHER REACTIVE ATYPIAS

Viral endocervicitis, radiation damage and marked inflammation with reactive atypia are all pitfalls, and need to be excluded before the cytological diagnosis of EGD is given (Michael et al 1984). Atypical reparative cells are notoriously difficult to interpret on cytology smears, and careful attention needs to be applied to the morphology (Giersson et al 1977).

A last problem area is related to the presence of reserve cell hyperplasia which may accompany immature squamous metaplasia. Interpretation of these small, somewhat crowded cells may be difficult on a cervical smear and this change has variously been diagnosed as AIS, CIN_3 (small cell) and exfoliated endometrial cells (Michael et al 1984).

1.4 DIAGNOSIS OF PRECURSOR LESIONS OF ENDOCERVICAL ADENOCARCINOMA

Diagnosis remains problematic particularly as symptomatology is usually absent. Cervical cytology may be useful as a screening test but false negative rates as high as 49% have been reported (Hurt et al 1977). The incidence of false positive and negative results first depends on the quality of the material submitted, cytobrush specimens yielding more abundant material (Trimbos & Arentz 1986, Dotters et al 1988, Weitzman et al 1988), and secondarily the diagnostic accuracy of the interpreters. Colposcopy likewise has a high false negative rate because of the high endocervical or deep cleft nature of most carcinomas (Bertrand et al 1987). Lastly endocervical curettage and cervical biopsy occasionally fail to reveal carcinoma when cytology shows malignant cells and cone biopsy may be necessary.

These problems no doubt contribute to the relative increase of adenocarcinoma, particularly in first world countries (Tasker & Collins 1974). Several articles note that adenocarcinoma and adenosquamous carcinoma now constitute a significant proportion of all invasive cancers of the cervix (table II). Other

factors are of course contributory including a relative decrease of squamous carcinoma as a result of successful screening activity

TABLE II:
PROPORTION OF ADENOCARCINOMA AND ADENOSQUAMOUS CARCINOMA IN TOTAL SERIES OF INVASIVE CARCINOMA OF CERVIX

Authors	percentage.
Gallup et al 1985	9.5%
Weiss & Lucas 1986	12.8%
Tamimi & Figge 1988	15.5%
Reagan & Ng 1988	16.0%
Horowitz et al 1985	16.0%
Berek et al 1985	18.2%
Shingleton et al 1981	18.5%.
Mayer et al 1976	20.0%
Julian et al 1977	28.0%
Davis & Moon 1975	34.0%

There is a prominent association between AIS and squamous CIN (Ill 4). The percentage of cases of AIS showing CIN 1-3 varies between 47,8% and 71,4% and the AIS: CIN_3 ratio varies from 1 : 26 up to 1 : 237 (Jaworski et al 1988). A common causative factor has been postulated (Alva & Lauchlin 1975, Maier & Norris 1982).

Most authors in discussing the subject of EGD, EGA, CIGN particularly in the cytology literature do not give definite criteria but others emphasize the spectrum of changes occurring in glandular epithelium with AIS considered the most severe lesion in the spectrum (Brown & Wells 1986, Ayer et al 1987, Jaworski 1990). As glandular epithelium can show "atypical" changes which are not necessarily "dysplastic" or "pre-neoplastic", this does not allow clarity of the problem.

Jaworski et al compared histologically the cytological and architectural appearance of AIS and EGD (Jaworski RC., 1990). Table III demonstrates these differences.

TABLE III:
CYTOLOGICAL AND ARCHITECTURAL APPEARANCE OF AIS AND EGD

	EGD	AIS
Nuclear enlargement	Present	Present
Nuclear chromatin	finely to moderately granular	Moderately to coarsely granular
Nucleoli	inconspicuous	micro but may be macro or inconspicuous
Nuclear shape	Oval	Oval or irregular
Nuclear pseudostratification	Minimal	Moderate to marked
Apoptosis	Present	Present(may be prominent)
Mitoses	Occasional (<1-2/glandular grouping	Frequent (>2/glandular grouping);
Glandular budding	May be present	+/- present
Tunnel clusters	May be present	+/- present
Papillary processes	May be present	+/- present
Cribriform glands	Absent	+/- present

A similar comparison of the features of AIS and EGA has not been attempted in the cytology literature but Lee et al 1991 compares cytological and architectural features between AIS and benign conditions. (Table IV)

TABLE IV:
AIS VERSUS BENIGN CONDITIONS (Lee et al 1991)

Presence of	% of smears: AIS	Benign conditions	P
Feathering	71	0	< .001
Extreme crowding	100	75	> .001
Rosettes	87	38	.002
Cilia	4	38	.003
Predominance of N/C ratios ≥ 1/2	56	0	.004
Predominance of groups < 50 cells	58	88	.043
Mitoses	36	0	.044
Strips with polarity	82	50	.045

1.5 GROOTE SCHUUR HOSPITAL STATISTICS

In recent years at Groote Schuur Hospital Cytology Department (GSH) there has been an increase in the number of so-called mild endocervical glandular atypias (EGA's) (see Table V). They have increased from 0,9% of the total number of Pap-smears screened in 1987 to 1,9% of the total in 1991. Whilst the total percentage of atypical smears has remained constant (4,4% in 1987 to 4,5% in 1991) the total number of EGA's within this group has increased from 25,3% in 1987 to 48,6% in 1991 and the only grade which has shown an increase has been the grade 1 or so-called mild endocervical glandular atypias. The other grades have remained relatively constant as a percentage of the total smears and total atypical smears.

The terminology used at GSH for endocervical glandular changes which do not fulfill the criteria for AIS is endocervical glandular atypia, which is graded as mild, moderate and severe. Where cytological features as mentioned earlier, suggest AIS, this diagnosis is given either suspicious of AIS or diagnostic of AIS, depending on the degree of certainty. For the purposes of this study these mild, moderate and severe endocervical atypias will be called Grade 1, 2, 3 EGA respectively and a diagnosis suspicious of AIS will be called Grade 4 EGA.

TABLE V:
GROOTE SCHUUR STATISTICS 1987-1991 SHOWING GRADES OF EGA

	1987	%	1988	%	1989	%	1990	%	1991	%
Grade 1 (Mild EGA)	592	78.1* 0.9◆ 19.7#	1102	79.2* 1.5◆ 28.0#	1335	81.4* 1.6◆ 28.9#	1147	86.4* 1.6◆ 27.4#	1381	85.0* 1.9◆ 41.3#
Grade 2 (Mod. EGA)	126	17.2* 0.2◆ 4.3#	223	16.0* 0.3◆ 5.7#	248	15.1* 0.3◆ 5.4#	164	12.4* 0.2◆ 3.9#	215	13.2* 0.2◆ 6.4#
Grade 3 (Severe EGA)	24	2.6* 0.0◆ 0.7#	47	3.4* 0.1◆ 1.2#	45	2.7* 0.1◆ 1.0#	12	0.9* 0.0◆ 0.3#	25	1.5* 0.0◆ 0.7#
Grade 4 (AIS)	11	1.5* 0.0◆ 0.4#	12	0.9* 0.0◆ 0.3#	2	0.1* 0.0◆ 0.0#	1	0.1* 0.0◆ 0.0#	1	0.1* 0.0◆ 0.0#
ADENOCA	5	0.7* 0.0◆ 0.2#	7	0.5* 0.0◆ 0.2#	11	0.7* 0.0◆ 0.2#	3	0.2* 0.0◆ 0.1#	3	0.2* 0.0◆ 0.1#
T.ENDO ATYPIA	758	1.1◆ 25.3#	1391	1.9◆ 35.4#	1641	2.0◆ 35.5#	1327	1.8◆ 31.7#	1625	2.2◆ 48.6#
T.ATYP	2998	4.4◆	3934	5.3◆	4622	5.5◆	4191	5.7◆	3342	4.5◆
TOTAL	67506		74364		83606		73811		74007	

Legend:
* % of total EGA,
◆ % of total smears,
% of total atypical smears.

The 100% increase in the proportion of grade 1 EGA in this five year period is of great interest because, it occurred in the absence of any similar increase in the higher grades. This has important workload implications as well as important implications for the patient. The aim of the study was therefore to analyse and appraise the grading system used by the Cytology Department of Groote Schuur Hospital in categorising endocervical atypical changes and to determine, if possible any factors which may be related to the development of these changes.

2. METHODOLOGY

All patient's with recorded atypical endocervical cells on Pap smears processed by the Cytology Laboratory at Groote Schuur Hospital in 1987 were investigated.

2.1 SELECTION CRITERIA

- Computerised patient records based on the SNOMED coding system were available. All cases designated mild, moderate, severe endocervical atypia and AIS were extracted using this system.

- Patient's initial and subsequent Pap smear request and report forms were manually drawn from the Record Files.

  These records included :

  - The original cytological examination request form (appendix 1) containing details of the patients age, LMP, exogenous hormonal treatment, contraceptive method, macroscopic appearance of the cervix including presence or absence of an ectropion.
  - The original cytological report for the above.
  - Follow-up details with copies of all subsequent request and report forms.

  In those cases where all the above details were available the original Pap smear was drawn from the slide file.

2.2 COLLECTION OF DATA

2.2.1 CLINICAL DATA

Clinical data was obtained retrospectively from the original cytology request forms. The following information was extracted:

the patient's name, age, last menstrual period, whether pregnant, whether the patient was post-menopausal, whether an erosion was present, contraceptive use, exogenous hormone use. This data was recorded on a specifically designed data collection form (appendix 2).

2.2.2 CYTOLOGICAL DATA

The initial grade of endocervical atypia as assessed by the GSH screening cytologist was obtained from the patient's original cytology report and recorded on the Clinical and Follow-up Data Collection Form.

Original smears were rescreened without consulting the records and the following characteristics were assessed (see appendix 3):

2.2.2.1 Architectural features of endocervical cells:

1.1. Quantity as determined by the number of groups of cells per slide.

1.2. Presence of extreme crowding, as determined by the degree of nuclear overlapping within groups.

1.3. The approximate number of cells per group, recording those where the majority of groups contained > 50 cells (Ill 6).

1.4. The presence of feathering of nuclei along the edge of the groups (Ill 6, 7, 10).

1.5. The presence of loss of polarity in strips.(Ill 11)

1.6. The presence of rosettes (Ill 12, 13).

2.2.2.2 Cytological and nuclear features of endocervical cells (Ill 14 - 21).

2.1 Anisonucleosis as determined by variation in nuclear size and shape.

2.2 Nuclear\cytoplasmic ratio greater than 0.5.

2.3 Thickness of nuclear membrane, recording those cases where a thickened nuclear membrane was present in most of the atypical cells.

2.4 Appearance of chromatin, recording if the chromatin was even, dense, or coarse.

2.5 Appearance of cytoplasm, recording the presence of foamy, vacuolated or homogeneous cytoplasm.

2.6 Presence and appearance of nucleoli, recording if in the majority of endocervical cells nucleoli were absent, micro or macro.(Illustrations..)

2.7 Absence or presence of mitoses.

2.8 Absence or presence of apoptosis.

2.2.2.3 Background features

3.1 The presence of blood covering the smear.

3.2 The presence of inflammatory exudate.

3.3 The presence of pathogens, indicating if candida, trichomonas, gardnerella, herpes, or chlamydia were seen (Ill 22 - 25).

2.2.2.4 Features of squamous cells (Ill 26 - 31).

4.1 Indicating if the squamous cells were atypical.

4.2 If atypical if there were changes to suggest CIN1, CIN2, CIN3, HPV or squamous carcinoma.

4.3 If there were atypical regenerative or inflammatory changes present.

If there were any other features of note, these were recorded separately. The presence of exfoliated endometrial cells was included under this category of miscellaneous abnormalities (Ill 32, 33).

This data was recorded on a specifically developed data collection form (appendix 3).

2.2.3 FOLLOW-UP DATA

Manual Cytology Department record files containing patient's initial and subsequent Pap smears request and report forms were perused. The outcome of the EGA's at the end of the five year follow-up period was assessed in terms of the following criteria:

1. Lost to follow-up.
2. Regressed on subsequent smears.
3. Persisted as the same grade of atypia.
4. Deteriorated to a higher grade.

An atypia was regarded as having:

Regressed when subsequent pap smears during the five year follow up period revert to normal without further deterioration to significant atypia.

Persisted when on subsequent pap smears, during the five year follow-up period, the same grade of EGA was recorded without evidence of either regression to normal or deterioration to a higher grade of EGA.

Deteriorated when on subsequent pap smears during the five year follow-up period a higher grade of EGA was recorded. Assessment of deterioration in the case of grade 3 EGA necessitated subsequent recording during the follow up period of grade 4 EGA(AIS). In the case of grade 4 EGA deterioration implies subsequent recording of an invasive lesion cytologically. The majority however, of these grade 3 and 4 EGA's where not followed up cytologically but where investigated colposcopically and histologically. This investigation did not mean the patient was lost to follow up and these patients where entered in the" persisted" group.

This data was recorded on the Clinical and Follow-up Data Collection Form.

2.2.4 COLLECTION OF HISTOLOGICAL DATA

If a cervical biopsy, fractional curettage, cone-biopsy or hysterectomy had been performed on the patient during the five year follow-up period, the histological diagnosis given on these specimens was recorded on the Clinical and Follow-up Data Collection Form.

2.3 COLLATION AND ANALYSIS OF DATA.

A specially designed computer programme was written in DBase4 to collate and analyse the data captured. The Epistat computer package was used to perform statistical testing as required.

3. RESULTS

3.1 SAMPLE AND GRADING.

There were 758 patients with atypical endocervical glandular cells present on their pap smears during 1987. Five of these cases were invasive adenocarcinomas, and were excluded for this reason from the study. Of the remaining 753 patients, 407 (53,7%) patients met the selection criteria. Table VI shows the reasons why the remaining patients (346) could not be included in the study sample.

TABLE VI:

REASONS WHY PATIENTS WERE NOT INCLUDED IN STUDY SAMPLE

Reason for exclusion	number
Original request form lost	174
Original request form incomplete	138
Original slides lost	34
Total not meeting inclusion criteria	346

Table VII shows the grading of endocervical atypias given to patients' Pap smears by the GSH cytologists in the total and sample populations.

TABLE VII:

GRADING OF PAP SMEARS IN TOTAL AND SAMPLE POPULATIONS

Grade	Total pop.		Study pop.		Excluded pop.	
	Number	%	Number	%	Number	%
1	592	78.1	304	74.7	288	83.2
2	126	17.2	68	16.7	58	16.8
3	24	2.6	24	5.9	0	0
4	11	1.9	11	2.7	0	0
Total	753	100	407	100	346	100

Table VIII shows the age range and grading of patients who met the admission criteria.

TABLE VIII:

NUMBER OF PATIENTS BY GRADE OF ENDOCERVICAL ATYPIA AND AGE.

Age range (Years):	<20	20-29	30-39	40-49	50-59	60+	Total	% of Total
Grade:	Patient numbers:							
1	46	117	86	27	18	10	304	74.7%
2	3	35	20	3	6	1	68	16.7%
3	1	10	9	2	1	1	24	5.9%
4	0	3	4	1	2	1	11	2.7%
Total	50	165	119	33	27	13	407	100%
% of total:	12.2%	40.5%	29.2%	8.1%	6.6%	3.2%	100%	

3.2 FIVE YEAR FOLLOW-UP OF PATIENTS

One hundred and twenty four patients (30.5%) did not return for follow up during the five year period from 1987 and were deemed lost to follow up. Table IX shows the percentage in each grade which were lost to follow up.

The remaining 283 (69.5%) who were followed up were evaluated according to the outcome criteria shown in Table X and Figure 1.

TABLE IX:

FOLLOW-UP OF SMEARS.

	Total smears	Lost to fol.-up	Followed up
Grade 1			
Number	304	108	196
%		35.5	64.5
Grade 2			
Number	68	13	55
%		19.1	80.9
Grade 3			
Number	24	3	21
%		12.5	87.5
Grade 4			
Number	11	0	11
%		0	100
Total	407	124	283
% of Total		30.5	69.5

TABLE X:

OUTCOME MEASURED IN TERMS OF PERSISTENCE, REGRESSION AND DETERIORATION

	Followed up	Persist	Regress	Deter.
Grade 1				
Number	196	51	145	0
%		26.0	74.0	0
Grade 2				
Number	55	31	22	2
%		56.4	40.0	3.6
Grade 3				
Number	21	14	3	4
%		66.7	14.3	19.0
Grade 4				
Number	11	10	0	1
%		90.9	0	9.1
Total	283	106	170	7
% of Total		37.5	60.1	2.5

3.2.1 FACTORS INFLUENCING OUTCOME

Some of the factors which may have influenced outcome were assessed, including associated atypical features of squamous cells (Fig. 2 and Tables XI & XII).

TABLE XI:

ASSOCIATED ATYPICAL FEATURES OF SQUAMOUS CELLS RELATED TO PERSISTENT ENDOCERVICAL GLANDULAR ATYPIA

Grade	Total persisting in grade	% of persisting lesions in category CIN1	CIN2	CIN3	HPV	REGEN	Total squam les.
1	51	15.7%	2.0%	0.0%	39.2%	19.6%	39
2	31	3.2%	32.3%	35.5%	16.1%	6.5%	29
3	14	0.0%	7.1%	71.4%	0.0%	7.1%	12
4	10	0.0%	0.0%	3.0%	0.0%	0.0%	3

TABLE XII:

ASSOCIATED ATYPICAL FEATURES OF SQUAMOUS CELLS RELATED TO REGRESSING ENDOCERVICAL GLANDULAR ATYPIA

Grade	Total regressing in grade	% of persisting lesions in category CIN1	CIN2	CIN3	HPV	REGEN	Total squam les.
1	145	0.0%	0.0%	0.0%	8.9%	26.9%	52
2	22	9.0%	0.0%	0.0%	13.6%	27.0%	11
3	3	0.0%	0.0%	0.0%	0.0%	66.7%	2
4	0	0.0%	0.0%	0.0%	0.0%	0.0%	0

3.3 HISTOLOGICAL CORRELATION

The percentage in each grade in which histological correlation was available is recorded in Table XIII.

The histological diagnosis given in each of these cases is recorded in Table XIV.

TABLE XIII:

HISTOLOGICAL CORRELATION OF EGA'S

Grade	Number of patients with follow-up cervical histology	% of total within grade.
1	8	3%
2	23	34%
3	20	83%
4	11	100%
Total	62	15.5% (of total sample of 407)

TABLE XIV:

HISTOLOGICAL CORRELATION OF ALL GRADES OF EGA

Histological diagnosis	Grade 1		Grade 2		Grade 3		Grade 4	
	No.	%	No.	%	No.	%	No.	%
With or without HPV:								
CIN 1	0		0		0		0	
CIN 2	0		5	22%	1	5%	0	
CIN 3	0		10	44%	10	50%	2	18%
HPV without CIN	5	63%	5	23%	1	5%	0	
Squamous Carcinoma	0		1	4%	1	5%	0	
AIS	0		0		1	5%	4	36%
Adenocarcinoma	0		0		1	5%	2	18%
EGD	0		1	4%	1	5%	0	
MGH	0		1	4%	0		3	27%
Endocervical polyp	1	13%	0		1	5%	0	
Cervicitis	0		0		1	5%	0	
No abnormality	2	25%	0		2	10%	0	
TOTAL	8	100%	23	100%	20	100%	11	100%

3.4 THE CYTOLOGICAL RESULTS

The cytological aspects of the data collection form were analysed and Figures 2 to 10 demonstrate these results as well as Tables XV and XVI.

TABLE XV:
MITOSES AND APOPTOSIS

Grade	Mitosis		apoptosis	
	No.	%	No.	%
1	0	0	0	0
2	0	0	0	0
3	3	12.5%	1	4.2%
4	4	36.4%	3	27.3%

TABLE XVI:
INTERPRETATION OF ENDOMETRIAL CELLS ON CERVICAL SMEARS

Grade	Endometrial cells no.	Interpreted as ECA no.	%
1	18	10	55.5
2	2	2	100
3	0	0	0
4	0	0	0

Figure 1: Five year follow-up of patients with EGA's.

