CERVICAL SMEAR AT ANTENATAL BOOKING IN HUSM: IS IT WORTHWHILE?

By

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V ABBREVIATIONS

AGUS	Atypical glandular cells of undetermined significance
AIS	Adenocarcinoma in situ
ASCUS	Atypical squamous cells of undetermined significance
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in situ
HPV	Human papillomavirus
HSIL	High grade squamous intraepithelial lesion
HUSM	Hospital Universiti Sains Malaysia
IUCD	Intrauterine contraceptive device
LMP	Last menstrual period
LSIL	Low grade squamous intraepithelial lesion
O&G	Obstetric and Gynaecology
POG	Period of gestation
SCC	Squamous cell carcinoma

VI DEFINITION

Statement on specimen adequacy

a) Satisfactory for evaluation – Complete patient clinical information, clean smear, >75% of epithelial cells present with endocervical and/or transformation zone (Squamous metaplastic cell) present for interpretation.

b) Satisfactory for evaluation but limited by:-

i) Lack of patient clinical information.

ii) Partially obscured by blood, inflammation, thick areas, poor fixation, drying which precludes interpretation of 50 – 75% of epithelial cells.

iii) Lack of an endocervical/transformation zone component (this does not require a repeat smear. Location of transformation zone, age of pregnancy, previous therapy may limit the clinician's ability to obtain an endocervix sample (hence the clinician has to decide about repeat smear).

c) Unsatisfactory for evaluation

i) Lack of patient identification on the specimen and /or request form.

ii) A technically unacceptable slide i.e. obscurer by blood, inflammation, thick smear, poor fixation, air drying artifact etc, which precludes interpretation of 75% or more of the epithelial cells.

iii) Scant squamous cell component i.e. less than 10% of surface area of the slide.

Cervical Intraepithelial Lesion (CIN)

The spectrum of intraepithelial changes beginning as with a generally well differentiated neoplasm, traditionally classified as mild dysplasia and ending with invasive carcinoma. These changes, confined to squamous epithelium above the basement membrane, include nuclear pleomorphism, loss of differentiation as cells progress from the basement membranes to the surface epithelium. When one third or less of the distance from the basement membrane to the surface involved, the lesion are called grade I (CIN I); when more than one third but less than or equal to two thirds is involved, grade 2 (CIN II) and more than two thirds is involved or full thickness involvement, grade 3 (CIN III).

Low-grade squamous intraepithelial lesion (LSIL) – lesions caused by human papillomavirus and CIN I are placed in category called low grade squamous intraepithelial lesion (LSIL).

High-grade squamous intraepithelial lesion (HSIL) – consists of category of CIN II and CIN III.

Abnormal smear

Abnormal condition of the cervix identified in a smear can be divided into those which are definitely benign and those which are believed to have neoplastic potential. Benign abnormalities are relevant to the smear only if they prejudice its reliability as a test for the precancerous stages or if they are known to be associated with cervical intraepithelial neoplasia, as in the case of the human papillomavirus.

Inflammatory smear

Inflammatory changes are associated with cervicitis caused by trichomonas vaginalis, candidiasis or other organisms. They can be caused by degenerative or regenerative changes in the epithelial cells.

VII KELANTAN

Kelantan is one of thirteen states in Malaysia. This state covers an area of 14,929 km^2, consists mostly of lush rainforests and white beaches fringed by crystal clear waters.

There are ten districts in the state of Kelantan, viz Kota Bharu, Bachok, Pasir Putih, Tumpat, Pasir Mas, Machang, Kuala Krai, Tanah Merah, Jeli and Gua Musang. Kota Bharu, the capital is the major urban centre but there are plans to open the southern portion of the state under an ambitious multi-million dollar development project. There is a good system of roads in most parts of the state except in Gua Musang and in the interior region of Kuala Krai district.

Kelantan has a population of about 1.4 million, 95% is Malay with Chinese, Indians, Thais and Orang Asli making up the rest. Kelantanese are reknowned for their warmth and friendliness.

Kelantan's economy is chiefly agrarian with the land yielding padi, rubber and tobacco. Fishing along its 96 kilometres coastline is also an important economic activity while livestock rearing is gaining importance. Cottage industries which employ traditional skills such as batik painting, wood carving and songket wearing are also evident. In recent years, tourism has also become a major money spinner.

Business is indeed booming and it is the women folk who are actively exploiting this economic opportunity. One has only to stride leisurely in the Central Wet Market of

Kota Bharu to appreciate this. There, Kelantanese ladies will be haggling over the prices of fresh seafoods and fishes. Leafy vegetables and exotic herbs, fruit pickles and salted fishes. The women of this state are never the passive partner when it comes to running bussinesses. No wonder this cultural state is also known, historically as "Negeri Cik Siti Wan Kembang" which is literally means "a state governed by the Queen".

There are all together nine hospitals in the state – 2 in Kota Bharu district and one each in each of the districts except Bachok and Jeli. Previously, specialist services were only provided at Kota Bharu Hospital and University Science Malaysia Hospital at Kubang Kerian in Kota Bharu. Obstetric and gynaecology operations are only carried out at these two tertiary care hospitals. Recently starting from 1998, Kuala Krai Hospital was upgraded and at present Obstetrician and Gynecologist, Anaesthesiologist, Physician, Paediatrician, Surgeon and Pathologist are giving their specialist services to improve the health status of that district and surrounding areas.

