Laboratory Processes
Pathology of CIN
Role of HPV

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PLAN

- LABORATORY PROCESSES
- PATHOLOGY OF CIN
- ROLE OF HPV

Reception

- Request form
- PID
- History
- Clinical Data

The ThinPrep Process
Overcoming Pap Smear Limitations:

A Simple, Important Change

Cells are collected in a vial of preservative solution



The ThinPrep 2000 Processor



Fully Automated

Separates cells from blood, mucus, and non diagnostic debris.



1. Dispersion
2. Cell Collection
3. Cell Transfer

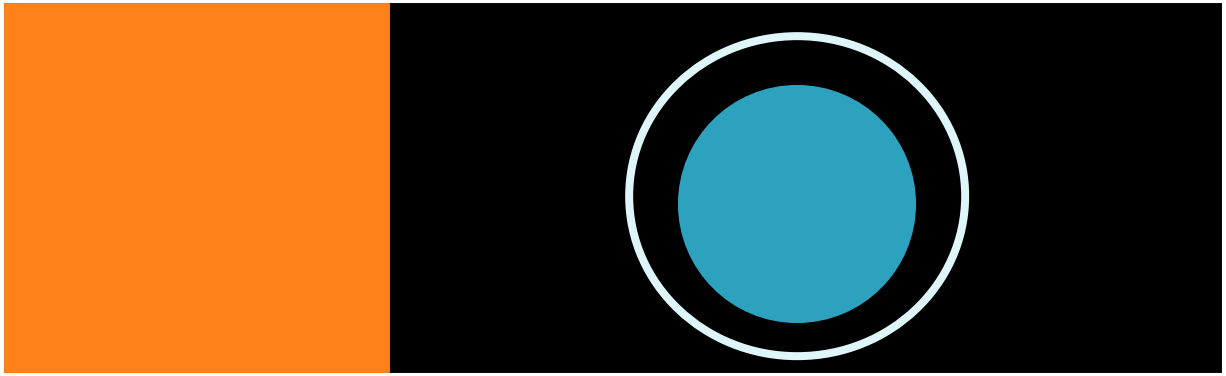
Turnaround Time
Unaffected by Proces

Screening:

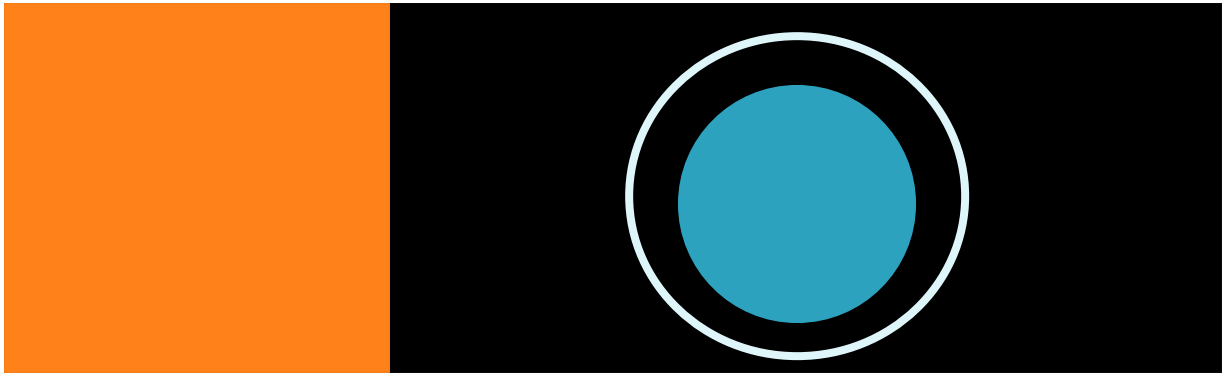
- involves the microscopic assessment of all the cells on a slide.

- it is done by starting at one corner and working systematically from one end to the other, overlapping each microscopic ‘field’ as you progress.

A ThinPrep Slide

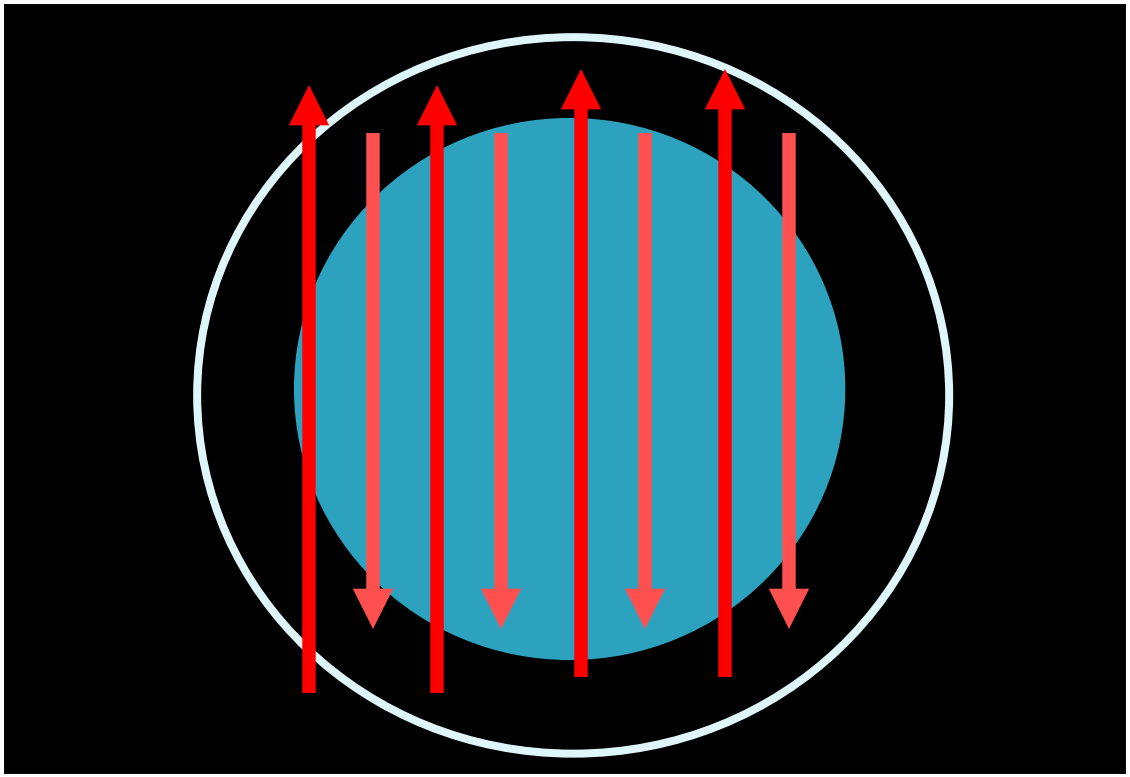


A ThinPrep Slide: Screening



10X Screening

A ThinPrep Slide: Screening



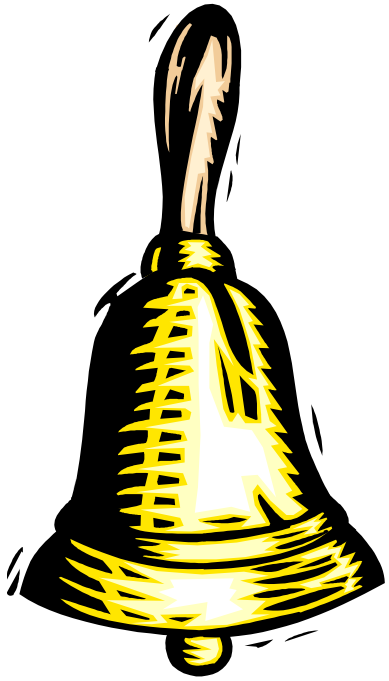
10X Screening

Cervical cytology report----structure

- Quantity of material
- Endos present or not
- Dyskaryosis or negative
  Squamous or glandular?
- Other eg infective agents
- Management recommendation