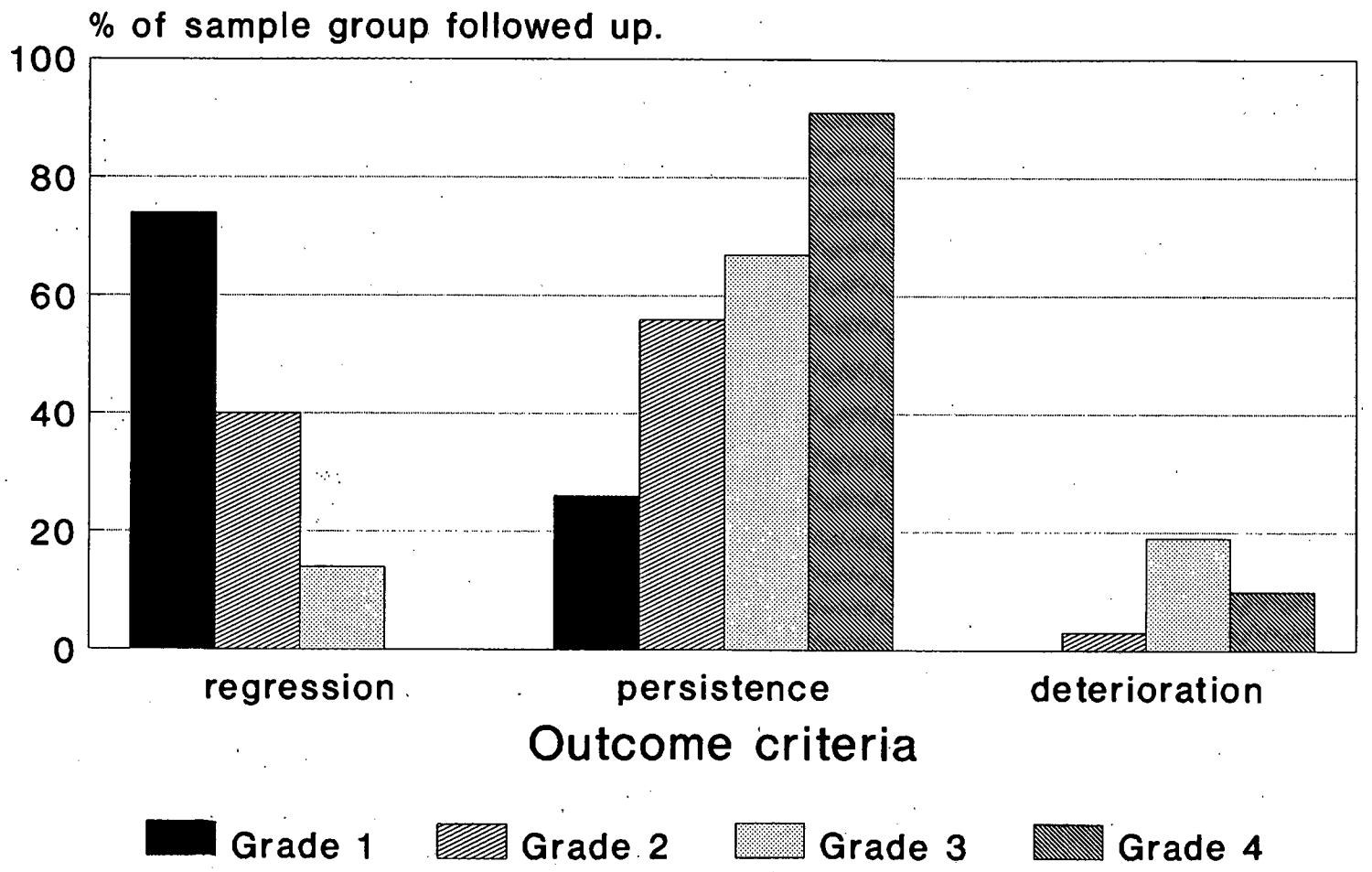


Figure 2: Features of squamous cells by endocervical grade.

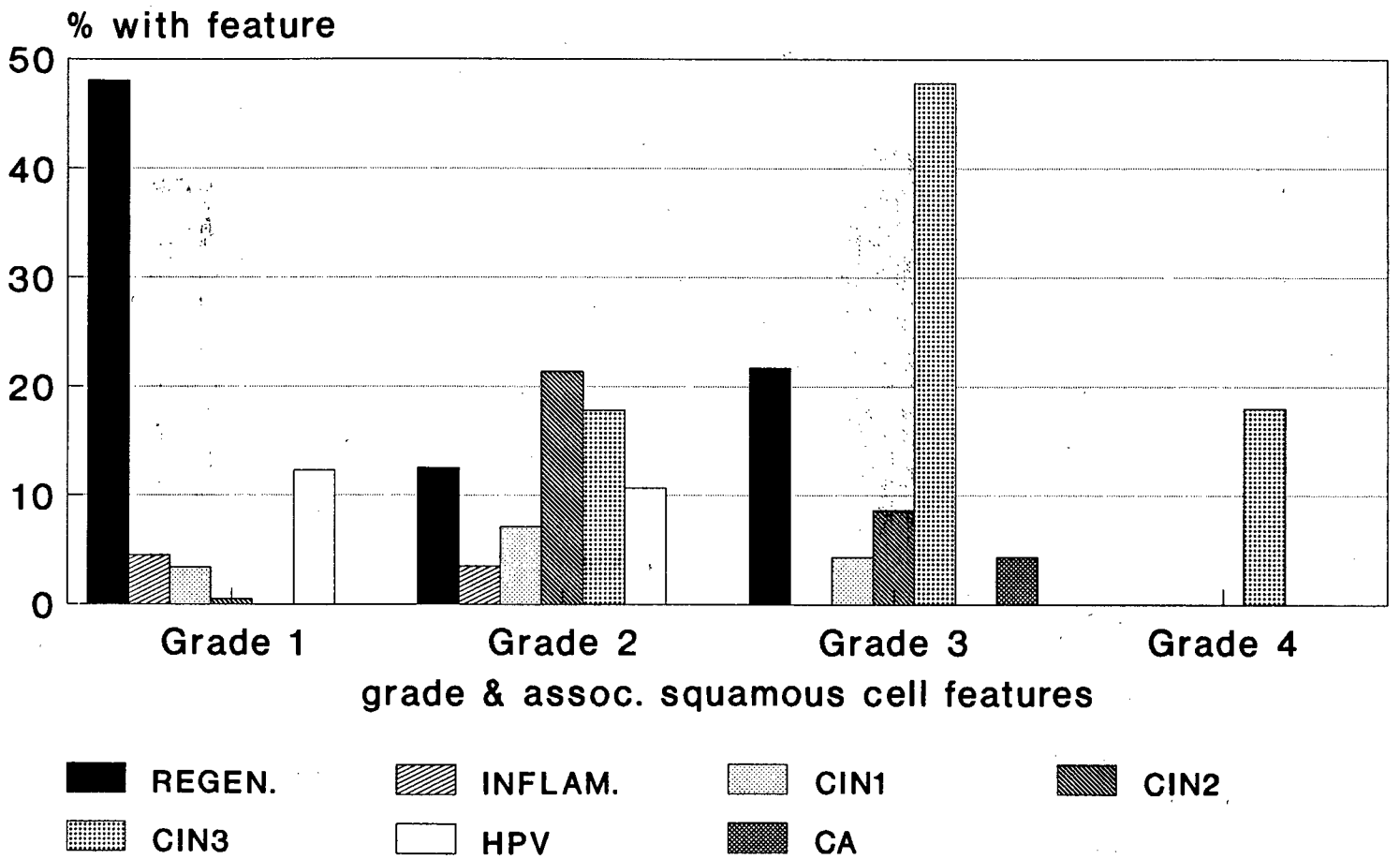


Figure 3:Factors which may be associated with development of endocervical atypias

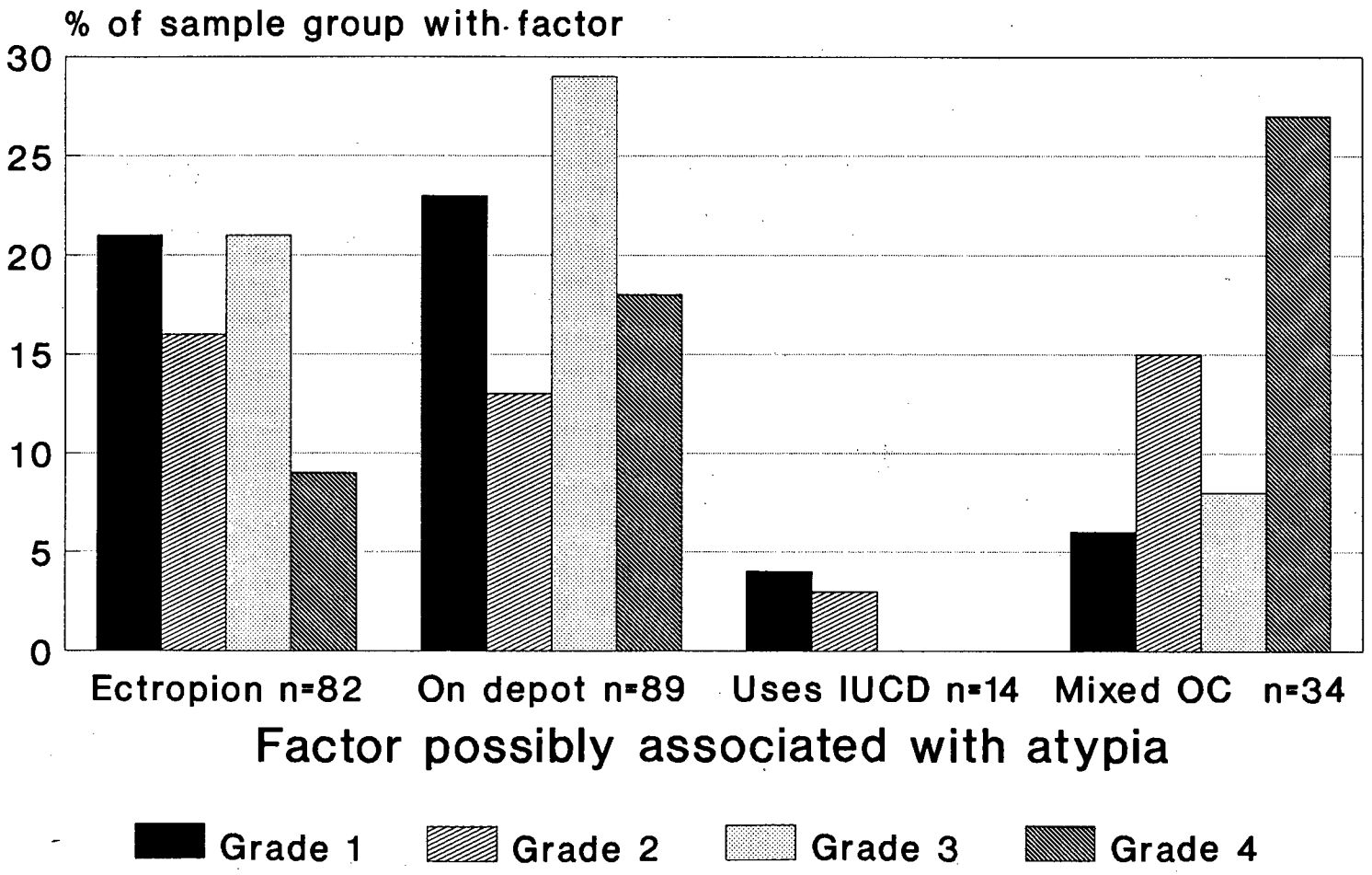


Figure 4:
Types of organism by grade

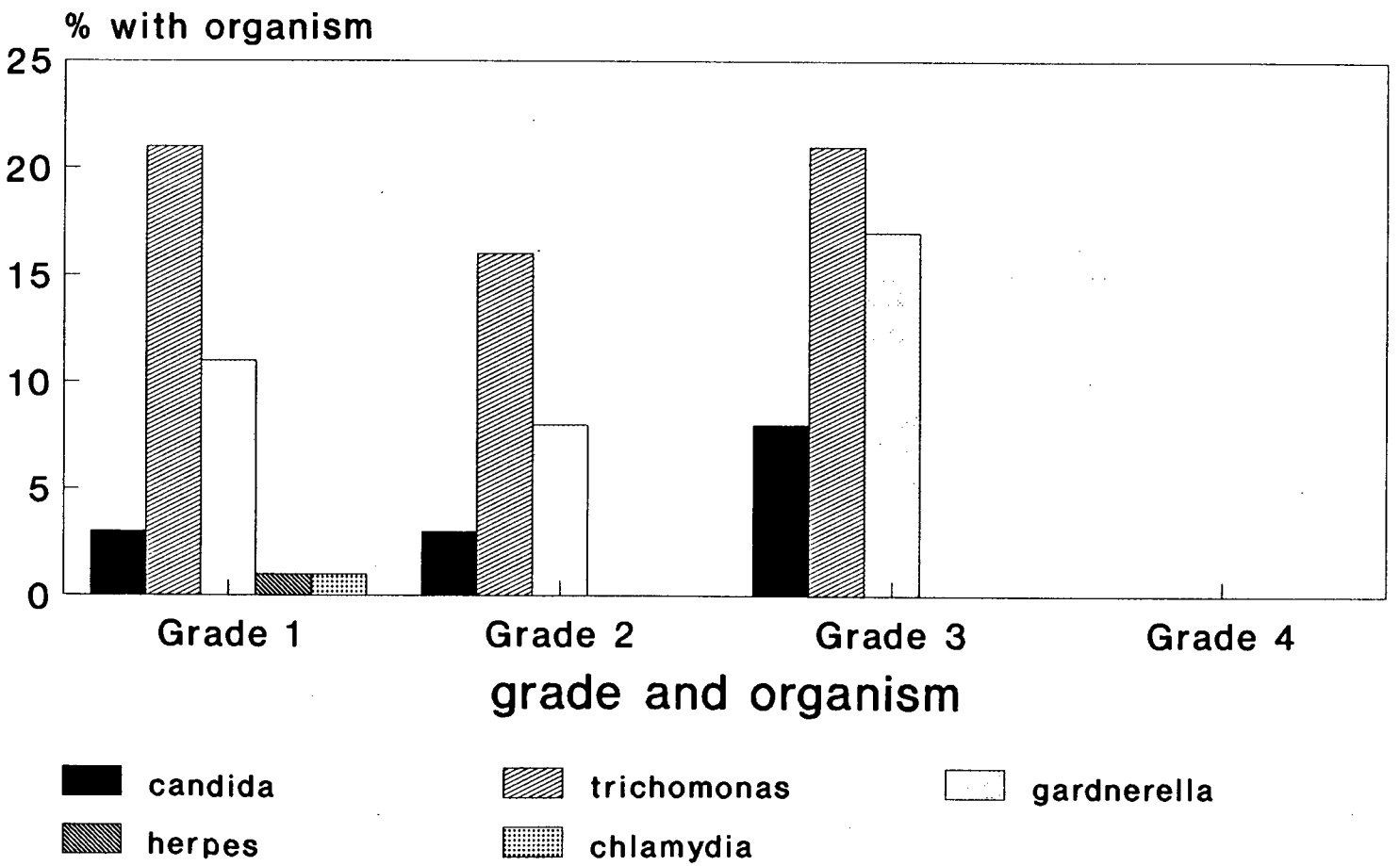


Fig. 5: Architectural features of endocervical groups

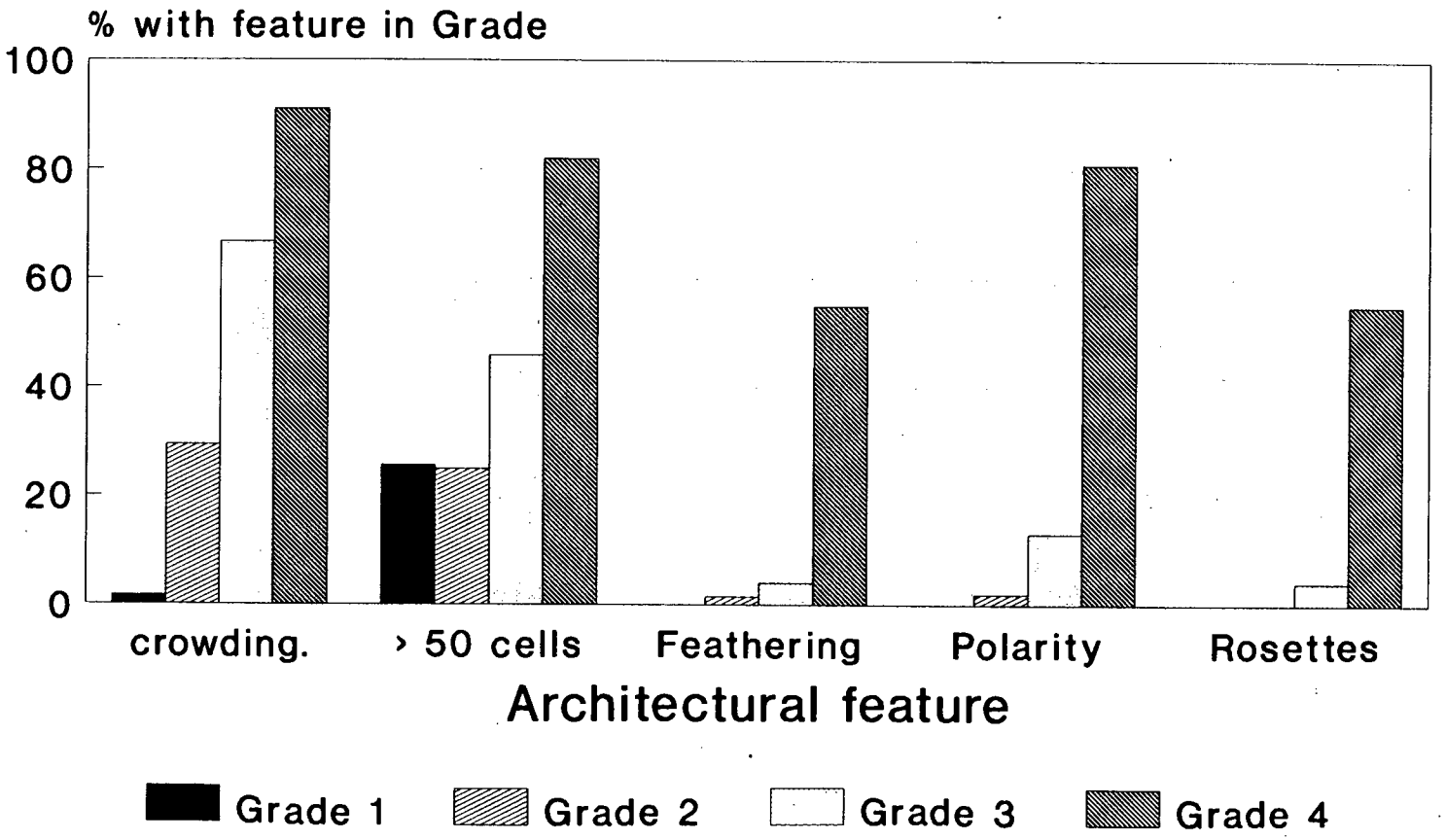


Figure 6:
Accumulative architectural features.