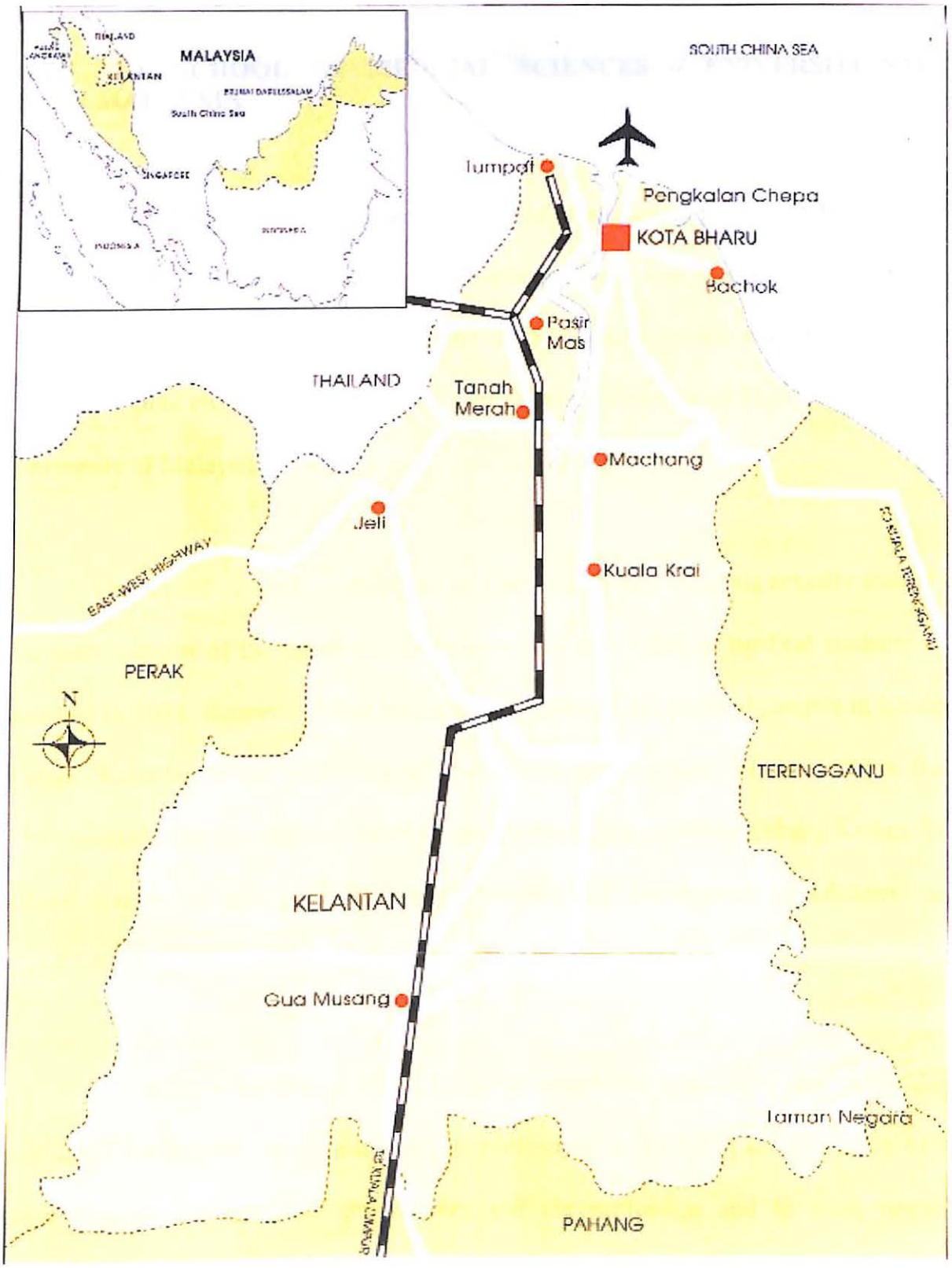

Figure 1. Map of Kelantan.

VIII THE SCHOOL OF MEDICAL SCIENCES – UNIVERSITI SAINS MALAYSIA

The School of Medical Sciences is the third medical school in Malaysia. It was set up in mid 1979. It is the first medical school which was built in a less developed eastern coast of West Malaysia, while the other two medical schools being located in the busy capital city of Malaysia, Kuala Lumpur viz. University of Malaya and National University of Malaysia.

The school of Medical Sciences of Universiti Sains Malaysia actually started in the main campus of the university in Penang. The first batch of medical students was enrolled in 1981. However, these pioneers only came to the medical campus in Kubang Kerian, Kelantan at the beginning of their fourth medical year. Further intakes from 1990 onwards had the students enrolled straight into the campus in Kubang Kerian. The school started its post-graduate programme for the Department of Obstetric and Gynaecology in 1991.

It is unique because it is the first to adopt our innovative and community orientated curriculum for its students. Its philosophy is to stress the relevance of its curriculum to the needs of the country and the profession and to work towards producing competent practitioners who would be able to identify themselves as part and parcel of the health care system of the country.

IX HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM)

Hospital Universiti Sains Malaysia (HUSM) is the teaching hospital for the school of Medical Sciences, Universiti Sains Malaysia. It was built in 1976 under the Third Malaysia Plan and is located at Kubang Kerian Town about 6.4km from the state capital Kota Bharu. The construction was completed in 1984 and was officially opened by the Royal Highness Al-Sultan Kelantan on 26th August 1984. The first patient was admitted on the 21st January 1984 and the first baby was born in April 1984.

Besides teachings and research, the University Hospital also provides adequate medical services for the population. It also serves as the referral center for the state and the neighbouring states in the East Coast of Peninsular Malaysia.

The hospital has a total of 675 beds for both medical and surgical disciplines. All the departments are adequately staffed and the hospital has backup services by the blood bank, laboratory and radiological units.

X THE DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

This is one of the departments in the School of Medical Sciences. The department staff consists of 11 lecturers, 5 registrars, 16 medical officers/trainee lecturers and 10 house officers. The postgraduate program was started in 1991 and the first Master of Medicine candidates in Obstetrics and Gynaecology graduated in June 1995.

The department of Obstetrics and Gynaecology occupies the first floor of the old hospital building and the ground floor, first floor and second floor of the new building. There are two gynaecology ward in the first floor of the old building with a total of 62 beds, and two obstetrics wards in the new hospital building with a total of 80 beds (40 beds antenatal and 40 beds postnatal).

The labour ward is situated on the first floor, adjacent to the obstetrics ward with an adjoining operating theatre. The Obstetrics and Gynaecology clinics situated at the ground floor, which are equipped with ultrasound machine, cardiotocography (CTG) machine and Colposcope. The clinics runs as follows:-

Table 1: O & G Clinic Schedule in HUSM.

DAY	MORNING	AFTERNOON
SATURDAY	ANTENATAL	BOOKING CLINIC
SUNDAY	ANTENATAL OUTPATIENT CLINIC	GYNAECOLOGY OUTPATIENT CLINIC
MONDAY	1) COMBINED MEDICAL CLINIC 2) MENOPAUSE CLINIC 3) OUTPATIENT ULTRASOUND CLINIC	1) ONCOLOGY & MOLAR CLINIC 2) COLPOSCOPIC CLINIC
TUESDAY	ANTENATAL OUTPATIENT CLINIC	GYNAECOLOGY OUTPATIENT CLINIC
WEDNESDAY	FERTILITY AUGMENTATION CLINIC	1) FAMILY PLANNING CLINIC 2) POSTNATAL CLINIC

The doctors are divided equally into four teams to manned these clinics as well as the wards and duties during normal working days as well as on call days.

XI ABSTRACT

Objectives: The study is aimed to determine the prevalence of abnormal cervical smear in pregnant women attending booking clinic at Hospital Universiti Sains Malaysia. Secondly, we aimed to determine the pregnancy-related physiologic changes associated with normal cytological cervical smear in the study population. Lastly, we planned to compare the cytological cervical smears between pregnant and non-pregnant women in Hospital Universiti Sains Malaysia.

Methodology: It is a prospective cross-sectional study, was carried out at the Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan between July 1999 till June 2000. A total of 321 pregnant women agreed to participate after counseling and verbal consent was obtained.

Results: From 321 cervical smears collected, 310 (96.6%) were satisfactory smears and 11 (3.4%) were unsatisfactory. The detail results of satisfactory smear, normal smear were 55 (17.7%), non-specific inflammatory smear were 193 (62.3%), infection of Gardnerella were 4 (1.3%), Actinomyces infection was 1 (0.3%), *Trichomonas vaginalis* infection was 1 (0.3%), Candidiasis infection were 45 (14.5%), ASCUS were 5 (1.6%), AGUS were 3 (1.0%), LSIL (HPV & CIN I) were 3 (1.0%) and no HSIL or carcinoma.

From the study, the pregnancy-related changes associated with normal cervical smears in the study population consist of 285 (91.9%) navicular cells, 308 (99.3%) Lactobacilli, 207 (66.8%) squamous metaplastic cells, 3 (1.0%) decidual cells and no trophoblastic cells or "Arias-stella" changes cells. Navicular cells and abundant

lactobacilli are characteristic of pregnancy-related changes in the cervical smear that are commonly seen. Decidual cells, trophoblastic cells or "Arias-stella" cells are rarely seen in pregnancy.