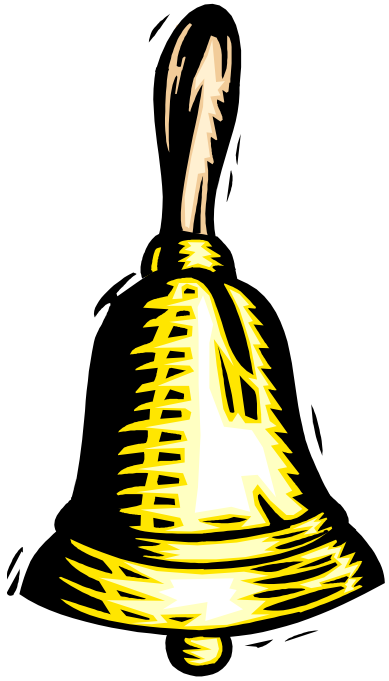
Laboratory Quality Assurance

- IQC
- EQA



IQC

- RECEPTION
- STAINING
- MICROSCOPY
- REPORTING
- PERFORMANCE MONITORING



EQA

- PHE EQA Proficiency Testing
- NEQAS technical scheme
- UKAS UK

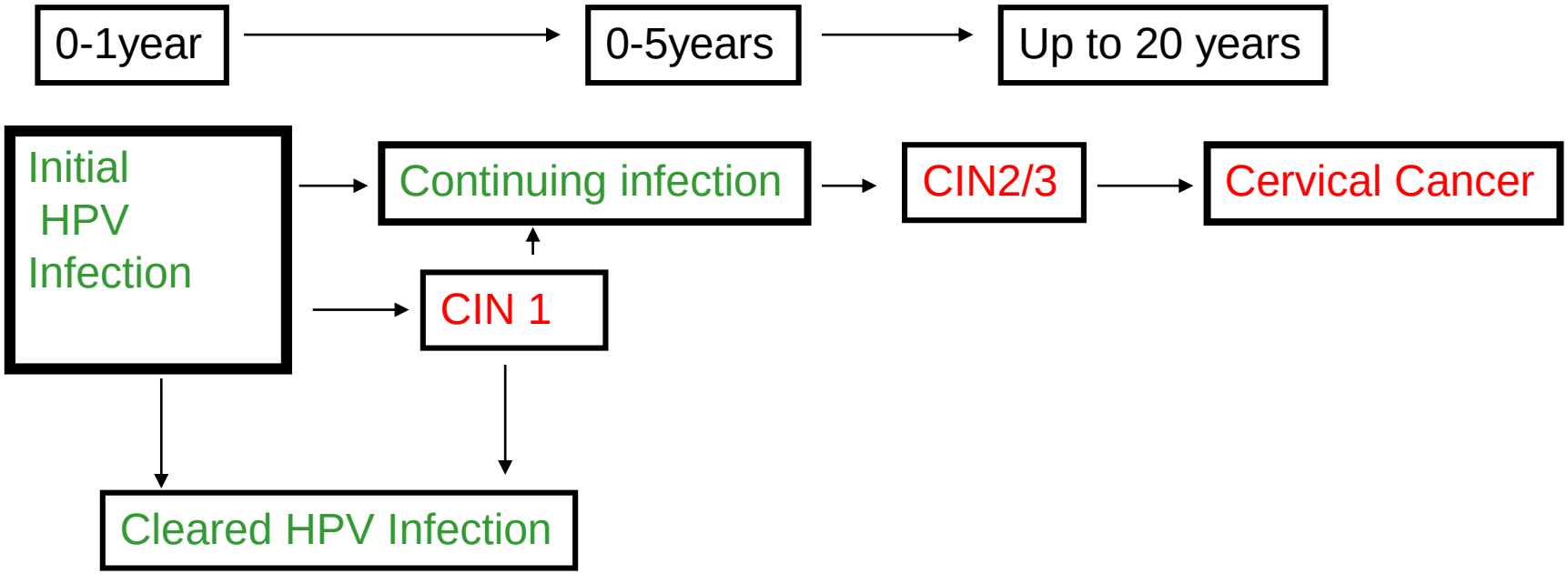
- YPAST DATA surveillance
- Smear Takers Performance



FAILSAFE

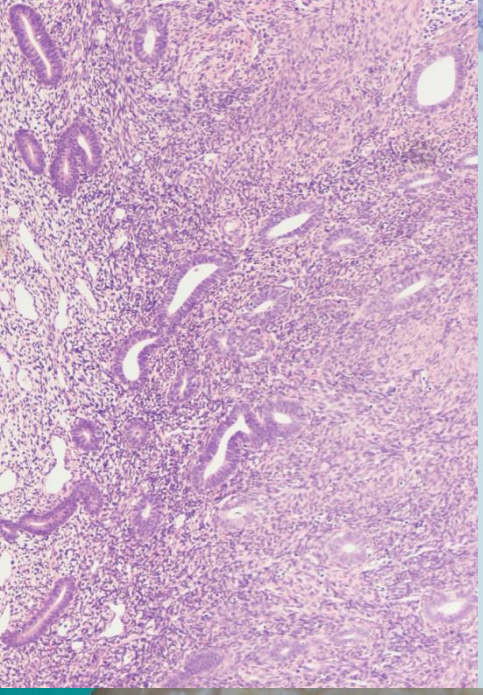
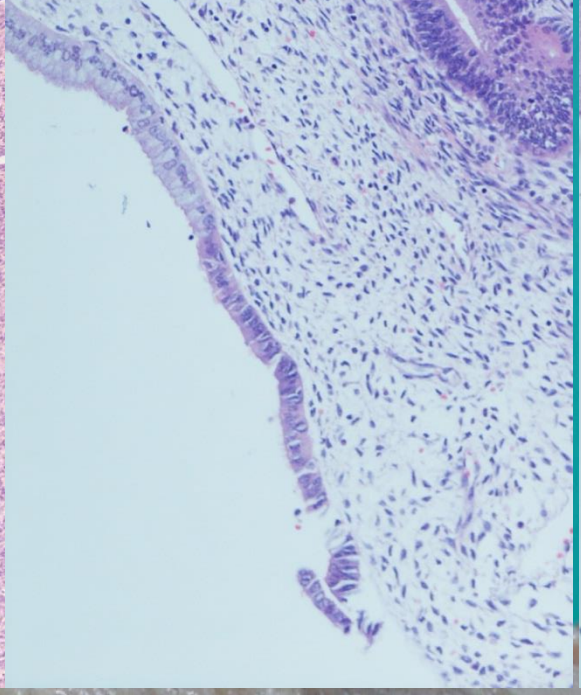
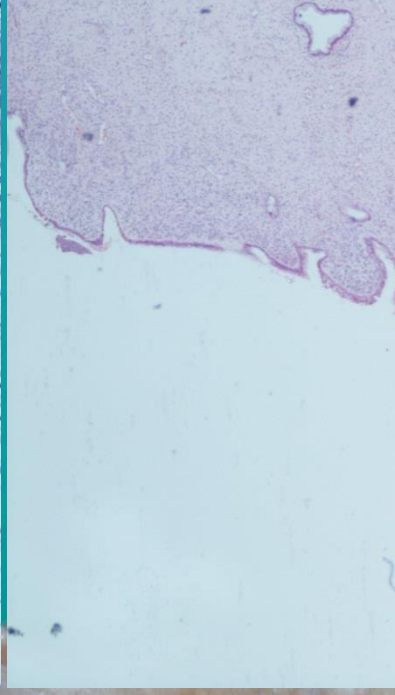
- Labs must operate failsafe system for all abnormal smears
- NHSC Trust failsafe database
- Annual Audit failsafe
- NHSC Trust Cervical Screening QA Group
- YPAST report

