

The Anal Canal: an Important Transition Zone for Rectal Microbicide Development

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Plan

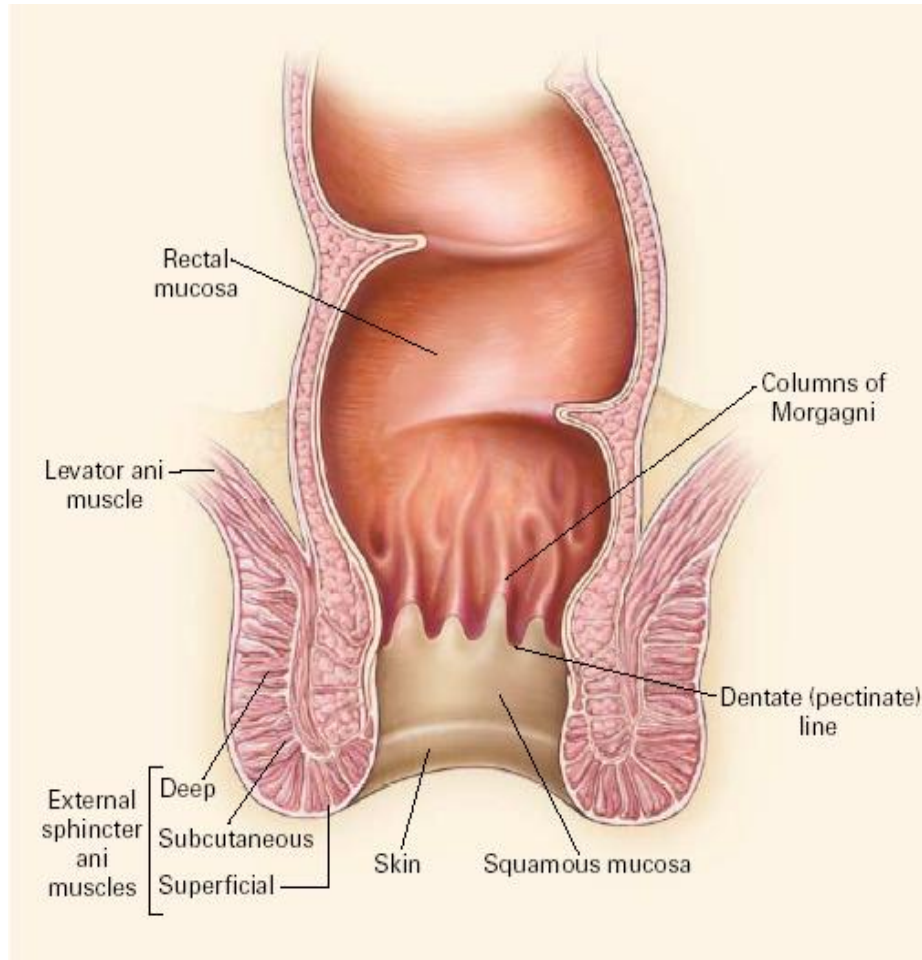
- Anal canal
- Comparison with the rectum
- Common anal conditions
- Human papillomavirus infection and the anal canal
- The anal canal in the context of rectal microbicide development