When comparing the cervical smear of pregnant women with the cervical smear of non-pregnant women, the results were statistically not significant at p-value <0.05 for normal smear, non-specific inflammatory smear, *Gardnerella vaginalis* infection, Actinomyces infection, Chlamydia infection, ASCUS, AGUS, LSIL, HSIL, Squamous cell carcinoma and adenocarcinoma of the cervix. The result was statistically significant at p-value <0.05 for candida infection. Candida infection is more prevalent in pregnant women.

In non-pregnant population, we are able to detect more abnormal cervical smears such as LSIL (2.0%), HSIL (1.1%), Squamous cell carcinoma (0.3%) and adenocarcinoma of the cervix (0.3%). This is probably because the samples are larger, a total of 831 cervical smears.

Conclusion: We conclude that we are able to detect abnormal cervical smears, whether the smears are taken during pregnancy or non-pregnant state and larger samples are required in pregnant population to get statistically significant results. Physiological pregnancy-related changes that may be present in the cervical smears such as decidual cells, trophoblastic cells or "Arias-stella" changes cell which can mimic malignant cells are rarely seen in pregnant population and we can differentiate the cells with meticulous and expert interpretation of the smears by the cytopathologist.

XII ABSTRAK (Malay Version)

***Objektif**: Kajian dijalankan untuk menentukan prevalens kes-kes calitan serviks abnormal dalam wanita hamil yang menghadiri klinik "booking" di Hospital Universiti Sains Malaysia. Tujuan kedua adalah untuk menentukan sitologi calitan serviks yang normal yang berkaitan dengan perubahan fisiologi semasa hamil di dalam populasi terlibat. Akhir sekali, kajian ini bertujuan untuk membezakan sitologi calitan serviks di antara populasi wanita hamil dan wanita yang tidak hamil.*

***Metodologi**: Kajian ini adalah kajian bersilang prospektif yang telah dijalankan di Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan dari bulan Julai 1999 hingga bulan Jun 2000. Seramai 321 wanita hamil telah bersetuju untuk menyertai kajian ini setelah kaunseling dan kebenaran telah diberi dengan persetujuan secara lisan.*

***Keputusan**: Dari 321 calitan serviks yang diambil, jumlah calitan yang "satisfactory" adalah 310 (96.6%) dan yang "unsatisfactory" adalah 11 (3.4%). Dari calitan yang "satisfactory", keputusan terperinci adalah 55 (17.7%) normal, 193 (62.3%) yang "non-specific inflammatory", 4 (1.3%) infeksi Gardnerella vaginalis, 1 (0.3%) infeksi Trichomonas vaginalis, 1 (0.3%) infeksi Actinomyces, 45 (14.5%) infeksi Candida, 5 (1.6%) "ASCUS", 3 (1.0%) "AGUS", 3 (1.0%) "LSIL", dan tiada "HSIL" atau karsinoma.*

Dalam kajian ini, sitologi calitan serviks yang normal yang berkaitan dengan perubahan fisiologi semasa hamil adalah dengan adanya ciri-ciri seperti kehadiran sel "navicular" sebanyak 285 (91.9%) calitan, "Lactobacilli" sebanyak 308 (99.4%), sel

skuamus metaplasia sebanyak 207 (66.8%), sel "decidual" sebanyak 3 (1.0%), tiada sel "trophoblastic" atau sel yang ada perubahan "Arias-stella".

Kehadiran sel "navicular" dan "lactobacilli" adalah merupakan ciri-ciri perubahan fisiologi semasa hamil yang biasa didapati dan sel "decidual", sel "trophoblastic" serta sel yang ada perubahan "Arias-stella" adalah jarang didapati.

Dengan membezakan keputusan calitan serviks wanita hamil dan wanita tidak hamil, tiada perbezaan statistik yang signifikan yang didapati pada "p-value" <0.05, untuk calitan normal, calitan non-specific inflammatory, infeksi Gardnerella vaginalis, infeksi Actinomyces, infeksi Chlamydia, "ASCUS", "AGUS", "LSIL", "HSIL", karsinoma sel skuamus dan "adenocarcinoma". Tetapi keputusan mempunyai perbezaan statistik yang signifikan pada "p-value" <0.05 untuk infeksi Candida. Infeksi candida adalah lebih prevalens dikalangan wanita hamil.

Dalam populasi wanita tidak hamil, kita dapat mengesan lebih banyak calitan serviks yang abnormal seperti "LSIL" (2.0%), "HSIL" (1.1%), sel skuamus karsinoma (3.0%) dan "adenocarcinoma" (3.0%). Ini mungkin kerana sampel calitan serviks bagi wanita tidak hamil adalah lebih besar, jumlahnya adalah 831 calitan serviks.

***Kesimpulan**: Sebagai kesimpulan, kita dapat mengesan calitan serviks abnormal, sama ada calitan itu diambil semasa wanita itu hamil atau tidak hamil, dan sampel yang besar diperlukan untuk populasi wanita hamil untuk mendapatkan keputusan yang signifikan secara statistik. Calitan serviks yang berkaitan dengan perubahan fisiologi semasa hamil seperti kehadiran sel "decidual", sel "Trophoblastic" atau sel perubahan "Arias-stella" yang boleh mimik sel malignan adalah jarang dijumpai pada populasi wanita hamil dan kita boleh membezakan sel-sel tersebut dengan pemerhatian dan kepakaran "cytopathologist" yang meinterpretasikan calitan serviks.*

1 INTRODUCTION

Cancer of the cervix is the second most frequent malignancy in women. Recent estimates of the number of new cases appearing each year worldwide, representing 15% of all cancers diagnosed in women (Parkin DM, Laura E, Muir CS, 1980). About 80% of these cases occur in developing countries, where it is the most common of all cancers among women. There have been reports of increasing rates of cervical cancers in younger populations, particularly under 35 years of age (Elliot PM, Tattersall MHN et al, 1989).

It is well accepted that conscientious and widespread use of cervical cytology will significantly decrease the incidence and mortality rates of cervical cancer. Cervical smear is routinely taken at antenatal booking visit in most developed-countries but not in our centre, cervical smear is only taken when patient has symptoms such as per vaginal discharge, post-coital bleeding or bleeding during pregnancy.

The screening programme usually involved the non-pregnant women at reproductive age groups and post-menopausal women. Pregnancy represents an ideal time for an opportunistic cervical cancer screening in order to increase the coverage of screening programme. Direct visual inspection of the cervix, cervical cytology and bimanual examination should form part of routine antenatal examination at booking.

Therefore this gave me the interest to highlight screening by the cervical smear on the antenatal women in HUSM, in order to have the local data regarding the prevalence and incidence of abnormal cervical smears in pregnant population and more

women can be screened because this is the best time for them to see doctors and be examined.

At University Hospital, University of Malaya the incidence of abnormal smears in pregnancy was 1:294 deliveries (Sivanesaratnam et al 1998). The incidence of carcinoma in situ in pregnancy has been reported to be 1 in 770 pregnancies and that of invasive carcinoma, 1 in 2,200 pregnancies (Hacker et al, 1982). At University Hospital, University of Malaya, the incidence of invasive carcinoma in pregnancy is 1 in 4,077 pregnancies (Sivanesaratnam et al 1991,1998). One to nine percent of patient with cervical carcinoma are said to be pregnant within six months when the diagnosis was established (DiSaia and Creasman, 1989, Creasman 1970).