Natural History of Infection : Cervical Cancer

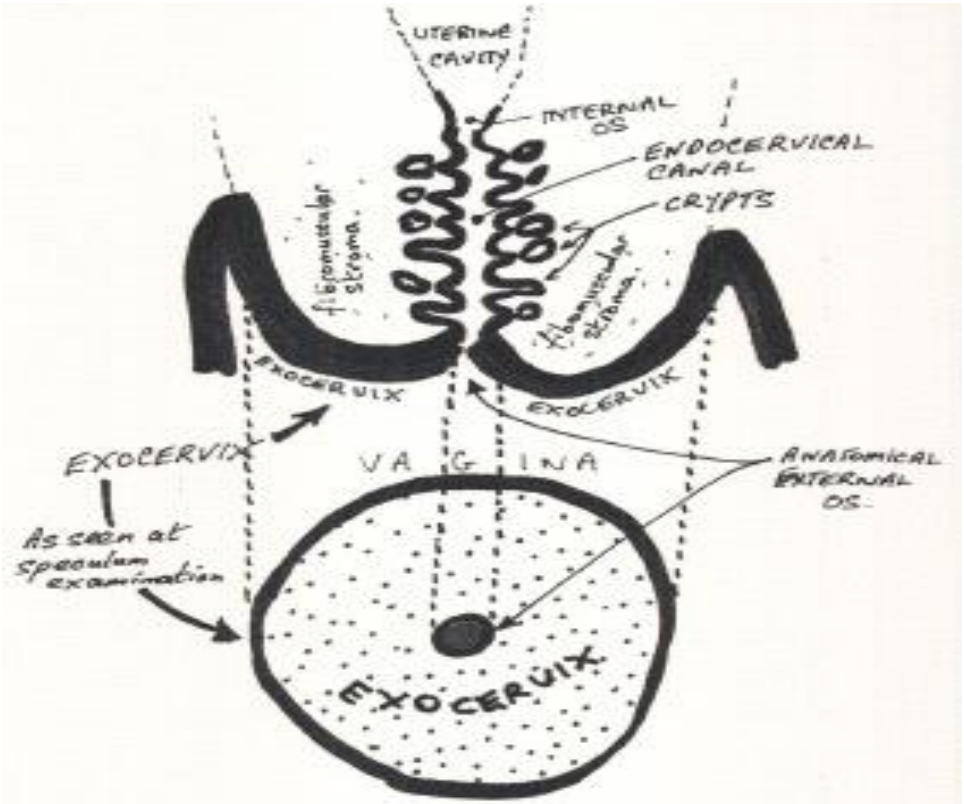


CIN 1-3 Cervical intraepethelial neoplasia

01950

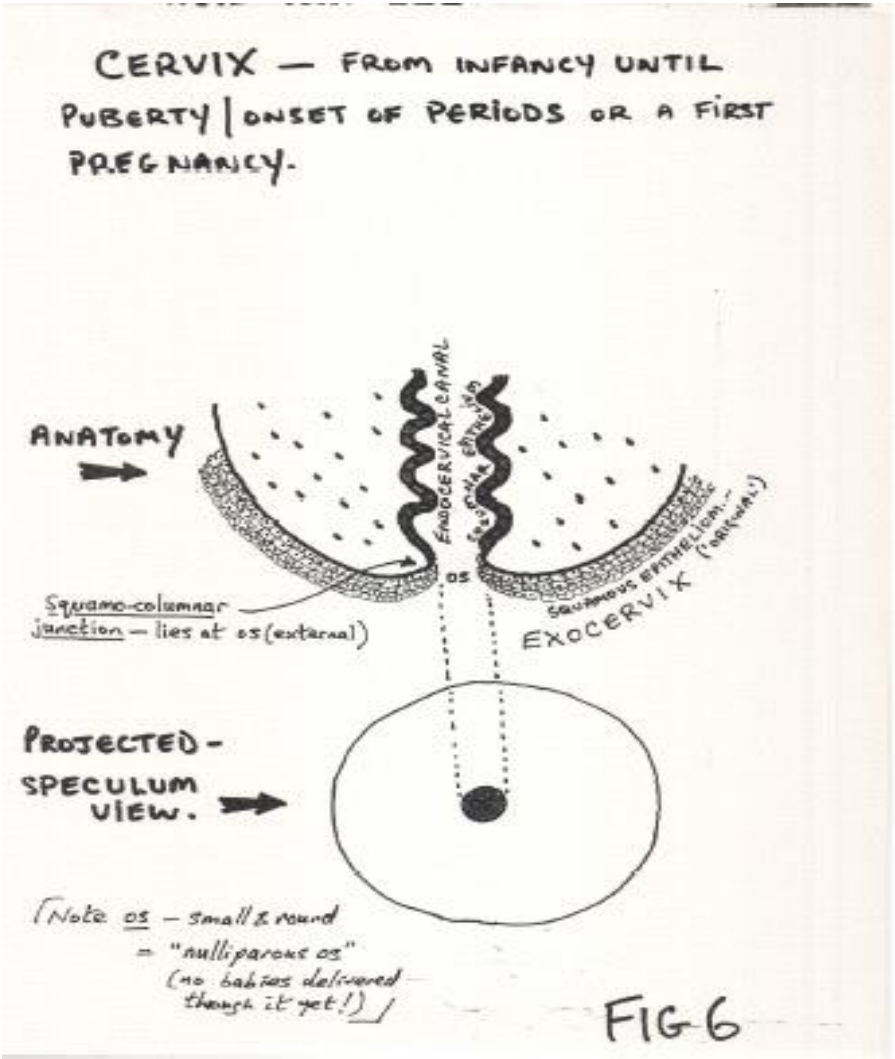




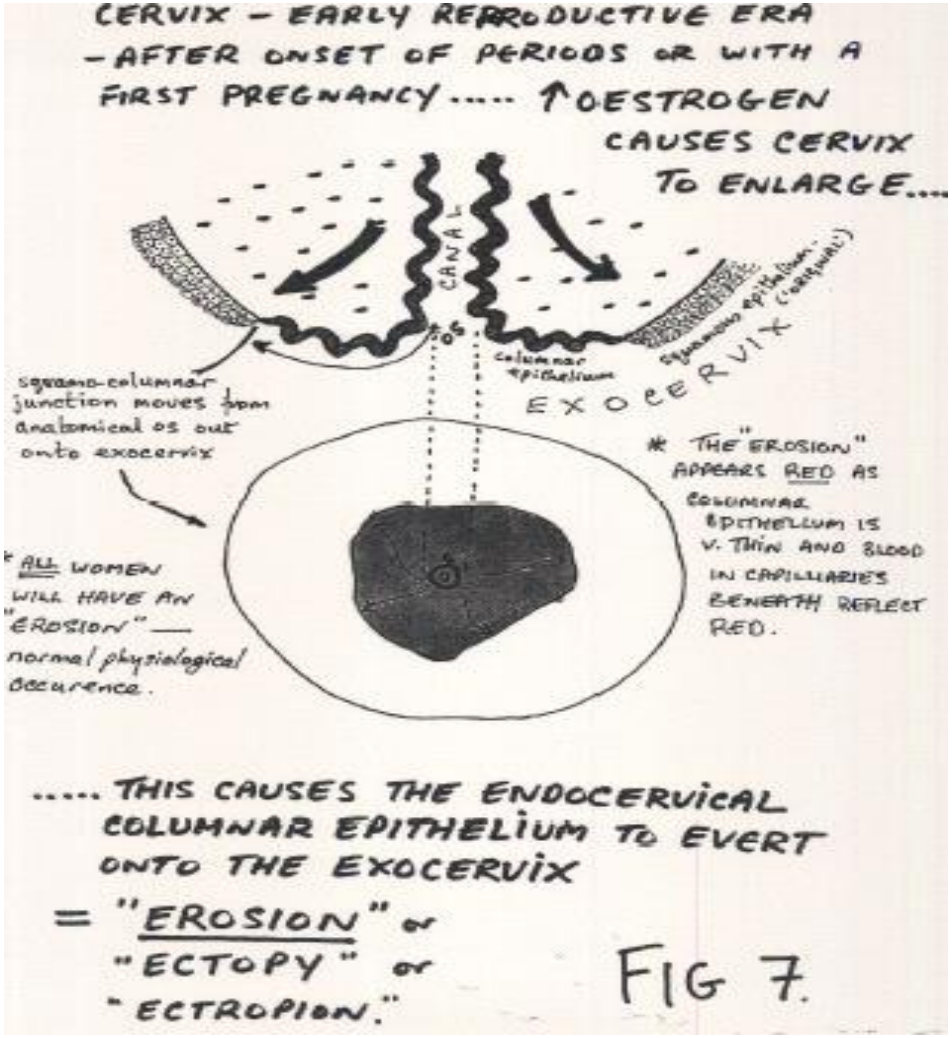
1950

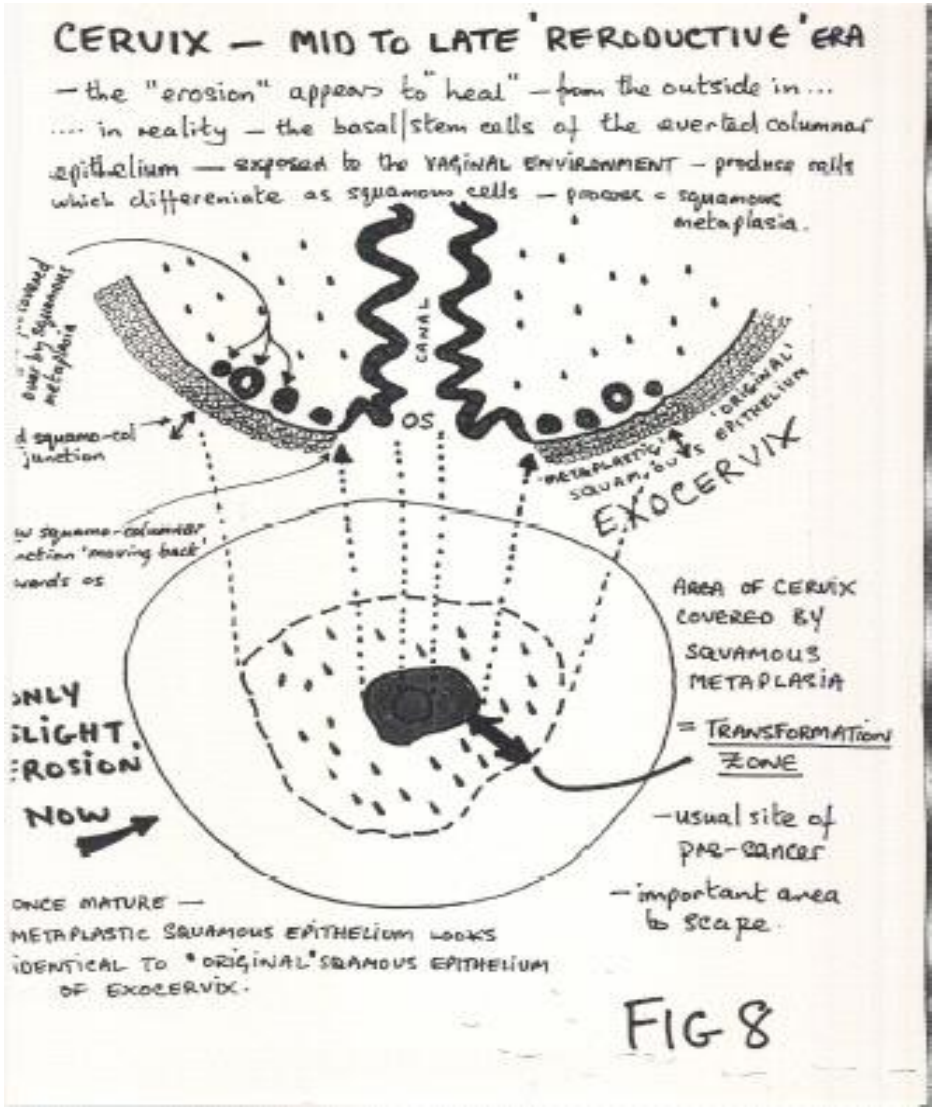


ANATOMY OF CERVIX
—PROJECTION TO SHOW APPEARANCE AT SPECULUM EXAMINATION.