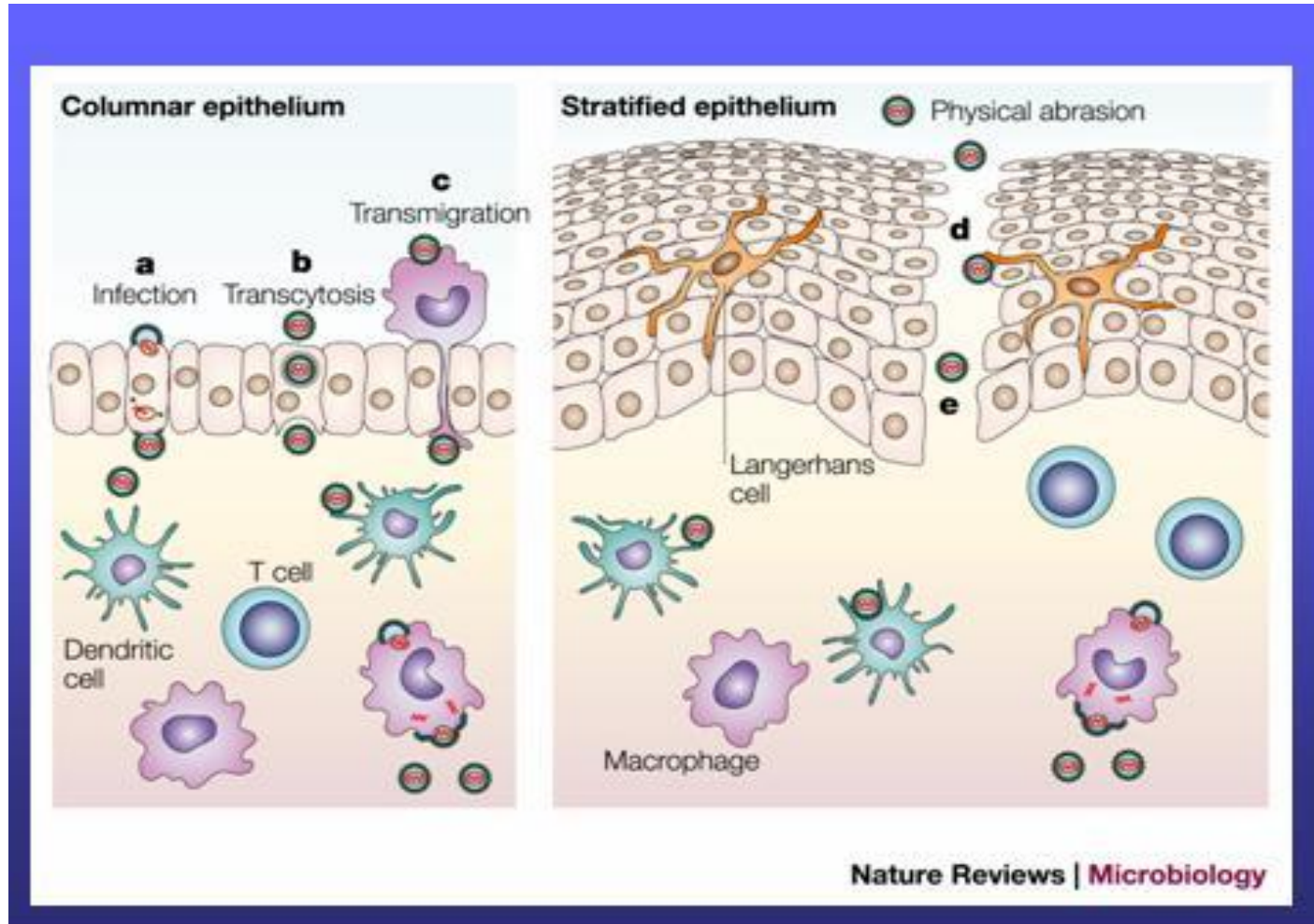
Anus versus rectum



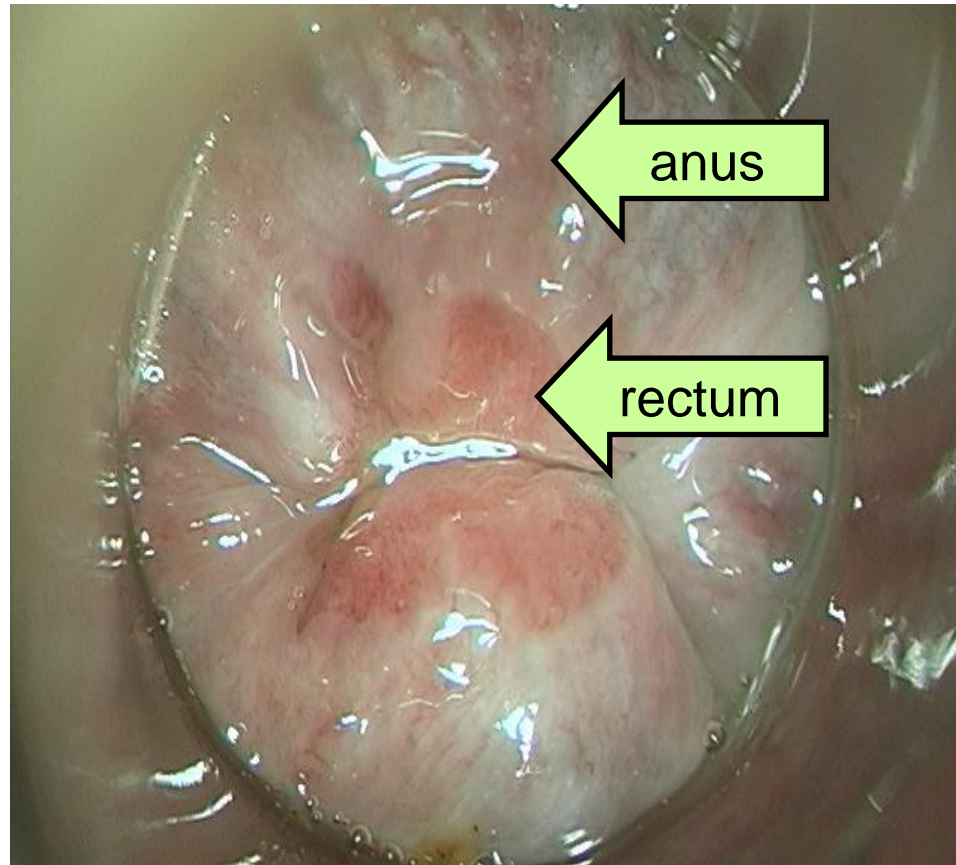
The anorectum



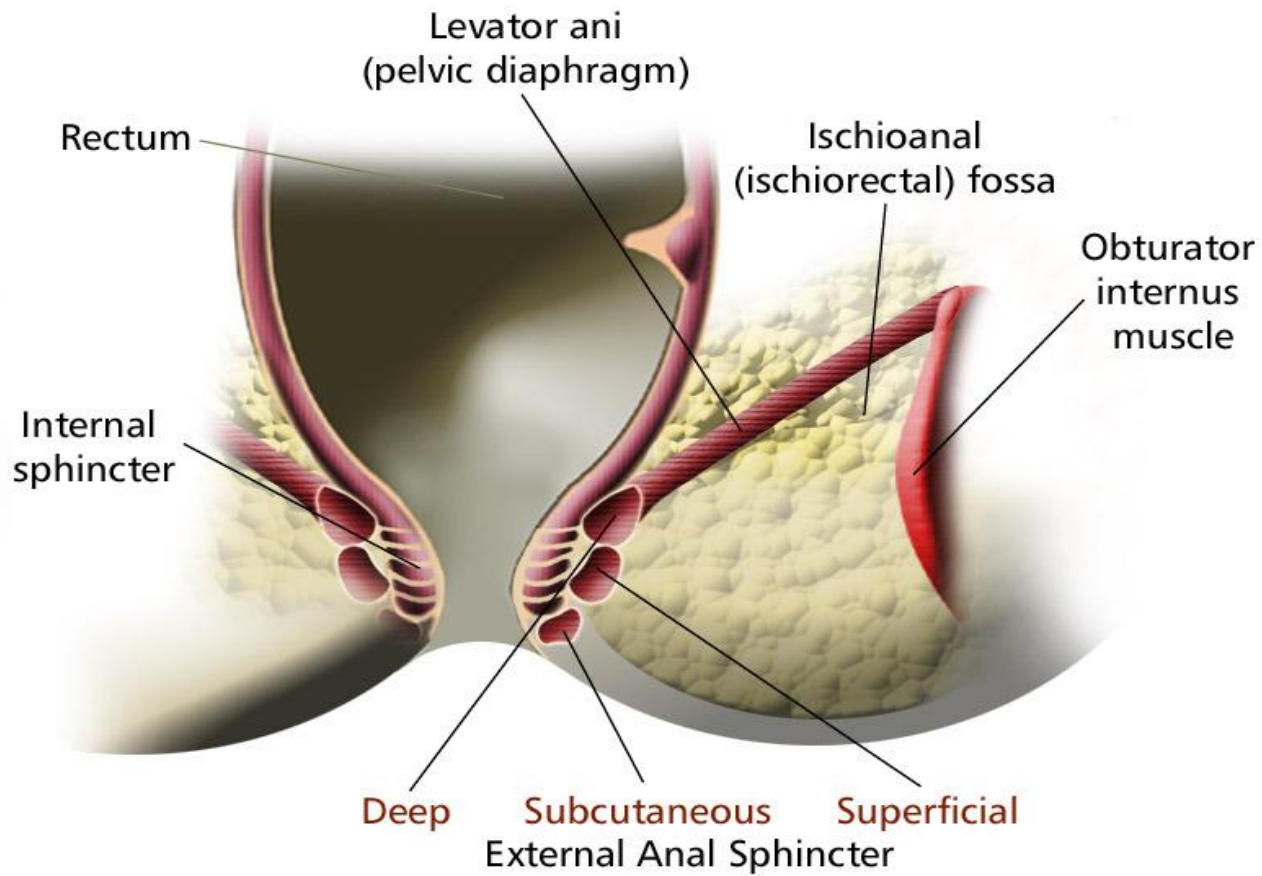
Rectal and anal epithelium



Anorectal transition zone



Anorectum



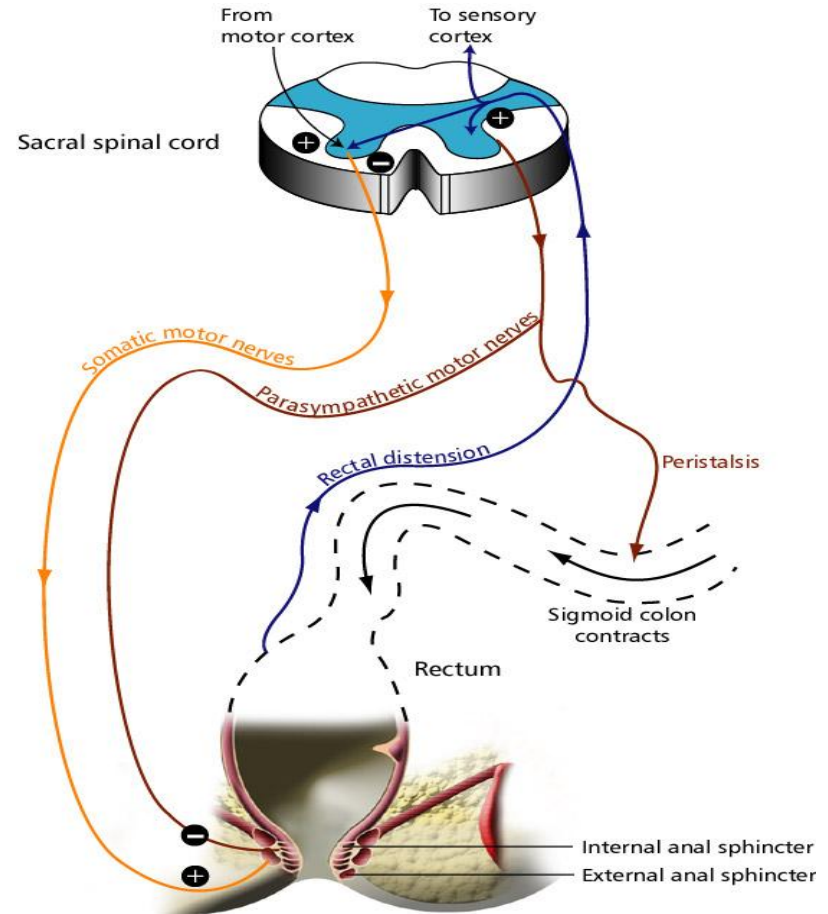
Manometry

Test site	Resting (mmHg)	Squeeze (mmHg)
Anal (male and female)	56	178
Anal no RAI male	91	152
Anal RAI male	71	177
Pelvic floor (nulliparous)	-	15-20



Anal and rectal sensation

- **Anal canal:**
Sensation to touch, pin-prick, heat, and cold
- **Rectum:**
The rectum is only sensitive to distention by receptors in the rectal wall and pelvic fascia or surrounding muscle



Common anal conditions



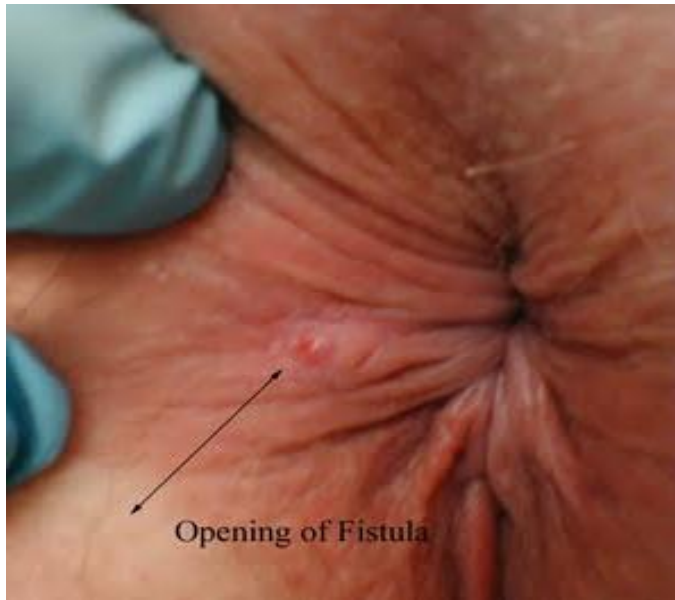
