

# Using Mucosal Tissue to Evaluate Effectiveness

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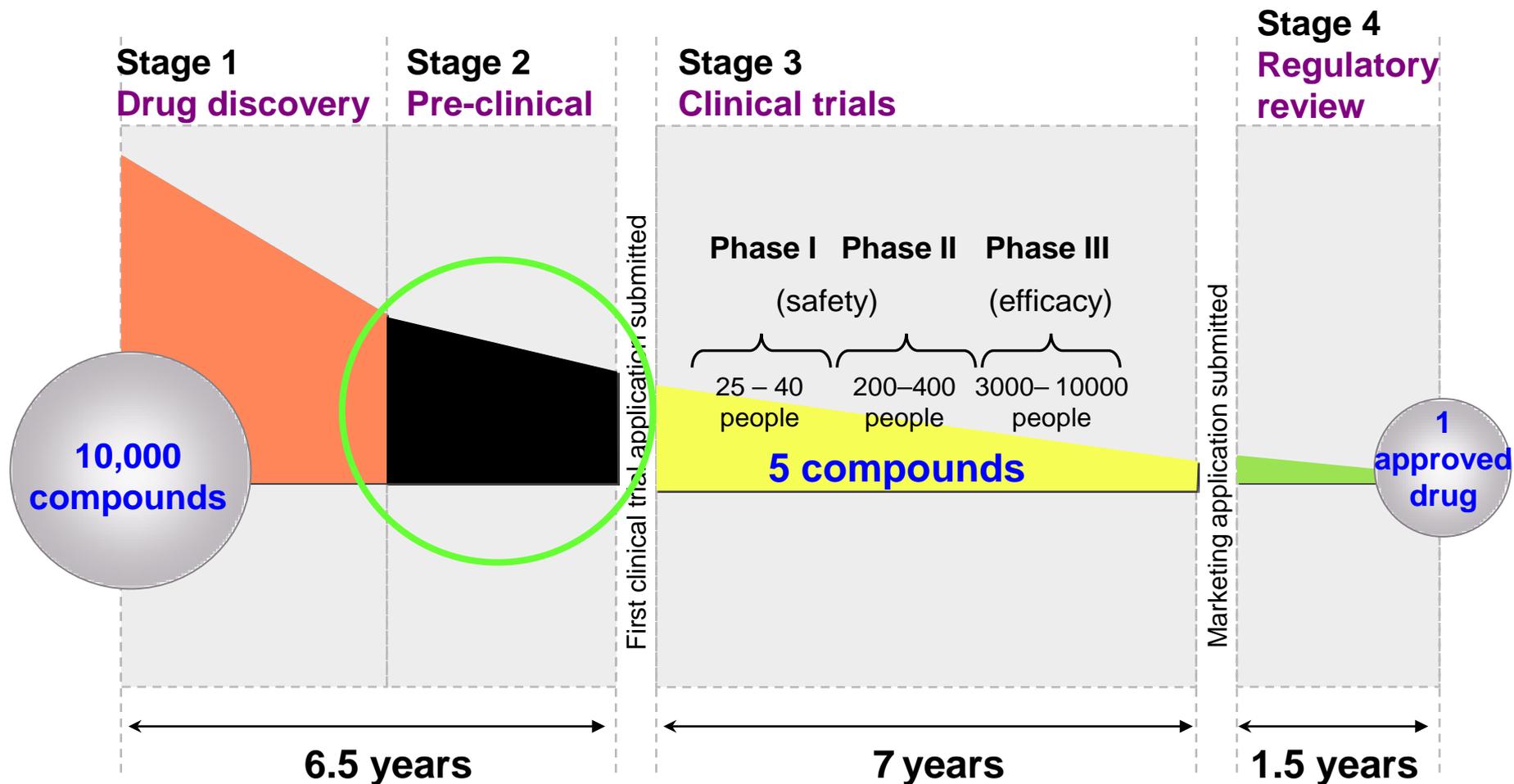
University of Pittsburgh

MTN Annual Meeting

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# Drug development pathway





# What we'll talk about...

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- Pre-clinical microbicide testing
- Tissues used for explant cultures
- What can we do with tissue explants?
- Moving toward validation



# Pre-clinical testing

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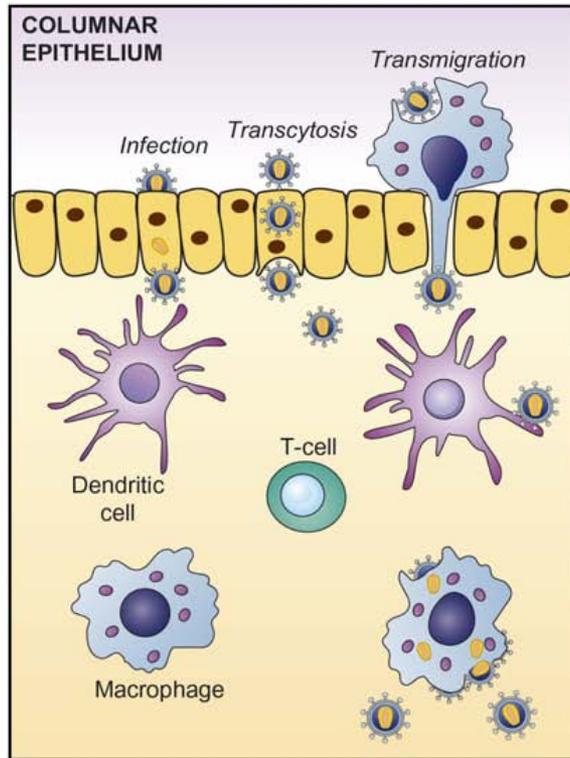
- Traditionally done by testing the compound for anti-HIV activity and cellular toxicity
- Primary immune cells or cell lines for high throughput testing
- Handful of laboratories using mucosal tissue explant cultures

# Why use mucosal tissues?

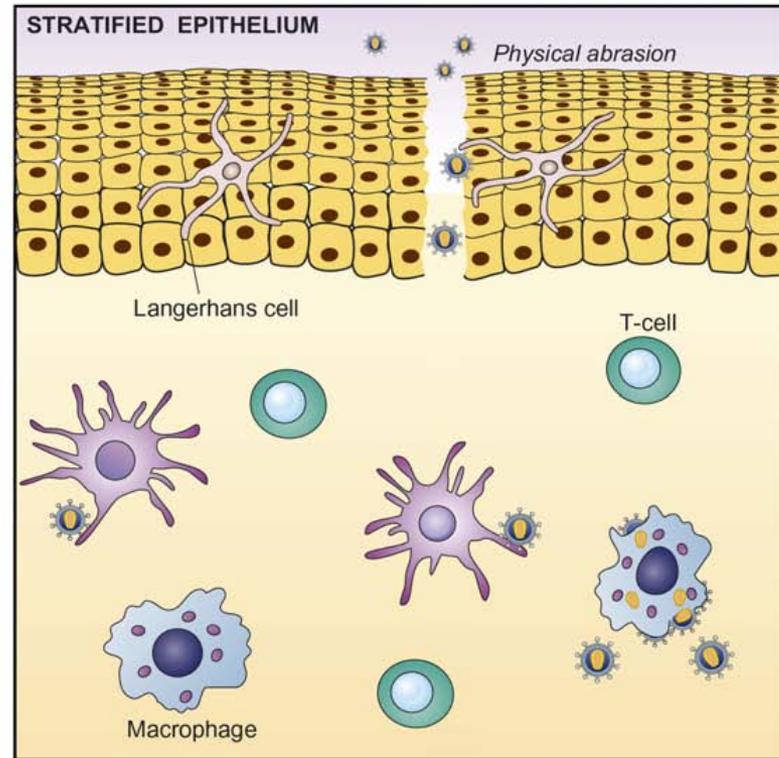
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- Mucosal tissue is where the virus enters and where the product will be applied
- Tissue consists of relevant cell types (HIV targets) in biologically appropriate ratios
- Surgical remainders
  - do not require patient recruitment
  - obtained within a few hours of surgery
- Endoscopic/colposcopic biopsy
  - require patient recruitment
  - obtained within minutes of scoping and convenient