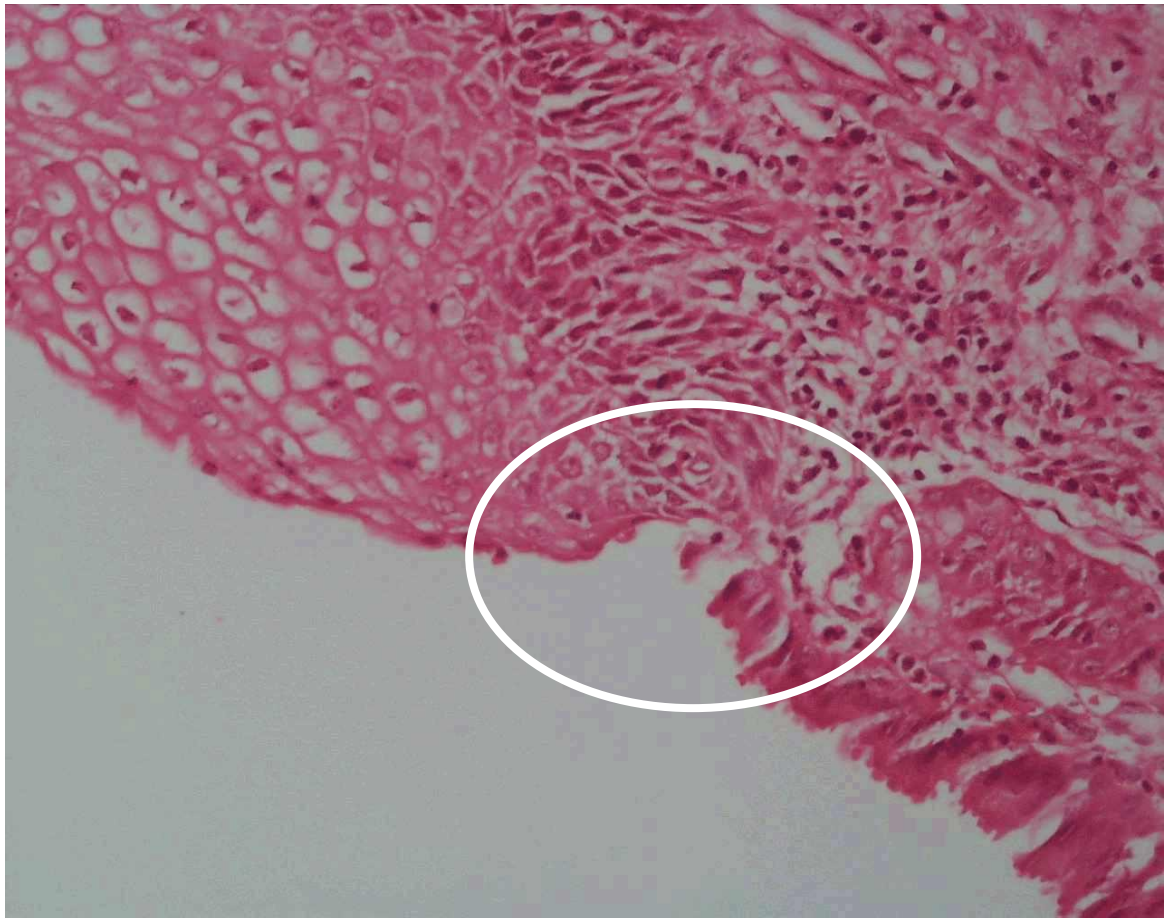
FIG. 3.


CERVIX — FROM INFANCY UNTIL PUBERTY | ONSET OF PERIODS OR A FIRST PREGNANCY.
ANATOMY
ENDOCERVICAL CANAL
OS
Squamo-columnar junction — lies at os (external)
SQUAMOUS EPITHELIUM (VAGINAL)
EXOCERVIX
PROJECTED - SPECULUM VIEW.
Note os — small & round = "nulliparous os" (no babies delivered though it yet!)
FIG 6