1



2



3



4



1



2



3



4



Clinical presentation

- Pain
- Mass
- Itch
- Discharge
- Bleeding



Human papillomavirus



Anogenital HPV types

Anogenital HPV (~40)

**High-risk types
(HPV 16,18)**

**Low-risk types
(HPV 6,11)**

- low-grade dysplasia
- high-grade dysplasia
- anogenital cancers

- low-grade dysplasia
- genital warts
- respiratory papillomatosis



Anal HPV prevalence

- 50-70 % of the general population exposed to anogenital HPV (serology)
- Anal HPV by PCR
 - 61 % HIV-neg MSM
 - 42 % HIV-neg female
 - 93 % HIV-pos MSM



Anal HPV infection

Risk factors:

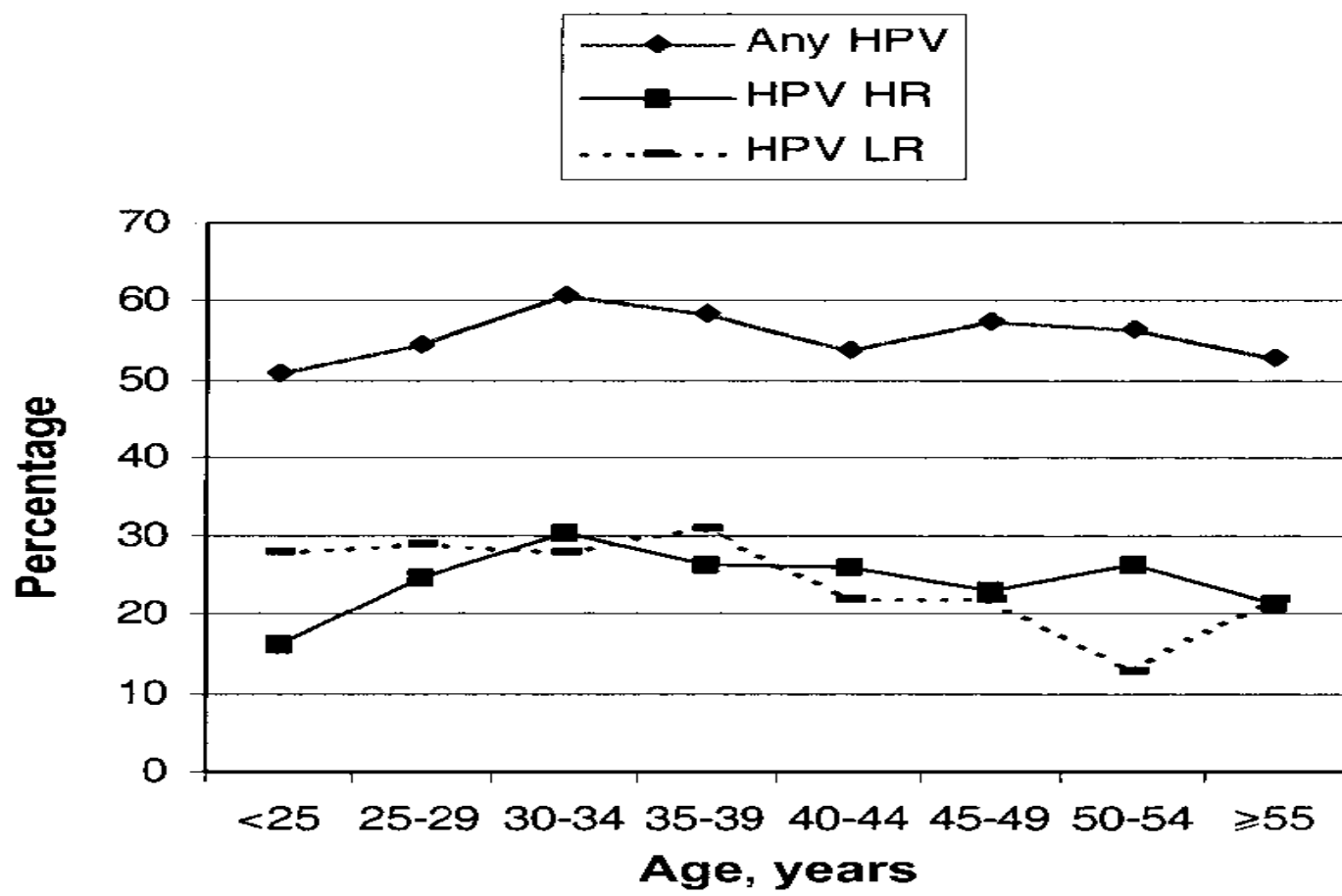
- Receptive anal sex
- History of anal warts
- HIV infection
- Smoking history
- Younger age