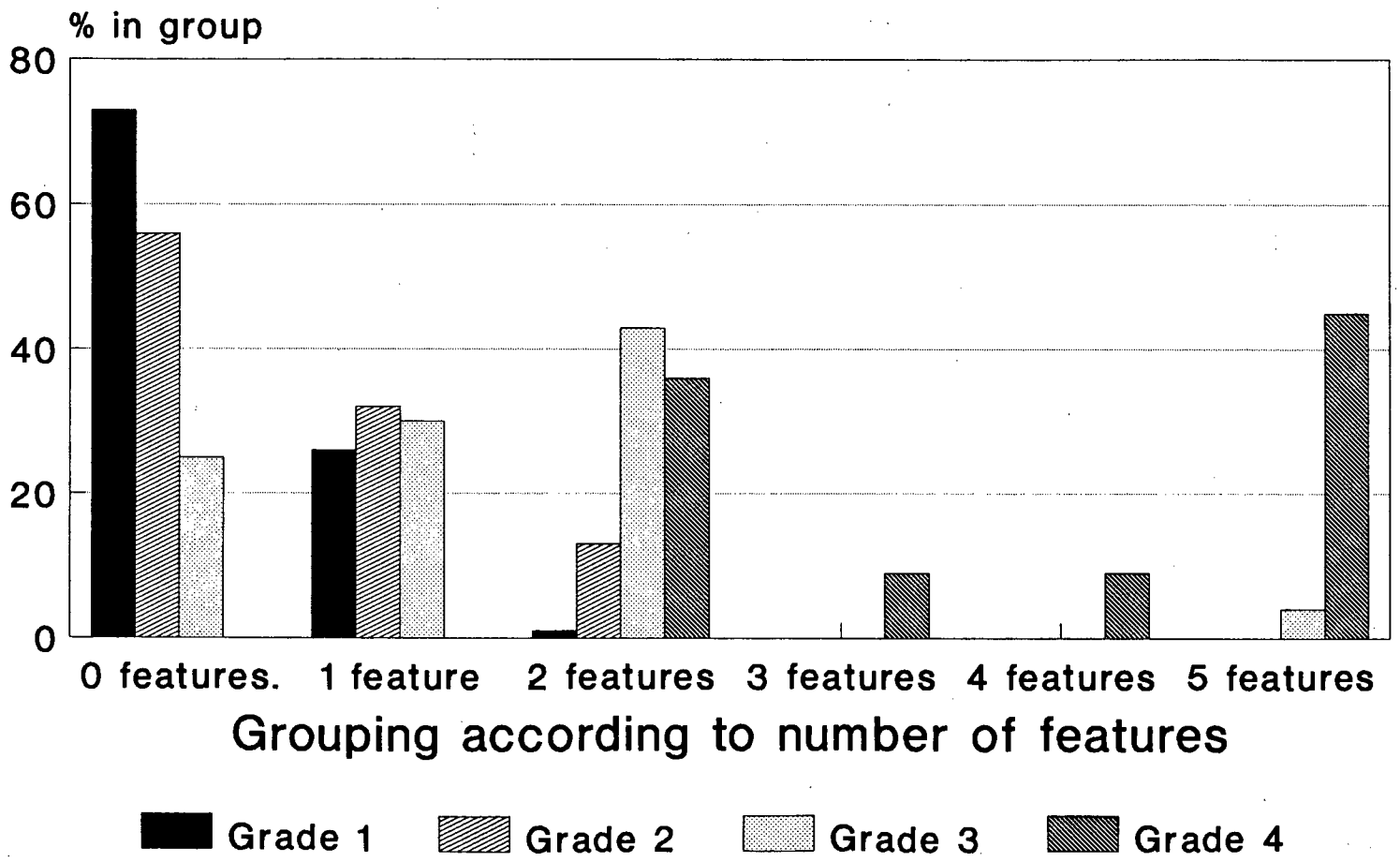


Figure 7: Cytological & nuclear features of endocervical cells

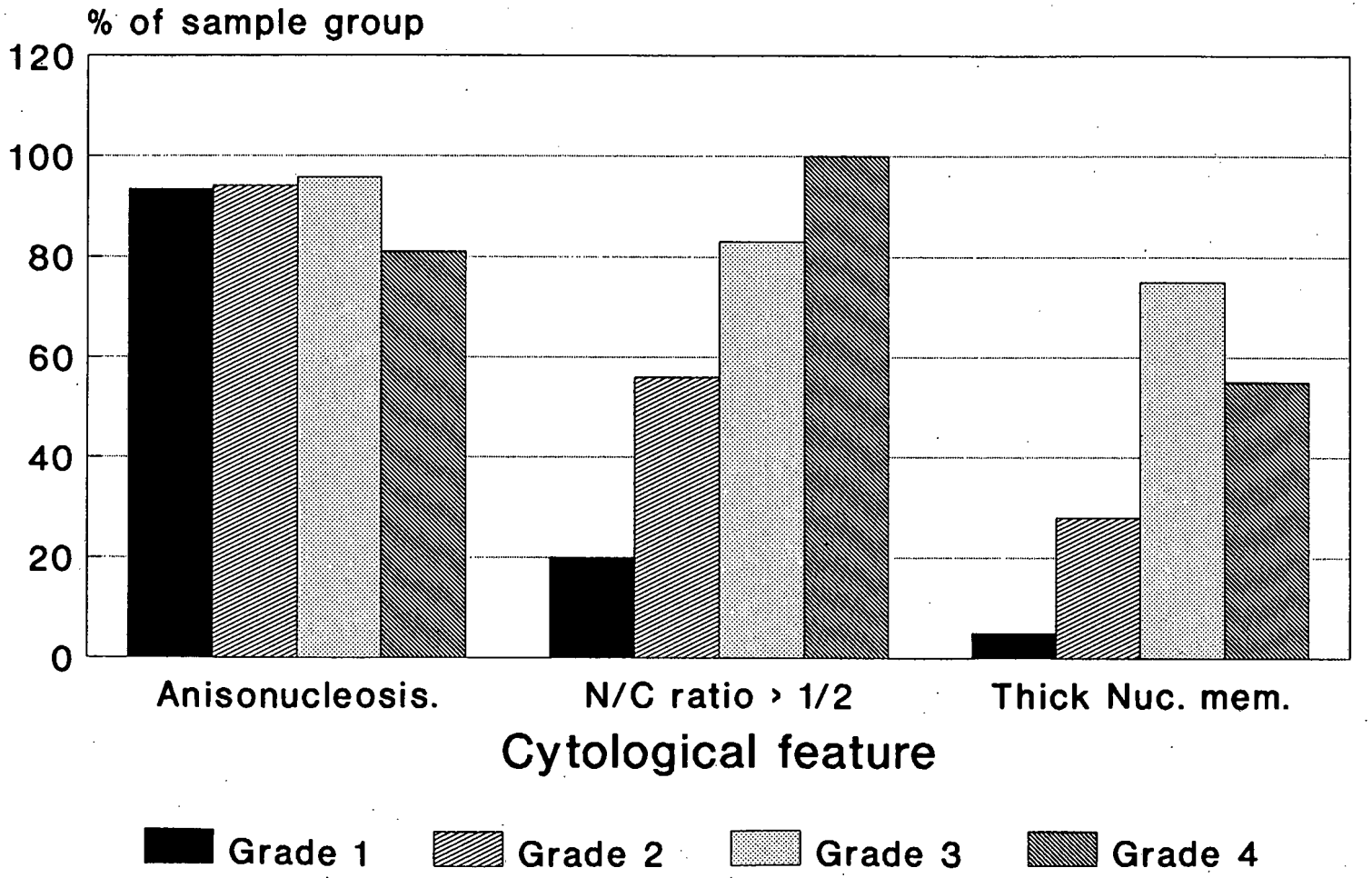


Figure 8: Cytological & nuclear features of endocervical cells

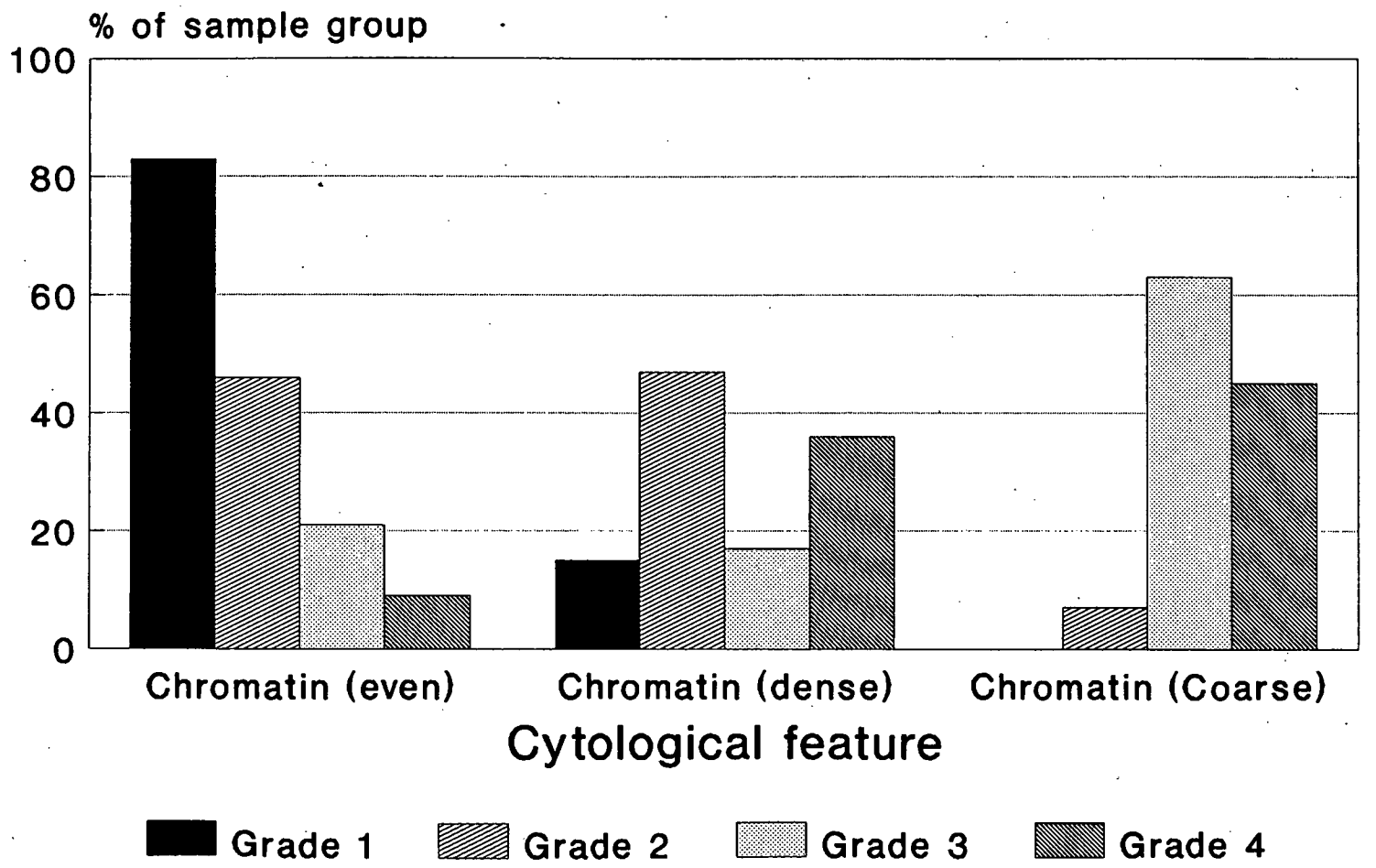


Figure 9: Cytological & nuclear features of endocervical cells

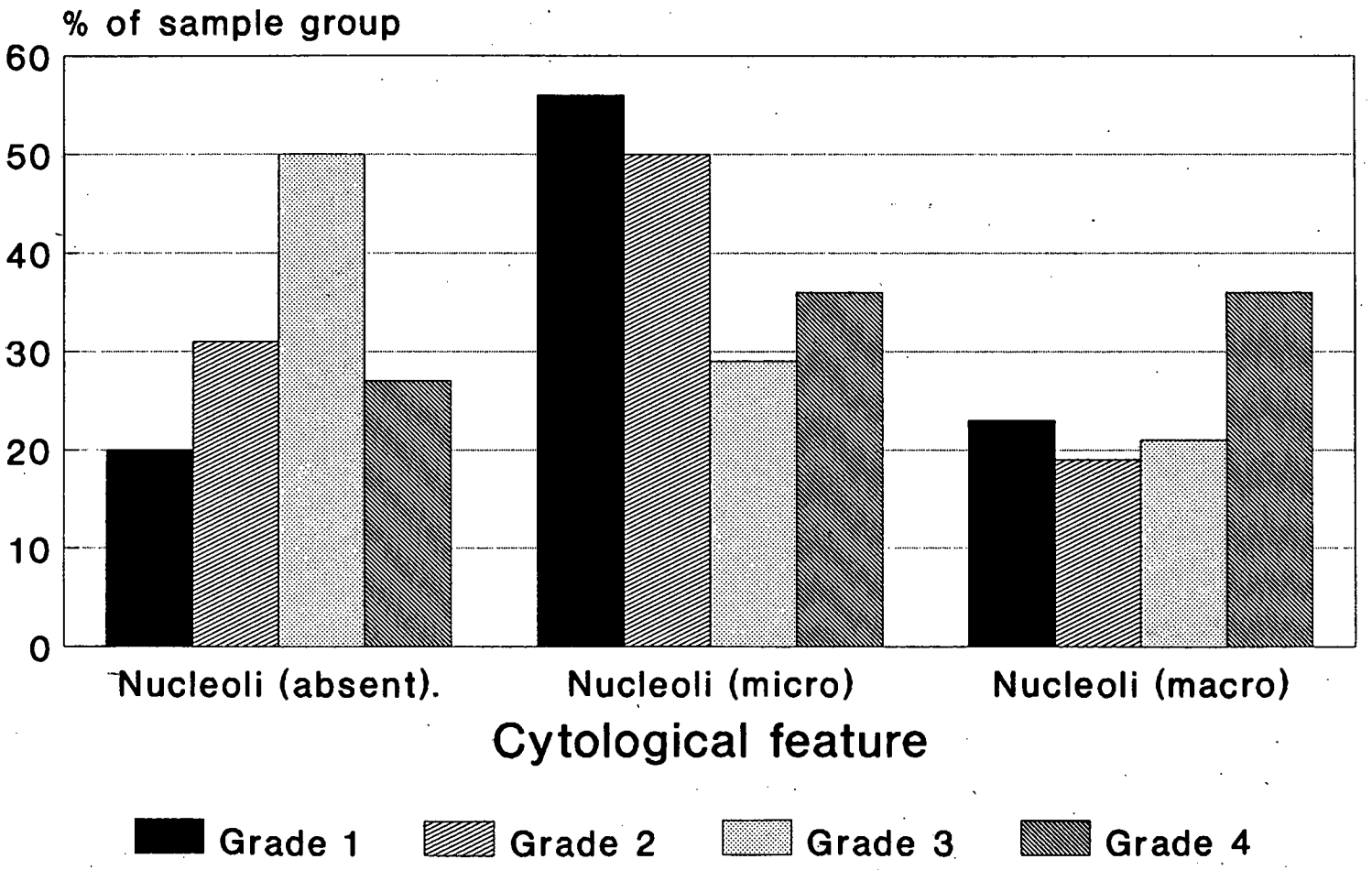
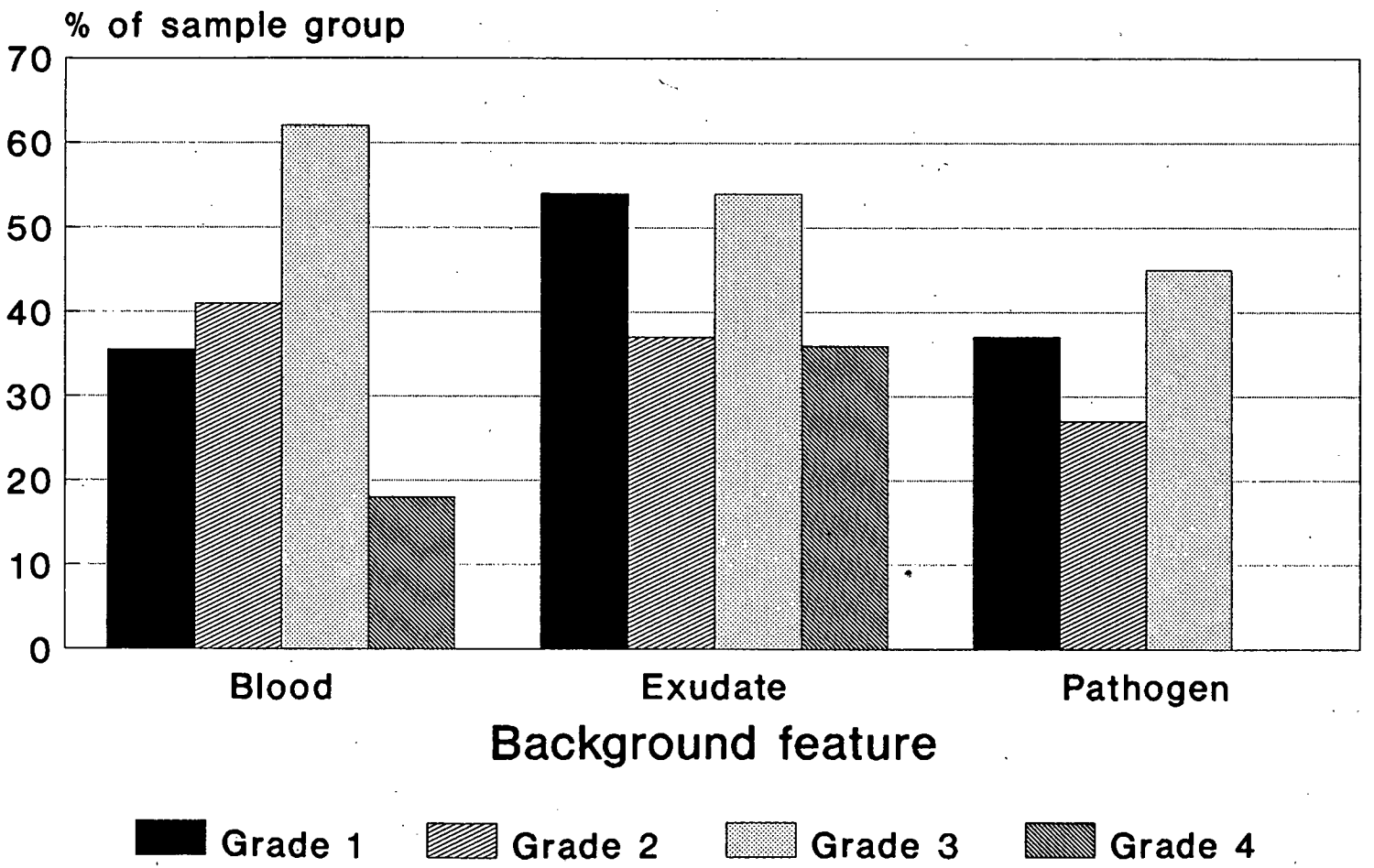
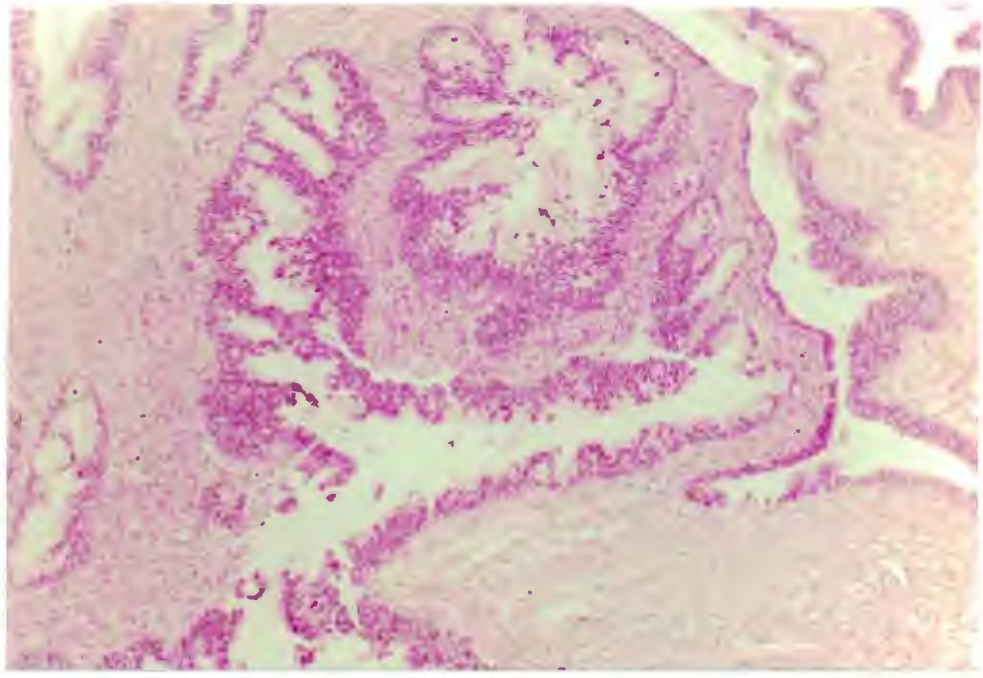