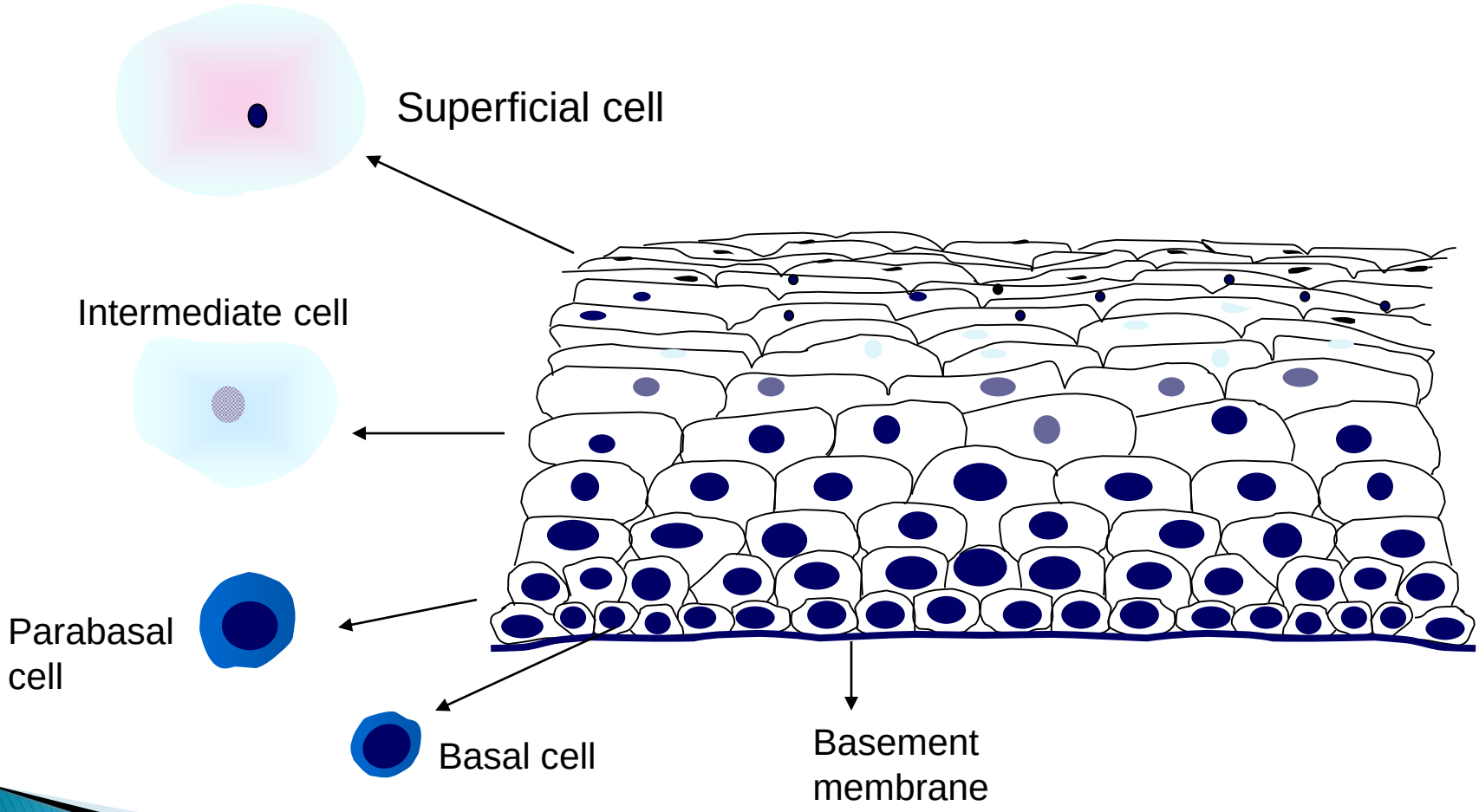

CERVIX – EARLY REPRODUCTIVE ERA
– AFTER ONSET OF PERIODS OR WITH A FIRST PREGNANCY ..... ↑OESTROGEN CAUSES CERVIX TO ENLARGE....
CANAL
OS
squamous epithelium (pink)
columnar epithelium
EXOCERVIX
squamo-columnar junction moves from anatomical os out onto exocervix
* ALL WOMEN WILL HAVE AN "EROSION" — normal physiological occurence.
* THE "EROSION" APPEARS RED AS COLUMNAR EPITHELIUM IS V. THIN AND BLOOD IN CAPILLARIES BENEATH REFLECT RED.
..... THIS CAUSES THE ENDOCERVICAL COLUMNAR EPITHELIUM TO EVERT ONTO THE EXOCERVIX
= "EROSION" or "ECTOPY" or "ECTROPION."
FIG 7.


CERVIX — MID TO LATE 'REPRODUCTIVE' ERA
— the "erosion" appears to "heal" — from the outside in ...
.... in reality — the basal/stem cells of the everted columnar epithelium — exposed to the VAGINAL ENVIRONMENT — produce cells which differentiate as squamous cells — process = squamous metaplasia.
CANAL
OS
'ORIGINAL' EPITHELIUM
EXOCERVIX
AREA OF CERVIX COVERED BY SQUAMOUS METAPLASIA
= TRANSFORMATION ZONE
— usual site of pre-cancer
— important area to scope
ONCE MATURE — METAPLASTIC SQUAMOUS EPITHELIUM LOOKS IDENTICAL TO 'ORIGINAL' SQUAMOUS EPITHELIUM OF EXOCERVIX.
FIG 8