Consequences:

- Nil
- Condyloma accuminata
- Anal dysplasia
- Anal malignancy



Anal HPV by age in HIV-neg MSM



Risk factors for anal dysplasia

- HPV infection
- Receptive anal sex
- HIV infection (esp. CD4 <200)
- Genital wart diagnosis
- History of anal discharge
- Current cigarette smoking
- High-grade CSIL/VSIL or cancer
- Iatrogenic immunosuppression



Anal dysplasia as an anal cancer precursor

- Comparing anal dysplasia/cancer with cervical dysplasia/cancer:
 - Same HPV risk types
 - Similar pathology
 - Same chromosomal abnormalities
 - Frequently diagnosed simultaneously



Prevalence of anal dysplasia

- San Francisco: 36% HIV-pos versus 7% in HIV-neg MSM
- Los Angeles: 69% HIV-pos clinic cohort of MSM
- EXPLORE: 20% in HIV-neg MSM
- Thailand: 33.9% HIV-pos versus 12.5% HIV-neg MSM



Incidence of high-grade anal dysplasia

- Incidence of high-grade anal dysplasia over 4 years from normal or low-grade dysplasia was 49% in HIV-pos and 17% in HIV-neg MSM
 - HIV infection
 - CD4 < 200
 - Multiple HPV types
 - High-level high-risk HPV infection



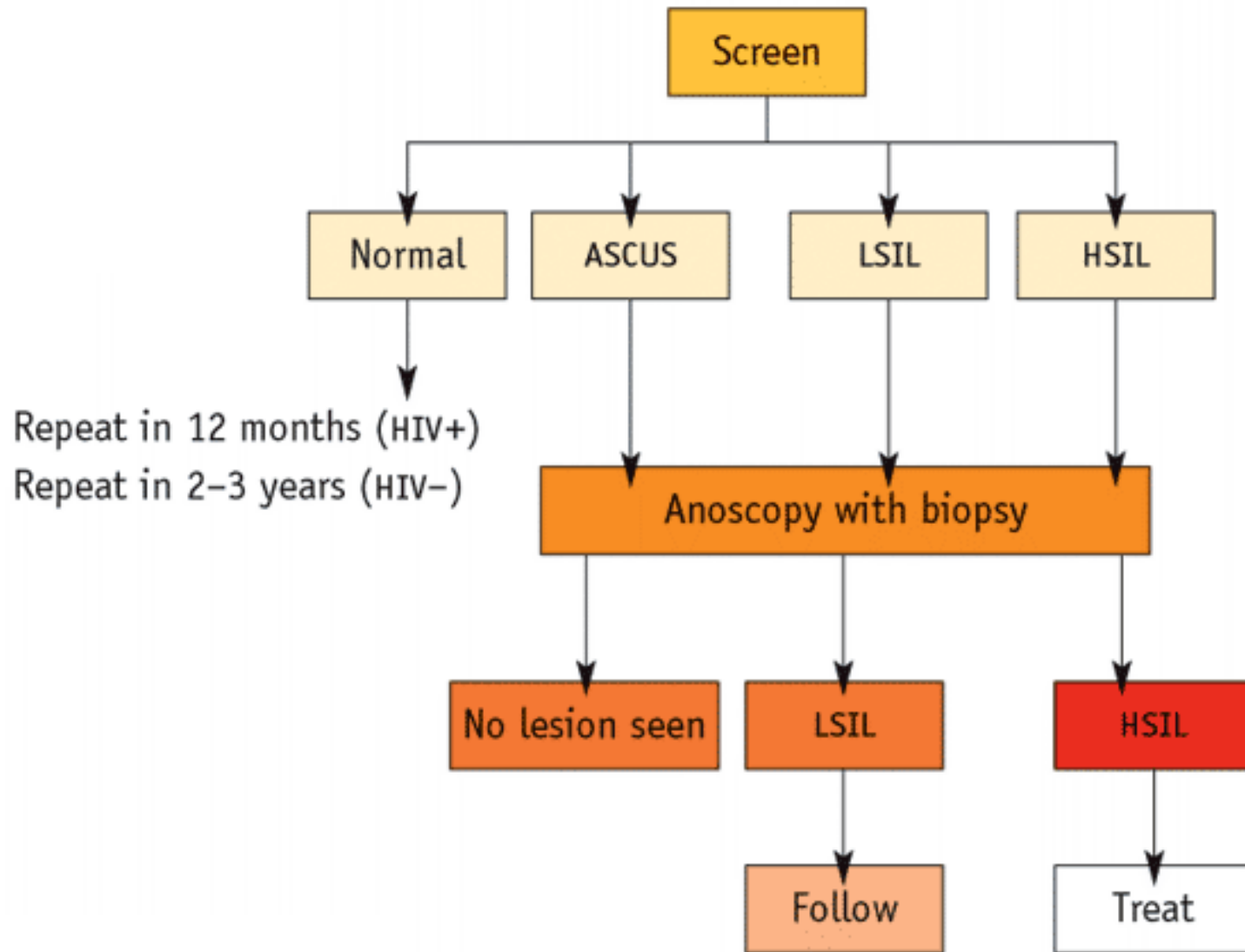
Anal cytology screening

- Sensitivity similar to cervical cytology
- Poor correlation of anal cytology with anal biopsy
- Anal histopathology is mandatory to grade disease
- Main aim of screening is to detect any abnormality
- No national/international guidelines



Palefsky 1997, Cranston 2007, Panther 2004





Anal cytology alternatives

- Education
- Regular digital rectal examination
 - Focal pain
 - Bleeding
 - Nodularity/thickening
- No cytology without a referral route for high-resolution anoscopy with Rx capability



High-resolution anoscopy (HRA)

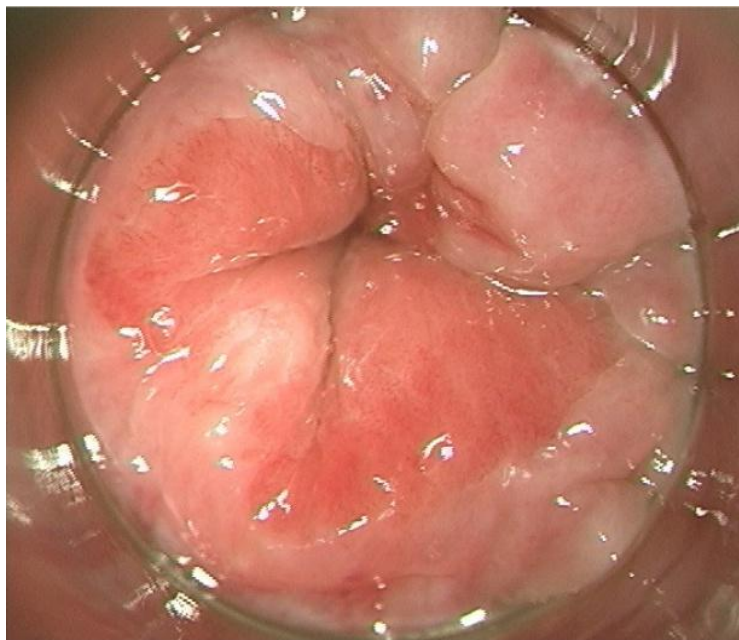
- Peri-anal inspection
- Digital examination
- Soak anal canal in 3% acetic acid
- Visualize transitional zone using a high-resolution anoscope (colposcope) with x 16 magnification