CYTOLOGY OF NORMAL CERVIX

1.1 Cell Content of Normal Cervical Smear

In the absence of pathologic conditions, the smears contain mainly squamous and columnar cells. The squamous epithelium responds to ovarian hormonal production. Three types of squamous cells can be distinguished. The superficial cells, the intermediate cells and the parabasal cells (Wied GL, Bibbo M 1988).

The superficial cells are large, flat, polygonal and mostly eosinophilic. They contain a pyknotic nucleus, which is small (5μm to 6μm in diameter), dark and featureless.

The intermediate cells are somewhat smaller than the superficial cells, are usually flat and polygonal, and contained a vesicular nucleus with finely granular or reticular chromatin, a smooth nuclear membrane, and occasionally a small nucleolus and/or an X-chromatin body (about 1 μm in diameter, firmly adherent to the inner aspect of the nuclear membrane). The cytoplasm is cyanophilic and may contain glycogen which stains yellowish with Papanicolaou technique.

The parabasal cells are small, rounded and cyanophilic. They contained a large vesicular nucleus, similar to the nucleus of intermediate cells.

Squamous cells are found singly or in small clumps but show no tendency to form any special groupings.

Columnar endocervical cells are often shed in sheets or strips. They are elongated and cyanophilic with a clear cytoplasm. The eccentric vesicular nucleus contains a finely distributed chromatin, a small nucleolus, and often an X-chromatin body. When present in sheets or strips, the endocervical cells conserve their polarity, meaning that their orientation recalls their histologic structure. Endocervical cells may display a certain amount of anisokaryosis.

Endometrial glandular cells are observed normally during the first ten days of the cycle. They are smaller than endocervical cells, with scant, sometimes vacuolate, cyanophilic cytoplasm and characteristically are grouped in tight three-dimensional clusters. The nuclei are vesicular and contain finely distributed chromatin, usually

without visible nucleoli. From day 6 to day 10 of the cycle, endometrial glandular cells form tight balls surrounded by one or more layers of larger cells with pale cyanophilic cytoplasm, originating in the endometrial stroma. This typical formation has been called "exodus" by Papanicolaou.

Small histiocytes can be observed at the end of the menstrual cycle and during the first 10 days of the proliferative phase. They are thought to originate in the endometrial stroma. They are similar in size to parabasal cells, but their cyanophilic cytoplasm is foamy, their cellular outlines are indistinct and their nucleus is often notched or kidney or bean-shaped with prominent chromatin pattern. There may be two or more nuclei. When the nuclei are numerous, histiocytes can become quite large and are then designated as multinucleated giant cells. They may contain phagocytozed material (hemosiderophages, for instance) in which case, they are called macrophages. In the absence of inflammation, macrophages are scavengers, removing debris and blood after menstruation.

Granulocytes are commonly observed at the beginning and the end of the menstrual cycle and do not indicate the presence of infection or inflammation. Lymphocytes and plasma cells are rarely observed on the normal smears. Psammoma like concretions are occasionally observed and probably represent inspissated mucus (Wied GL, Bibbo M, 1988).

1.2 Hormonal Changes of Normal Cervical Cytology

The general pattern of the cells varies depending on the stage of the menstrual cycle, during pregnancy or puerperium and also prepubertal girl and postmenopausal woman.

1.2.1 At Birth

Gonadal hormones are produced in large amounts during pregnancy and pass readily through the placenta into the fetal circulation. The squamous epithelium of the cervix and of the vagina of the newborn girl responds to this strong hormonal stimulation with a marked maturation. It will contain a clear predominance of superficial cells, with a general pattern very similar to that of a mature woman at mid cycle (Butler EB, Taylor DS 1973, Uyanwah PO 1985).

1.2.2 In Childhood

Within a few days to 1 week after birth, the maternal hormones are eliminated and the genital crisis set in, the more mature layers of squamous epithelium are desquamated and the smears will contain mostly parabasal cells. This atrophic pattern persists until the onset of puberty.

1.2.3 At Puberty

Even before the first menstrual period occurs, the smear begins to change, intermediate cells replace the parabasal cells of childhood and a few scattered superficial cells reflect the onset of oestrogen secretion. This pattern may appear several months before clinical menarche.

1.2.4 During the Reproductive Years.

Day 1 of cycle is the first day of menstruation. The first 5 days are characterized by a smear containing blood, cellular debris, inflammatory cells, and endometrial glandular cells and stroma cells. Endometrial glandular cells are often found in compact groups, with little nuclear detail. Endometrial stroma cells which resemble small histiocytes are shed as clusters or discrete cells. There are many superficial cells, but there is often a false eosinophilia, ie the cytoplasm of intermediate cells stains pink.

From day 5 to day 10, the smear usually contains a predominance of intermediate squamous cells, which may display a slightly wrinkled cytoplasm. The cells may occur in clusters but are mostly isolated. Endometrial glandular and stromal cells are found in tight groupings, which Papanicolaou called "exodus". The stromal cells form the inner part of the cluster; they are tightly grouped and somewhat hyperchromatic. The glandular cells have more cytoplasm than the stromal cells, and form the periphery of the clump. The smear cleans up and by day 10 there are few if any inflammatory cells and no more blood seen.

Day 11 to 15 comprise the ovulatory phase, characterized by very clean smears with many superficial squamous cells, mostly isolated and quite flat. Just before or at time of ovulation, the endocervical mucus is very abundant and can be stretched outside the vagina when grabbed with a forceps; this is called "spinnbarkeit". When this mucus is spread on the glass slide and allowed to air-dry without fixation or staining, it will reveal a special fern-like crystallization under microscope.

The secretory phase begins around day 15 of the cycle. During the first few days the cells tend to group together and to lose some of their flatness. Eventually the cellular membrane becomes crinkled and the cells form dense aggregates in which the cellular outline are indistinct. The predominant cell during secretory phase is intermediate cells which contain large amount of glycogen.

At the end of the cycle, leucocytes and histiocytes in varying numbers may be seen. With the degeneration of the corpus luteum, progesterone secretion ceases, but estrogen levels are maintained by developing follicles, so that the predominant cells are of the intermediate type, with scattered superficial cells (Meisels A, 1988).

Anovulatory cycles can be detected by the absence of signs of progesterone activity during the second half of the cycle. For this purpose a smear taken on days 22 to 24 of 28-day cycle is particularly useful (Meisel A, 1965). Contraceptive pills mostly contains oestrogen-like substances associated with progesterone-like chemicals, which causes smears to resemble those observed during the secretory phase (Heber KR 1975, Reyniak JV 1969).

In amenorrheas, the cytohormonal smear may reveal whether the ovaries are functional. If the smear shows atrophy, then there is no hormonal production. This finding is of prognostic significance, particularly in primary amenorrheoa.

1.2.5 During Pregnancy.

During the first trimester of pregnancy the hormonal support is supplied by the corpus luteum, which produces increasing amount of oestrogen and progesterone. At the end of 14th week, the placenta takes over the function of corpus luteum, which degenerates and ceases to function.