# What tissues are used?



Colorectal  
Endocervix  
Endometrium



Ectocervix  
Vagina  
Penis  
(glans, foreskin)  
Anus

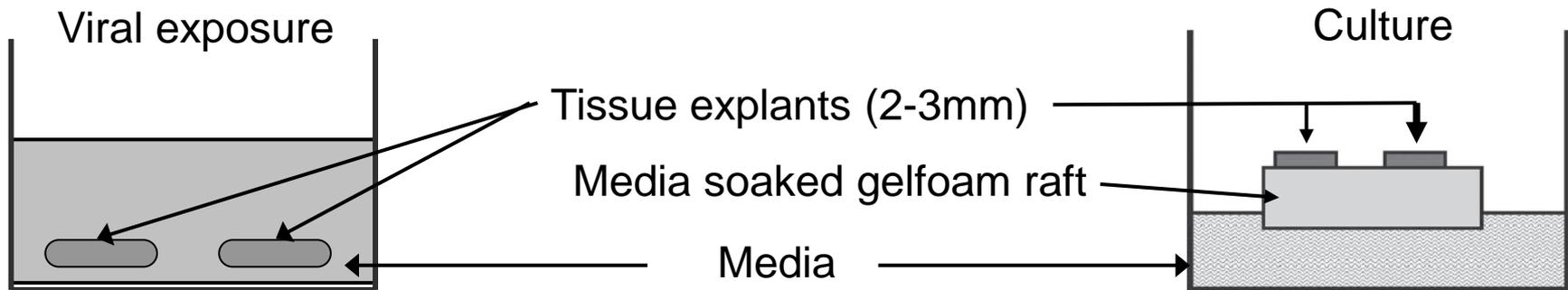


# Colorectal and ectocervical models

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- Colorectal tissue – obtained from surgical resections or endoscopic biopsies
  - Non-polarized (Anton/McGowan, Shattock)
  - Polarized (Dezzutti)
- Ectocervical tissue – obtained from pre-menopausal women undergoing hysterectomies
  - Non-polarized (Asin, Shattock)
  - Polarized (Dezzutti, Gupta [with T cell co-culture])

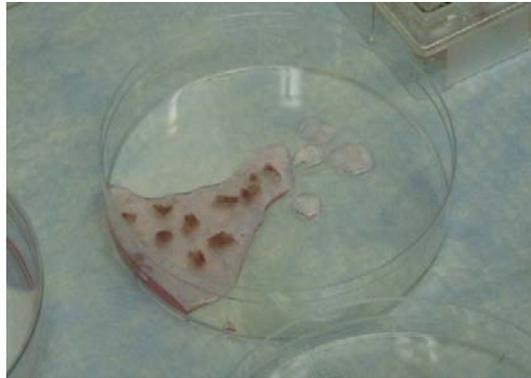
# Non-polarized colorectal explants



- 2 mm piece of colorectal tissue
- Explants exposed to virus/microbicide while submerged in media (96 well plate)
- Explant placed on a media-soaked gelfoam raft in a 24-well plate following viral exposure
- Cultured in DMEM/pen/strep/ $\pm$ 10% FCS
- Infection determined by presence of p24 in culture supernatants (10-14 days post exposure)

# Non-polarized colorectal explants

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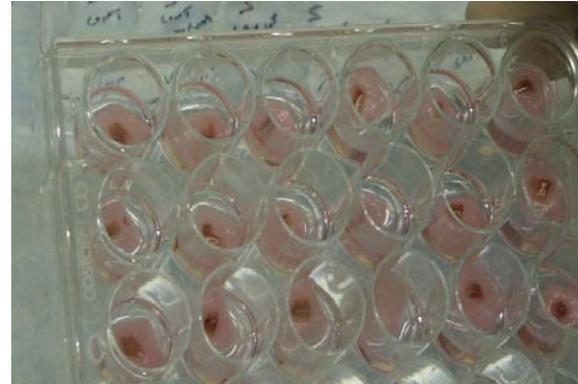
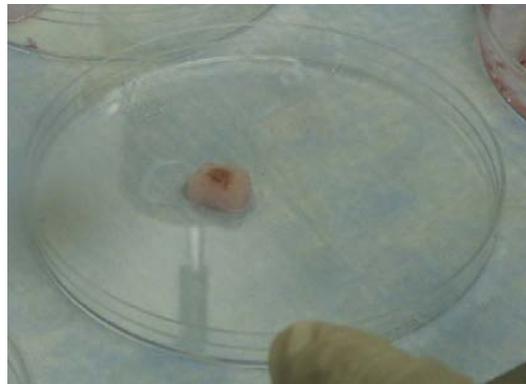


**Endoscopic biopsies**

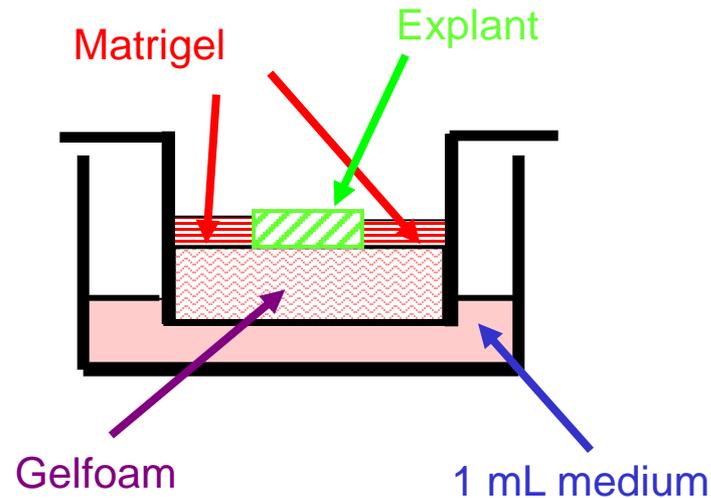


**Absorbable gelatin sponge**

+



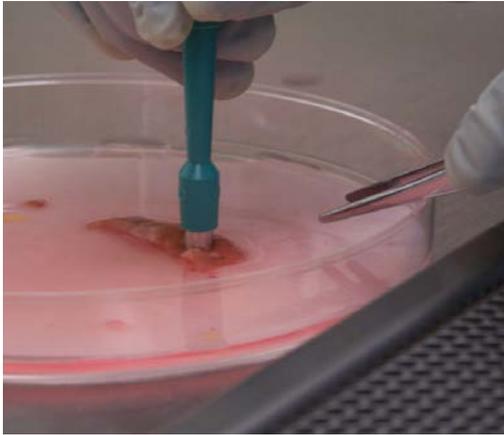
# Polarized colorectal explants



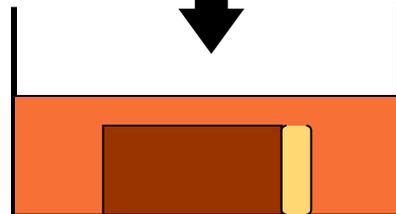
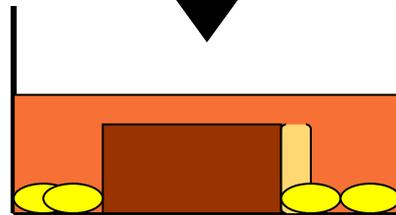
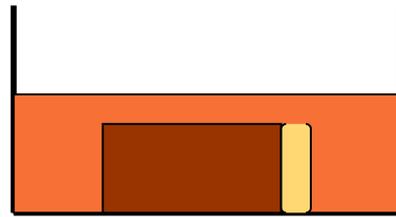
- 5 mm circular piece of colon (biopsy punch) and muscle is excised
- Explant is placed (epithelium on top) on presoaked gelfoam inserted into a transwell
- Explant is sealed with Matrigel around the epithelium

# Polarized colorectal explants

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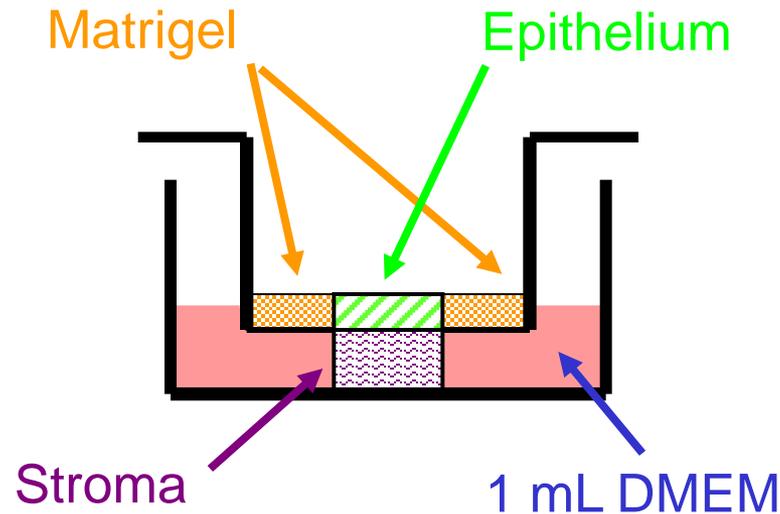
# Non-polarized ectocervical explants



- Human cervical tissue: cut into 2-3mm “explants” (complete RPMI)
- Submerged tissue exposed to virus (2h)
- Wash
- Culture overnight