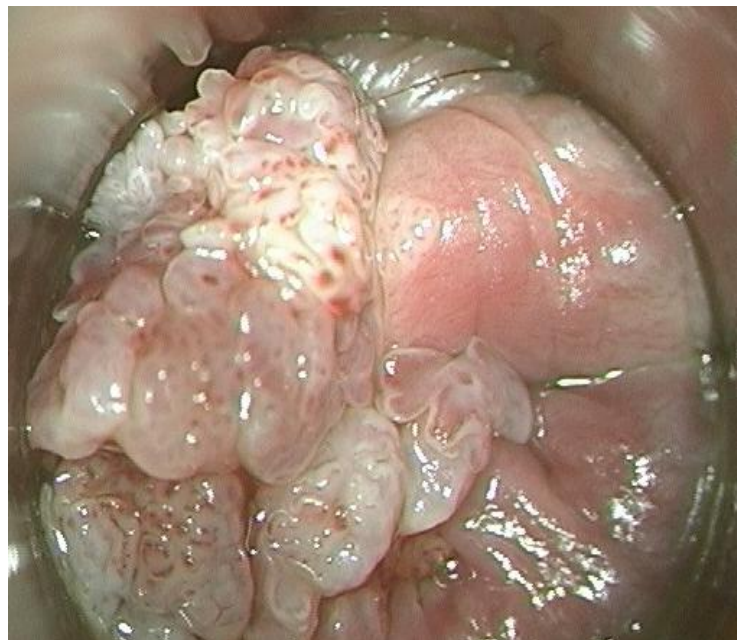
HRA and image capture



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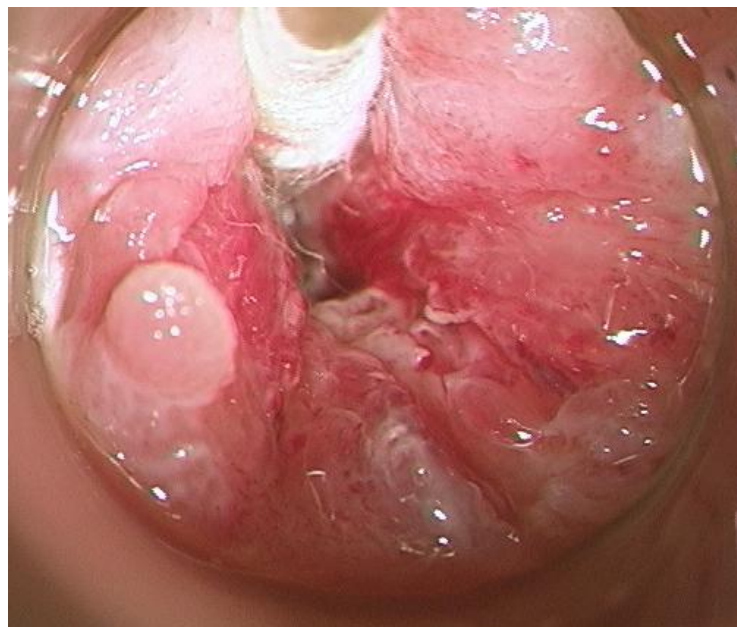
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Anal high-grade dysplasia treatment

- Medical: Trichloroacetic acid
Imiquimod
Infra Red Coagulation
- Surgical: Electrofulguration
Cold scalpel excision
Laser ablation



Anal cancer rates

- General population: 2 : 100 000
- MSM 'pre-HIV': 35 : 100 000
- HIV+ MSM 70-140 : 100 000



Anal canal and rectal microbicide development



Anal STI

- HSV-2
 - Ulceration
 - Recruitment of activated CD4 cells
 - Increase in immune mediators of inflammation (cytokines)
 - Associated with incident HIV infection
 - High prevalence in MSM
- HPV
 - Friable, easily bleeding internal anal lesions
 - Inflammation
 - Associated with incident HIV infection
 - High prevalence in MSM

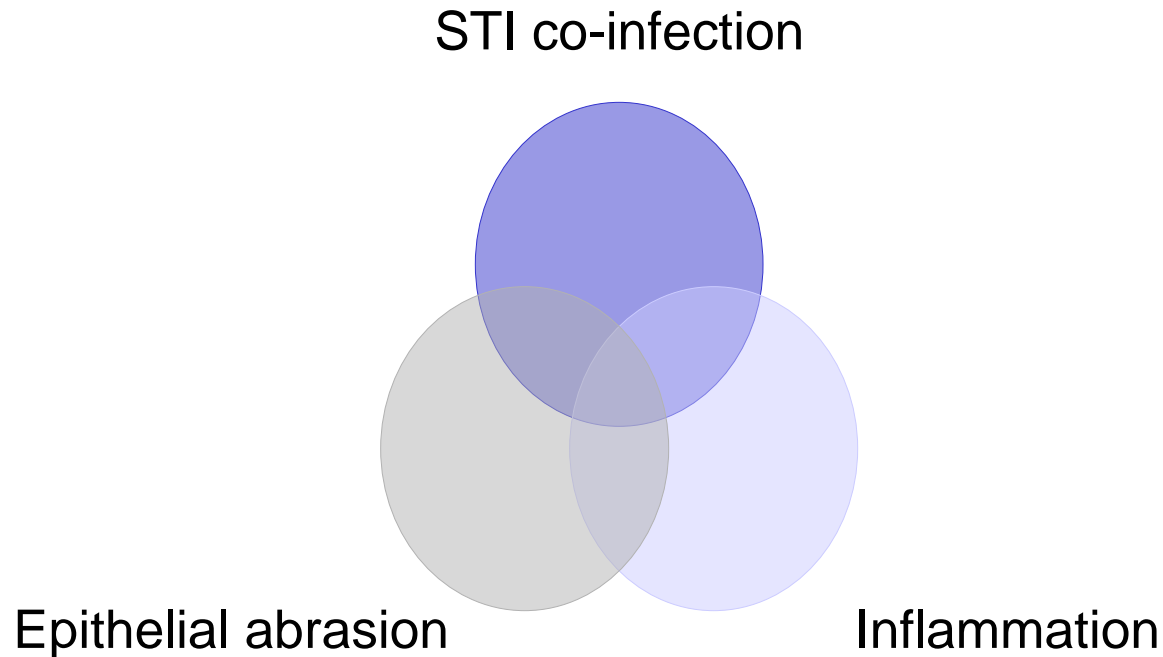


Effect of RAI

- Inflammation
- Ulceration
- Increase in inflammatory conditions (fissure, fistula, abrasion)