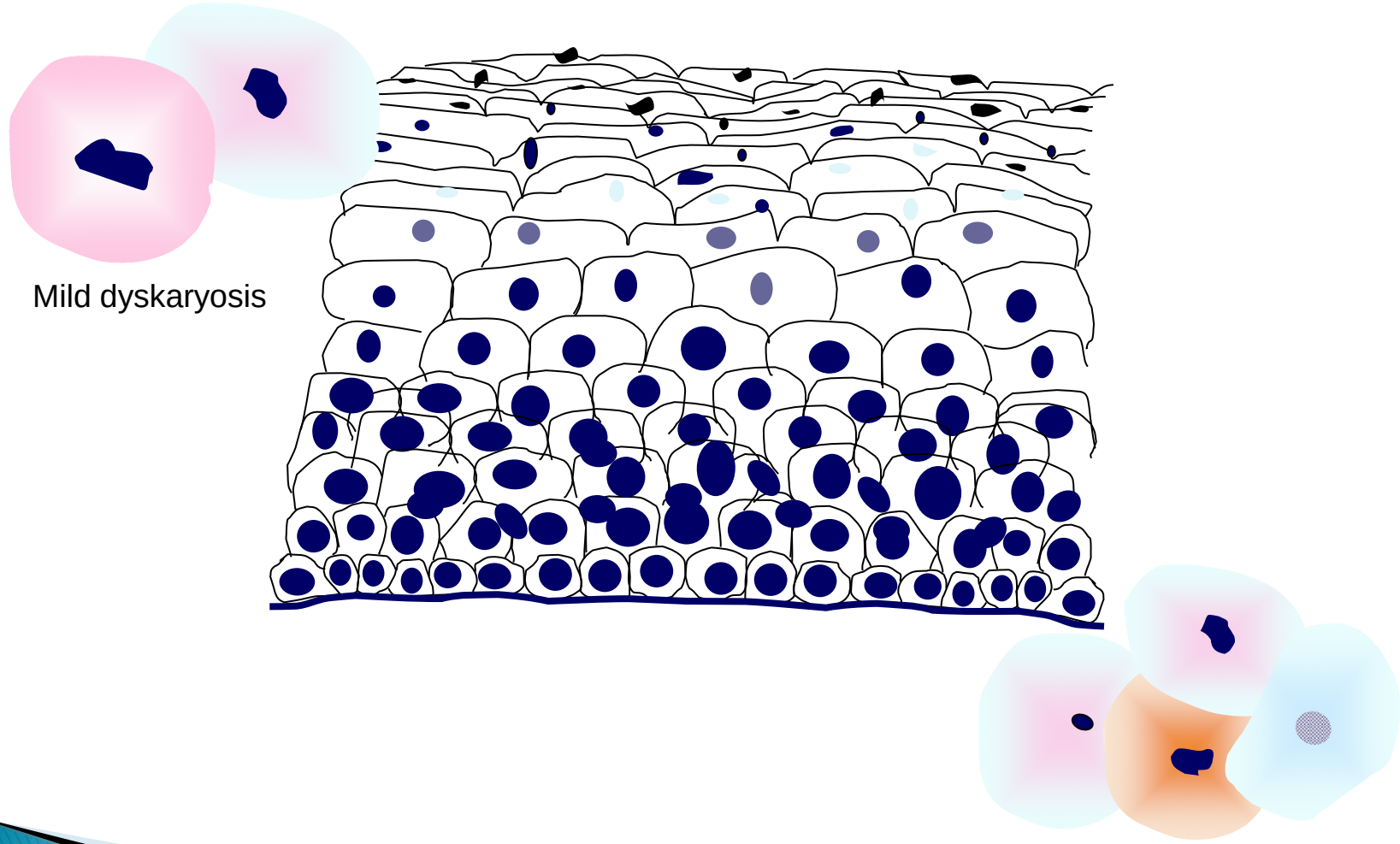
Squamocolumnar Junction



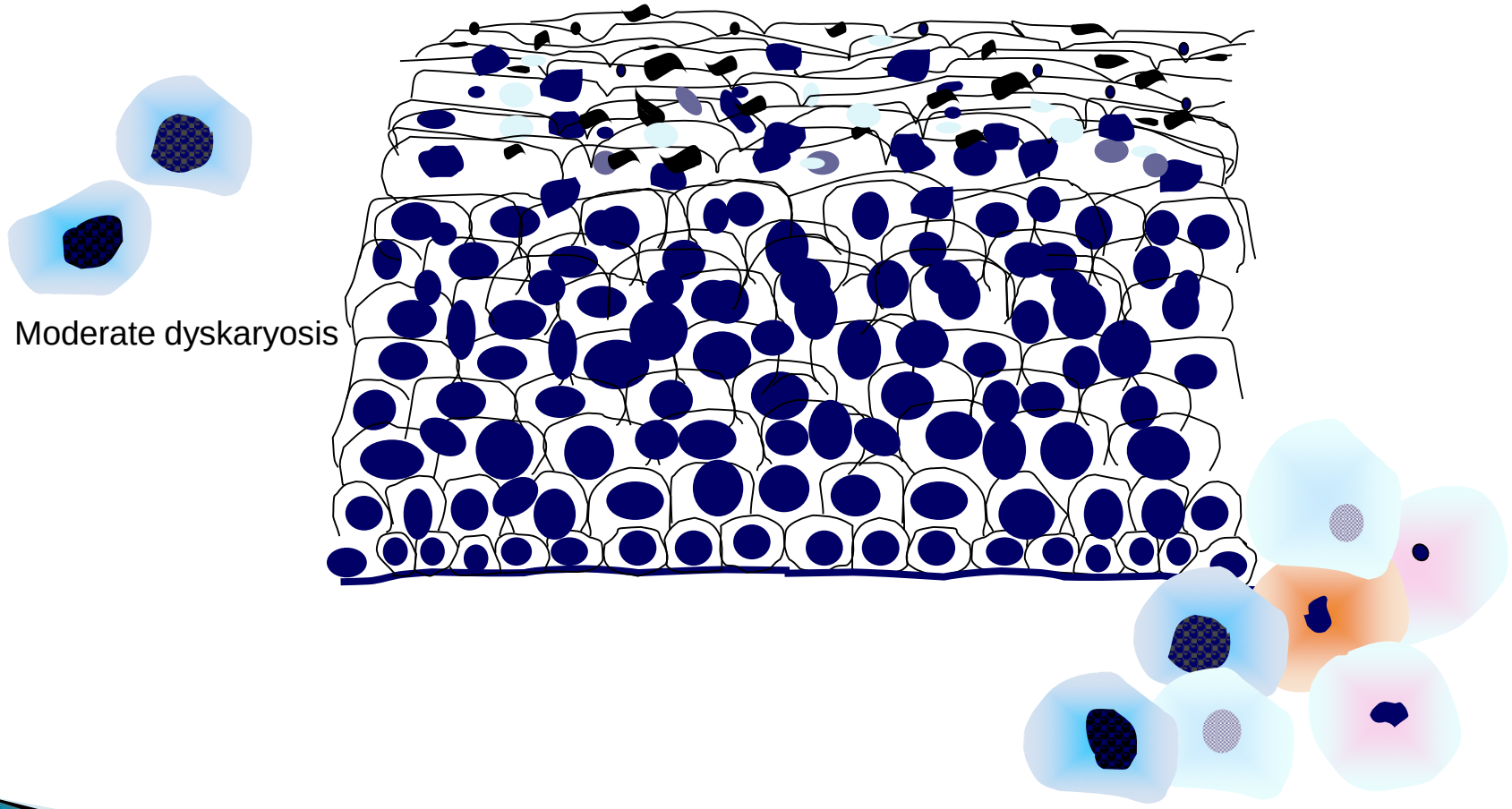
Normal squamous epithelium



Cervical Intraepithelial Neoplasia I

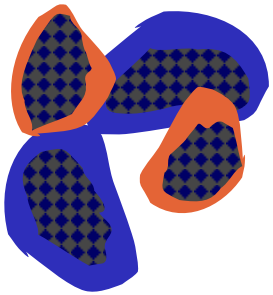


Cervical Intraepithelial Neoplasia II

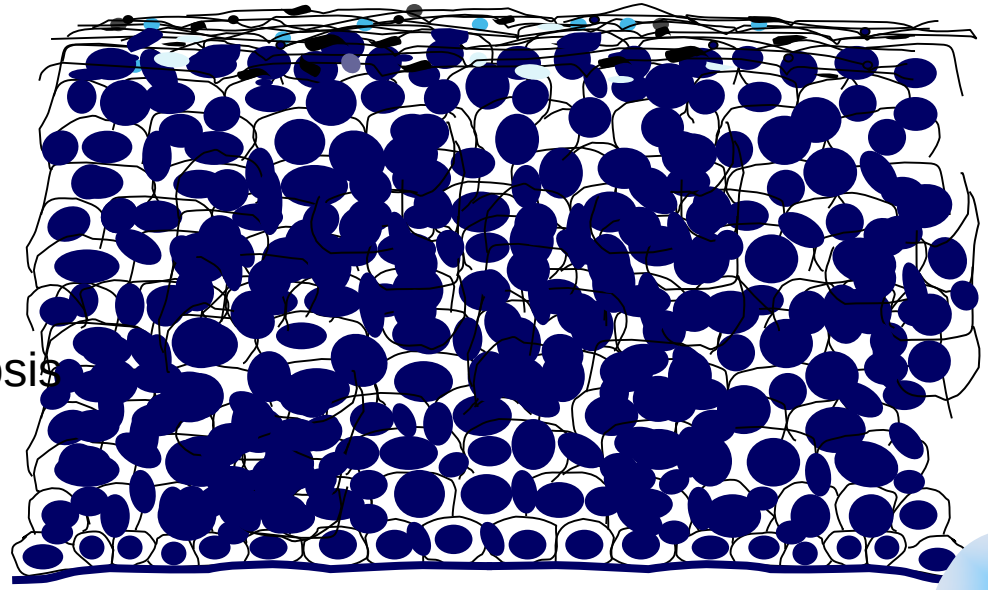


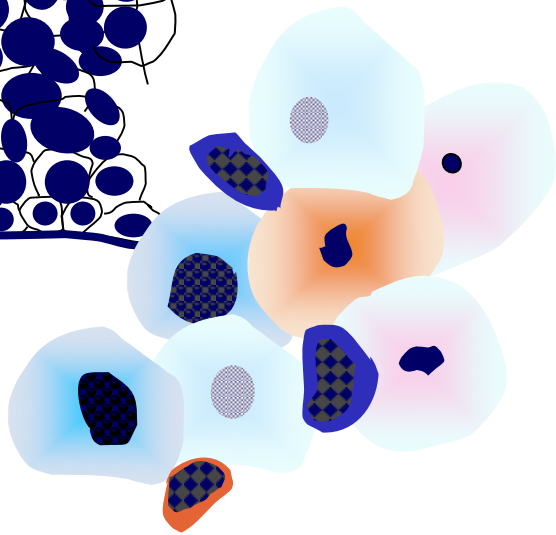
Moderate dyskaryosis

Cervical Intraepithelial Neoplasia III

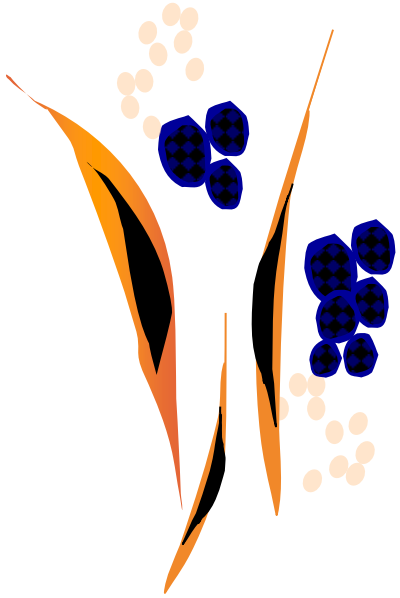


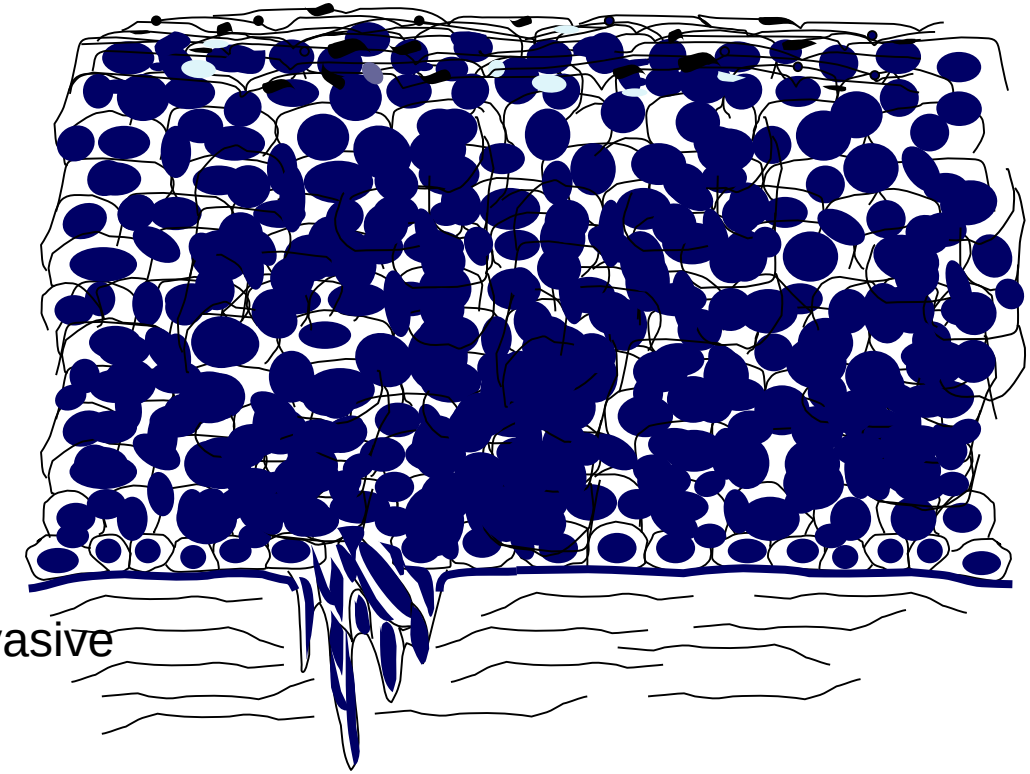
Severe dyskaryosis





Microinvasive / Invasive Carcinoma of Cervix





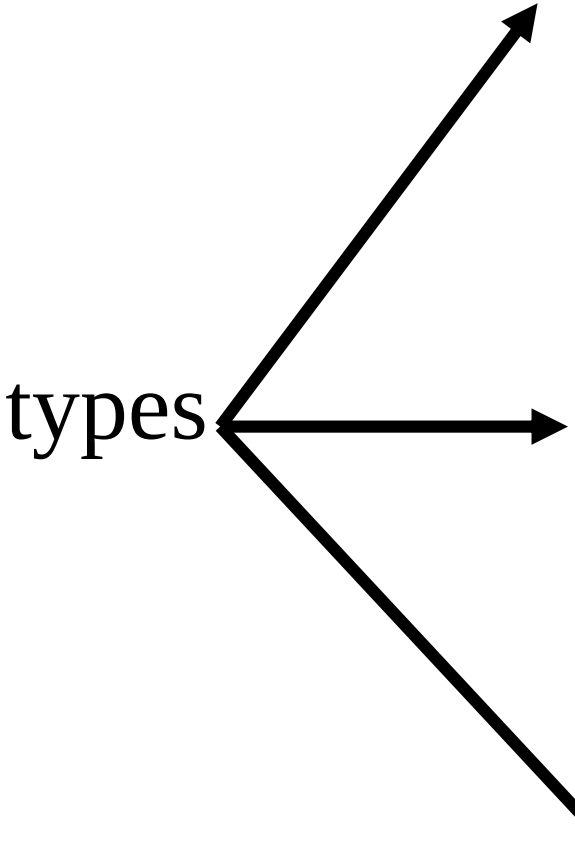
Severe dyskaryosis ? Invasive

Human Papilloma Virus

HPV