Figure 10:
Background features of smear

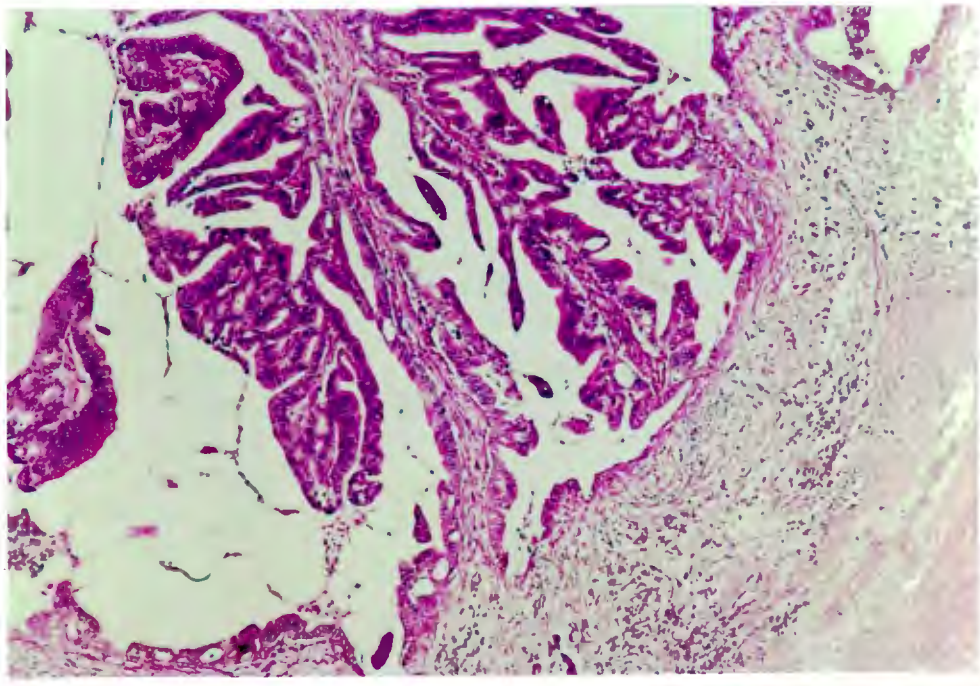


4. ILLUSTRATIONS

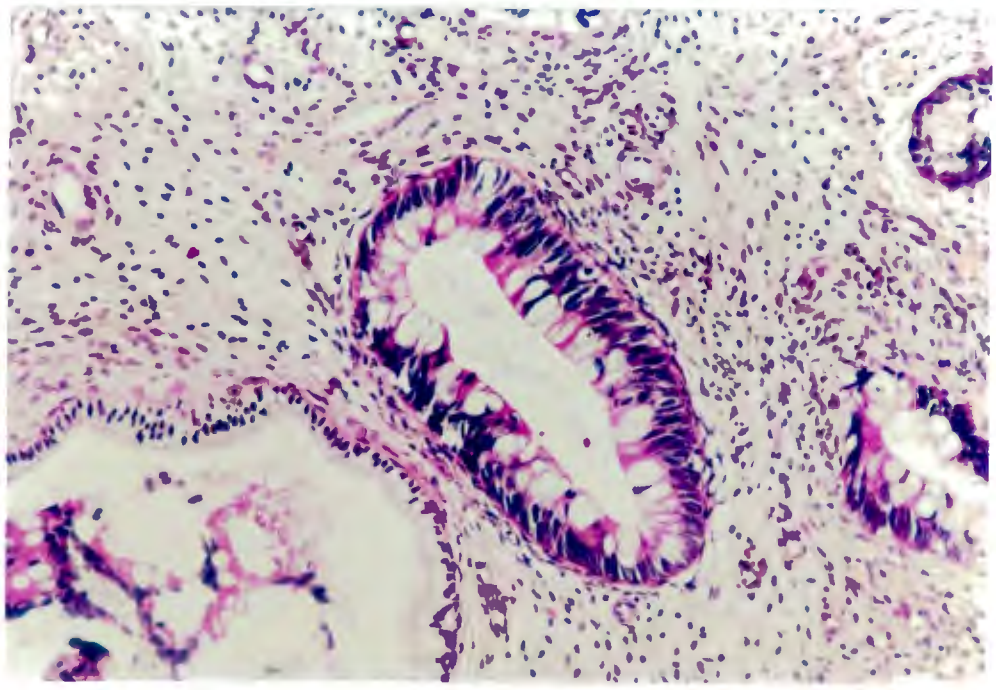
ILLUSTRATIONS



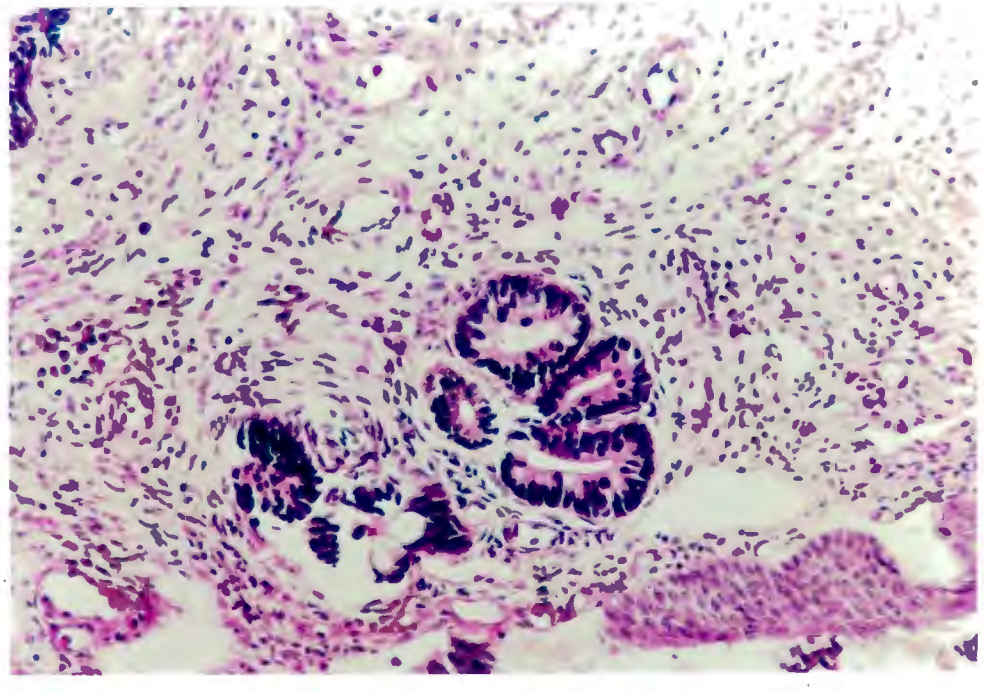
Ill 1: Endocervical AIS characterized by nuclear stratification and glandular budding histologically (H & E, X 200)



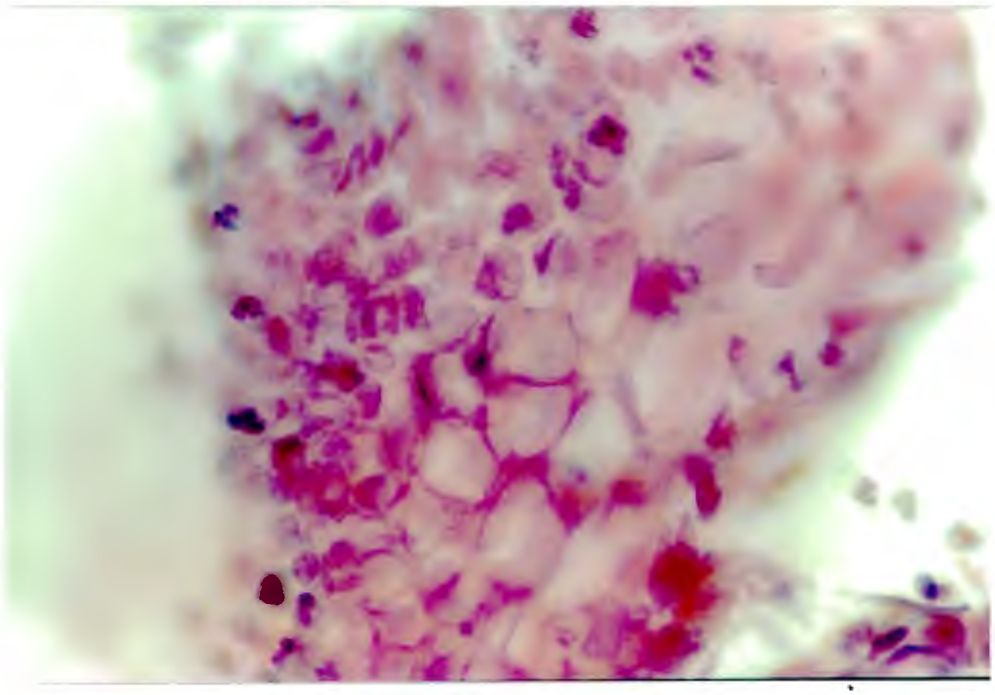
Ill 2: Endometroid AIS characterized by marked nuclear stratification and lack of juxtaluminal cytoplasm histologically (H & E, x 200)



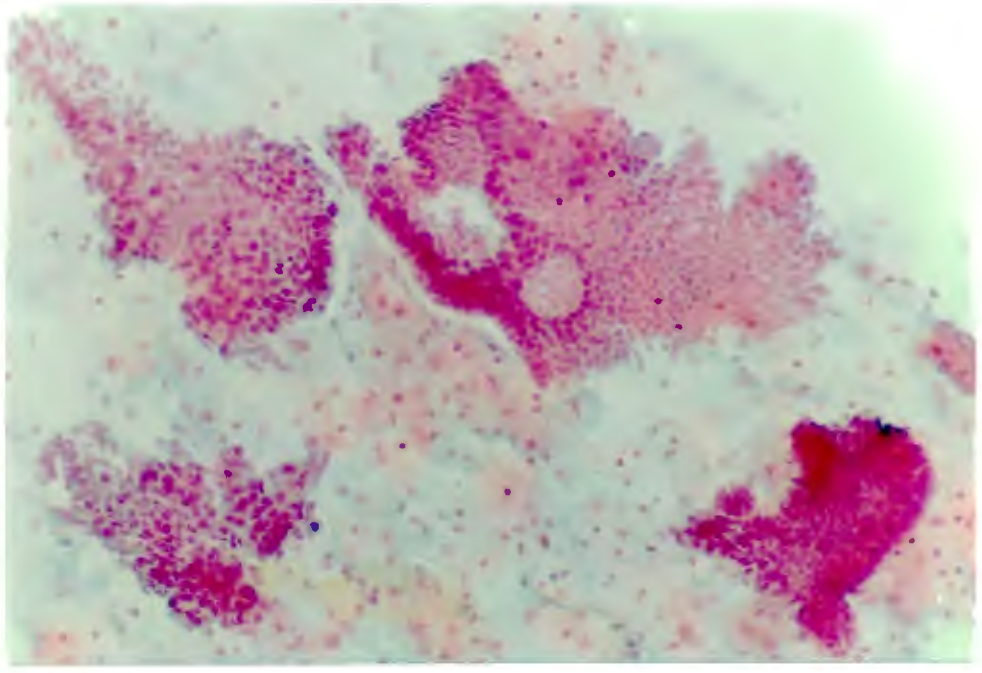
Ill 3: Intestinal AIS showing abundant goblet cell metaplasia with nuclear stratification. A normal endocervical gland is present at the left corner (H & E, x 400)



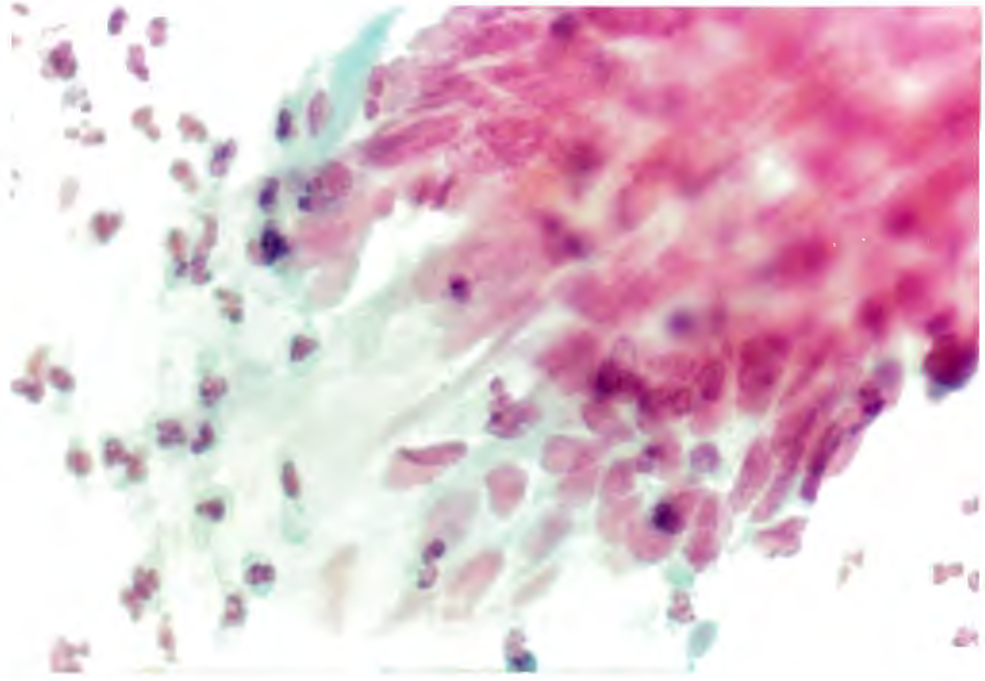
Ill 4: The presence of concommitant CIN_3 with AIS is noted at the right corner (H & E, x400)



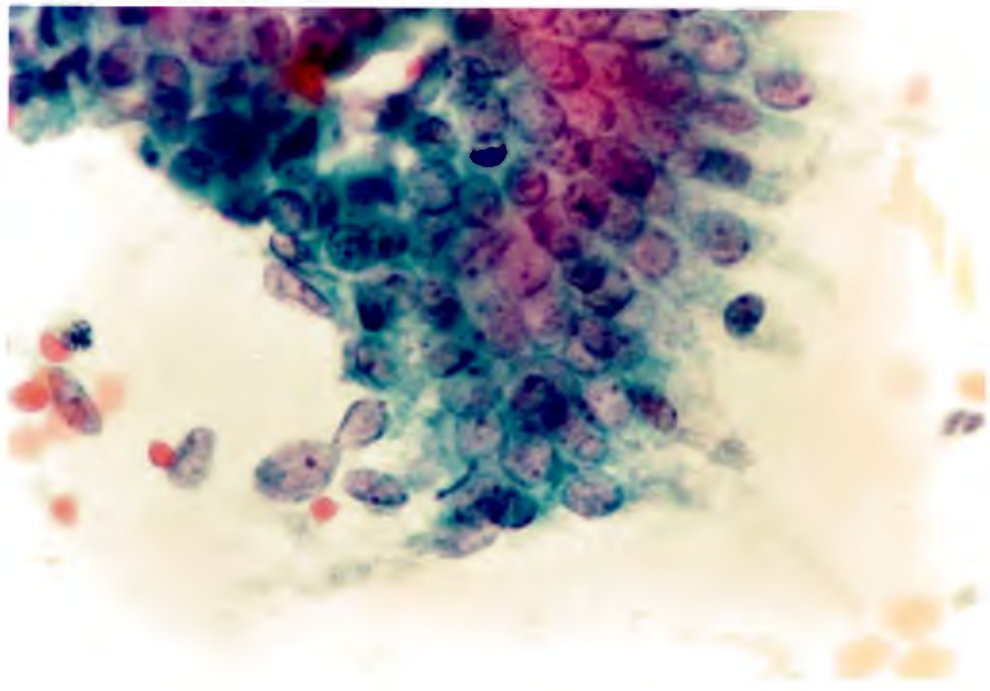
Ill 5: Goblet cells can be seen in this crowded sheet of glandular cells depicting intestinal AIS. (Pap x800)



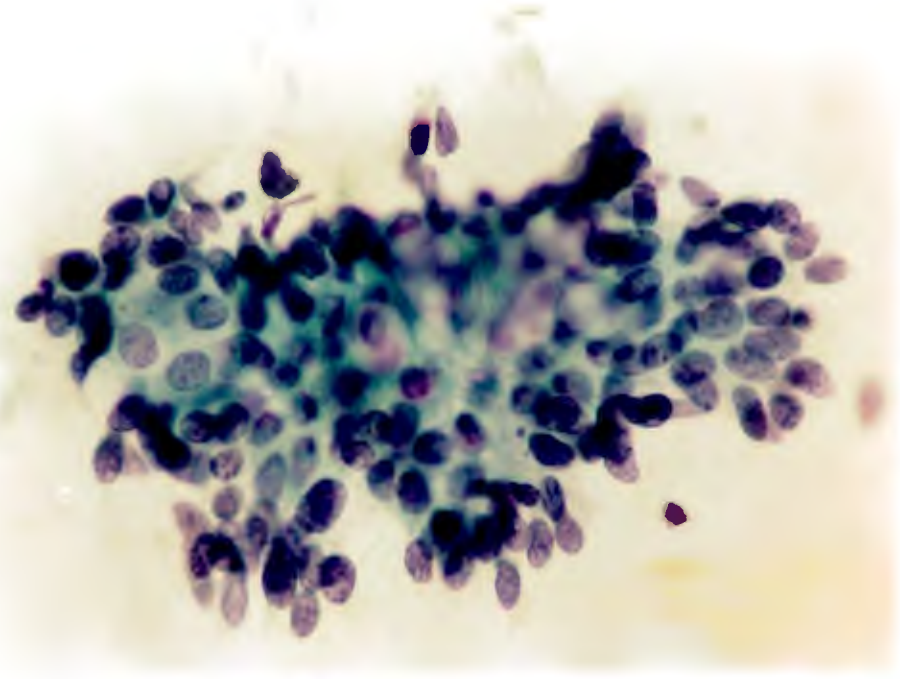
Ill 6: Numerous, crowded hypercellular groups of endocervical cells with peripheral feathering - typical of AIS cytologically (Pap x 200)



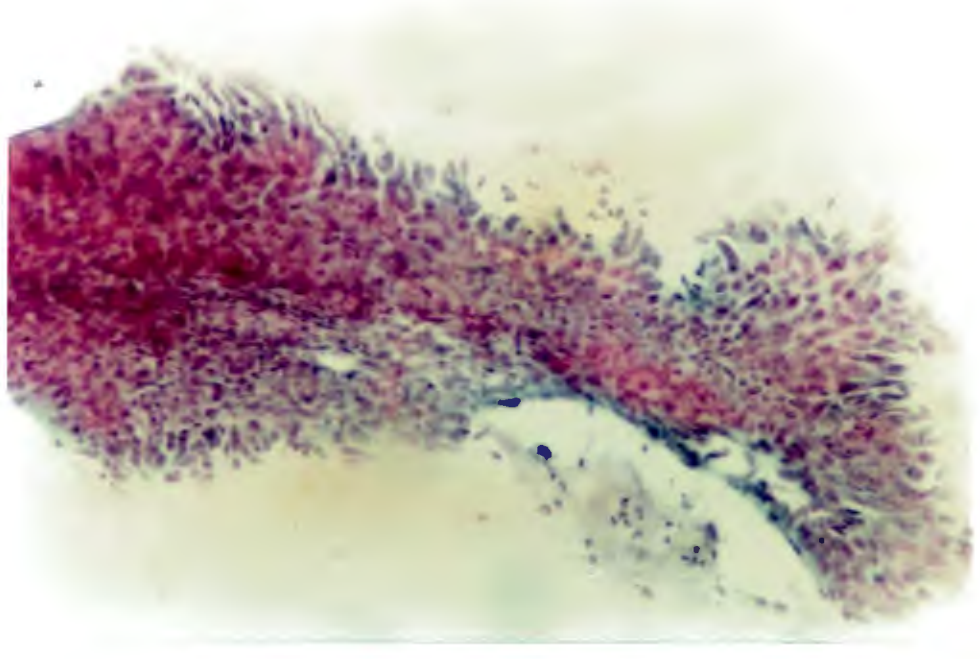
Ill 7: Extreme crowding of endocervical nuclei and associated feathering - the diagnosis of AIS was confirmed (Pap, x 800)



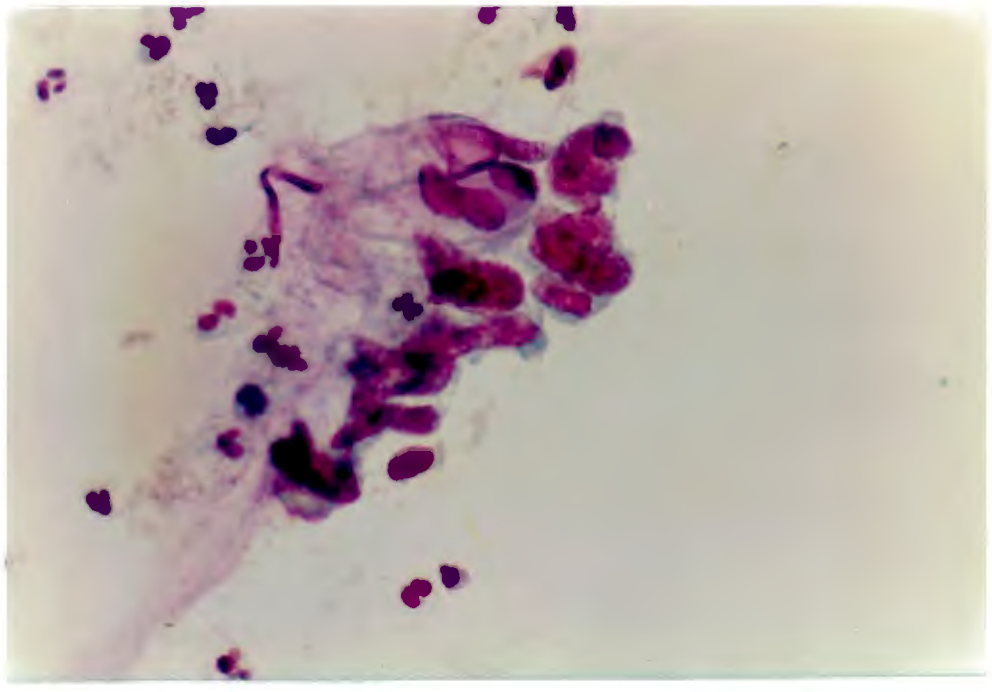
Ill 8: Moderate nuclear crowding without feathering - the diagnosis of cervicitis was made histologically (Pap, x 800)