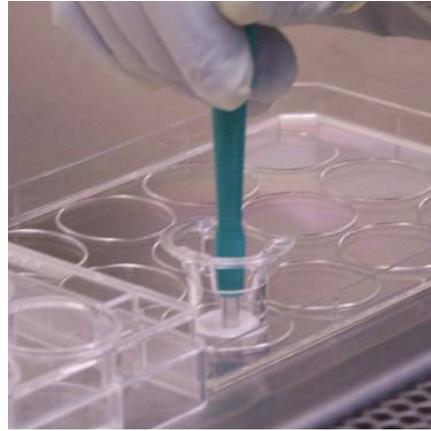
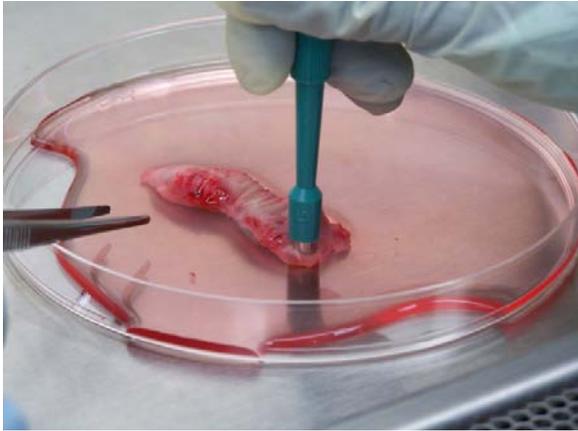
- Transfer explants to fresh plates
- Culture for 12-14 days to determine infection of explants (p24 in culture supernatant)

# Polarized ectocervical explants



- 5 mm circular piece of ectocervix (biopsy punch) and muscle excised
- Explant inserted through a hole in filter of a transwell insert and sealed with Matrigel around the epithelium

# Polarized ectocervical explants



# Non-polarized vs. polarized

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## Non-polarized

### Pros

- Utilize all tissue
- Perform more replicates
- Models “worst case” scenario

### Cons

- Can not evaluate transmission events
- Can not evaluate formulations

## Polarized

### Pros

- Biologically relevant
- Allows apical application of product/virus
- Model topical and systemic application

### Cons

- Limits tissue utilization
- Limited replicates



# What can we do with explants?

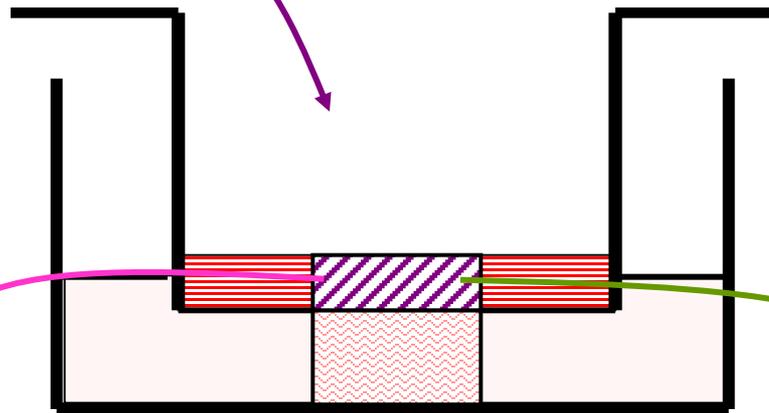
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- Evaluate drug and/or formulation safety
  - MTT assay
  - Histology
  - Drug permeability
- Determine product efficacy
- Ex vivo product testing

# Product safety – formulated



Microbicide

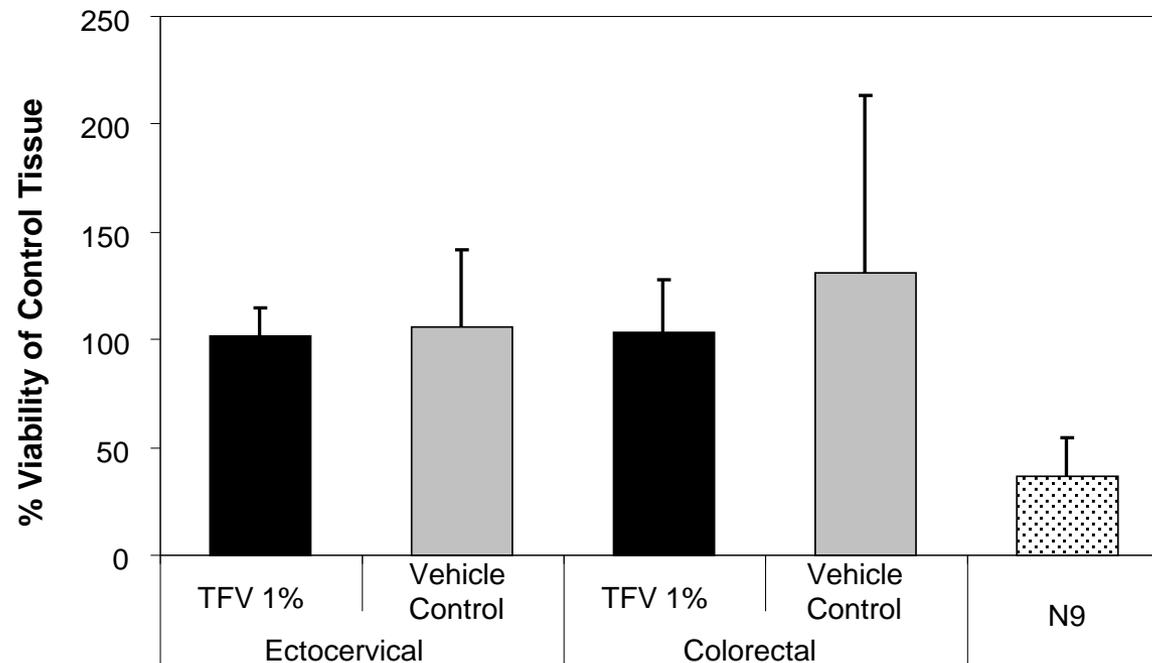


MTT assay  
(mitochondrial activity)