The cervical smear pattern in normal pregnancy does not vary significantly from that seen in the normal secretory phase. The pregnancy smear has two characteristic features:-

1) the presence of numerous "navicular" cells, so called by Papanicolaou because of their shape; these small intermediate cells contain a large amount of glycogen, which pushes the cytoplasm out to the periphery where it becomes dense and cyanophilic; the nucleus is eccentrically located; and
2) a marked cytolysis related to the abundance of lactobacilli, which thrive in the glycogen-rich environment.

Decidual changes, on rare occasions, occuring in the cervix may give rise to large cells in the cervical smear. These are round, pale centrally located large vesicular (one or two) nuclei with prominent nucleoli. Since these findings are unusual, the decidual cells may be mistaken for dyskaryotic cells (squamous carcinoma).

Arias-Stella changes, cytologically the large cells with irregularly shaped and hyperchromic nuclei, associated with Arias-Stella may be confused with dyskaryotic cells.

Trophoblasts may appear on gynecologic smears during pregnancy or in the weeks following delivery. Cytotrophoblasts have one, or rarely two, nuclei with finely distributed, regular chromatin and a prominent nucleolus, and a cyanophilia, sometimes vacuolated cytoplasm. They occur singly, or more frequently, in clusters in which the cellular boundaries are indistinct. Syncytiotrophoblasts form clumps of varying size and shape. The cytoplasm is dense, cyanophilic, and contains a variable number of darkly stained nuclei with coarse chromatin and small nucleoli.

Trophoblasts are rarely found on routine cervical smears from non-pregnant women. When they are numerous, or if they are present with morphologic abnormalities, the possibility of a trophoblastic tumour (hydatidiform mole, invasive mole or choriocarcinoma) should be taken into consideration.

Metaplastic cells present when the cervix becomes everted to expose the columnar epithelium of the canal. This is most marked at puberty, during pregnancy and women with high-oestrogen oral contraceptives. The acid pH of the vagina stimulates replacement of columnar epithelium with squamous metaplasia. The area in which this initially occurs is known as the "transformation zone". If the squamous metaplasia has matured completely, it cannot be recognized on cellular samples; the cells are identical to the mature squamous cells of the cervix. Only cells from immature metaplasia can be identified with certainty. Such cells are parabasal cells with a normal nuclear/cytoplasmic (N/C) ratio with round or oval nucleous containing finely granular or reticular chromatin; the nuclear membrane is smooth and thin, small nucleoli may be present. The cytoplasm is dense and cyanophilic. Metaplastic cells show great

uniformity of size and shape. They may occur singly or in small groups or chain, rarely in loose sheets. Because of the cytoplasmic processes, they are called "spider" cells.

Presence of endometrial cells in a cervical smear during pregnancy is an abnormal finding (Meisels A, 1991).

The histological and cytological interpretation of material taken from a cervix during pregnancy is often said to be more difficult than that in the non-pregnant state. This is misleading because, once the changes described above are eliminated, there is no differences between the histological or cytological appearances of epithelial abnormalities in the features associated with CIN in pregnancy from those seen in the non-pregnant state (Creasmann et al 1970, Sivanesaratnam 1991).

1.2.6 After Menopause

The cellular pattern after the menopause depends on the amount of residual oestrogen secreted by the ovarian stroma and the adrenal cortex. Superficial cells may persist on the smear for many years. Eventually the cellular pattern becomes atrophic, when and if oestrogen production effectively ceases. The smear then consists mostly parabasal cells.

1.3 Nonspecific Cytological Changes In Inflammation

In the presence of active inflammation smears usually contain many polymorphonuclear leucocytes and histiocytes followed by lymphocytes and plasma cells if inflammation persists. The epithelial cells show various inflammatory changes and cell debris may be found. The composition of the cell population also changes when the upper layers of epithelium are damaged, parabasal cells from the lower layers of the epithelium are found. This phenomenon is even pronounced when there is regeneration of already injured epithelium. On the other hand in some circumstances inflammatory irritation may increase the maturation of the squamous epithelium leading to a smear pattern with predominantly superficial cells and sometimes even with anucleate squamous due to keratinization. A smear is identified as inflammatory when some or all of the following changes are found :-

- There is a marked increase in inflammatory cells, beyond the physiological mild to moderate leucocytosis seen in normal women.
- The epithelial cells are covered by an exudate of polymorphs, especially if polymorphs can be seen permeating the cytoplasm of epithelial cells.
- Lymphocytes may present in significant numbers, with or without plasma cells.
- Reactive and degenerative changes are seen in epithelial cells with altered maturity of cells (Gray W, 1995).

1.4 Inflamatory Changes In Squamous Epithelial Cells (Gray W 1995).

Cytoplasm and nucleus may show changes. Cytoplasmic abnormalities includes:-

1) Vacuolation; small vacuoles may appear in the cytoplasm, sometimes merging into a single large vacuole pushing the nucleus to one side. The thin border of cytoplasm can usually be identified as that of a squamous cell because it retains its compact appearance. Leucocytes may be seen within vacuoles in the epithelial cells, a process known as leucophagocytosis.

2) Perinuclear haloes:- the cytoplasm immediately surrounding the nuclear membrane is lighten in colour. The boundary of the halo is often vague.

3) Changes in staining reaction, with certain types of inflammation, intermediate parabasal and metaplastic cells show eosinophilia, staining pink instead of green.

4) Premature or excessive keratinization of squamous cells may occur.

The following changes can be seen in the nucleus:

The nucleus commonly enlarges with pale staining due to fluid absorption. This results in considerable variation in the nuclear size. Wrinkling of the nuclear membrane may occur, with selective condensation of chromatin at the nuclear margin and cleaning of the nucleus centrally. There is retention of nuclear symmetry and the nucleolus may disappear altogether. Multinucleation is common.

Condensation of chromatin, known as pyknosis, may occur in dying cells, or the nucleus may disintegrate in a process known as karyorrhexis. Dissolution of the nucleus

or karyolysis is another mode of cell death which is sometimes seen in inflammed smear. Nucleoli may be prominent and multiple.

1.5 Inflammatory Changes in Endocervical Cells

Sometimes the delicate cytoplasm loses definition and lies in taffers around the nucleus or is permeated by polymorphs. In other cases mucus vacuolation of cytoplasm is prominent as in goblet cell hyperplasia.

Nuclei vary markedly in size, but the shape is always round to oval. The nucleus sometimes has a large nucleolus, occasionally even two. The chromatin may be somewhat coarsened but never markedly so. Nuclear overlapping, as occasionally seen in malignant or dyskaryotic columnar cells, does not occur except when due to multinucleation, which is common. When regeneration supervenes, mitotic figures are seen in the endocervical epithelium, but the mitosis are always morphologically normal. Occasionally, numerous anucleate small ciliated tuffs are found, consisting of the terminal plate and pink or red cilia, mingling with vacuolated endocervical cells without cilia resembling histiocytes. Pyknotic nuclear remnants are also usually seen. The phenomenon, called ciliocytophthoria, was originally thought to be due to viral damage, but is now recognized as non-specific degenerative change (Muller K 1975).