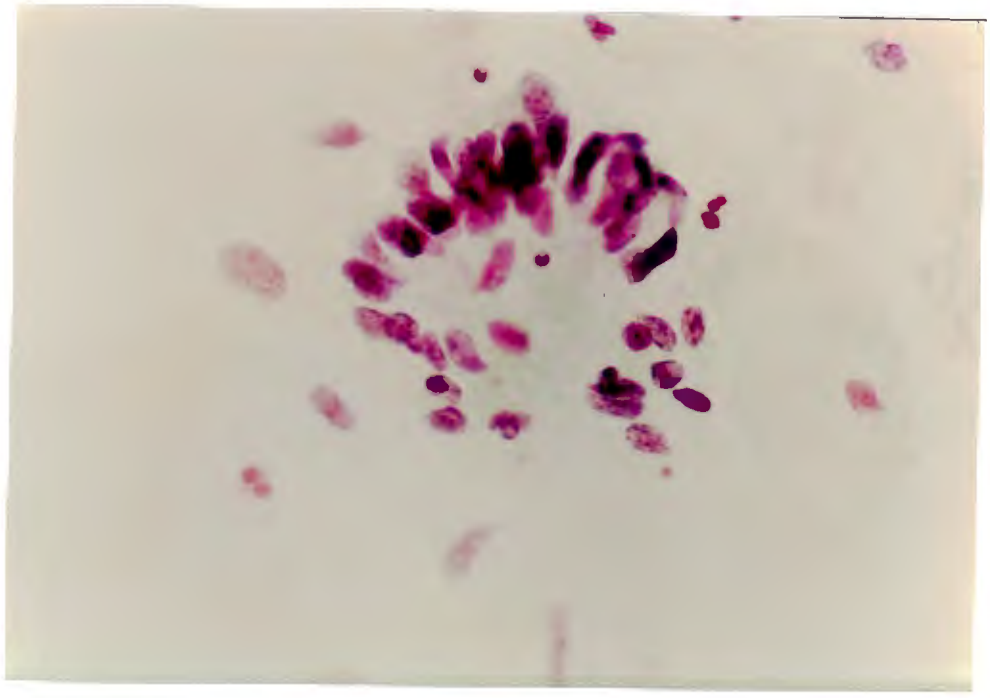
Ill 9: Although nuclear crowding and hyperplasia is present, there is no true feathering - the histological diagnosis of microglandular hyperplasia was made in this case (Pap, x 800)



Ill 10: A hypercellular group with crowding, loss of polarity at the periphery with feathering, in a histologically confirmed example of AIS (Pap, x 200)

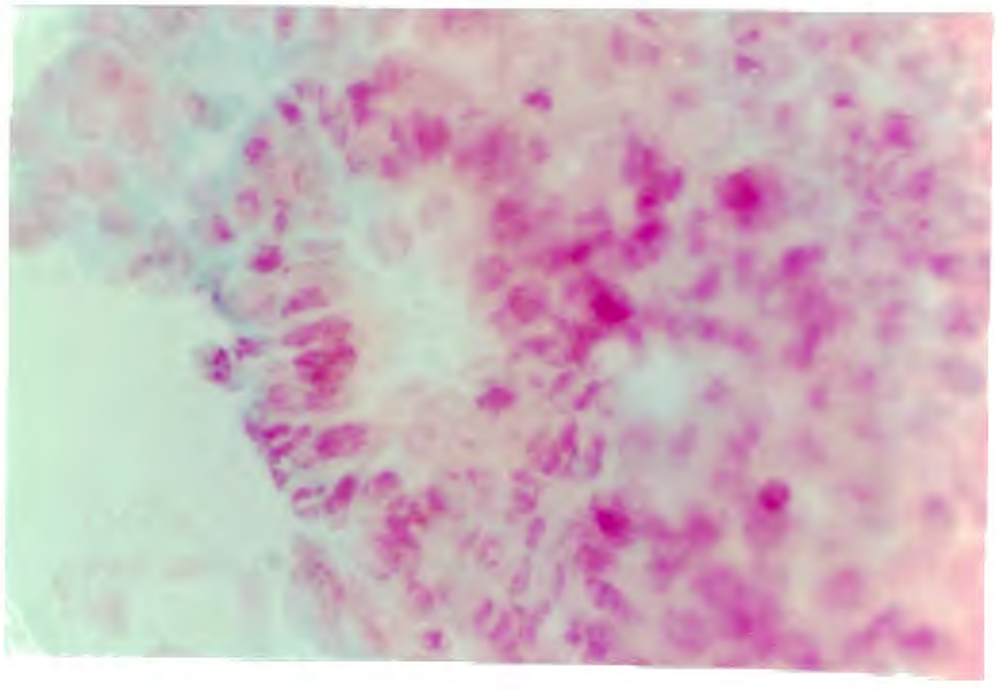


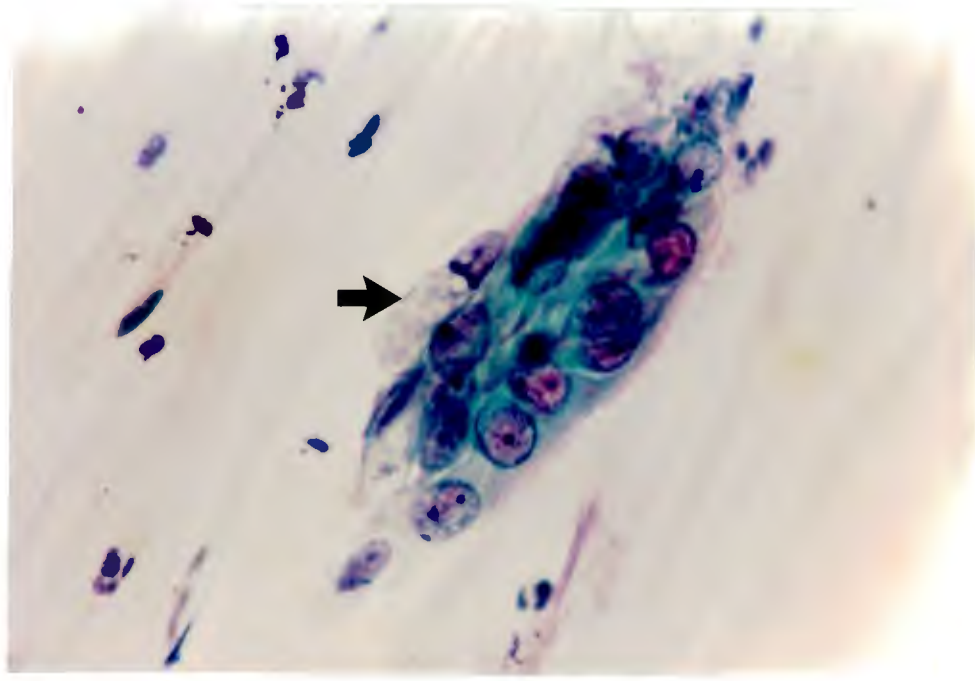
Ill 11: Loss of polarity in short strips of endocervical cells is another important architectural feature. (Pap, x 800)



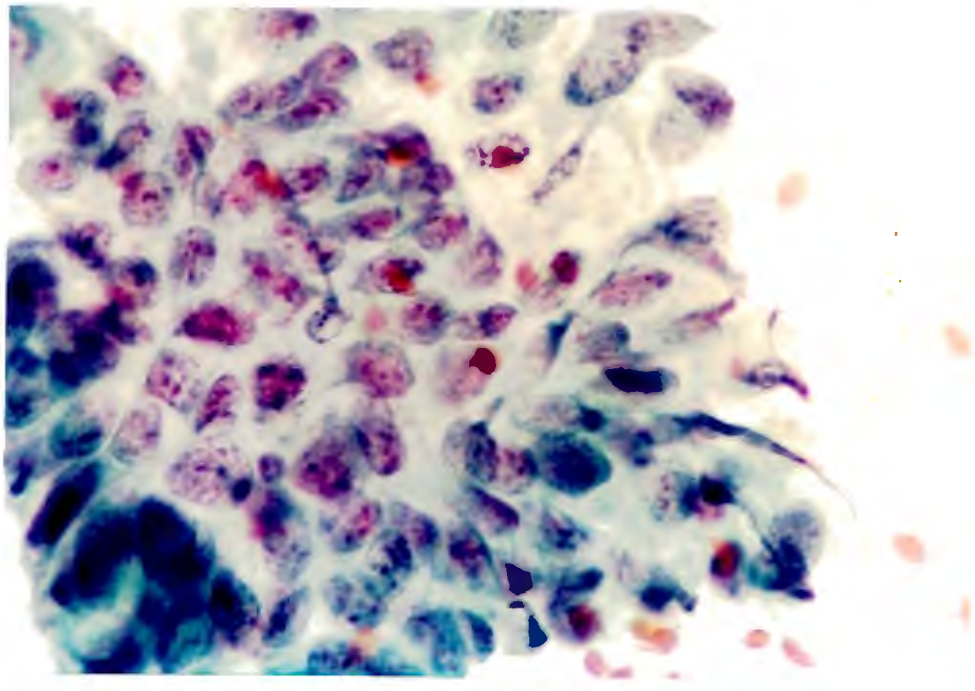
Ill 12: Rosette formation may either be seen in small groups of cells with stratified nuclei or as below (Ill 13) within larger groups of crowded nuclei (both Pap, x 800)

Ill 13:

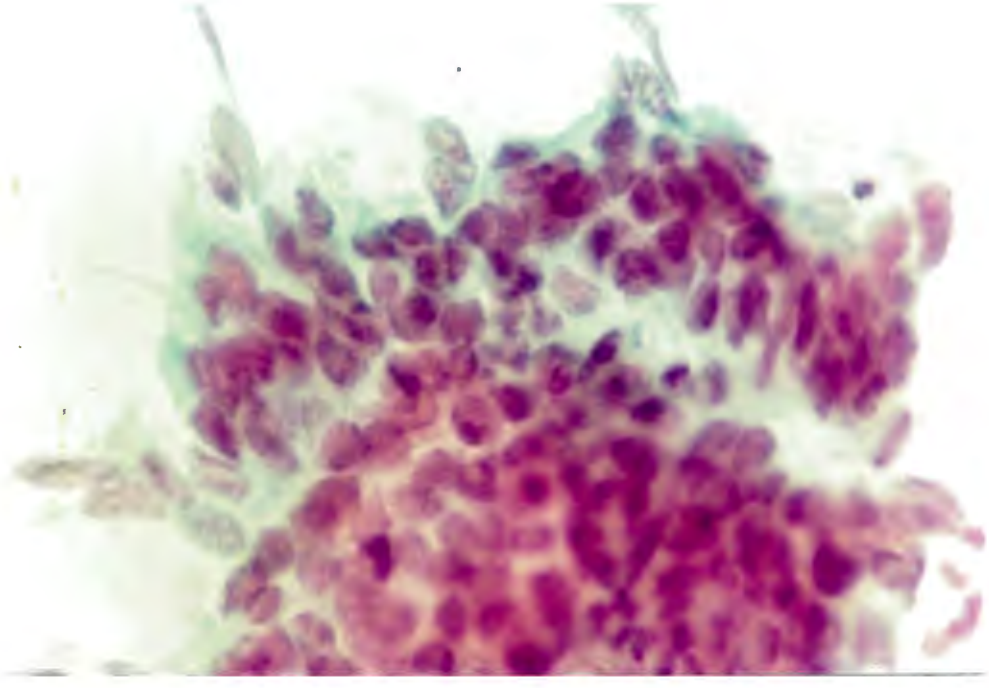




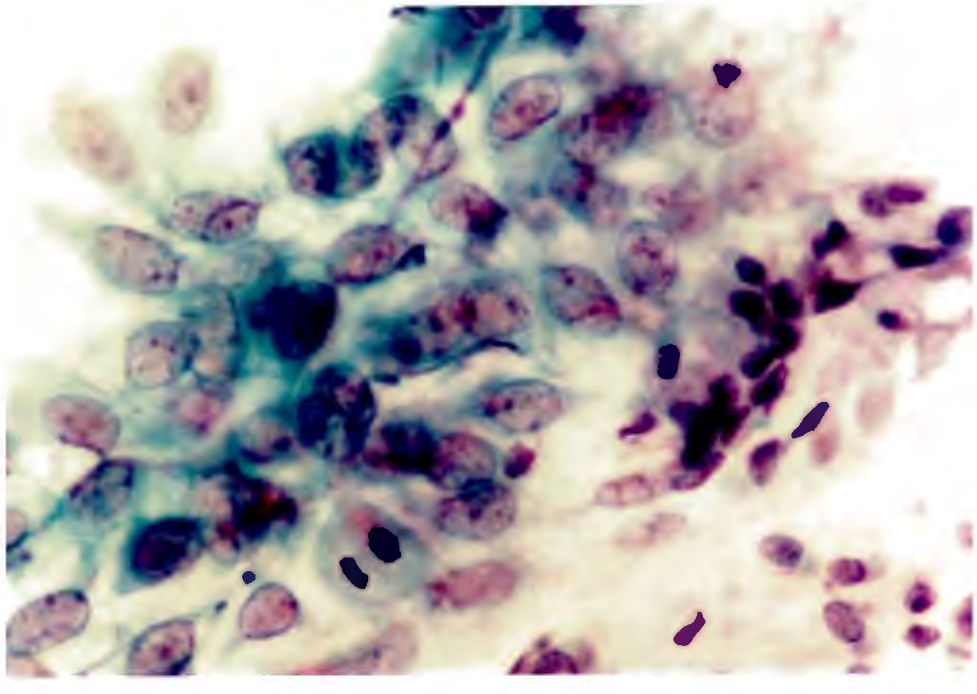
Ill 14: Small groups of cells with some anisonucleosis and prominence of nucleoli are typical of reactive endocervical cells (Pap, x 800). (The columnar cell, as indicated, has a foamy cytoplasm)



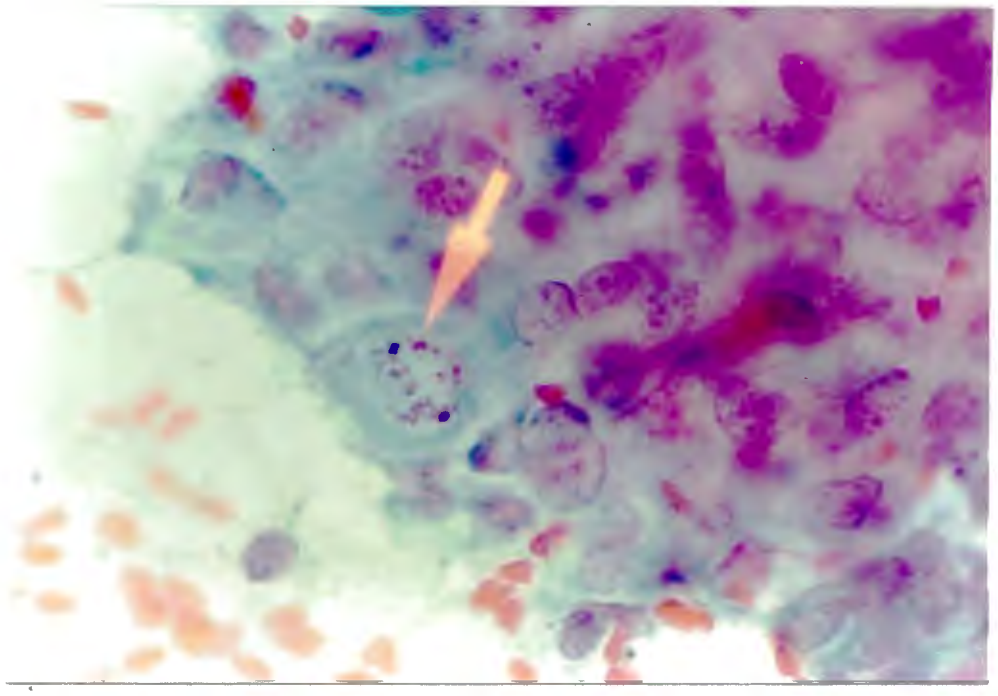
Ill 15: By comparison as well as anisonucleosis and occasional nucleoli, there is chromatin coarseness and nuclear crowding in this example of adenocarcinoma (Pap, x 800) (Differentiation from atypical repair may at times be difficult).



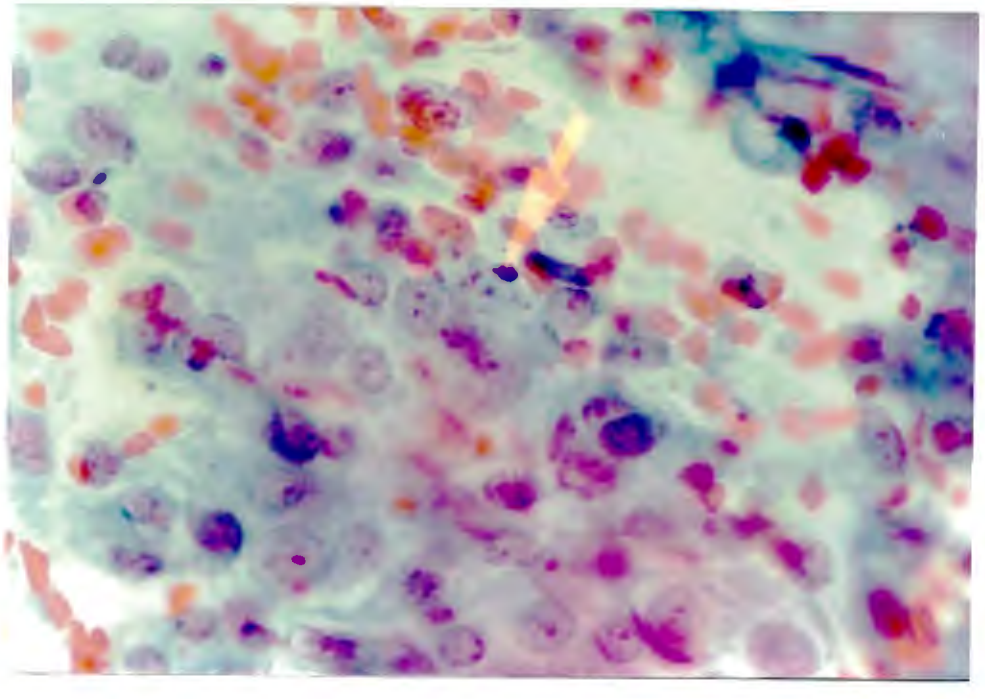
Ill 16: Chromatin tends to be dense but not coarse in *in situ* adenocarcinoma (Pap, x 800)