- STABLE ,UBIQUITOUS AND WELL ADAPTED TO HOST
- 20 YRS AGO SHOWN TO BE ASSOCIATED WITH CANCER OF THE CERVIX
- INFECTS SQUAMOUS AND MUCOSAL EPITHELIUM

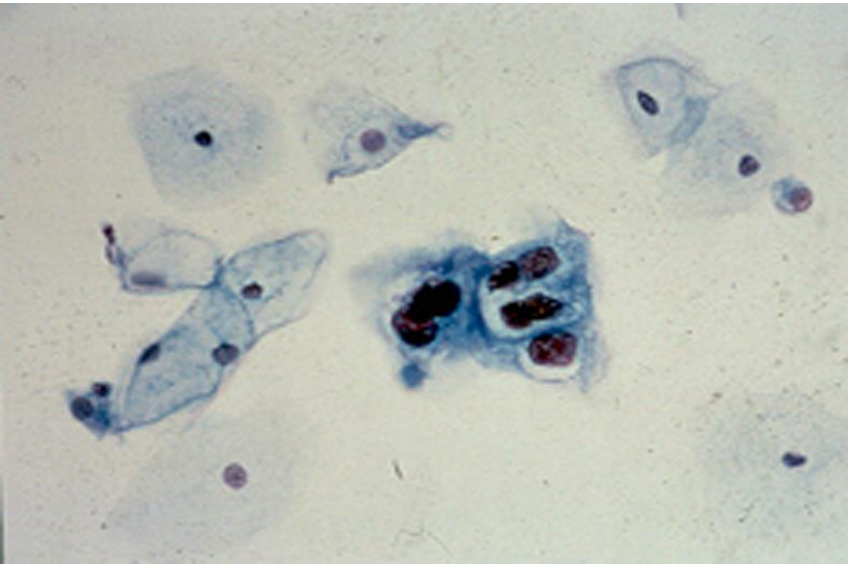
Non-oncogenic (1-4) cutaneous



LR-HPV assoc with genital warts(6-11)

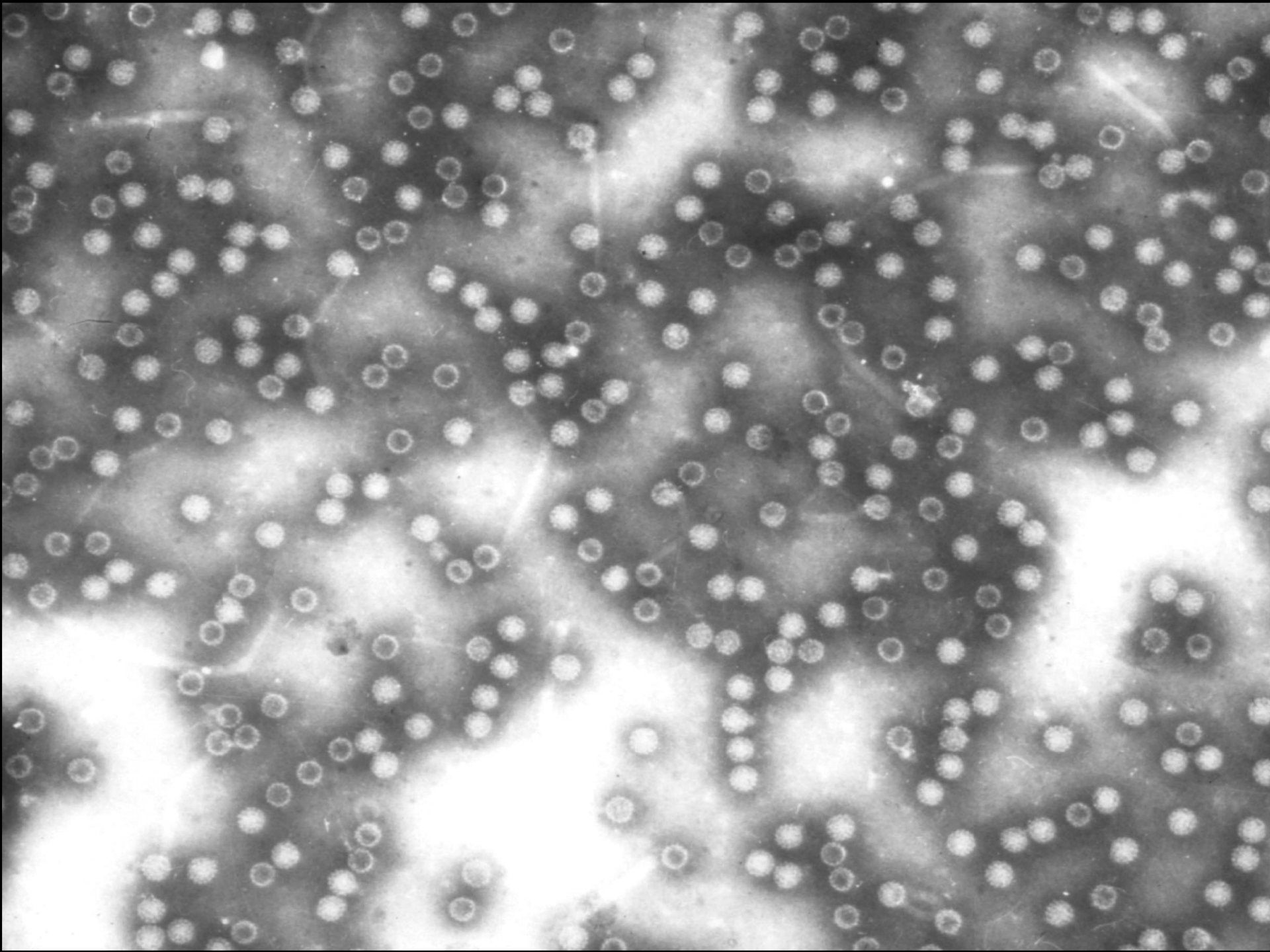
HR-HPV progression to cancer (16,18,31,51,33,45)

Cytology of HPV



- Koilocytes
- Keratinised plaques
- Multinucleated cells
- Dyskeratosis





HPV

- Strong evidence that PERSISTENT INFECTION is necessary for oncogenic effect ie not the acquisition of the virus but its persistence and then only a small no will progress to cancer
- From acquisition of HPV to invasive cancer not fully detailed