The perfect storm of RAI



Anal and rectal tissue study

- Presence of HIV-1 co-receptor (CCR5 and CXCR4) positive T cells in anal and rectal tissue
- Cytokine environment in anal and rectal tissue
- Can anal and rectal explants can be infected with CCR5 and/or CXCR4 tropic HIV-1
- To quantify HIV-1 mucosal viral load in anal and rectal tissue



Study population

- Population: Chronically infected HIV-positive participants with PVL <50 or >10,000 copies per mL, and HIV-negative participants, over 18 years of age
- Screening: Rectal GC/CT by culture, HSV-2 by culture and Ag detection, HIV-1 PVL
- Biopsies: Paired visually normal anal and rectal tissue biopsies

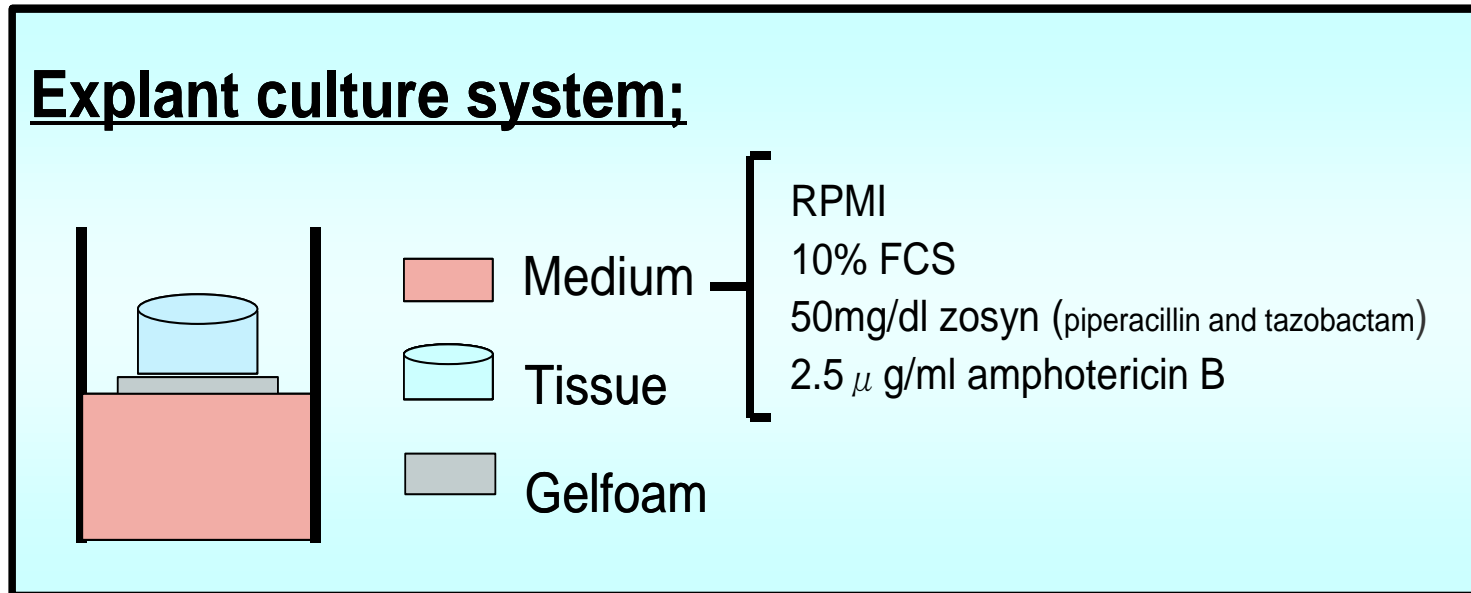


Assessment of mucosal T cell phenotype

- Tissue fragments were digested with collagenase II
- Mucosal mononuclear cells were quantified by Trucount (BDIS) and analyzed by FSCSCalibur using Cellquest software
- Cells were gated for CCR5, CXCR4, and both CCR5 and CXCR4 on CD4+CD45+ lymphocytes
- N = 5 (3 HIV-neg, 2 HIV-pos)



Explant infection using gel-foam rafts



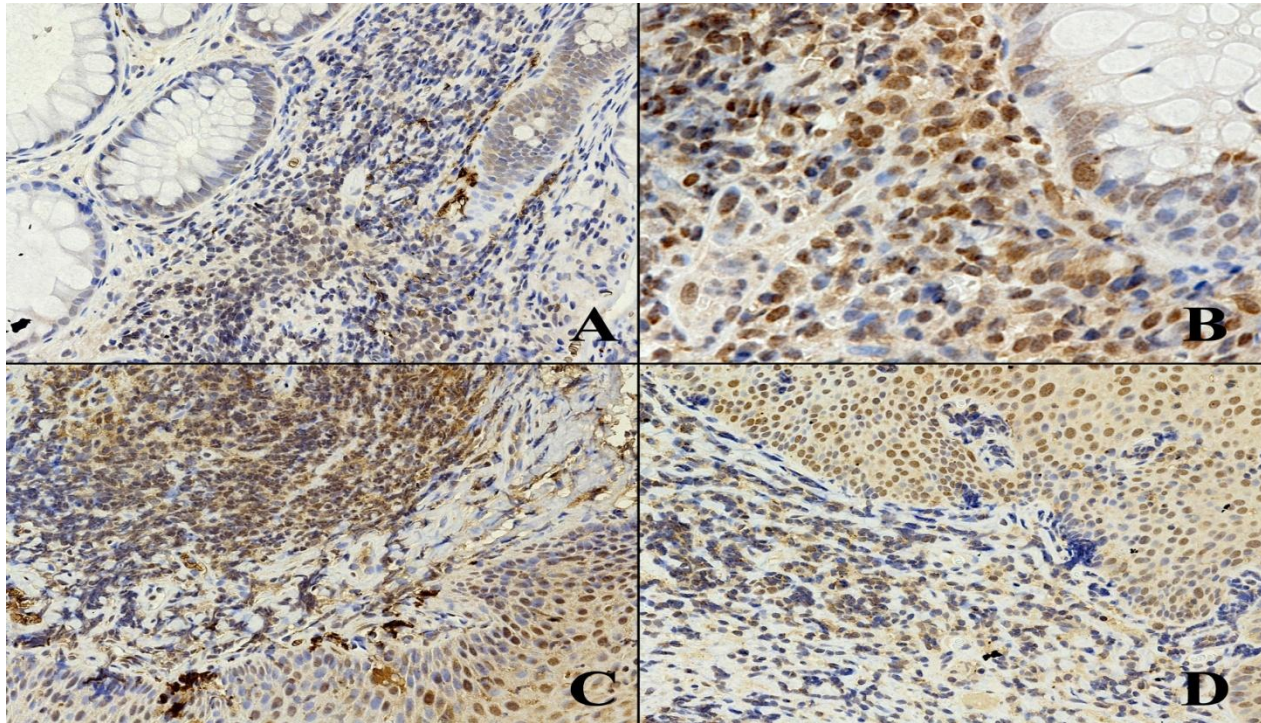
- Biopsies were placed on rafts and immersed in Dulbecco's modified Eagle culture medium
- Infected with HIV-1 Bal/Lav for 2 hours, then washed and rafts replaced
- Assessed every 3-4 days for infection by quantification of P24 Ag by ELISA