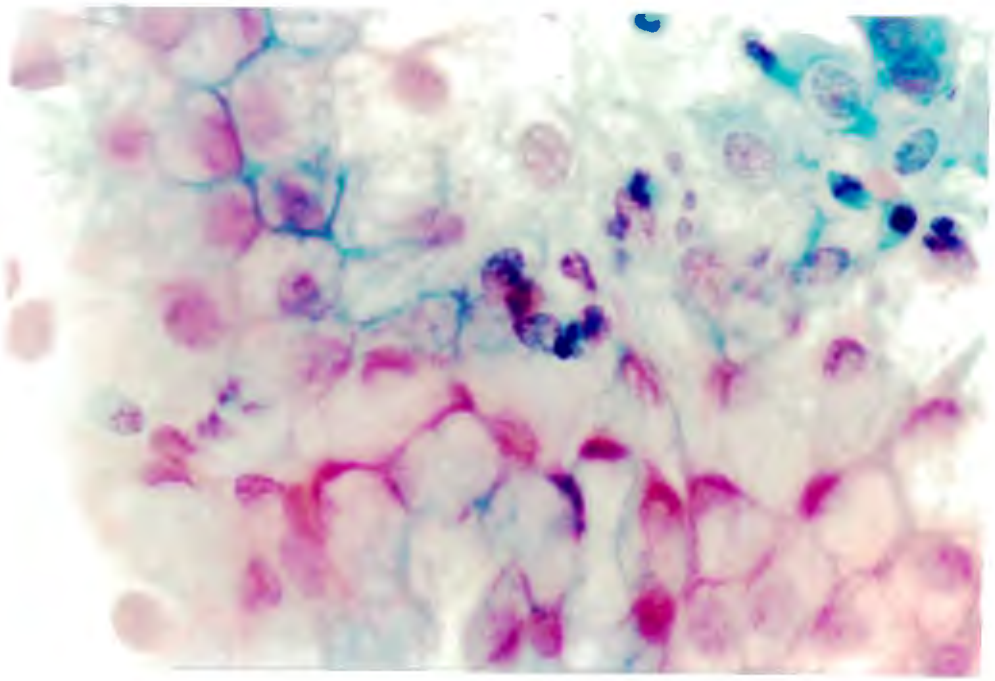
Ill 17: Mitoses are occasionally seen in groups of reactive/reparative endocervical cells (Pap, x 800)



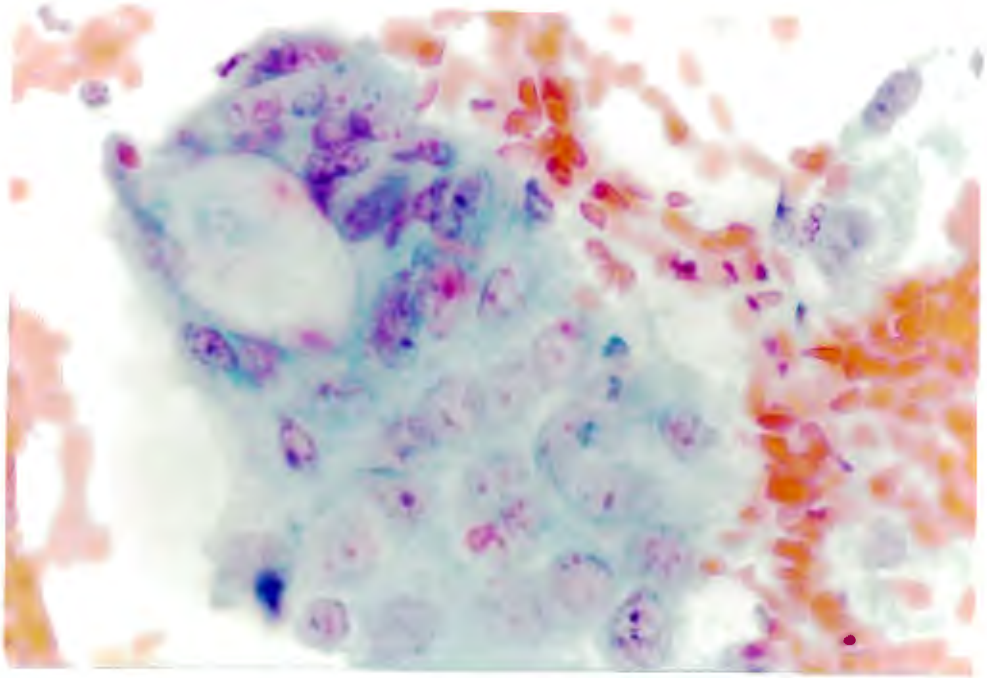
Ill 18: Mitoses can be more frequently, but not invariably, seen in cases of *in situ* adenocarcinoma (Pap, x 800)



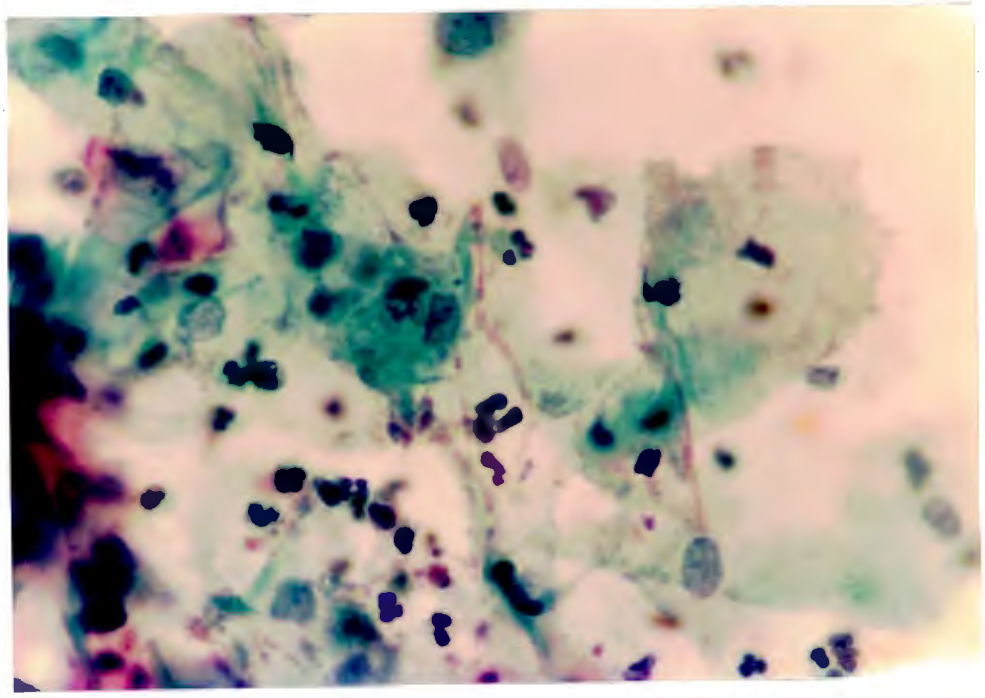
Ill 19: Apoptosis is also a feature of *in situ* adenocarcinoma (Pap, x 800)



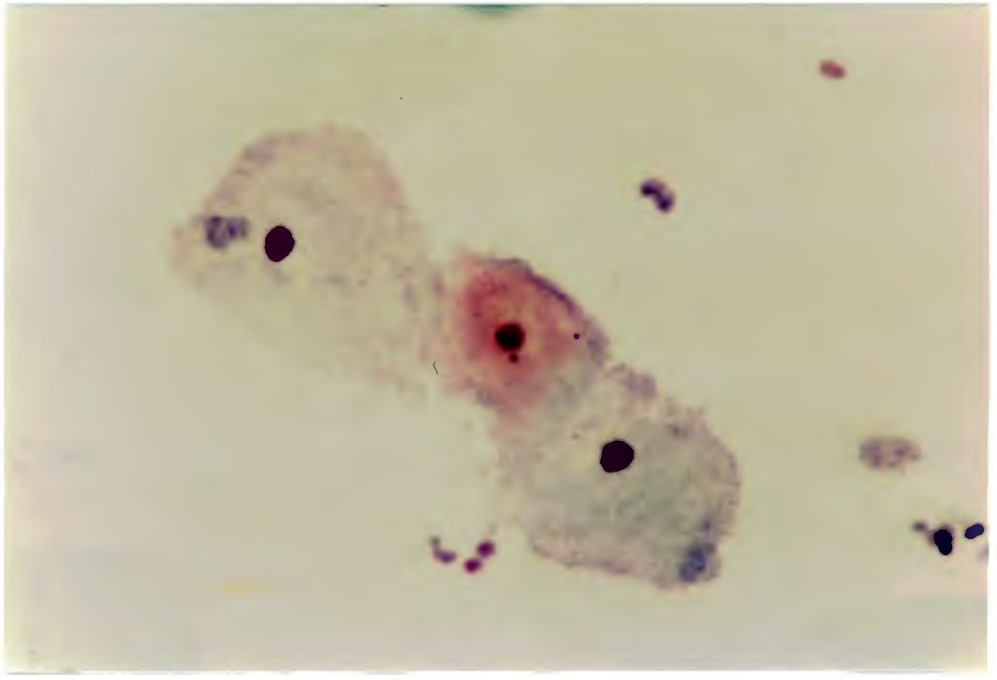
Ill 20: Vacuolated mucin filled endocervical cells are not infrequently seen, sometimes associated with microglandular hyperplasia, as in this case (Pap, x 800)

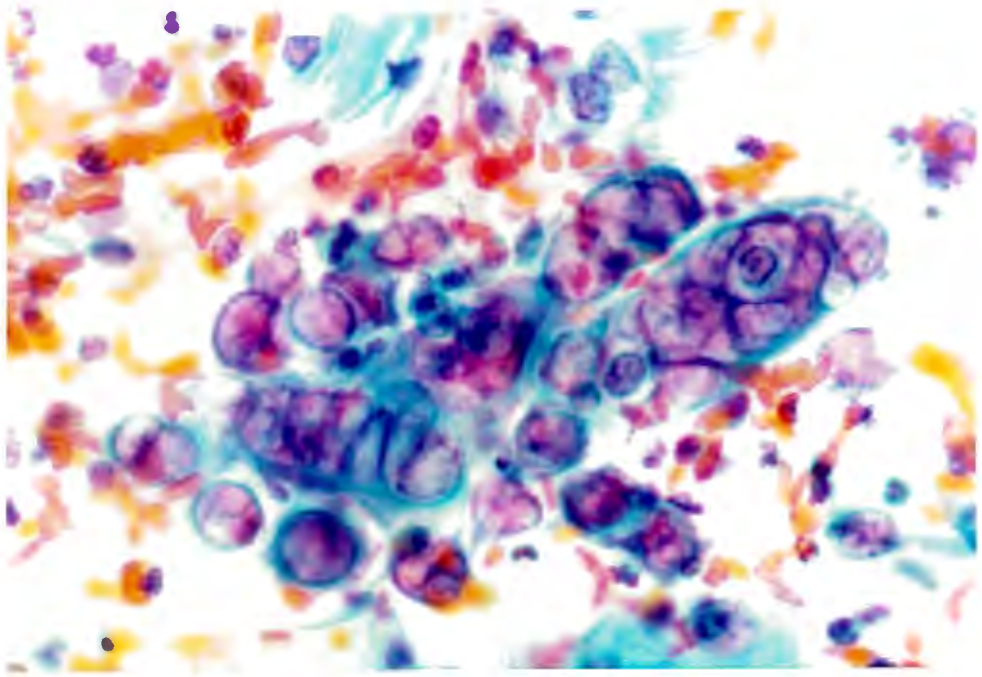


Ill 21: More homogeneous cytoplasm and nuclei with macronucleoli. This case was diagnosed histologically as invasive adenocarcinoma. Coarse chromatin, anisonucleosis and nuclear crowding is also seen (Pap, x 800)

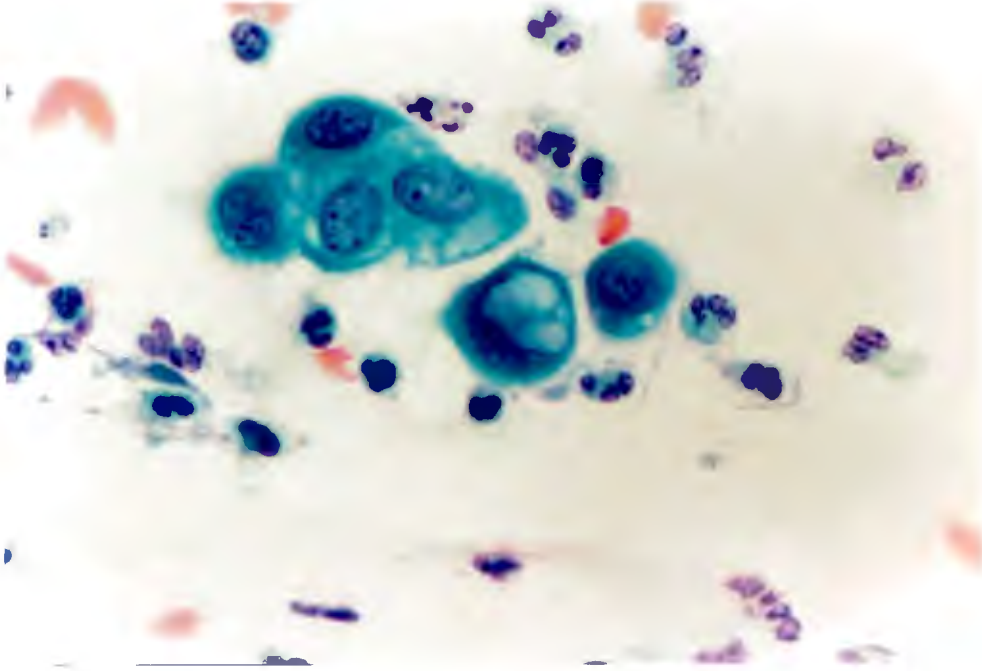


Ill 22: Pathogens included Candida as seen above (Pap, x 800)
and
Ill 23: Gardnerella with clue cell formation as seen below (Pap, x 800)

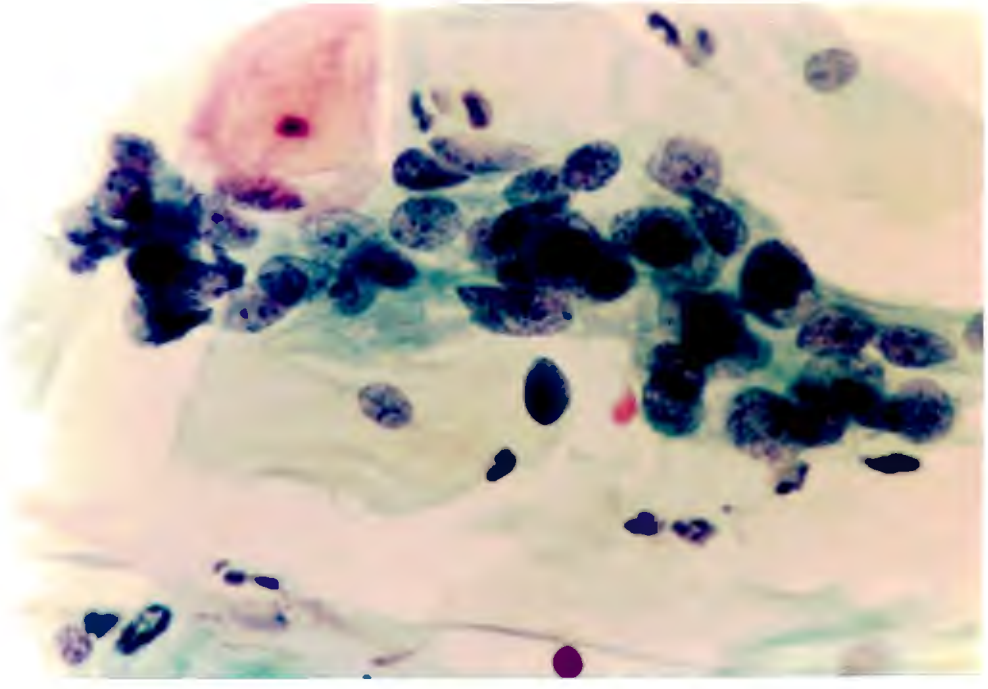




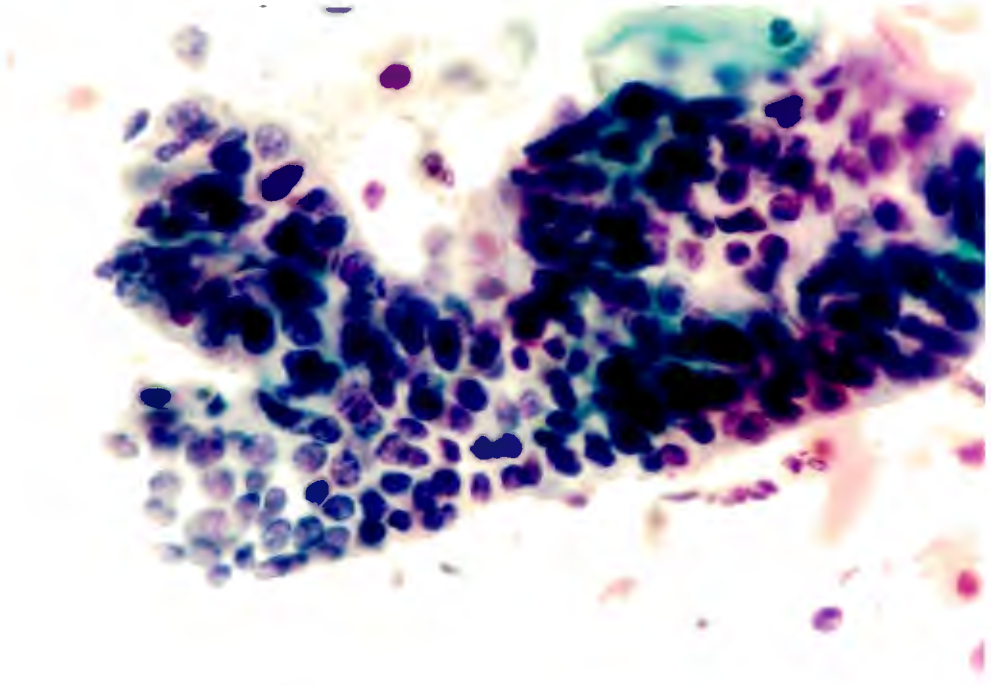
Ill 24: Multinucleation, cowdry inclusions and margination of chromatin, typical of herpes virocytes were seen in the one example (Pap, x 800)



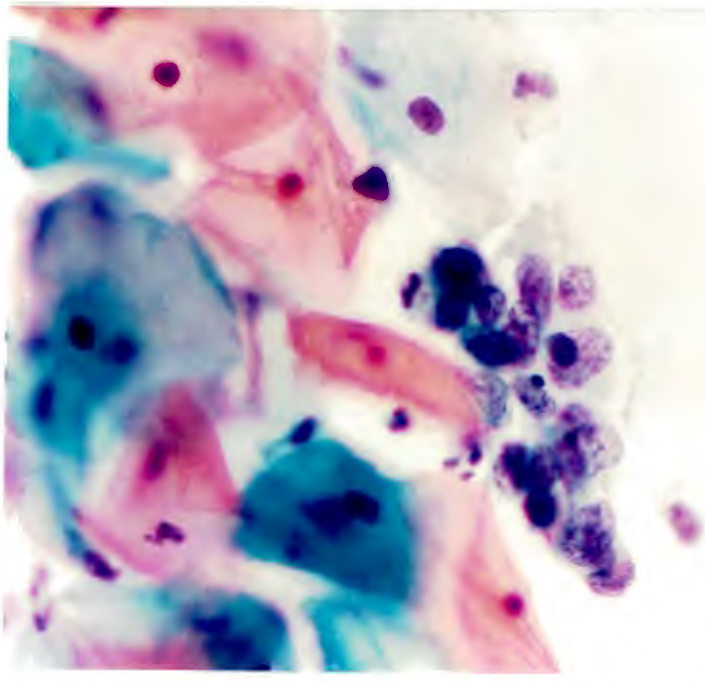
Ill 25: An inflammatory smear with vacuolation of metaplastic cells is a feature of chlamydia (Pap, x 800)



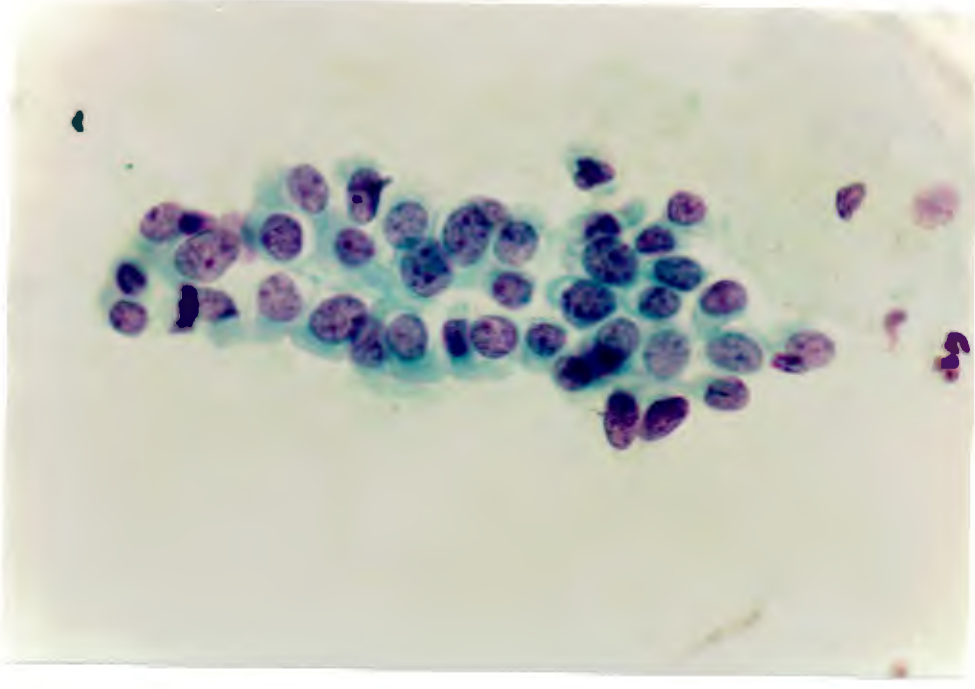
Ill 26: Anisonucleosis, coarse chromatin and syncytial groupings are features of CIN_3 (Pap, x 800)