Histology

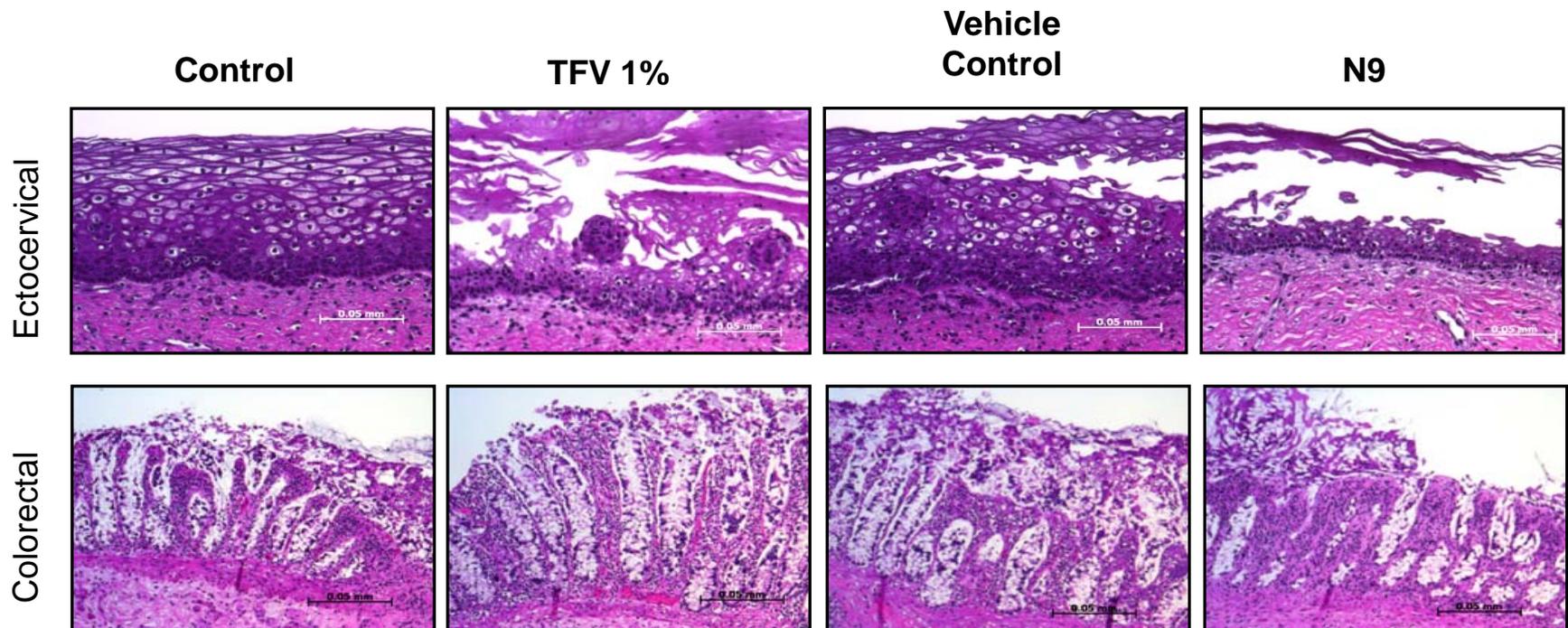
# Product safety – formulated

MTT assay



# Product safety – formulated

## Histology



3 of 5 tissues exhibited facture or sloughing of the epithelium

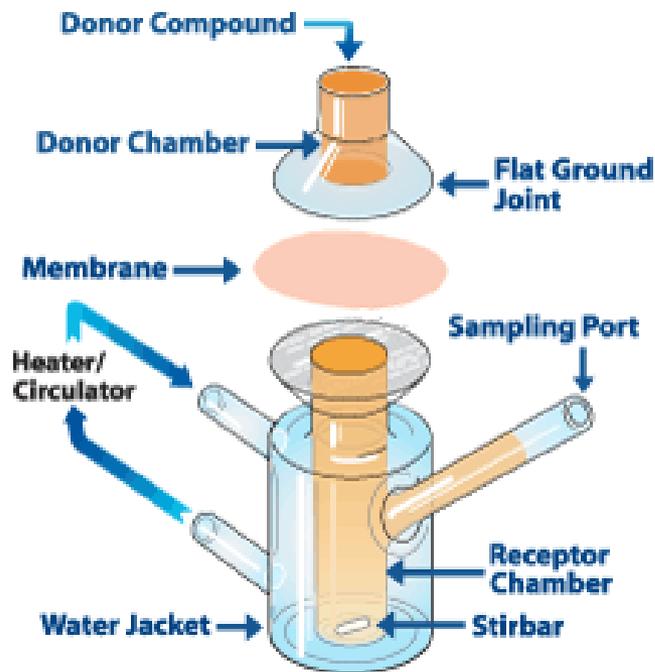


# What can we do with explants?

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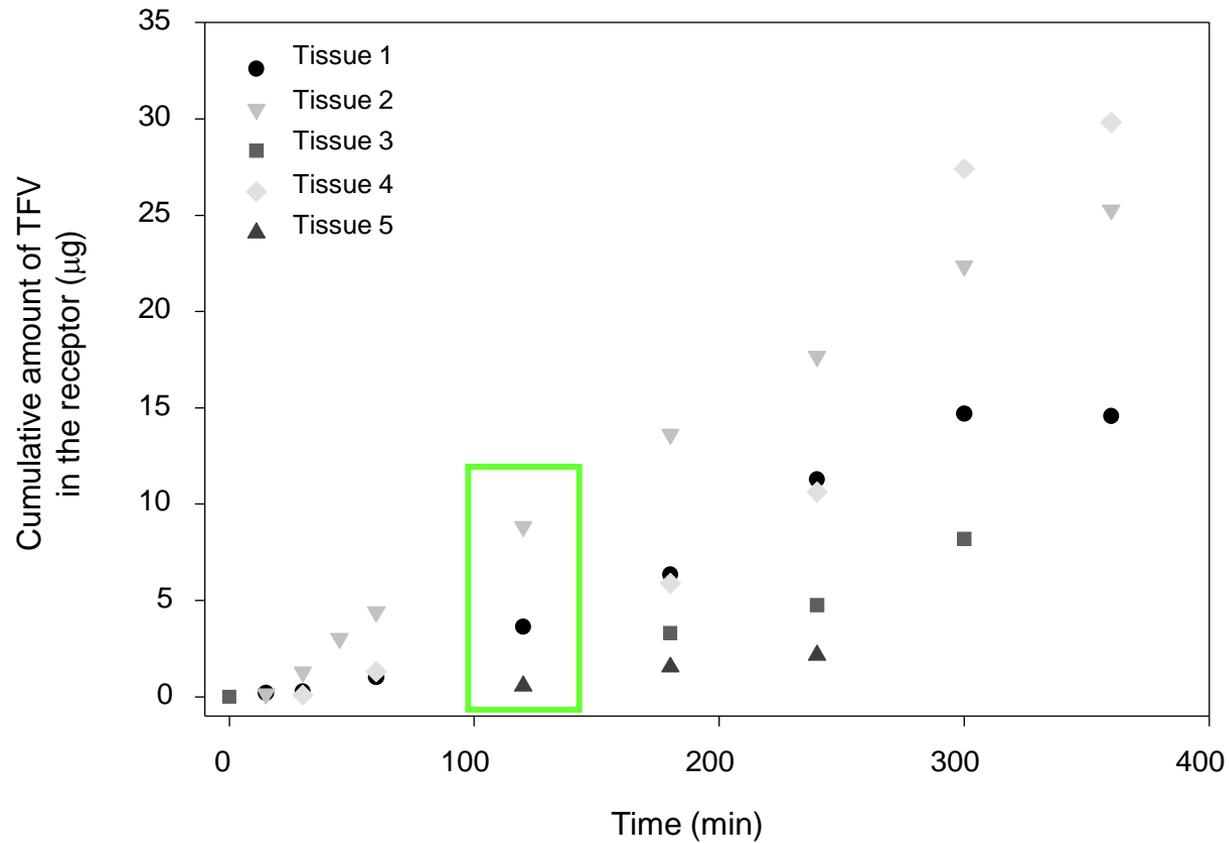
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# Drug permeability



- Tissue is placed between donor and receptor chambers
- Product is added to donor chamber
- Receptor chamber is sampled at designated time points