1.6 Cytological Changes in Infection

1.6.1 Bacteria

Lactobacillus of Doderlein has been considered to represent the 'normal' flora, it is found in only about 20% of patients screened (Barlett JG, 1978). It is a rod of variable length, usually associated with cytolysis. Cytolysis occurs when, in the presence of lactobacilli, the cytoplasm disappears, resulting in the presence of "naked" nuclei. The Doderlein bacillus feeds on glycogen, which is found in the intermediate cells and is more abundant during the second half of the menstrual cycle and during pregnancy. The naked nuclei represent the remains of intermediate cells. Cytolysis occurs with a "clear" background, as opposed to autolysis, which is the destruction of the cytoplasm by the action of other agents, and which is accompanied by numerous inflammatory cells.

Gardnerella vaginalis (previously Haemophilus vaginalis or Corynebacterium vaginalis) adhere to the surface of squamous cells, forming a uniform grainy cyanophilic cover known as "clue cells". Gardnerella vaginalis can be cultured in the vast majority of cases when clue cells are present (Bibbo M, Wied GL 1988).

Chlamydia trachomatis is an extremely small intracellular microorganism responsible for trachoma and inclusion conjunctivitis, lymphogranuloma venereum, and sexually transmitted infections of the genital tract that may cause pelvic inflammatory disease and infertility. Chlamydia infection of the cervix is diagnosed by culture, monoclonal antibodies and enzyme-linked immunoassays. The Papanicolaou smear is

unreliable. The cellular changes that have been described consist of small eosinophilic coccoid bodies found within the cytoplasm and fine vacuoles appear with larger cyanophilics inclusions. The vacuoles are of uniform size and have distinct outlines. They are grouped around the nucleous and tend to mold to each other (Johanisson G, Lowhagen GB, Lyche E 1980).

1.6.2 Protozoa

Trichomonas vaginalis appears on smears as a pale, greyish pear-shaped body, which resembles partially lyzed cytoplasm. It often contains a small, dense, accentric nucleus. The cytoplasm displays small, round, eosinophilic granulations. Trichomonads may occur without signs of inflammation but frequently produce a marked reaction, trichomoniasis with abundant inflammatory exudate. Mature squamous cells often display perinuclear halos in the presence of trichomoniasis, and the smear is poorly stained, most cells being faintly eosinophilic. In 1960s, Bibbo reported finding Trichomonas vaginalis in about 13% of women screened, in 1970s the ratio was reduced to 6% and presently it is in order of 2% to 3%. This rate varies widely from one area to another and the University of Chicago Clinic in 1987, reported 15% positive smear (Bibbo 1988).

A thin, elongated bacterium known as ***Leptothrix*** is often found associated with trichomonads and can be easily recognized on smears. It varies in length and does not seem to have much clinical significance when present without other pathogens.

1.6.3 Fungi

Actinomyces are occasionally found on smear in patient wearing an intrauterine contraceptive device. They are recognized by their characterictic "dust-ball" appearance: small, round, poorly delineated formation of microorganisms from which thin filaments radiate in all directions.

Candida are large yeast cells with elongated segmented forms. They are identified by their budding and pseudomycelia. Squamous cells on the smear are often poorly stained, with a pale, sometimes eosinophilic vacuolated cytoplasm. The presence of fungi on smears should be reported but it is difficult to be specific because these microorganisms closely resemble each other (Eriksson G, Wanger L 1975).

1.6.4 Viruses

The most common viral infection of the genital tract is produced by ***Human papillomavirus*** (HPV). When present the cytologic findings are characteristic (Naif ZM 1966). Koilocytosis are mature squamous cells (intermediate or superficial) characterized by a large perinuclear cavity. This cavity has sharply cut borders and appears very lightly stained, usually cyanophilic. Peripheral to this cavity, the cytoplasm is very dense and may stain intensely eosinophilic or cyanophilic. When eosinophilic, it sometimes appears as dense as hyaline. The nucleus of koilocytes display various degree of degeneration, depending on the stage of HPV infection. The nuclear membrane is usually not apparent. There are neither nucleoli nor inclusion

bodies. Binucleation is frequent and multinucleation can be observed, but without molding of the nuclei. Koilocytes are pathognomonic, their presence is definite proof of HPV infection. However, the Papanicolaou stain must be of excellent quality, so that the finer characteristics of cytoplasmic amphophilia may be discerned.

Dyskeratocytes are keratinized squamous cells, staining a brilliant orange with the Orange-6 staining (OG-6) of the Papanicolaou technique. They occur mostly in thick, three-dimensional clusters, characterized by the presence of vesicular nuclei with indistinct chromatin detail, identical to the nuclei of koilocytes. This features are also pathognomonic for HPV infection. In many cases, dyskeratosis is the only signal of HPV infection even in the total absence of koilocytes (Byrne P, Woodman C, Meanwell C, Kelly K, Jordan K, 1986).

1.7 Squamous Intraepithelial Lesions.

1.7.1 Low Grade Squamous Intraepithelial Lesion (LSIL)

Low Grade Squamous Intraepithelial Lesion (LSIL) category of the Bethesda System encompasses cellular changes due to HPV infection and mild or slight dysplasia (Cervical Intraepithelial Neoplasia – CIN I).

1.7.2 CIN I

Classical description of CIN I refer to mature superficial or intermediate cells containing enlarged, granular or reticular chromatin. Nucleoli are not visible. The nuclear membrane may be slightly convoluted, but generally appears smooth. Binucleation can be seen. The nuclei/cytoplasmic (N/C) ratio is moderately increased. The cytoplasm often appears normal; sometimes the staining is amphophilic and a perinuclear halos can be observed occasionally (Meisels A, Morin C, 1991).

1.7.3 High Grade Squamous Intraepithelial Lesion (HSIL)

In the Bethesda System, high-grade squamous intraepithelial lesion (HSIL) encompasses the cellular changes previously known as moderate dysplasia (CIN II) and severe or marked dysplasia and carcinoma in situ (CIN III). HSIL is characterized on the smears by the presence of immature cells of the parabasal type, with an increased N/C ratio, containing enlarged, somewhat irregular, hyperchromatic nuclei. The chromatin is either granular or reticular, or displays irregular chromatin bands and chromocenters. Nucleoli are small or absent. The nuclear membrane is often unevenly stained and its outline may be jagged. The cytoplasm is dense and cyanophilic, rarely eosinophilic. These cells are found isolated or more often, forming small groups. The pattern is quite uniform, the degree of anisokaryosis is slight and the background in absence of coexisting inflammation is clean (Meisels A, Morin C 1991).

1.7.4 Atypical Squamous Cells of Undetermined Significance (ASCUS).

It was meant to be used sparingly and expressively excluding reactive inflammatory and reparative changes and including only "puzzling", inconclusive smears for which a distinction could not be made between CIN and the unusual reactive changes (Schiffman et al 1993). Three to ten percent of women screened will present with this type of smears.

1.7.5 Atypical Glandular Cells of Undetermined Significance (AGUS)

AGUS consist mostly an enlargement of the nuclei with some degree of anisokaryosis and presence of nucleoli. The endocervical glandular cells are found mostly in clusters or small groupings, without much overlapping. These changes may be reactive, or in some cases, may be precursors to more advanced lesions like adenocarcinoma in situ (AIS).