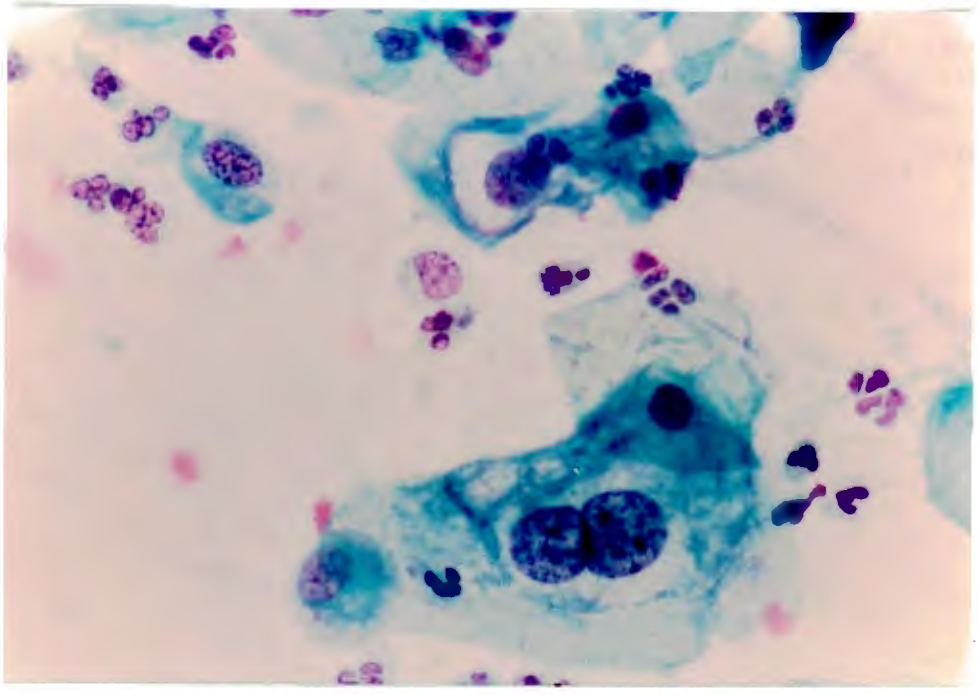
Ill 27: Dysplastic immature metaplastic cells may acquire a somewhat honeycomb grouping reminiscent of AIS. Note lack of extreme crowding and feathering (Pap, x 800)



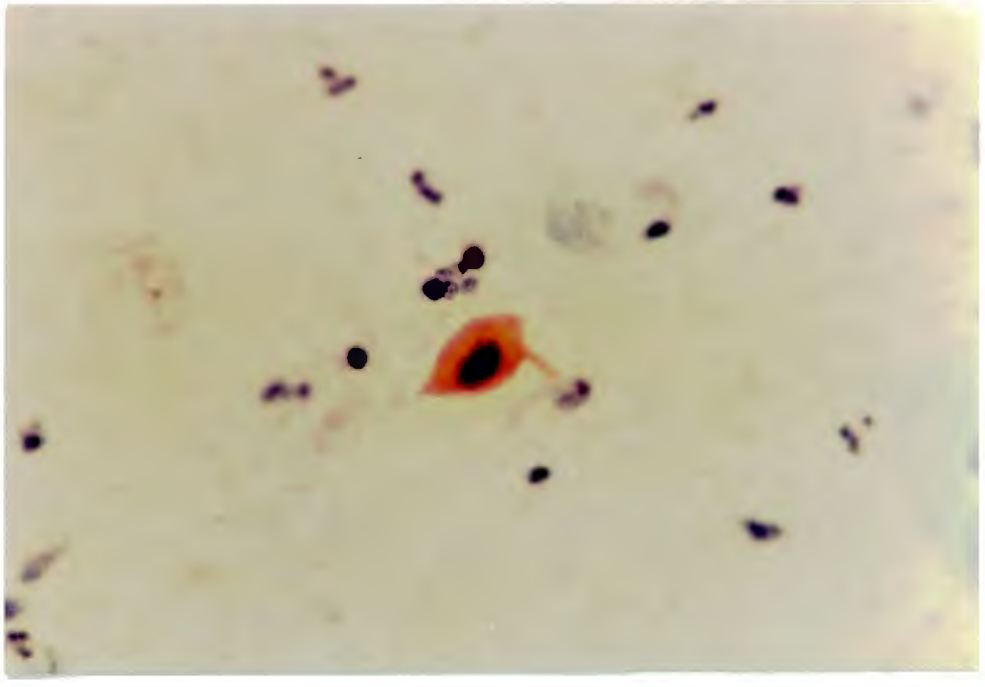
Ill 28: Dysplastic nuclei exhibited marked anisonucleosis, with coarse chromatin which lack nucleoli (Pap, x 800)



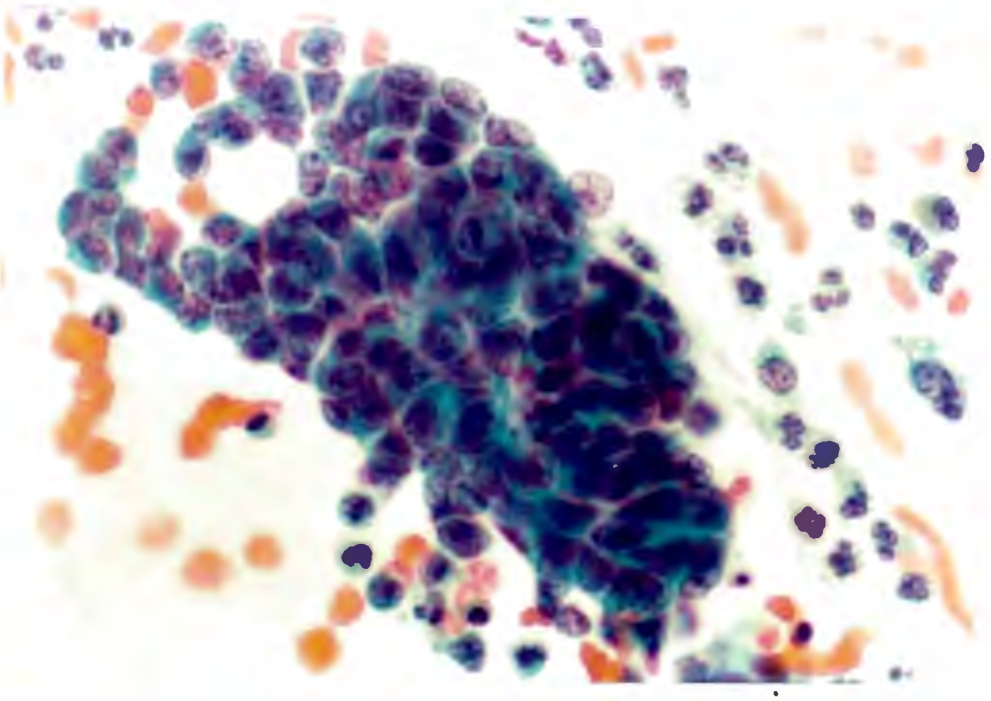
Ill 29: Reactive endocervical cells with some anisonucleosis, even chromatin and micronucleoli (Pap, x 800)



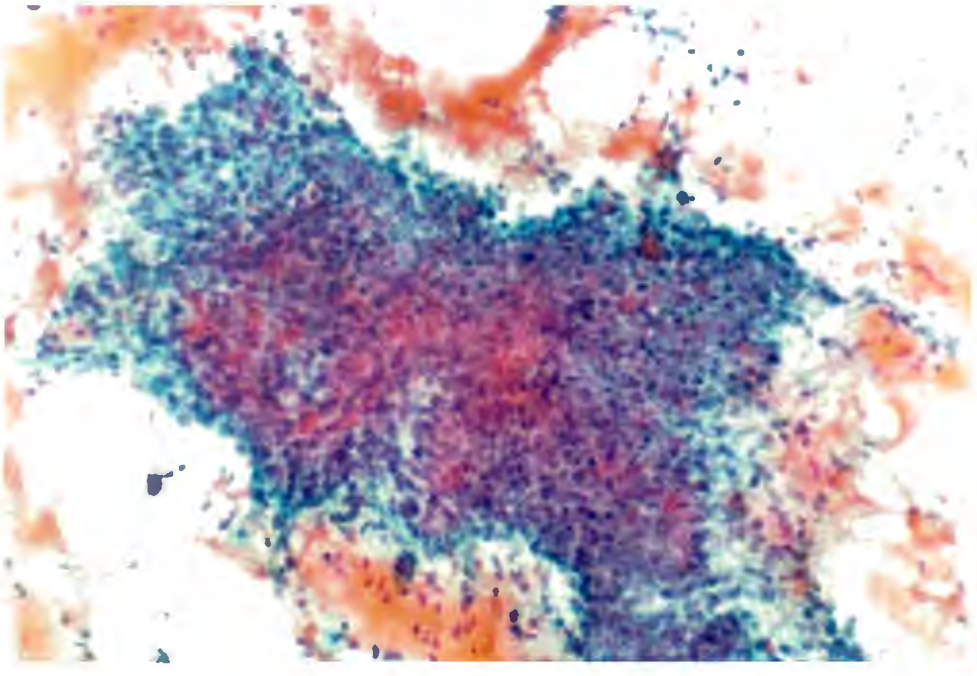
Ill 30: Koilocytosis and binucleation associated with HPV infection (Pap, x 800)



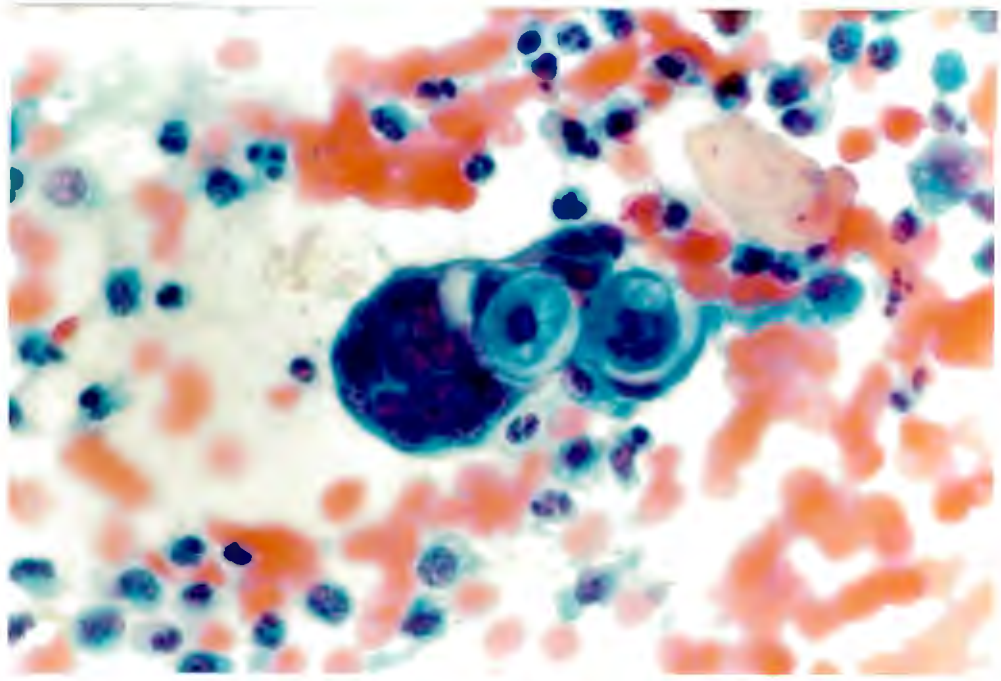
Ill 31: Dyskeratotic cells are also a feature of HPV infection (Pap, x 800)



Ill 32: Exfoliated endometrial cells tend to cluster as a 3 dimensional group and the apparent hyperchromasia is due to nuclear degeneration (Pap, x 800)



Ill 33: A flat sheet of endometrial cells with background stroma from lower segment endometrium (Pap, x 200)



Ill 34: Vacuolated atypical glandular cells in an inflammatory background related to IUCD usage (Pap, x 800)

5. DISCUSSION OF RESULTS

5.1 SAMPLE

Three hundred and forty six patients (46.3%) did not meet the selection criteria due to inadequate filing of original records. Although computer generated reports were available in many instances, these did not contain adequate clinical and patient related information and could not be used for the purposes of this study. Table VII shows that the sample population and the total population are similar, in terms of proportion of patients within each grade of EGA except that none of the grade 3 or 4 EGA's were excluded from the sample. The small numbers within these groups did not significantly alter the percentages within the other groups.

The age of the patients in the study population ranged from 14 years to 72 years, the majority (79.7%) occurring in the 20 to 39 year age group (Table VIII).

Ninety one percent of patients were categorised into grades 1 and 2. As noted in Table V, these patients represent an important component of the work load of the GSH cytology laboratory.

5.2 FIVE YEAR FOLLOW-UP OF PATIENTS WITH EGA's

5.2.1 LOST TO FOLLOW-UP

Thirty point five percent (124) of the patients in the sample group did not present for follow-up pap smears (Table XI). Of the 124 patients lost to follow-up. 121 (97.6%) were in groups 1 and 2. Many of those patients lost to follow-

up (53.7%) were pregnant patients whose screening pap smear was performed at an antenatal clinic.

5.2.2 FIVE YEAR FOLLOW-UP

Two hundred and eighty three (69.5%) of patients had been followed-up during the five year period from 1987 to 1991. The outcome of these patients with EGA's was assessed according to whether their EGA's regressed on subsequent smears, persisted as the same grade of atypia or deteriorated to a higher grade.

5.2.2.1 Grade 1 EGA's

Figure 1 and Table X show that 145 (74%) of patients with grade 1 EGA's regressed. The remaining 51 (26%) EGA's persisted. Table XI shows that 39 (76.5%) of these persisting lesions were associated with atypical squamous epithelial cells. The remaining 12 patients with persisting grade 1 EGA's were not associated with any specific features of note. Follow-up smears from these patients were rescreened and although anisonucleosis and regenerative changes were noted there was no other significant abnormality.

5.2.2.2 Grade 2 EGA's

Of the 55 (80.9%) of the grade 2 EGA's which were followed up, 96.4% either regressed or persisted. Of the 56.4% which persisted, 93.5% were associated with squamous lesions (Table XI). Two patients atypias deteriorated. These were found to be associated with squamous CIN3 extending into crypts on subsequent follow up pap smears and on cervical biopsy and one of these patient's had an area of microinvasive carcinoma. Review of the original smears in both cases showed scanty evidence of CIN3 with atypical immature

metaplastic cells possibly having been misinterpreted as EGA by the original screening cytologist. The distinction cytologically of primary EGA from squamous CIN extending into crypts can on occasions be difficult (Staymates 1982, Lee et al 1991) and many of the cases of persistent and deteriorating EGA's appear on rescreening to have been misinterpreted in this way .

5.2.2.3 Grade 3 EGA's

Twenty one (87.5%) of these patients were followed-up. Here the majority (85,7%) either deteriorated or persisted. Of the 66.7% of atypias which persisted, 85.7% were associated with atypical squamous cells. The majority (71.4%) where associated with squamous CIN3 . Histological correlation in these cases (Table XIV) confirms CIN3 extending into crypts, in none of these 10 cases was EGA or AIS noted histologically. However, in 2 of the 4 cases which deteriorated, atypical squamous cells were not seen cytologically, but histological investigation demonstrated AIS and invasive adenocarcinoma respectively. Further, discussion will follow as to the important differentiating cytological features. Of the remaining 2 cases which deteriorated one was found to be a squamous carcinoma histologically and the hysterectomy specimen of the other surprisingly showed HPV but neither CIN or EGA.

5.2.2.4 Grade 4 EGA's

As expected in this severe grade, the EGA's either persisted or deteriorated. Those recorded as "persisted" had histological correlation as well as cytological follow up in all cases. Only 3 of these cases (26.3%) were associated with atypical squamous cells cytologically, and these atypical changes were assessed as CIN3 both cytologically and histologically. In only one of these cases was concomitant AIS found histologically. The remainder showed architectural and

cytological features suggestive of a pure AIS. The results of histological examination in these 11 cases where AIS was suspected cytologically is presented in Table XIV. AIS was confirmed in 4 cases (36,3%). In a further 2 cases (18,1%) invasive adenocarcinoma was found. In the remaining 3 cases (26,3%) microglandular hyperplasia was the only endocervical abnormality found at hysterectomy. This is a known pitfall in the cytological diagnosis of AIS (Nichols & Fidler 1971, Lee et al 1991,) and the cytological features which may be useful in differentiating these lesions are discussed below.

The association between grade 1, grade 2 and grade 3 EGA's and abnormal squamous cells found in this study is important, because it may have important implications for the modification of the system of cytological diagnosis of EGA's used by GSH Cytology Department. The distinction between atypical immature metaplastic cells involved in CIN or tissue repair and atypical columnar glandular cells may be difficult and misinterpretation may occur. In these cases, attention should be given to the detail of the architectural groupings and to the shapes of the cells. In pure glandular atypia the groupings should retain a honeycomb configuration (Ill 6, 7) Focusing into crowded groups of hyperchromatic nuclei is necessary to visualise this configuration. The cells within these groups, and strips of cells should be columnar, rather than cuboidal and mucin production may be visible (Staymates 1982, Coleman & Evans 1988)

Where the squamous lesion is the predominant abnormality, the smear should be classified as a squamous lesion and managed as such with follow-up or treatment according to the grade of squamous abnormality. Squamous and glandular lesions can however on occasions occur simultaneously (Brown & Wells 1986), and the presence of CIN3 does not exclude coexistant AIS. The fact that the majority

(74%) of the grade 1 EGA's regressed, as did 40% of grade 2 EGA's (FIG. 1), implies that these lesions, when not associated with squamous CIN or HPV infection, are related to benign reactive rather than preneoplastic processes (see Table XII).

5.2.2.5 Other factors possibly associated with the occurrence of EGA's

The study was also designed to test the possible relationship of exogenous contraceptive hormones, intrauterine contraceptive device use and the presence of ectropions with the occurrence of EGA's, but as Fig. 3 shows, these factors occurred randomly and were not associated with the grade of EGA.

Ectropions were found to be present in between 8% and 22% of each grade of EGA. These lesions are not known to cause cervical pathology and are essentially physiological changes. There are however cytological features which can be ascribed to ectropions. These are:

> an increased number of endocervical cells present on the pap smears associated with ectropions (frequently more than 50 cells per group) and a propensity to bleeding during exfoliation.

If in addition to these above features, there are benign reactive changes with anisonucleosis, a cytological diagnosis of EGA may mistakenly be made. A diagnosis of EGA should only be made if in addition to the above findings, other abnormal architectural features are present (see section on cytological features).

Medroxyprogesterone (depoprovera) as a form of contraception was used in 89 patients in the sample. Depoprovera has been implicated with a number of

cervical abnormalities , the most important, related to the endocervix, being microglandular hyperplasia (MGH). MGH is characterised by glandular hyperplasia and thus increased numbers of cells, as well as cellular crowding cytologically (Ill 9). Nuclear abnormalities are usually minimal, but if present can lead to confusion with AIS and EGA (Lee 1988, Young & Clement 1991). Three cases of grade 4 EGA diagnosed cytologically were found on histological assesment to be MGH. This emphasises the importance of MGH as a pseudoneoplastic glandular lesion and thus a diagnosis which must be considered in all patients receiving progesterone stimulation. Although it has been postulated that there is a hormonal factor related to the etiology of endocervical carcinoma (Dallenbach-Hellwig 1984), this has not been adequately demonstrated. MGH is also found in a minority of oral contraceptive users. In this study the number of people using oral contraceptives was small and no significant relationship between grade of EGA and O.C. use was found.