# TFV permeability



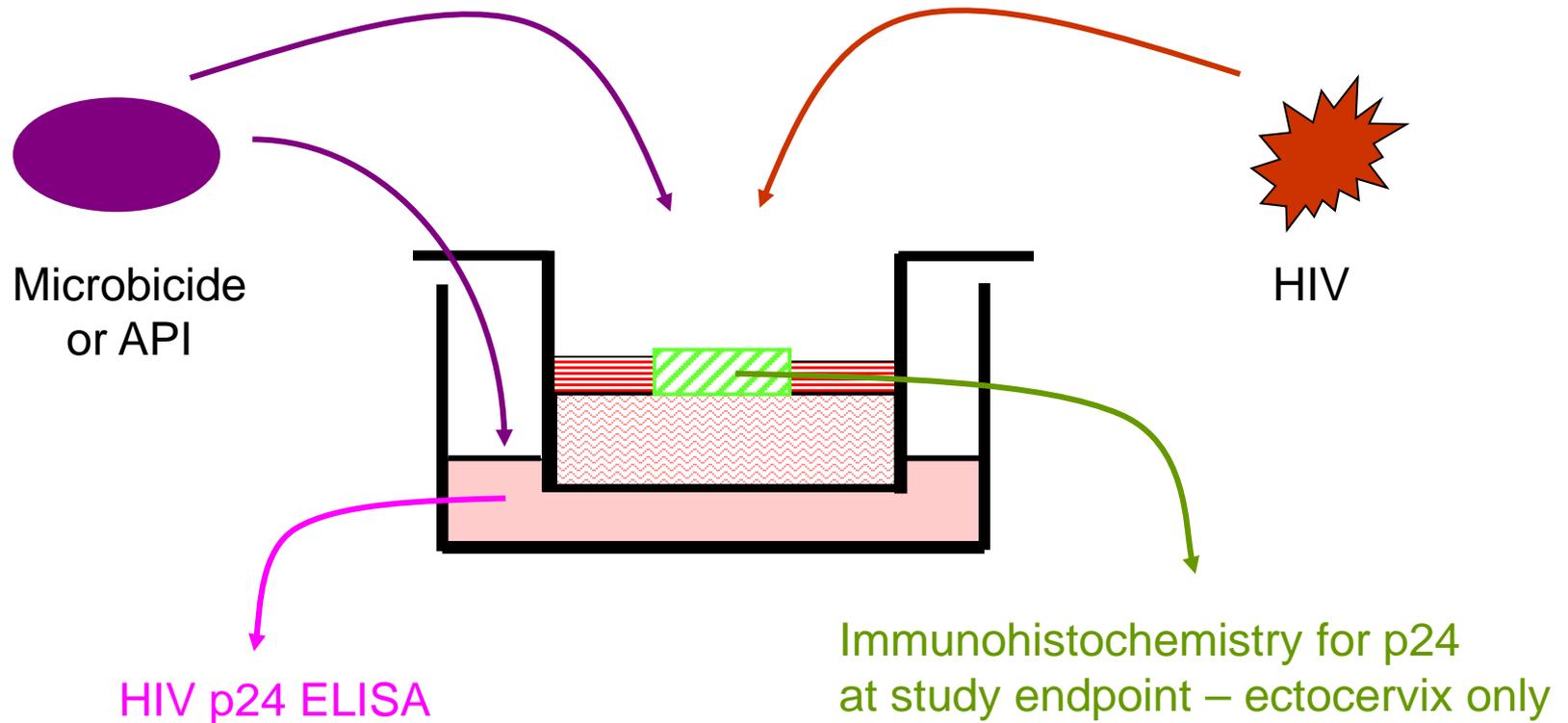


# What can we do with explants?

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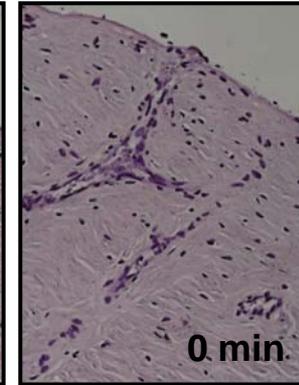
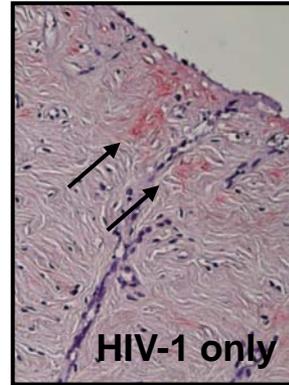
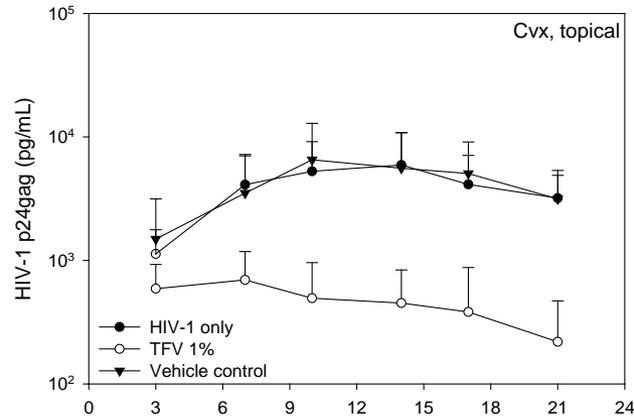
- Evaluate drug and/or formulation safety
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# Product efficacy – ~~to be evaluated~~

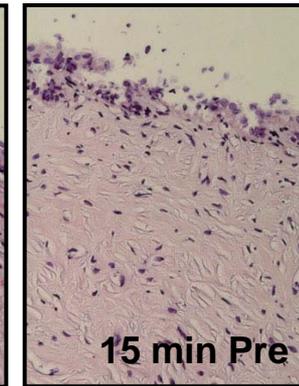
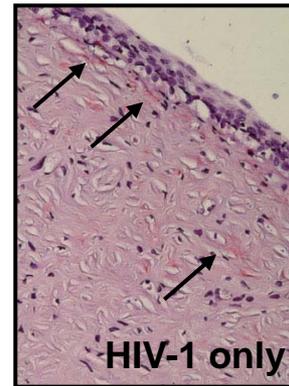
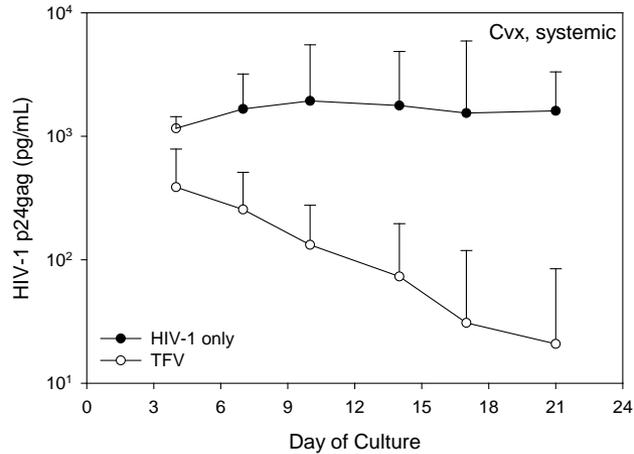


# Product efficacy

Topical application



Systemic application





# Product efficacy

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- In the presence of semen
- Model coital independent product use

# Testing with semen

	pH	Osmolality mmol/kg	Viscosity (cps)
100% semen	8.14	321	3.96
50% semen*	8.11	311	2.19
20% semen*	8.10	310	1.56
1% TFV gel	4.45	3347	3979.3