Tissue HIV-1 quantification

- Anoscopic biopsies were snap frozen in liquid nitrogen
 - Undetectable VL (N =12)
 - Detectable VL (N = 12)
- Total RNA isolated by TRIzol technique
- RT-PCR performed followed by real time PCR using Geneamp 5700 sequence detection system
- The mean of 3 quantifications was calculated and expressed as copies per microgram of total tissue RNA

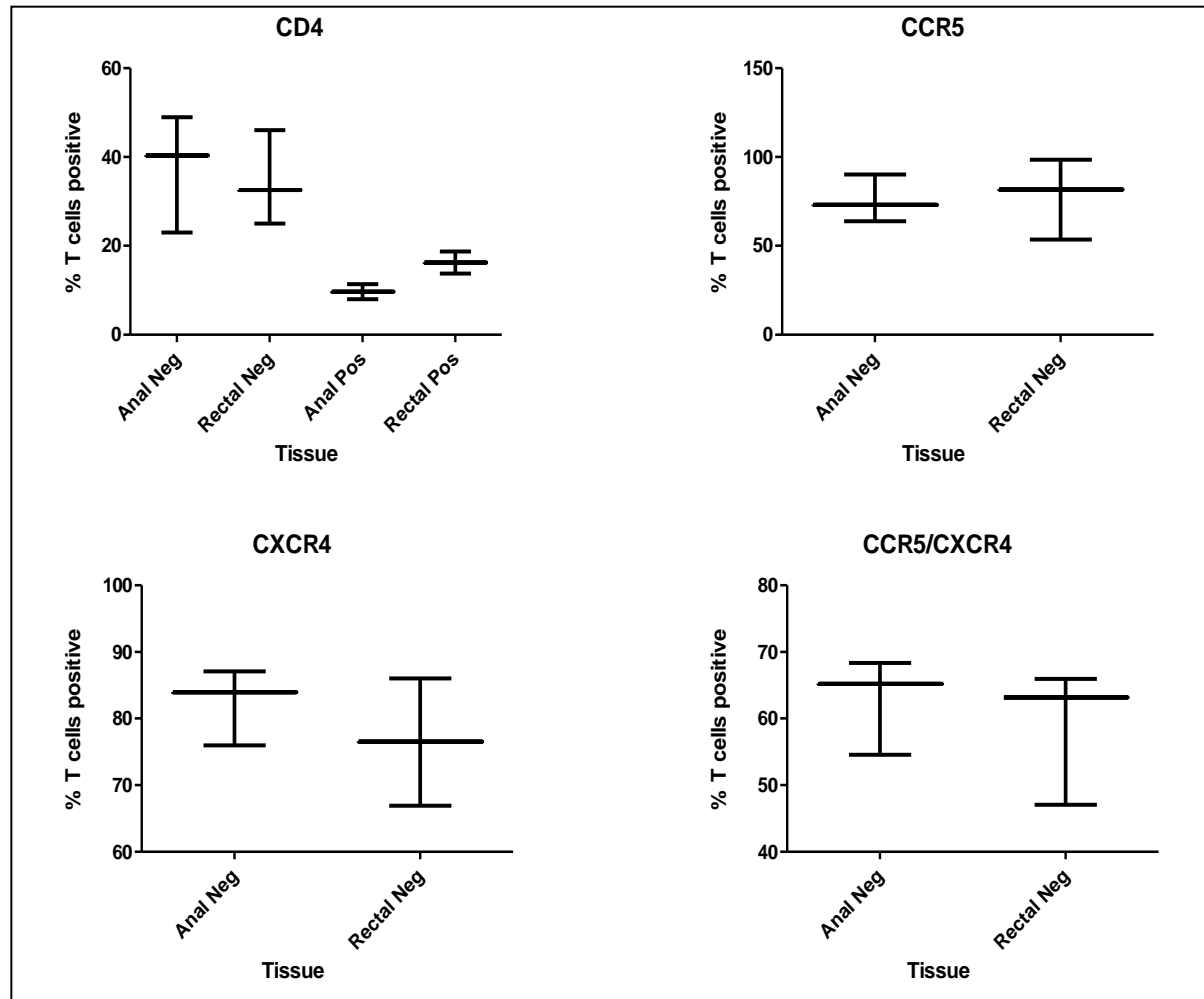


Immunostaining

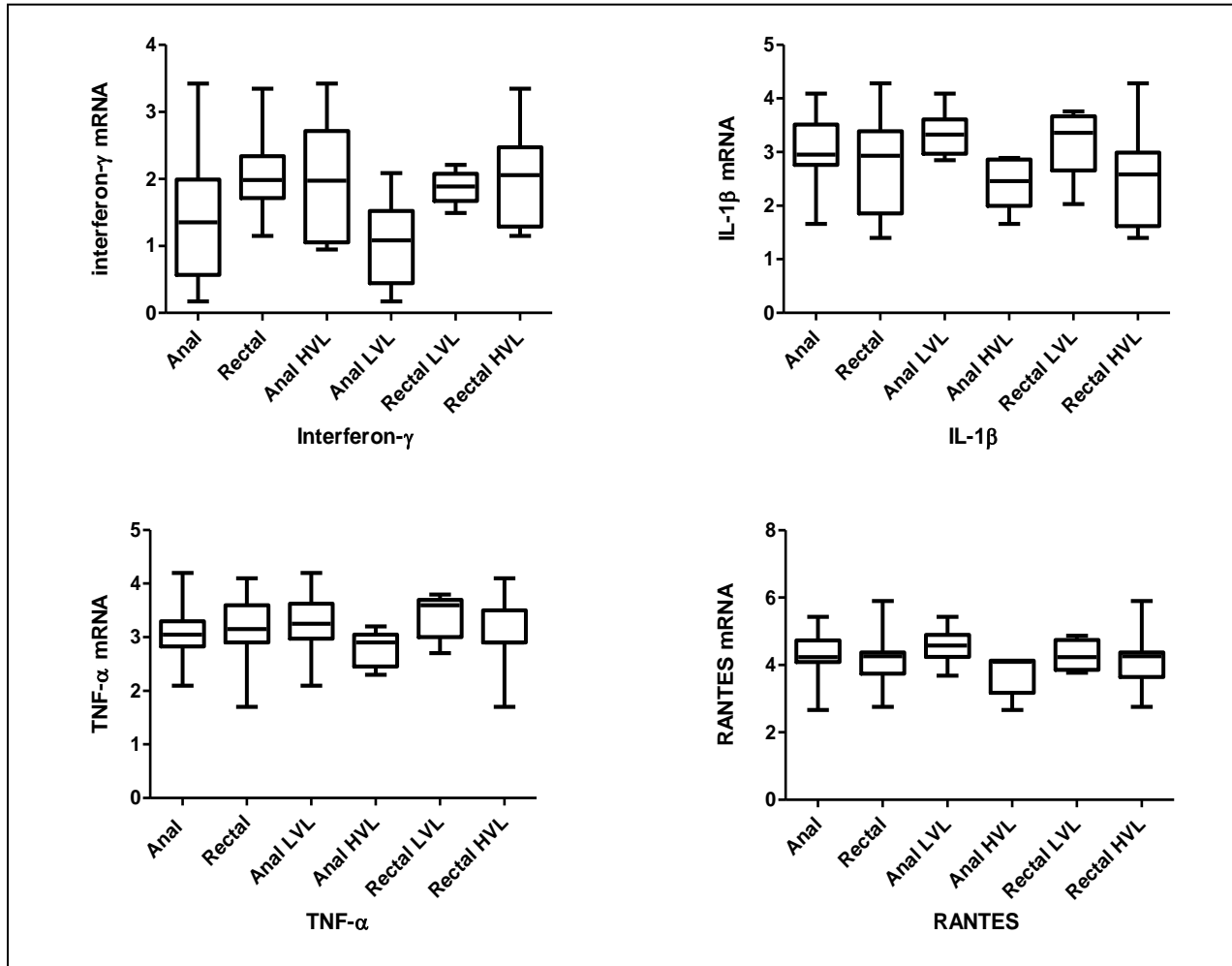


A: Rectal tissue stained for CXCR4 shows positive staining lymphocytes within the lamina propria B: Rectal tissue stained for CCR5 shows positive lymphocytes beneath the rectal epithelium C: Anal tissue stained for CXCR4 reveals positive lymphocytes in the lamina propria D: Anal tissue stained for CCR5 reveals positive lymphocytes beneath the squamous epithelium.