IUCDs were used by only 14 of the sample group. With this small number of cases no relationship could be demonstrated between the grading of EGA's IUCD's. Usage of the IUCD however, is related to endocervical glandular changes as a result of chronic irritation by the IUCD thread (Gupta et al 1978, Risse et al 1981). It is important to properly recognise these morphological changes since they may be confused with glandular neoplastic lesions (Ill 34).

The presence of HPV on the original pap smear was assessed by koilocytosis and dyskeratosis (Ill 30, 31). HPV types 16 and 18 have been demonstrated in a number of cases of endocervical adeno carcinomas (Cooper et al 1992). Figure 2 shows the features of the associated squamous cells in each grade of EGA. There was no significant difference between the frequency of occurrence

of HPV in association with grade 1 and grade 2 EGA's. ($p > 0.05$). However, the morphological features of HPV were not found to be associated with grade 3 or 4 EGA's.

It has been found that HPV DNA is present in 3 morphologically distinct forms in the nuclei of CIN and squamous carcinoma by in situ hybridisation techniques (Cooper et al 1991). The integration of virus type 16 and 18 may be of importance in carcinogenesis and the morphologically distinctive features may be lost. Whereas with non-integrated episomal virus, the morphological features of koilocytosis and dyskeratosis are preserved (Cooper et al 1991). The findings in this study do not disagree with this theory .

Organisms, such as herpes virus type 2 (Ill 24), as suggested by the Zur Hausen hypothesis (Zur Hausen 1982), may act as co-carcinogens. However, in this study only one case of herpes virus infection was found and possible association between herpes virus infection and EGA's could not be investigated.

A number of other organisms were also found in the cervical smears (Figure 4). Trichomonas was found to be the most frequently occurring organism, followed by Gardnerella and Candida. No consistent association with either grade 1, 2 or 3 and type of infection was found. No organisms were found in any of the grade 4 cases. The reason for this is unclear, but may be related to the small number (11) of cases in the group and thus no significance should be placed on this finding.

(Ill 22-25 demonstrate the cytological features of some of these organisms).

Both squamous and endocervical cells contained in these inflammatory pap smears showed a high proportion of regenerative changes (Fig.2). Such regenerative changes, which are associated with the above infections, in particular trichomoniasis (Gupta 1991) tend to regress on follow-up as shown in Table XII) and should not be diagnosed as atypical.

5.3 CYTOLOGICAL FEATURES OF ORIGINAL PAP SMEARS

There have been only a few comprehensive descriptions of the cytological aspects of adenocarcinoma *in situ* and related lesions (Lee et al 1991, Ayer et al 1987, Ayer et al 1988, Krumins et al 1977, Bousfield et al 1980). Endocervical glandular dysplasia is a lesion described as showing cytological and architectural atypia similar to adenocarcinoma *in situ* (AIS) but of lesser degree (Barter & Waters 1970, Jaworski 1990).

The word atypia means not normal, or not typical for a normal cell of this particular tissue. Vooijis 1991, suggests that the use of the word atypia without further specification should be avoided since it can be used too often as a substitute for a careful description and definition. Commonly the word atypia is used as a descriptive diagnosis when indicating minimal to slight aberrations from the normal. Features most frequently causing such a diagnosis of slight to minimal atypia are nuclear enlargement and aberrations from the normal configuration of the cell. The most often causative processes of this abnormality are inflammation and regenerative reactions. Bearing these definitions in mind, and considering the features discussed in the introductory chapter the cytological findings of each smear with EGA rescreened are presented below.

The results of the analysis of those features present within each grade of EGA, are grouped into architectural, cytological and background features. Related features such as pathogens and squamous cell abnormalities have already been mentioned in the preceding discussion, but where relevant will be discussed.

5.3.1 ARCHITECTURAL FEATURES

The architectural features chosen for analysis were those assessed to be of diagnostic significance by Lee et al 1991 (see Table IV) in differentiating AIS from benign conditions. Figure 5 shows that Grade 1 EGA's did not show feathering, loss of polarity in strips or rosette forms. Five cases (1,6%)) showed some nuclear crowding, but the only architectural abnormality which occured with any frequency (25,7%) was the presence of >50 cells per endocervical glandular group. Those cases which showed nuclear crowding were all cytobrush smears. Interpretation of cytobrush smears can at times be difficult because of the smearing artifact which can distort the morphology, particularly if forceful smearing has occured. Other authors (Novotney et al 1992) have also noted this difficulty in interpreting cytobrush smears.

Grade 2 EGA's did show some nuclear crowding (29,4%)) and 25% had > 50 cells per group. Again the other 3 features were not demonstrable. Some of the cases which showed nuclear crowding were again cytobrush smears.

The original article on the subject of the cytological diagnosis of AIS by Krumins et al (1977) and subsequent articles on the subject (Ayer et al 1987, Lee et al 1991) emphasise the architectural features and in particular extreme nuclear crowding, feathering, rosette formation and loss of polarity in strips as being the

most important diagnostic features of AIS. A later article by Bousfield et al 1980, again emphasised these same features and on the subject of endocervical glandular dysplasia remarked that in their 3 cases crowded sheets, columnar strips and rosettes were also seen.

As abnormal architectural features were found in the more severe grades of EGA it can be assumed that these were the dominant features noticed by the screening cytotechnologist. Subsequent histological examinations carried out in these cases do not correlate well with the original cytological diagnoses. Table XIV shows that only 54,6% of the cases suspected of being AIS (Grade 4 EGA's) were in fact adenocarcinomas, either invasive or *in situ.* In those cases where there was a positive correlation, at least 4 of the 5 architectural features were present. This was not the case where there was no positive correlation between histology and cytology.

Figure 6, emphasises the above finding. Accumulative architectural features are of more significance in the diagnosis of AIS than any other criterion.

5.3.2 CYTOLOGICAL FEATURES

Figure 7, demonstrates that the occurrence of anisonucleosis on its own is not a useful feature in the cytological diagnosis of AIS and EGD because, in 95% of cases of Grade 1 EGA, it was the only abnormal finding and 75% of grade 1 cases regressed.

By comparison the higher grade EGA's showed, in addition to anisonucleosis, a predominance of abnormal cells with an NC ratio > 1/2, and an increased number

cases with a thickened nuclear membrane. Many of these cases had related squamous CIN lesions and these abnormalities are possibly related to involvement of endocervical crypts by squamous metaplasia and intraepithelial neoplasia. Consequently, the diagnosis should rather have been CIN with endocervical crypt involvement instead of EGA.

Figure 8, demonstrates the chromatin features of the various grades. The Grade 1 EGA's had even chromatin distribution in 83% of cases. Although the nuclei appeared dense and hyperchromatic in 15% of cases, the chromatin was not coarse. Conversely, in the higher grades a higher percentage (63% and 45% of Grades 3 and 4 respectively) of cells showed coarse clumping of the nuclear chromatin which is a cytological feature of neoplasia. The majority of the grade 3 EGA's were histologically confirmed CIN3 lesions and the coarse chromatin distribution within nuclei is consistent with this. The observation of the architectural features in addition to the cytological features is important in distinguishing squamous CIN3 from severe EGA.

In some cases however, normal or slightly atypical endocervical cells persisted at the edge of sheets of dysplastic cells, leading the screener to assume the sheets were glandular in origin.

Many of the histologically confirmed cases of AIS in fact had hyperchromatic dense nuclear chromatin rather than coarse clumping and this feature has been observed by other authors (Nguyen & Jennot 1984, Lee et al 1991).

The types of *nucleoli* as demonstrated in Figure 9 are of little value in separating grade of EGA. Micronucleoli are generally present in any reactive

endocervical cell and when inflammation and repair are present macronucleoli are frequently seen, so this feature is not useful in separating neoplastic from benign conditions. (Ill 14, 15)

Mitosis and apoptosis was infrequently demonstrated (Figure XV) and were only present in Grade 3 and 4 EGA's. Jaworski (1990) places significant value on their presence in histological sections, particularly when architectural features suggestive of AIS are seen. Other authors (Lee et al 1991, Ayer et al 1987) encountered mitoses and apoptoses less frequently and in the context of cytological evaluation they appear to be less useful criteria.

5.3.3 BACKGROUND FEATURES OF SMEARS

Figure 10 demonstrates the similar relative frequencies between all grades of EGA indicating that background features are not helpful in the diagnosis of precursor lesions of malignancy. This is confirmed by other authors (Jaworski 1990, Lee et al 1991).

5.3.4 MISCELLANEOUS FEATURES OF SMEARS

5.3.4.1 Exfoliated endometrial cells

Although no separate category had been supplied on the data collection form for the presence of endometrial cells on the cervical smears, it was noted on rescreening that when exfoliated endometrial cells were present on the smears a high proportion of these were interpreted as " atypical glandular cells of endocervical origin". Although endometrial cells were present in only 20 cases of the total sample, 12 (60%) were interpreted as EGA. Classically endometrial cells

tend to "ball-up" into three dimensional clusters and the rather hyperchromatic degenerate nuclei can lead to misinterpretation. (Ill 32). In two of the 20 cases flat sheets of endometrial cells were directly exfoliated from the lower segment of the uterus by an endocervical brush in a post conised cervix. (Ill 33). Endometrial cells under these circumstances tend to be more closely packed, and hypercellular. The presence of both glands and stroma aids in interpretation of these cases.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

This analysis of the grading system of endocervical glandular atypias at present in use at Groote Schuur Hospital Cytology Laboratory, has shown:

6.1.1 GRADE 1.

The diagnosis of mild endocervical atypias (Grade 1) has been inappropriately applied in the majority of cases, due to the following reasons:

a) The majority (74.8%) of cases regressed and no cases deteriorated over the five year follow-up period. In those cases which regressed, it was found that anisonucleosis was the most frequently occurring feature. In addition, many of the smears were inflammatory and showed regenerative changes. These features should not have led to the diagnosis of "mild atypia" but a descriptive diagnosis should rather have been given.

b) Grade 1 cases which persisted were frequently associated with squamous epithelial abnormalities, in particular human papilloma virus infection. These changes should be reported as HPV alone.

The diagnosis of mild cervical atypia should only be used when no causative process is evident.

6.1.2 GRADE 2:

The data in this group also shows that the diagnosis of "atypia" was inappropriate in many cases. Forty percent of cases regressed in this heterogeneous group. Persistence of abnormalities again appears to be related to squamous atypias, in particular CIN. Histological correlation, where available, confirms this impression. Many of the so-called moderately atypical endocervical cells appear to be immature metaplastic cells showing dysplastic changes, and should be interpreted and managed as such. Where there was no squamous abnormality, apart from regeneration, the atypia regressed in the majority of cases.

6.1.3 GRADE 3:

Similar features are noted in this severe endocervical atypia group. Although 2 of the 20 cases which received histological correlation were endocervical glandular neoplasms, the majority were squamous CIN_3 lesions extending into crypts.

This difficulty in interpretation has been observed by other authors (Staymates 1982, Coleman & Evans 1988). Abnormal architectural features, and particularly accumulative architectural features, are necessary for the reliable cytological diagnosis of AIS and similarly EGD. Without these architectural features, which have been well described in the comprehensive descriptions available, the diagnosis should not be suggested.

6.1.4 GRADE 4:

This most severe group of cases where the diagnosis of AIS was suggested in the original cytology report is small, but conforms to the pattern described in the literature. Endocervical hyperplasia, in particular microglandular hyperplasia, presents a problem in cytological interpretation. Further research is required in this area.

The Bethesda System emphasises the need to classify cervical Pap-smear abnormalities in terms of causation, if possible stipulating whether a benign reactive process is favoured rather than a neoplastic or pre-neoplastic process. There is also a need to determine clearly if possible which cells are showing the abnormality. The results of this study confirm these recommendations.

6.2 RECOMMENDATIONS

1. The diagnosis of mild atypia should not be used indiscriminately.

2. Cervical Pap-smear abnormalities should, if possible, be classified in terms of causation, stipulating whether a benign reactive process or a neoplastic process is favoured. This requires the diagnostic cytologist to have a good working knowledge of normal endocervical cells under a wide range of conditions.

3. Only when the glandular abnormalities correspond to those described as relating to AIS, should this diagnosis be made. It should be remembered

that accumulative features are of more diagnostic significance than single features.

4. Where squamous epithelial abnormalities predominate, the diagnosis of CIN etc. should be made rather than endocervical atypia. However, if the described glandular abnormalities are in addition present, AIS/EGD coexistant with CIN should be considered.

5. The Bethesda system provides a diagnostic category termed atypical glandular cells of undetermined significance and wherever none of the above can be applied, this diagnosis should be considered. An invariably accurate prediction is not always possible on cytology, particularly when smears are obscured by inflammatory exudate or blood. Repeat smears and/or colposcopy may be necessary for further evaluation.

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APPENDIX 1

PD 99

CERVICAL CYTOLOGY REQUEST FORM

GROOTE SCHUUR HOSPITAL/UNIVERSITY OF CAPE TOWN

LAB SPECIMEN NO.

TO BE COMPLETED IN FULL FOR EVERY REQUEST

SURNAME

FIRST NAME

DATE OF BIRTH

ADDRESS.....

.....

PHONE NO. .....

DATE SMEAR TAKEN

RACE

2= WHITE
4= COLOURED
8= BLACK

FOLDER NO.

REQUESTING HOSPITAL

LOCATION

PREVIOUS CYTOLOGY ○ YES

PREVIOUS CYTOLOGY ○ NO

NUMBER.....

DATE.....PLACE.....

PREVIOUS RESULTS.....

.....

ORIGIN OF SMEAR

○ CERVICAL
○ VAGINAL
○ OTHER (SPECIFY)

DIAGNOSIS.....

.....

OTHER INFORMATION.....

.....

.....

SMEAR TAKEN BY (BLOCK LETTERS).....

DATE OF L.M.P. .....

DATE LAST PREGNANCY.....

PARITY

CONDITION OF CERVIX

HEALTHY

YES	1
NO	2

(if no, specify).....

CA. CERVIX CLINICALLY SUSPECTED

YES	1
NO	2

PELVIC IRRADIATION

DATE.....

TYPE.....

YES	1
NO	2

I.U.C.D.

DATE.....

TYPE.....

YES	1
NO	2

HORMONES WITHIN LAST THREE MONTHS

1=NONE
2=OESTROGEN (e.g. PREMARIN)
3=PROGESTERONE (e.g. DEPO PR.)
4=MIXED (e.g. O.C. PILL)
5=OTHER.....

PREGNANT

WEEKS.....

YES	1
NO	2

POST MENOPAUSAL

YEARS.....

YES	1
NO	2

ENDOCERVICAL CELLS

1	PRESENT
2	ABSENT

FLORA

1=NONE
2=TYPE I DÖDERLEIN
3=TYPE II DÖD & OTHER BACTERIA
4=TYPE III OTHER BACTERIA

PATHOGENS

1=NONE
2=TRICHOMONAS
3=LEPTOTHRIX
4=CANDIDA
5=G. VAGINALIS
6=OTHER

CATEGORY

A= MALIGNANT
B= SUSPICIOUS
C= ATYPICAL
D= BENIGN
E=NORMAL
F=INADEQUATE

RECOMMENDATION

.....

.....

FOR LABORATORY USE ONLY

SNOMED CODES

DECODE

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EXAMINED BY

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CHECKED BY

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STANDARD TEXT CODES (MAXIMUM 13)

DECODE

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APPENDIX 2

CLINICAL AND FOLLOW-UP DATA COLLECTION FORM

NO.	NAME	AGE	LMP PREGNANCY HORMONES	EXOGENOUS HORMONE NAME	EROSION Y or N	IUCD Y or N	FOLLOW-UP (DETAIL)	HISTOLOGICAL CORRELATION

Appendix 3
DATA COLLECTION FORM - ENDOCERVICAL STUDY

SPECIMEN NO. ..... **DATE** .....**AGE** .....

Follow-up (Specify)
.....

.....

.....

.....

1. Architectural features of endocervical cells.

1.1. Quantity *as determined by the number of groups of cells per slide.*
(A) (B) (C) (D) (E)
(< 5), (5 - 10), (10 - 20), (20 - 30), (30 - 40),
(F) (G) (H) (I)
(40 - 50), (50 - 60), (60 - 70) (70 - 80) *(Please ring the appropriate letter).*

In 1.2. to 1.6, insert "yes" or "no" as applicable.

1.2. Is there extreme crowding? [.....]

1.3. Are there > 50 cells per group? [.....]

1.4. Is feathering present? [.....]

1.5. Is there loss of polarity in strips? [.....]

1.6. Are rosettes present? [.....]

2. Cytological features:

In 2.1 to 2.3, insert "yes" or "no" as applicable.

2.1. significant variation in nuclear size/shape? [.....]

2.2. nuclear / cytoplasmic ration > 1/2 [.....]

2.3. a thin nuclear membrane is present [.....]

In 2.4 to 2.8, ring the appropriate choice as indicated

Is the: (A) (B) (C)
2.4. chromatin: (even), (dense) or (course)?
(A) (B) (C)
2.5. cytoplasm: (foamy),(vaculated) or (homogeneous)?
Are: (A) (B) (C) (D)

2.6. nucleoli: (absent),(micro),(macro) or (multiple)?

2.7. mitoses: (present) or (absent)? (Y/N)

2.8. apoptoses: (present) or (absent)? (Y/N)

3. Back ground features of the slide:
In 3.1 to 3.3, insert "yes" or "no" as appropriate

3.1. Is blood present? [.....]

3.2. Is there an inflammatory exudate? [.....]

3.3. Are pathogens present? [.....]

3.3.1. If pathogens are present, please indicate by ringing if: (canida), (trichomonas), (gardnerella), HSV), or (chlamydia) are present.(Input first two letters).*If "other" pathogens are present please specify:* .....
.....

4. Features of squamous cells.

Ring the appropriate choice in 4.1 to 4.3.

4.1 Are the squamous cells:(typical) or (atypical)? (Y/N)
(A) (B) (C) (D) (E)
4.2 If atypical are they:CIN1, CIN2, CIN3, HPV, C1HPV
(F) (G) (H)
CIN2HPV, CIN3HPV, SQ.CA?
(R) (I)
4.3 If atypical are they:Regenerative or inflamatory? (Use first 2 letters)

4.4 Are there other features of note: (yes) (no)?

4.4.1. *If there are other features of note, please specify what these are:*

.....

.....

.....

.....

.....

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.....

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Addendum 1 **THE 1991 BETHESDA SYSTEM**

ADEQUACY OF THE SPECIMEN
Satisfactory for evaluation
Satisfactory for evaluation but limited by ....(specify reason)
Unsatisfactory for evaluation .....(specify reason)

GENERAL CATEGORIZATION (OPTIONAL)
Within normal limits
Benign cellular changes: see descriptive diagnosis
Epithelial cell abnormality: see descriptive diagnosis

DESCRIPTIVE DIAGNOSES

Benign cellular changes

- Infection
  - *Trichomonas vaginalis*
  - Fungal organisms morphologically consistent with Candida sp.
  - Predominance of coccobacilli consistent with shift in vaginal flora.
  - Bacteria morphologically consistent with Actinomyces sp.
  - Cellular changes associated with herpes simplex virus
  - Other
- Reactive changes
- Reactive cellular changes associated with:
  - Inflammation (includes typical repair)
  - Atrophy with inflammation ("atrophic vaginitis")
  - Radiation
  - Intrauterine contraceptive device (IUD)
  - Other
- Epithelial cell abnormalities
  - Squamous cell
    - Atypical squamous cells of undetermined significance: qualify*
    - Low-grade squamous intraepithelial lesion encompassing HPV+ mild dysplasia/CIN 1
    - High-grade squamous intraepithelial lesion encompassing moderate and severe dysplasia, CIS/CIN 2, and CIN 3
    - Squamous cell carcinoma
  - Glandular cell
    - Endometrial cells, cytologically benign, in a postmenopausal woman
    - Atypical glandular cells of undetermined significance: qualify*
    - Endocervical adenocarcinoma
    - Endometrial adenocarcinoma
    - Extrauterine adenocarcinoma
    - Adenocarcinoma, not otherwise specified
  - Other malignant neoplasms: specify

Hormonal evaluation (applies to vaginal smears only)
Hormonal pattern compatible with age and history
Hormonal pattern incompatible with age and history: specify
Hormonal evaluation not possible due to ..... (specify)

---

* Atypical squamous or glandular cells of undetermined significance should be further qualified as to whether a reactive or a premalignant/malignant process is favoured.

\+ Cellular changes of human papilloma virus (HPV)(previously termed "koilocytosis", "koilocytotic atypia", or "condylomatous atypia") are included in the category of low-grade squamous intra-epithelial lesion.