Papillomavirus Replication

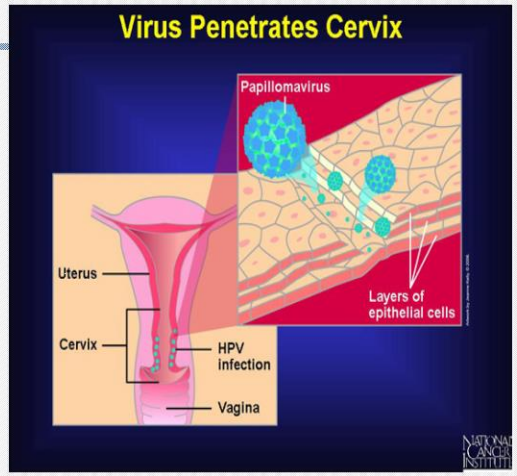
- Virus infects basal cells
- vDNA transported to nucleus and amplified 50/100 copies/cell
- Viral copy number maintained as limited cell replication MAINTENANCE REPLICATION
- As cells mature virus switches to PRODUCTIVE REPLICATION 1000's copies/cell

HPV

- Degradation of tumour suppressor proteins
- HPV infected cell undergoes continuous cell division (not stopping in response to DNA damage) to allow replication

- **Must have integration of viral DNA into cell**

HPV Infection



- Exact mechanism unknown
- HPV transmission is via intimate contact
- HPV infect basal layers
- Replication in keratinised cells

HPV

- Strong evidence that PERSISTENT INFECTION is necessary for oncogenic effect i.e. not the acquisition of the virus but its persistence and then only a small no will progress to cancer
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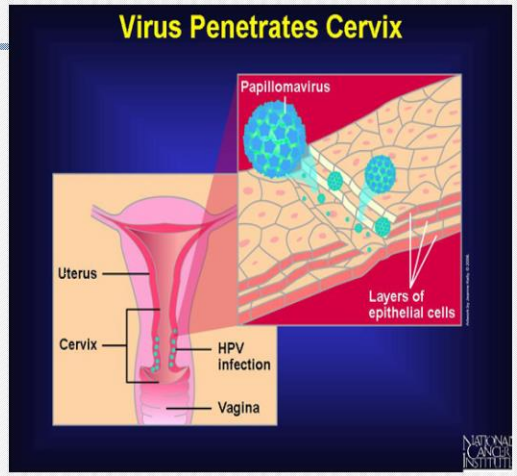
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What Governs Progression

- HPV type
- Persistence of HPV infection
- Loss of capacity to replicate viral DNA
- HPV integration

Patterns of Infection

- Clinical-visible to the naked eye
- Sub-clinical-not visible to the naked eye
- Latent- HPV only detectable by molecular means

- Condylomata acuminata(warts)
- Non-condylomatous (flat warts) Can be seen microscopically and at colposcopy







HPV

- 70-80% of population have had HPV at some point.
- 40%-70% prevalence in 20yr olds Transient Infection
- 20%-30% prevalence in 30yr olds Persistent Infection
- 80% show no morphological abnormality
- 20% develop CIN

HPV

- NOT AN STD IN THE TRADITIONAL SENSE. NOT STRICTLY A SEXUALLY TRANSMITTED DISEASE

HPV in Cervical Neoplasia

- HPV6/11 in benign and low grade lesions
- HPV 16/18(33,35) in high grade and invasive carcinoma
- HPV infection a precursor lesion to CIN
- HPV strong risk factor for the development of CIN and invasive cancer
- HPV16/18 found in high% of Adenoca

HPV Carcinogenesis

- Cofactors in Cancer-- Multistep
- Persistent HPV
- Immune Suppression
- Smoking
- Sarewski,et al lancet 1996; 347

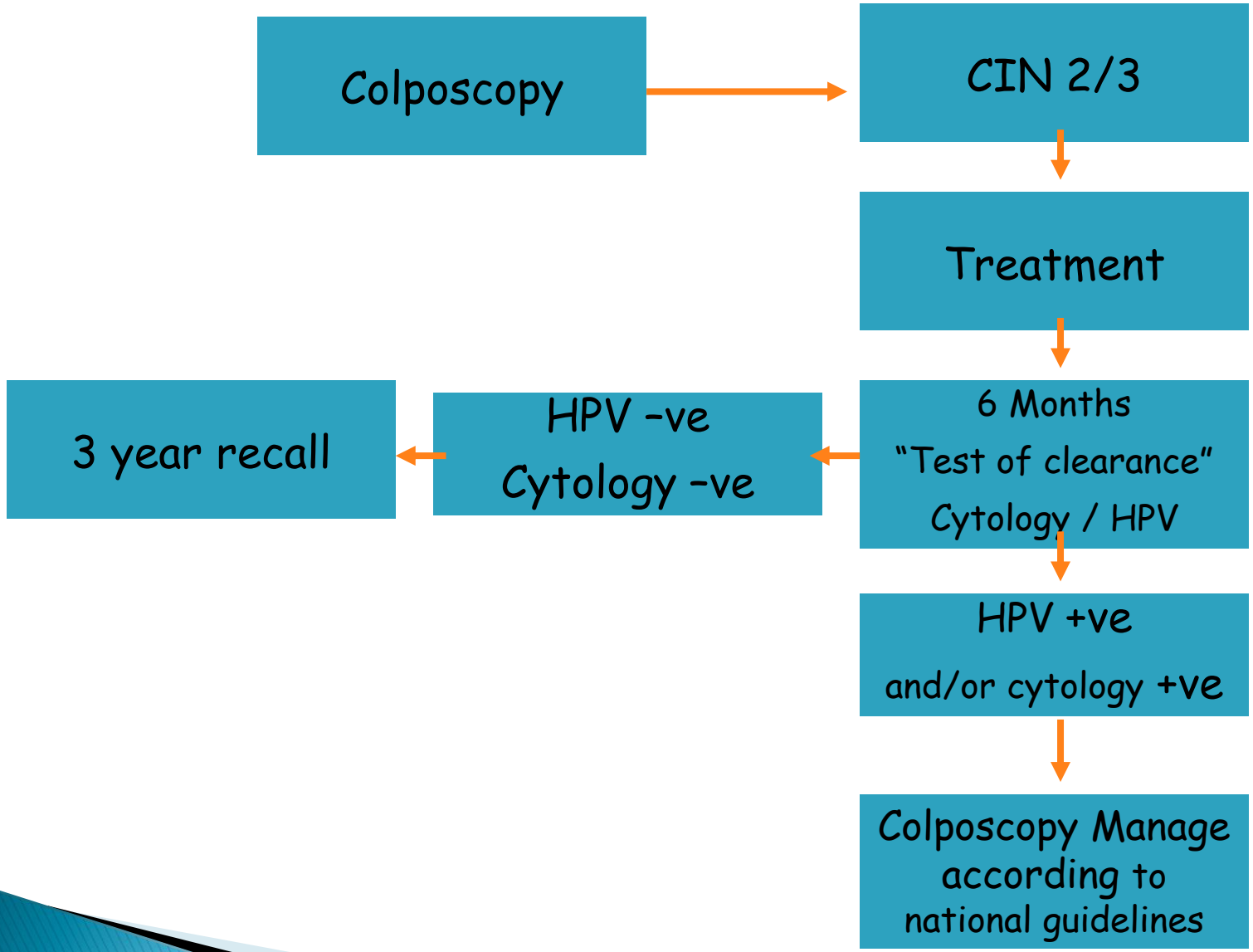
Detection Of HPV

- Morphological
  - E.M.
  - ISH
- Non-Morphological
  - PCR
  - COBAS 4800
  - Hybrid Capture (digene capture 2)

HPV Vaccination

- Therapeutic vaccination (*treatment of CIN and invasive cancer)*
- Prophylactic vaccination *(prevention of CIN and invasive cancer by preventing HPV infection)*
- *No effective treatment for HPV*

Test of Clearance



HPV Co testing

- Samples already prepared for screening
- Slides in backlog
- All samples tested for HR HPV
- HRHPV positive prioritised for screening
- Cytology negative HPV positive R12
- Cytology positive HPV positive Refer to colp
- Cytology negative HPV negative NR
- *Note TOC remains the same*

- Information and FAQs available

HR HPV primary screening

- Sample taking remains the same
- Information to women changes
- All samples will be tested for HR HP:V

- Only those that are HR HPV positive will have cytology

- Cytology positive ….HPV positive refer to colposcopy
- Cytology negative…..HPV positive repeat 12 months

Key Issues for Women

- HPV is very common in people who have been sexually active at any point in their lives
- Skin to skin contact
- The virus is asymptomatic
- May be undetected for years
- A partner may have acquired infection many years ago
- A positive HPV test need not imply promiscuity or infidelity
- NHSCSP implementation guide

Conclusion

- KEEP UP THE GOOD WORK !!