In AIS, the smear contain numerous endocervical glandular cells forming sheets, strips or rosettes with sometimes recognizable pseudostratification and loss of polarity. There is little or no overlapping of cells and sometimes certain borders may be seen on the periphery of the sheets or strips. The nuclei are larger than those of benign endocervical cells but they remain uniform and hyperchromatic. Small nucleoli are rarely present. The background is clear. When there is an associated SIL, the smear may contain predominantly cells from that lesion and the atypical endocervical cells may be entirely absent or overlooked (Bousfield L, Pacey F, Young Q 1980, Kumins I et al 1977).

1.8 Cytology of Invasive Squamous Carcinoma

Keratinizing carcinoma is identical on smears by presence of differentiated keratinized squamous cells, which may display bizarre shapes (tadpole like, fibroid etc). The enlarged nuclei are irregular, hyperchromatic sometimes pyknotic. They rarely contain a prominent nucleolus. They contain abundant keratin, keratin pearls and intercellular bridges.

Large cell carcinoma characterized by the presence of aggregates of large cells with cyanophilic cytoplasm and an increased nucleo-cytoplasmic ratio. The nuclei are increased in size and display marked anisokaryosis, their outline is irregular, the nuclear membrane appears irregularly thickened because of chromatin deposits on its inner aspect; chromatin is coarsely granular, with prominent bands and chromocenters, irregularly shaped macronucleoli are commonly seen. There are many mitotic figures. Monocellular keratinization is commonly seen but there are no keratin pearls.

Small cell carcinoma sheds, small round cells with very scanty cyanophilic cytoplasm, high nucleus/cytoplasm ratio. A hyperchromatic nuclei without prominent nucleoli, which are usually found isolated or forming small groups without much overlapping. The cells are slightly larger than lymphocytes. They resemble anaplastic small cell (oat-cell) carcinoma of the lung. No keratinization is seen in these tumours (Meisels A, 1991).

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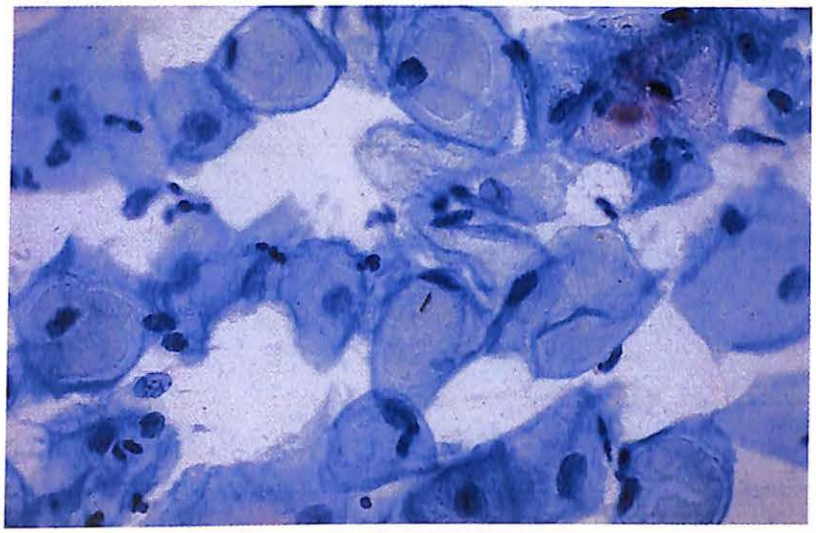

Figure 1.1 Navicular cells.

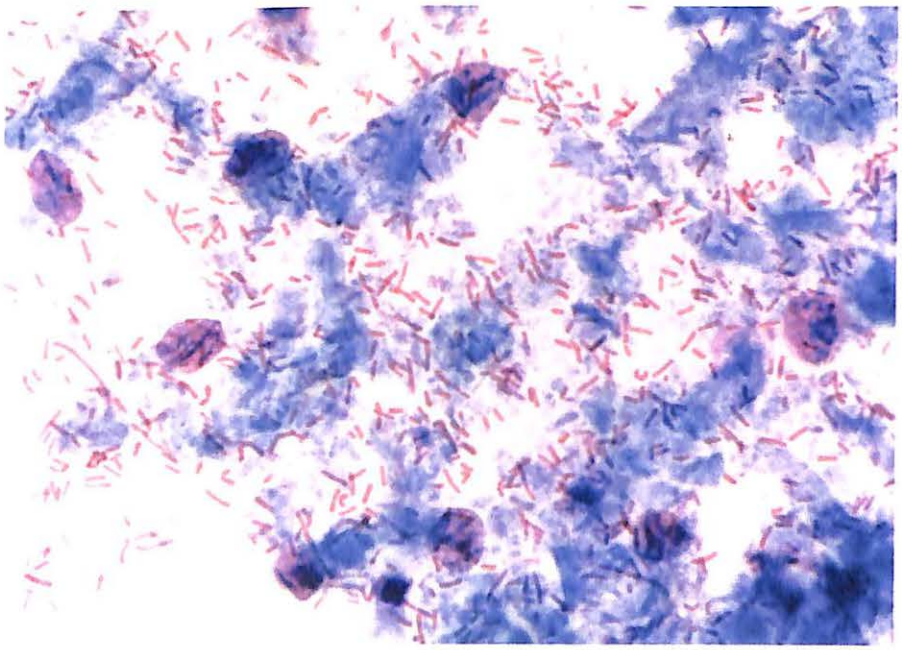

Figure 1.2 Abundant *Lactobacilli vaginalis* and cytolysis.

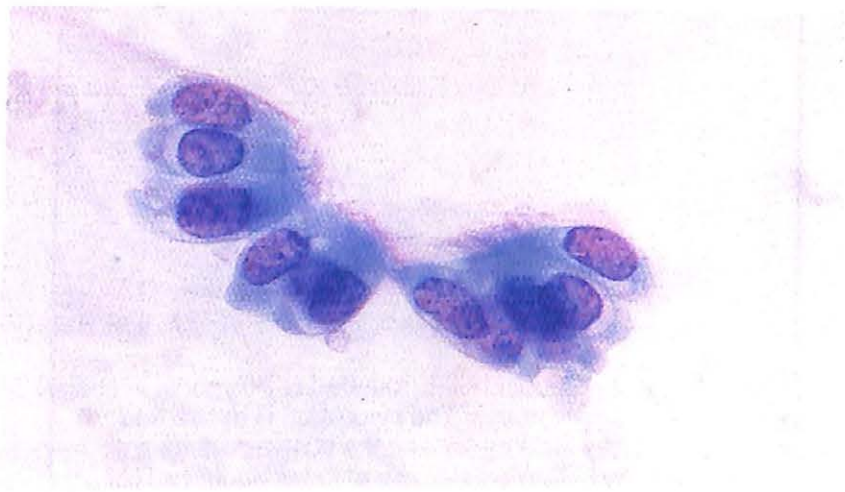

Figure 1.3 Endocervical cells.