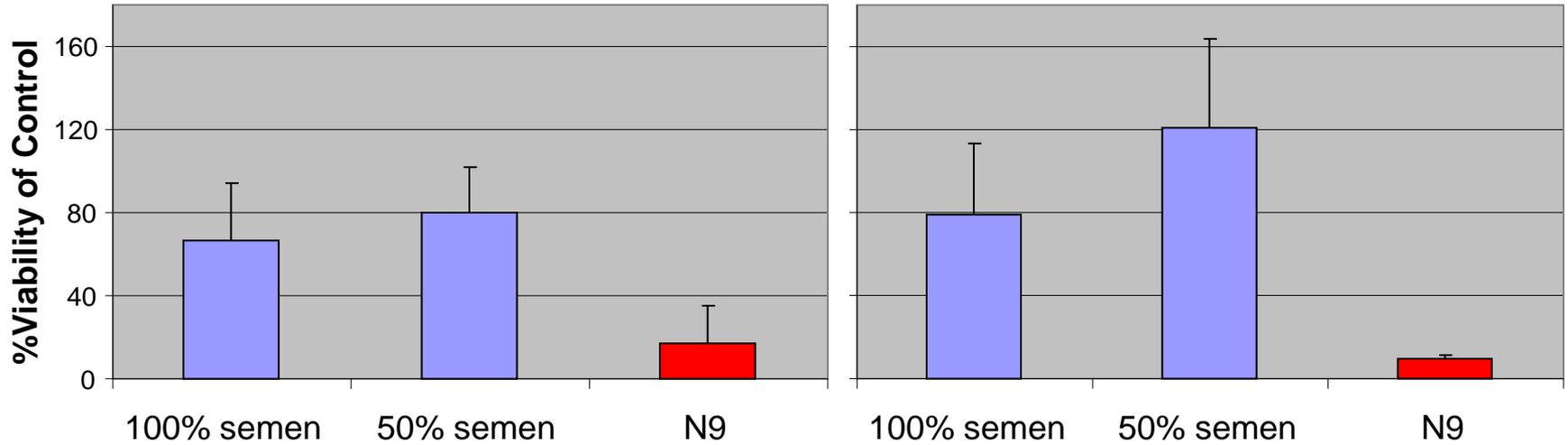
\*diluted in DMEM

# Testing with semen

MTT assay

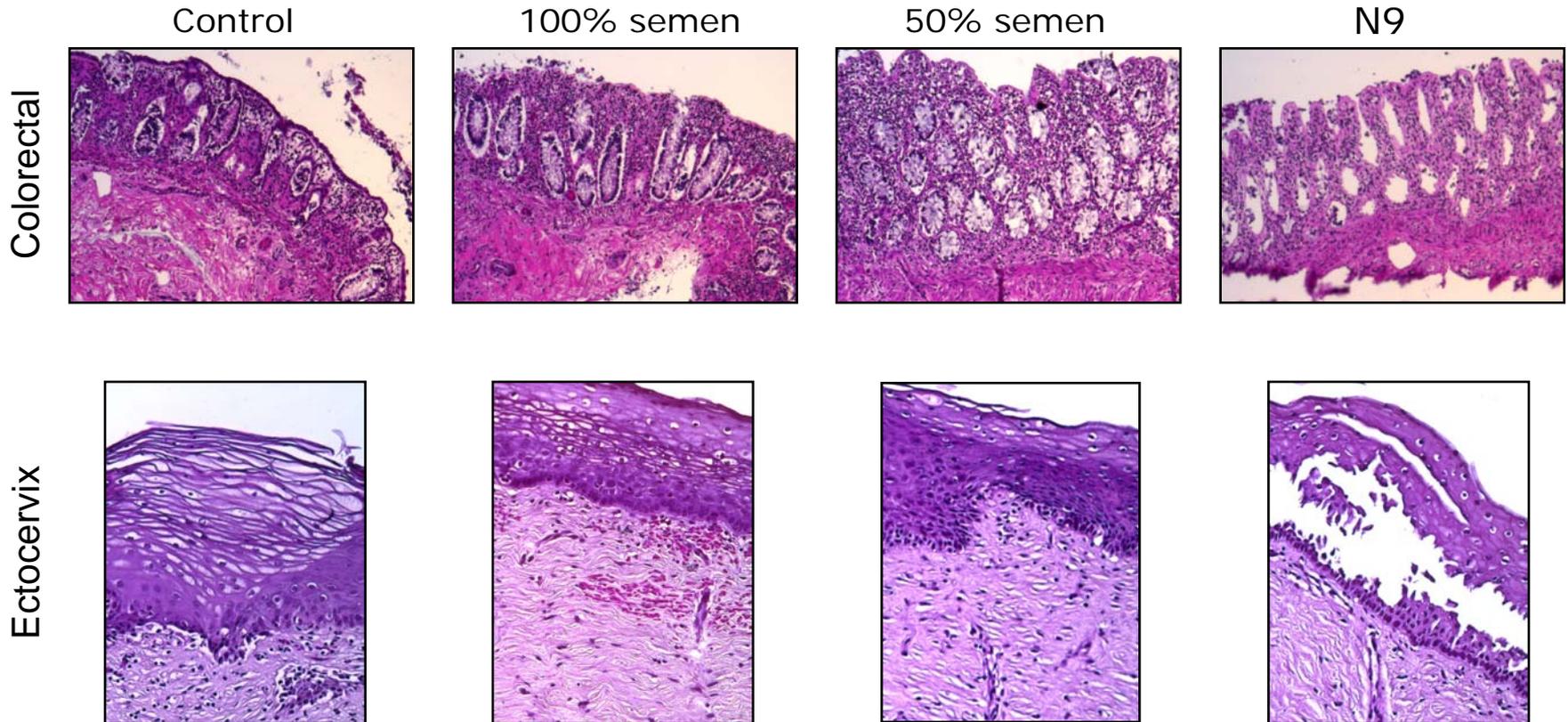
Colorectal explants

Ectocervical explants

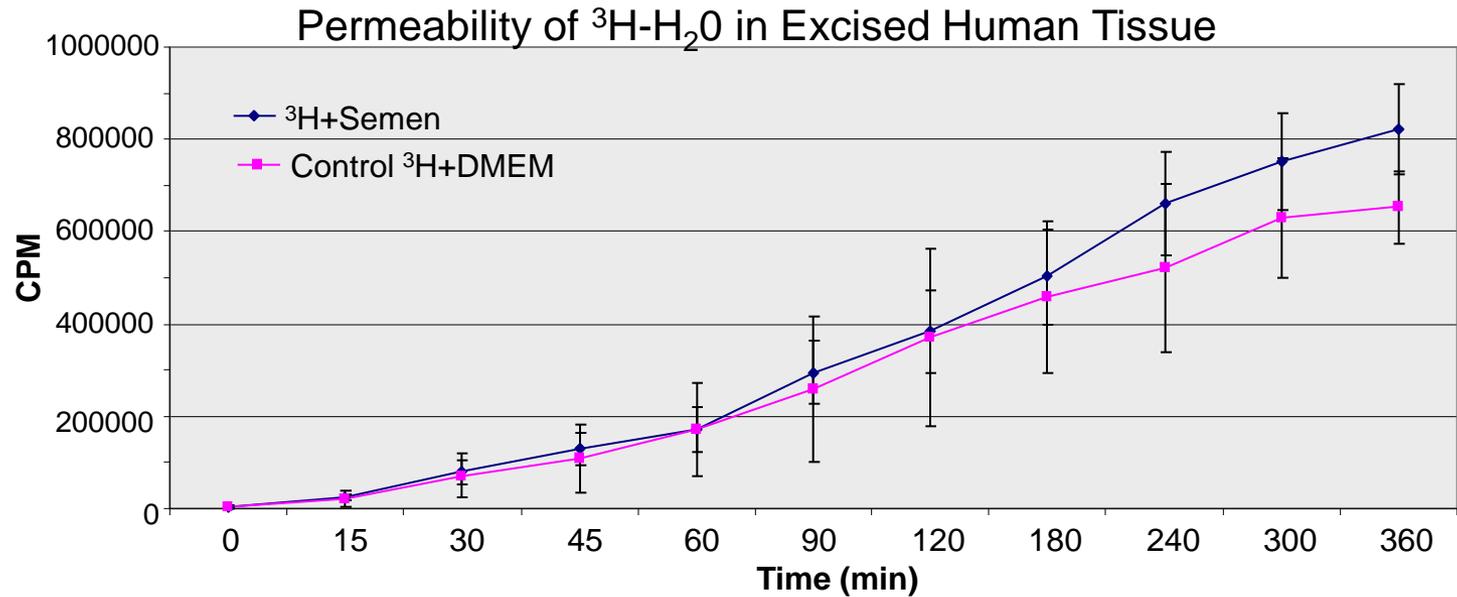


# Testing with semen

## Histology



# Testing with semen

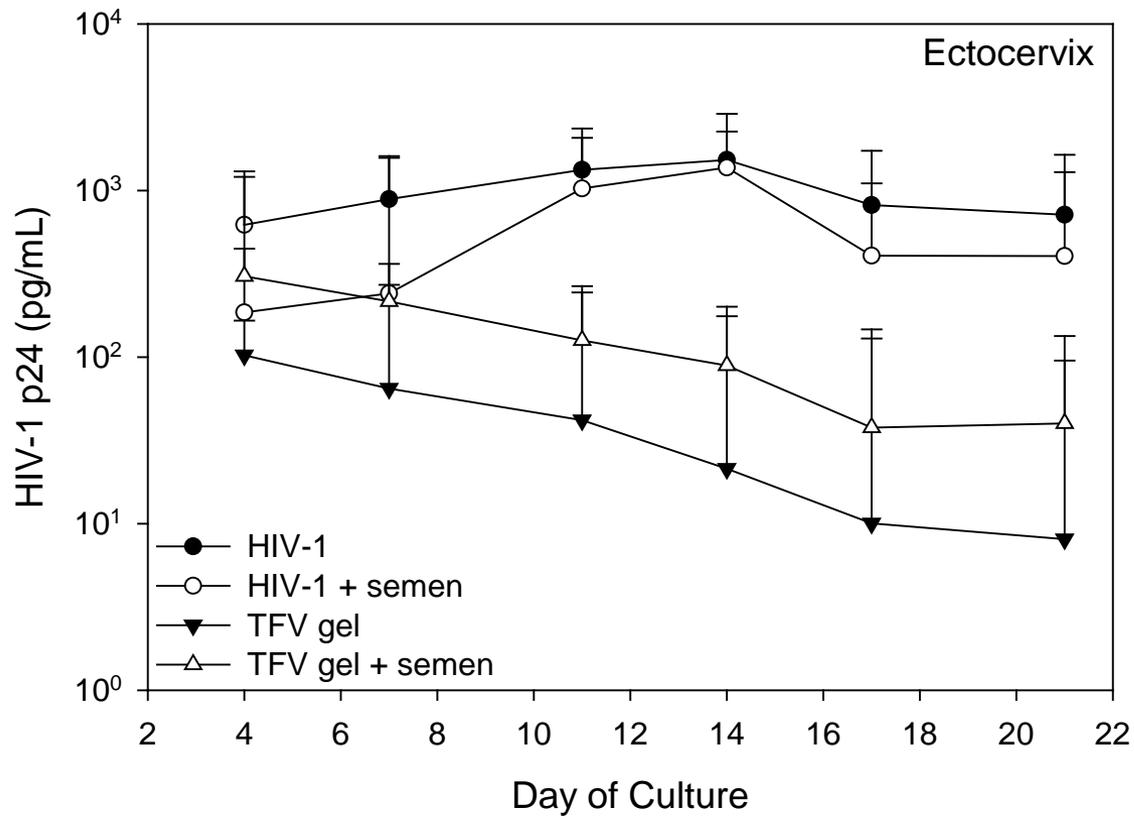


# Testing with semen

- No detectable semen enhancing viral infectivity (SEVI) effect

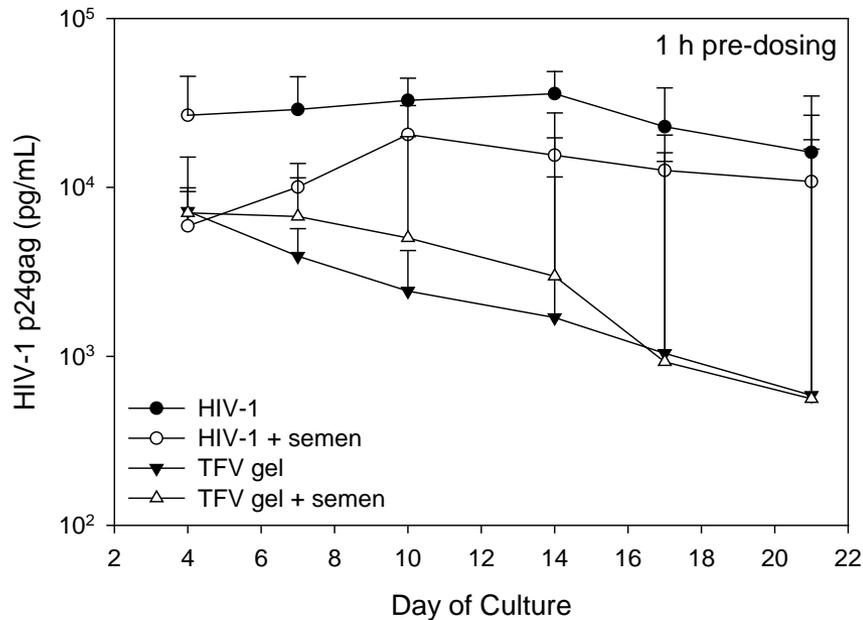


# Testing with semen

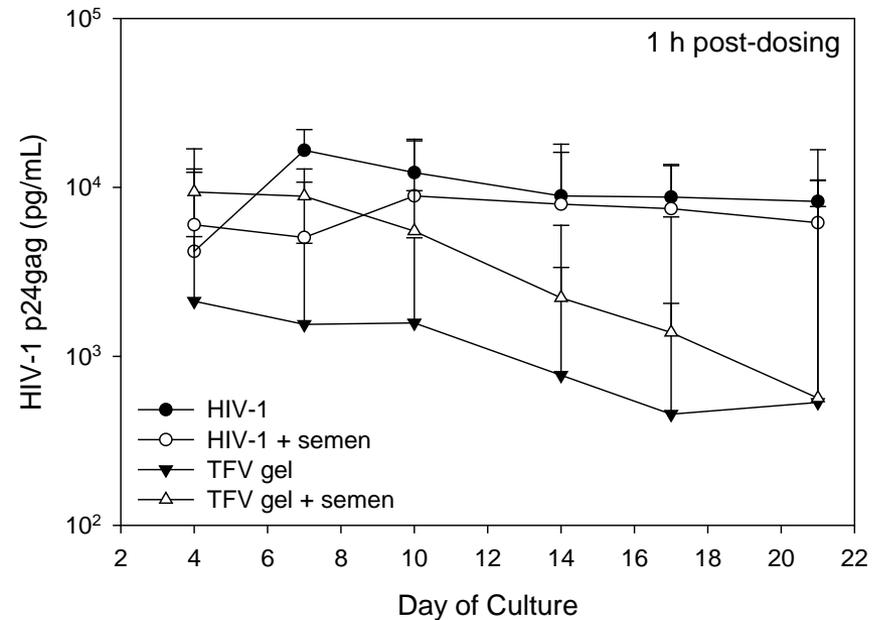


# Coital independent use

Gel is applied for 1 h, 24 h before (pre) or after (post) exposure to HIV-1



2 of 10 explants not protected regardless of semen



4 of 18 explants not protected regardless of semen

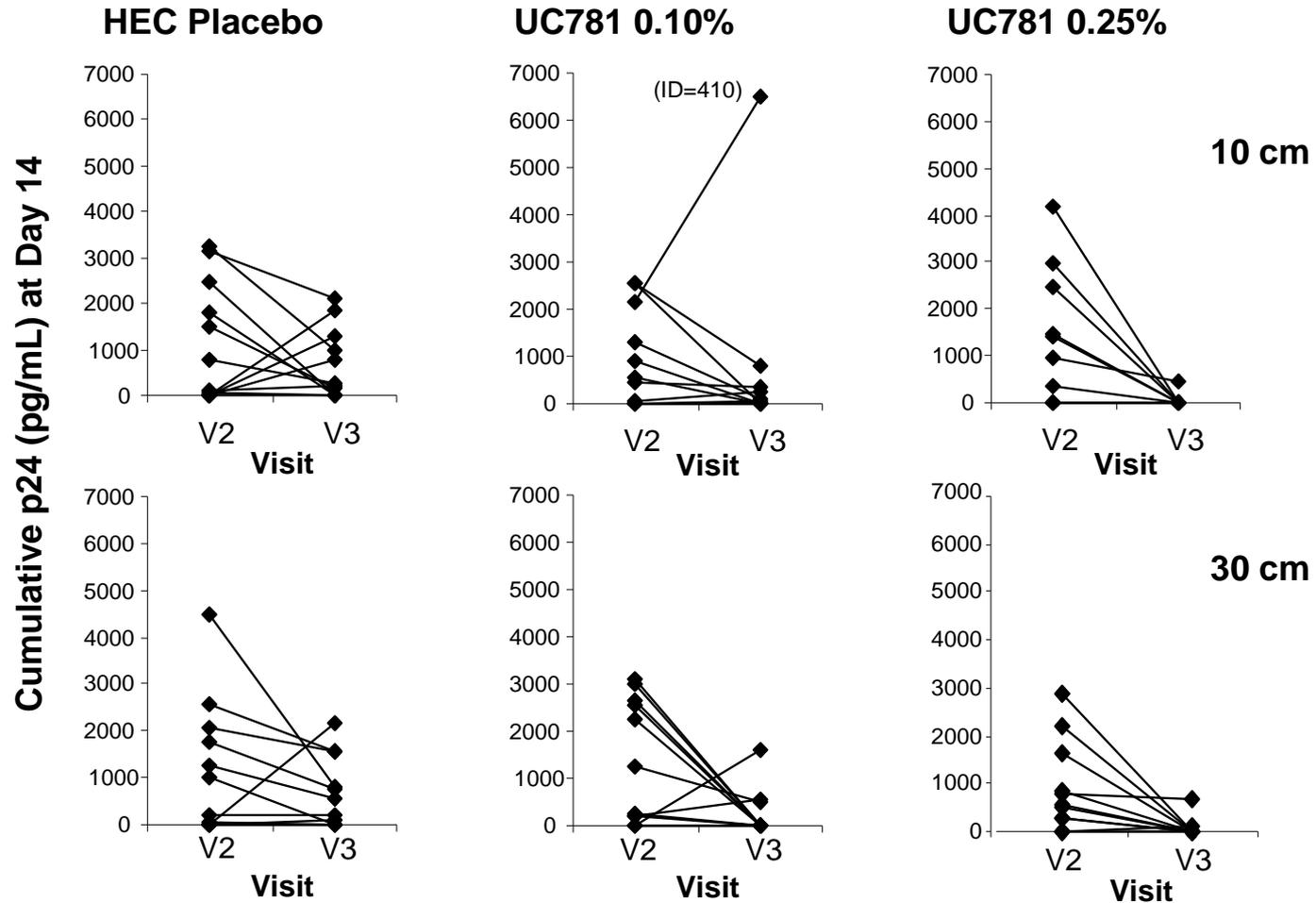