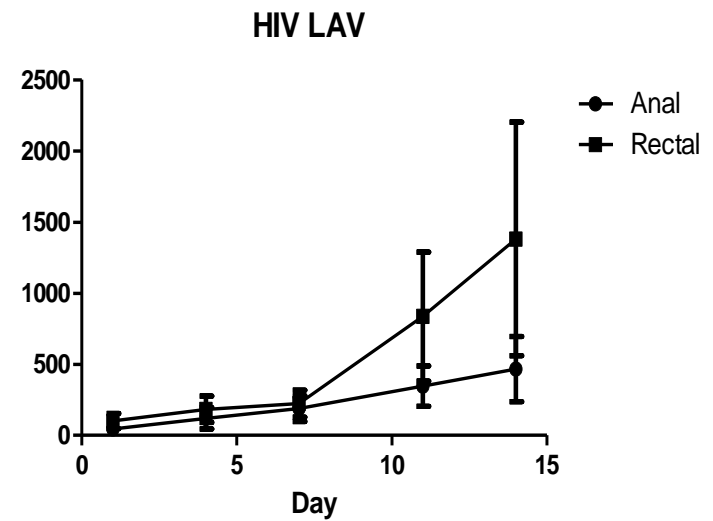
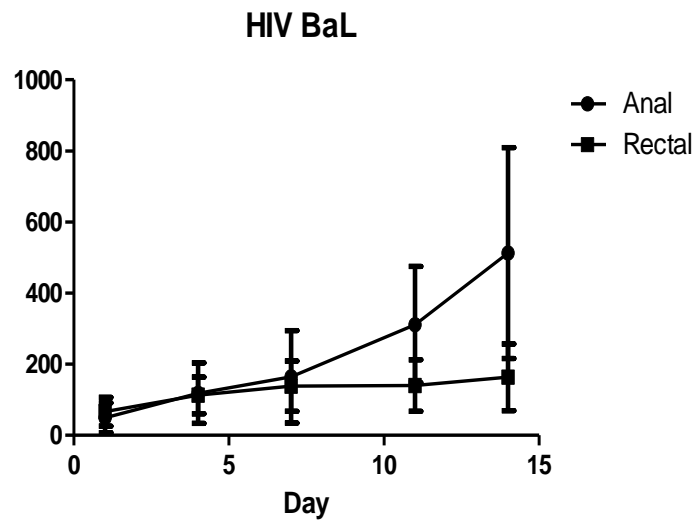
Anal and rectal T cell phenotype



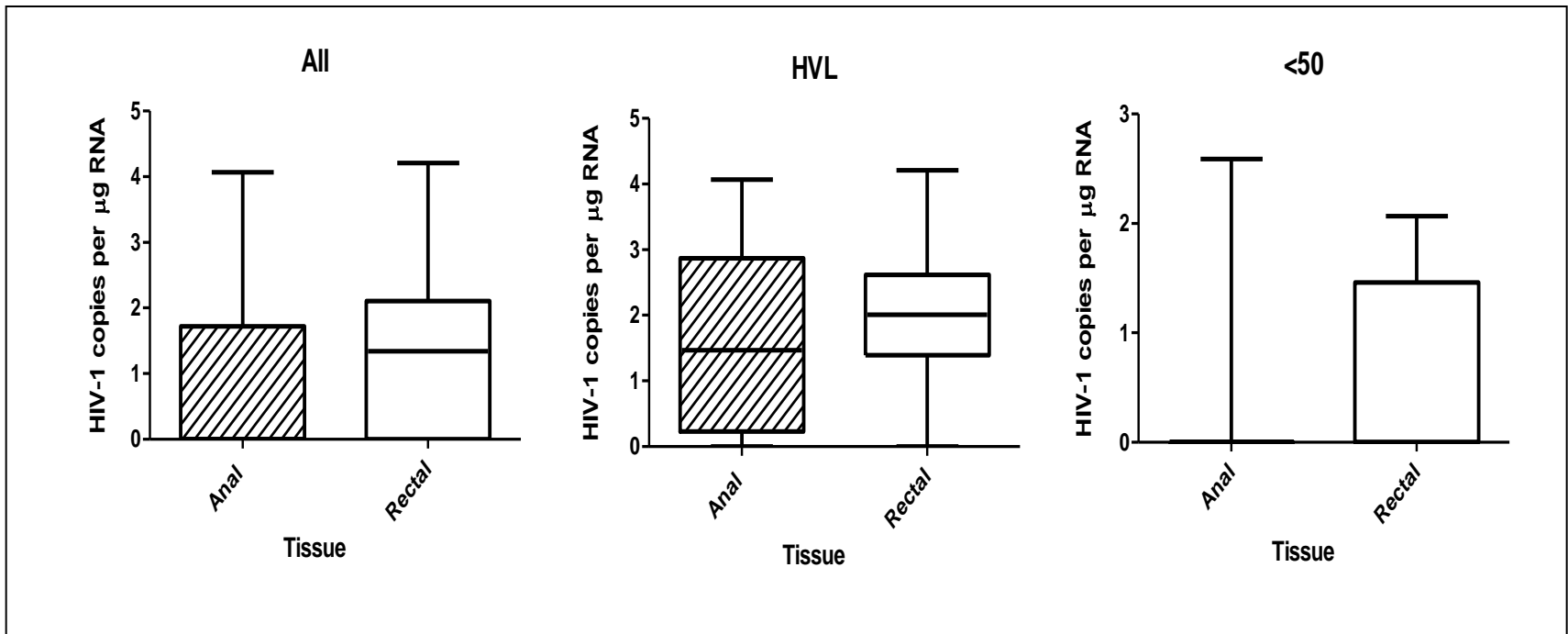
Anal and rectal cytokines



Anal and rectal explant infection



Anal and rectal HIV quantification



Conclusions

- CD4 T cells expressing both R5 and X4 co-receptors are present in anal and rectal epithelium
- Both anal and rectal explants can be infected with HIV-1
- The anal and rectal cytokine environment is similar
- The anal canal should be considered as a site of incident HIV-1 infection



Summary

- The anal canal is commonly affected by inflammatory conditions including STI
- RAI induces anal inflammation
- Individual practicing RAI are at risk of HPV associated dysplasia and cancer
- Anorectal health should be considered as an advocacy topic in rectal microbicide development





Thank You!