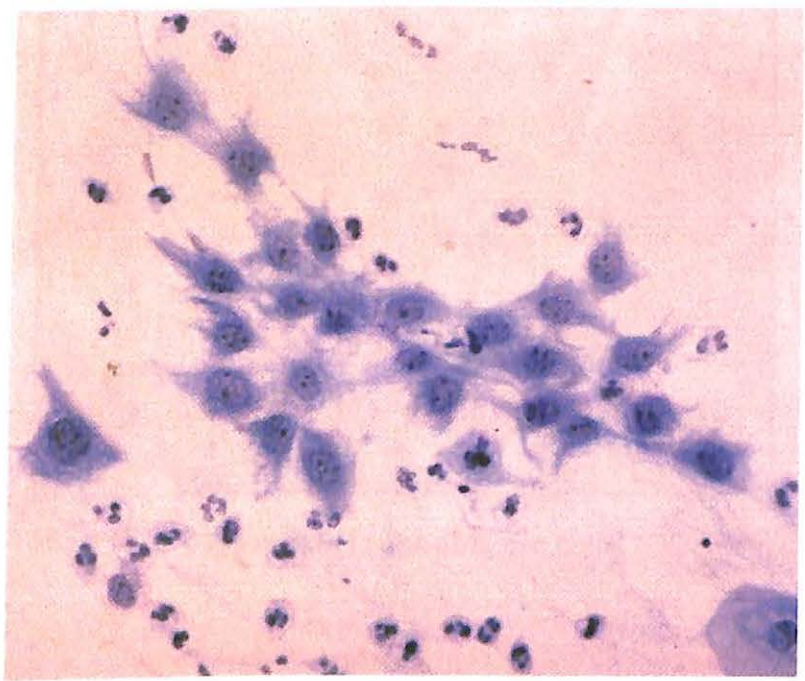

Figure 1.4 Squamous metaplastic cells ("Spider cells").

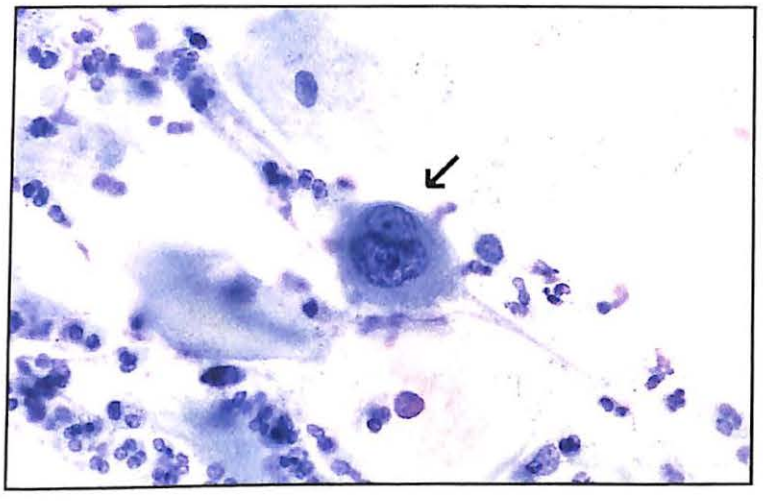

Figure 1.5 Decidual cells.

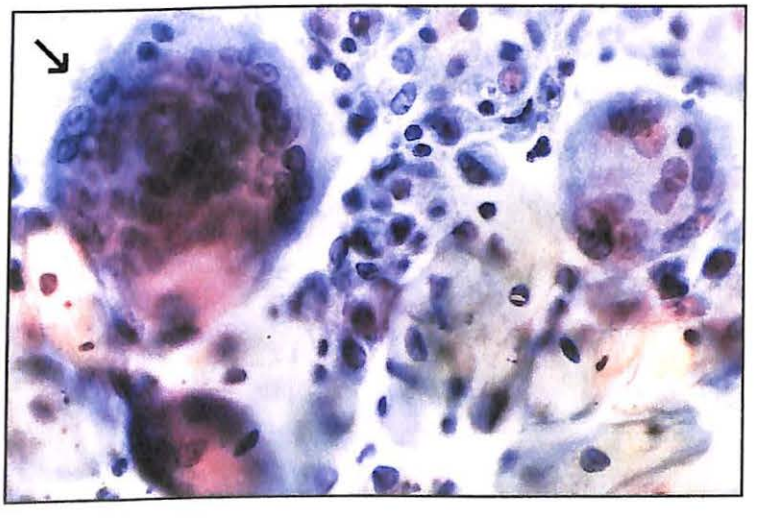

Figure 1.6 Trophoblastic cells.

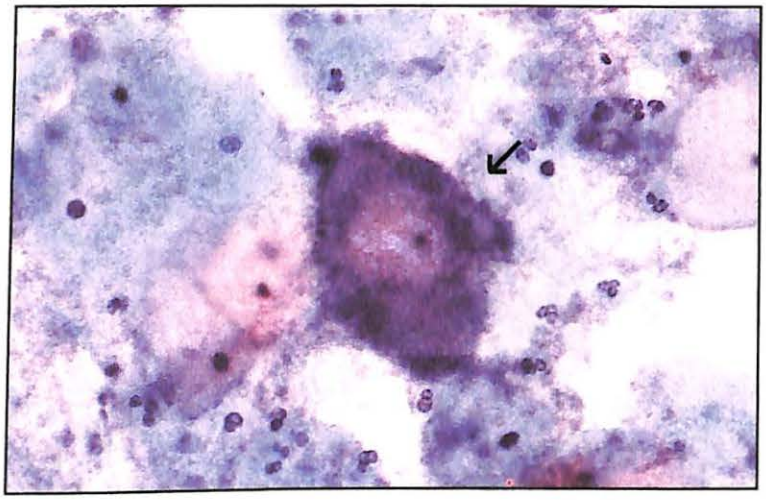

Figure 1.7 *Gardnerella vaginalis* infection ("Clue cells").

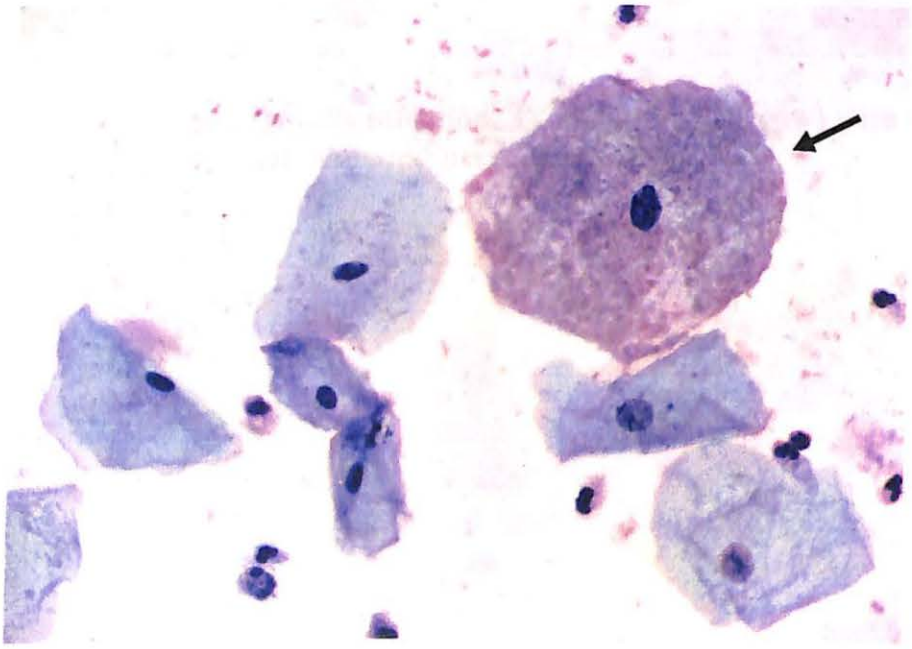

Figure 1.8 *Gardnerella vaginalis* infection ("Clue cells").