# What can we do with explants?

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- Evaluate drug and/or formulation safety
  - MTT assay
  - Histology
  - Drug permeability
- Determine product efficacy
- Ex vivo product testing

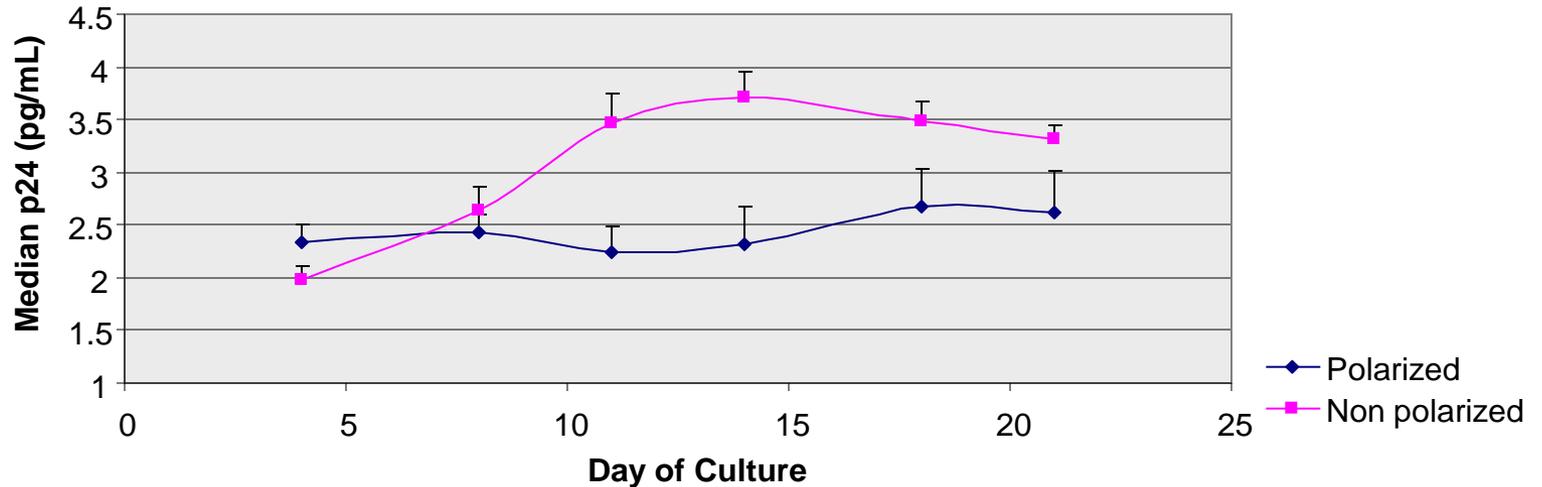
# Ex vivo testing



V2: Baseline; V3: 30 minutes post single dose

# Ex vivo testing

## □ Non-polarized vs. polarized explants



IHC positive: 88% non-polarized  
71% polarized



# Explants can be used to...

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- Evaluate microbicide safety and efficacy
  - Drug permeability
  - In the presence of semen
  - Multiple formulation types (gel, film, ring)
- Determine *ex vivo* efficacy
  - Surrogate for clinical efficacy?

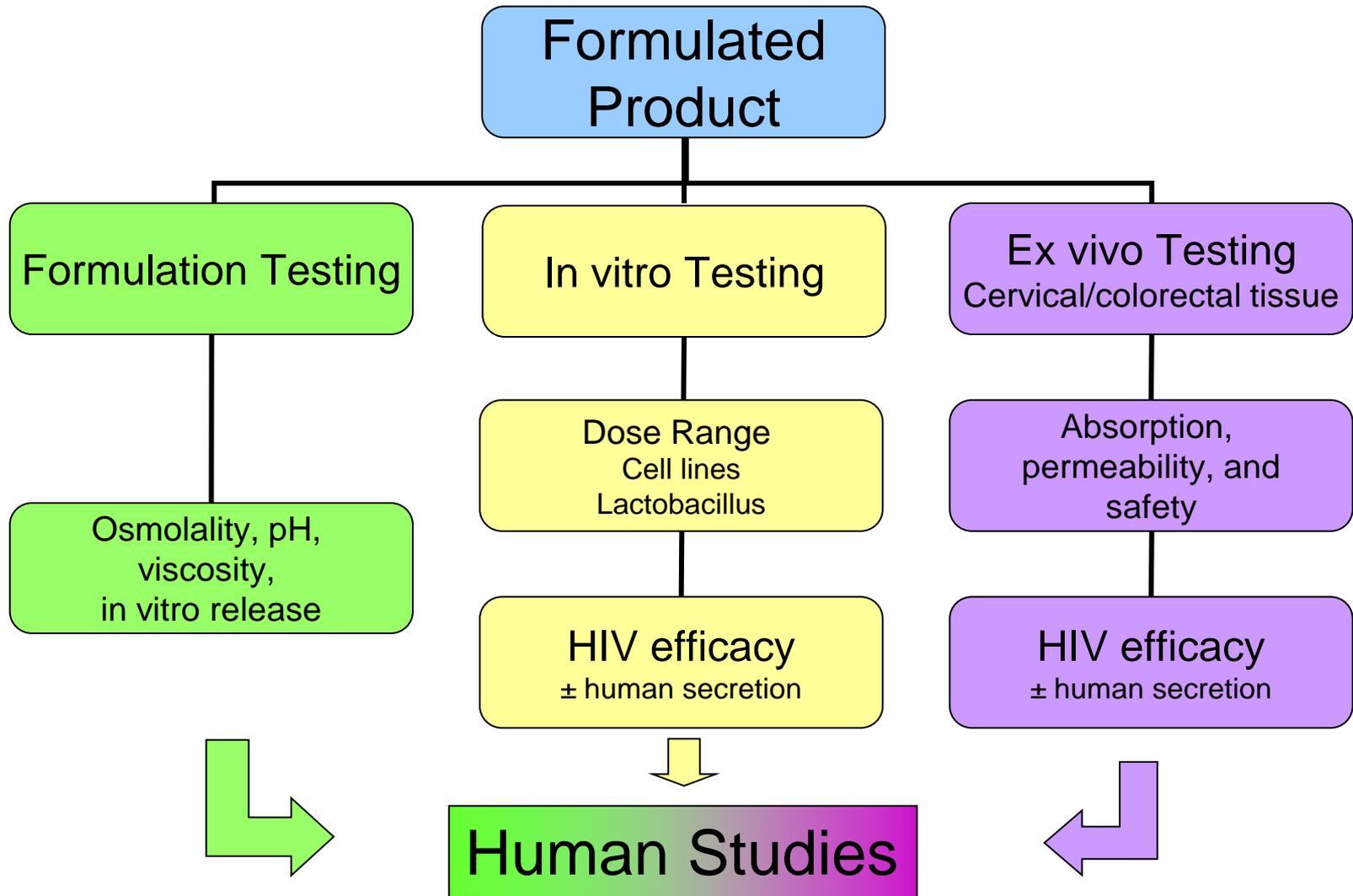


# Caveats of using explants

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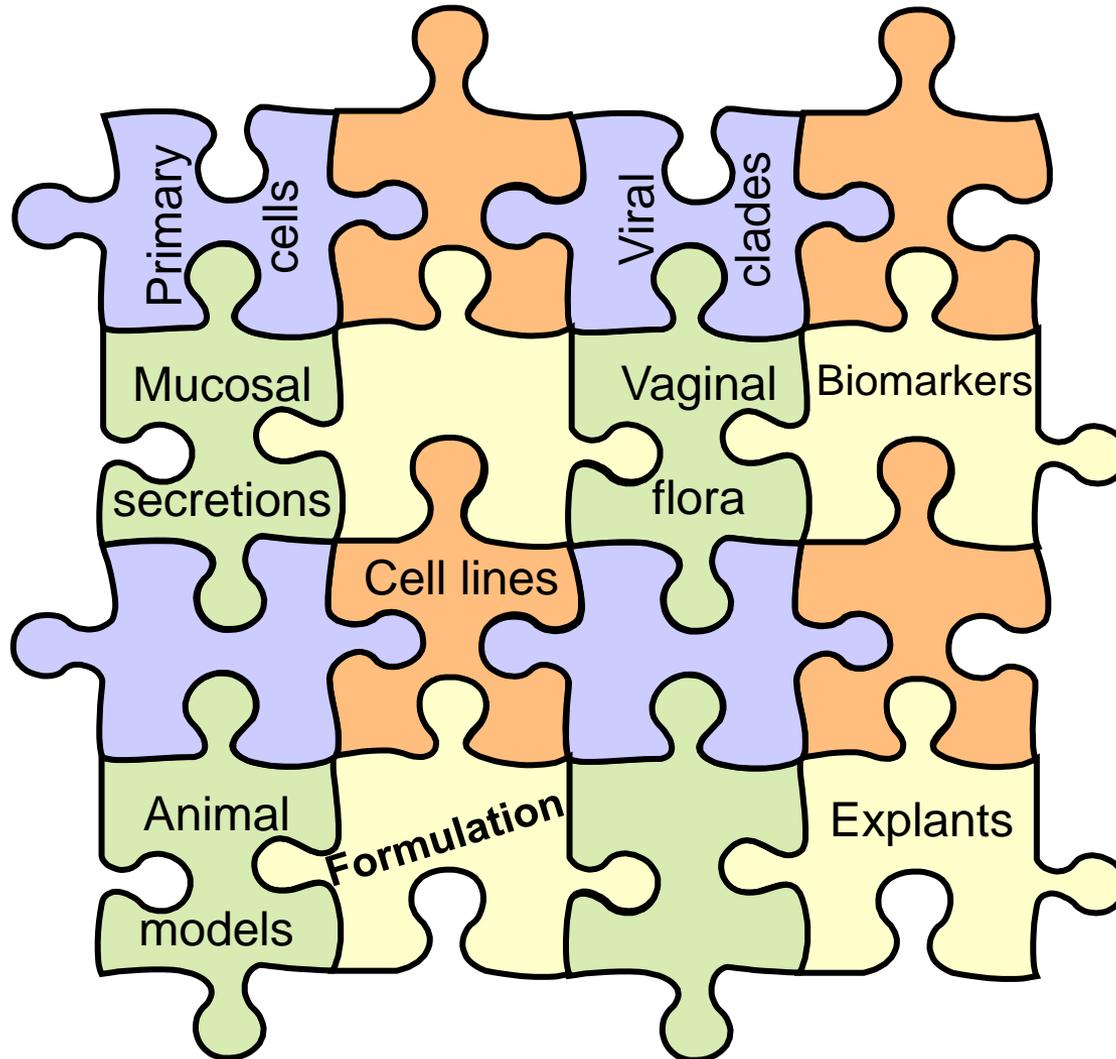
- Expensive and cumbersome to obtain
- Independent of hormonal control
- Inability to regenerate/repair
- No vascularization
  - No recruitment of immune cells
  
- Therefore, explants should be used
  - for the most promising candidates (not for screening)
  - as part of a comprehensive testing algorithm

# MTN pre-clinical algorithm



# Preclinical testing picture

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# Acknowledgements

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Thank you

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