Vaginal cytological characteristics as a biomeasure of estrogenization in a community-based population of older women

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Background

• Vaginal mucosa is a target tissue for a broad spectrum of estrogens.

• Vaginal atrophy
  – Indicates an estrogen deficit.
  – May interfere with sexual and urinary function.
  – Increases with age after menopause.
**Vaginal Epithelium**

### Three Types (Or Stages) of Vaginal Epithelial Cells

- **Parabasal** cell
- **Intermediate** cell
- **Superficial** cell

All scored to quantify estrogenization in the **Vaginal Maturation Index**

The Vaginal Maturation Index quantifies the relative proportion of the vaginal parabasal (P), intermediate (I), and superficial (S) cells presented as % P / % I / % S.

*Sources: Mills, Histology for Pathologists. 3rd Edition; LWW, 2006. Wheater, Functional Histology. 2nd Edition; Bibbo, 1997*
Quantifying Types of Desquamated Vaginal Epithelial Cells

Multichrome Papanicolaou stained cytology specimens obtained using vaginal self-swabs in NSHAP Wave 1.

McClintock lab:
• scored number and proportion of three epithelial cell types
• created a Maturation Index (MI).
Methods
National Social Life, Health and Aging Project (NSHAP) Wave 1

• Nationally representative sample of people 57 to 85 years of age (Wave 1, 2005 – 06).

• Demographic, health, sexual, physical, and biological measures collected in the home.

• Vaginal swabs were self-collected and used for the following measures:
  – Maturation Value (MV)
  – Vaginal Candidiasis (yeast)
  – Bacterial Vaginosis (BV)
  – High Risk Human Papilloma Virus (HPV)

The Institutional Review Boards of the University of Chicago and the National Opinion Research Center approved the protocol; all participants provided written documentation of informed consent.
Vaginal Swab Sample Collection

Illustrated by Rachel Seelen

Vaginal Swab Instructions

1. Choose a comfortable position. Either gently squat by bending at the knees, or sit on the toilet.

2. Remove swabs from packaging.

3. Hold swabs with the tip up. You may do all the swabs at once or one or two at a time.

4. Relax and gently insert the swabs into the vagina.

5. Stop when your fingers reach the vaginal opening or if you feel discomfort.

6. Turn the swabs inside the vagina as you count to "ten."

7. Place swabs and packaging directly inside the bag.

8. Return the bag to the interviewer.

STOP
Do not throw anything in the trash. Please place all packaging into the bag.
You may notice a small amount of discharge or blood on the swab. This is common and is not a cause for concern.
Flow chart of vaginal swabs collection

N=1550 Total Respondents

N=1028 Agreed
N=521 Refused
N=1 Subject mislabeled as male

N=811 Provided BV/VC and HPV swabs
N=1017 BV/VC swabs obtained in home

N=6 Provided BV/VC swab only
N=1013 HPV swabs obtained in home

N=2 Provided HPV swab only
N=9 Tried, but unable to provide any swab

N=1016 Cytology swabs received by McClintock lab
N=1016 Processed for cytology

N=469 Adequate for Ml analysis
N=147 Inadequate for traditional analysis
N=60 Inadequate for Analysis

N=59 Inadequate for Analysis
N=58 Other
N=1 No gel in tube
N=57 Adequate for Analysis

N=1016 BV/VC swabs received by MFRH
N=1016 Processed for BV/VC

N=1012 HPV swabs received by MFRH
N=2 Lost/Reused

N=1010 Processed for HPV 16/18 testing
N=1010 Adequate for HPV 16/18 analysis
Successful Vaginal Swab Collection

- 66% of women agreed to provide a self-administered vaginal swab specimen (N = 1,028 of 1,550)
- 85% were adequate for MI scores (N = 869 of 1,028)
- Non-responders to the vaginal swab protocol were:
  - older
  - <HS graduate
  - less likely to report a recent pelvic examination, menopausal prescription hormone use

Lindau et al., 2008
The Maturation Value
is a clinical measure of vaginal estrogenization
derived from the Maturation Index

Maturation Value =

1.0 x % superficial cells +  
0.5 x % intermediate cells + 
0.0 x % parabasal cells

Potential range: 0 – 100
Maturation Value of the Vaginal Epithelium

• suggested by Meisels in Acta Cytologica, 1965, who called it an “estrogenic value”

• a bioassay of functionally active estrogens counteracted by progesterone

• provides an integrated measure of hormonal bioactivity over many days

• based only on intermediate and superficial epithelial cells
Hypotheses

Vaginal epithelial estrogenization among post-menopausal women will be:

• Inversely associated with age and years since menopause
  Rationale: Results from historical clinical study (Meisels, 1966)

• Positively associated with obesity
  Rationale: Abdominal fat tissue can produce estrone

• Higher in African American women compared to other racial and ethnic groups. Rationale: Existing publications (McTernan, Wu, 2008; Setiawan, et al., 2006) and our own results from the NSHAP study suggest that African-American women have higher levels of free estradiol

• Positively associated with sexual function
Clinical Benchmark Study of Canadian Gynecology Patients

Meisels, 1966

Results
Distribution of Maturation Value in NSHAP Wave 1

![Graph showing the distribution of maturation value with bars representing different deciles ranging from 0 to 100 on the x-axis and fraction on the y-axis.]

![Bar graph showing the mean percentage of cells across different maturation value deciles, with columns representing superficial, intermediate, and parabasal types.]

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Maturation Value: (A) Age and (B) Hormone Therapy Use

A

- NSHAP population cohort (2005–6)
- Meisels clinical cohort (1961–5)

Mean maturation value (MV) vs. Age group

B

Mean predicted maturation value (MV) vs. Age

HT
No HT
Testing hypotheses using the maturation value

#1: Linear regression model to determine correlates (X) of the maturation value (Y).

\[ Y = \beta_0 + \beta_1 X_1 + \ldots + \beta_k X_k \]

Maturation value = \( \beta_0 + \beta_1 \text{hormone therapy} + \ldots + \beta_k X_k \)

Other covariates tested

Demographic characteristics:
Age, Race, Ethnicity, Education

Health characteristics:
Oophorectomy, Obesity, Hormone therapy past 12 mo, Sexual activity
Multiple linear regression model of the maturation value (MV), NSHAP Wave 1

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Parameter estimate</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.08</td>
<td>-0.21, 0.38</td>
<td>0.59</td>
</tr>
<tr>
<td>Years since last menstrual period</td>
<td>0.10</td>
<td>-0.05, 0.24</td>
<td>0.19</td>
</tr>
<tr>
<td>African-American race (vs white, ref.)</td>
<td>8.58</td>
<td>5.07, 12.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Some college/associates education (vs HS graduate, ref.)</td>
<td>-2.64</td>
<td>-5.25, -0.02</td>
<td>0.048</td>
</tr>
<tr>
<td>HT use in last 12 months</td>
<td>12.44</td>
<td>9.19, 15.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (inches)</td>
<td>0.58</td>
<td>0.34, 0.82</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Effects of other covariates in the model (Bilateral oophorectomy, Sexual activity past 12 months and Hispanic ethnicity) turned out to be non-significant.
#2: Logistic regression using the standardized maturation value as an independent variable (X) to predict symptoms/conditions (Y).

\[
\text{Prob.}(\text{symptom}) = \frac{1}{1 + e^{-(\beta_0 + \beta_1 \text{maturation value} + \beta_2 \text{age} + \beta_3 \text{HT})}}
\]

**Symptoms/Conditions examined:**

- Sexual Activity
- Pain during sex
- Vaginal dryness during sex
- Urinary incontinence

Bacterial vaginosis,
Yeast infection
High-risk HPV
Other urinary problems,
Logistic regression models of clinical and behavioral outcomes on standardized maturation value (MV), NSHAP Wave 1

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual activity (within the past 12 months)</td>
<td>0.88</td>
<td>0.73, 1.05</td>
<td>0.15</td>
</tr>
<tr>
<td>Pain during sex (for sexually active women)</td>
<td>0.80</td>
<td>0.55, 1.17</td>
<td>0.24</td>
</tr>
<tr>
<td>Problems lubricating (for sexually active women)</td>
<td>0.61</td>
<td>0.46, 0.82</td>
<td>0.001</td>
</tr>
<tr>
<td>Current bacterial vaginosis</td>
<td>1.54</td>
<td>1.26, 1.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current yeast infection</td>
<td>1.56</td>
<td>1.15, 2.13</td>
<td>0.005</td>
</tr>
<tr>
<td>High-risk HPV</td>
<td>1.08</td>
<td>0.78, 1.51</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Maturation value standardized by subtracting the sample mean and dividing by the sample standard deviation.
Conclusions
Hypotheses tested

Vaginal epithelial estrogenization among post-menopausal women will be:

- Inversely associated with age and years since menopause? **No**
- Positively associated with abdominal obesity: **Yes**
- Higher in African American women compared to other racial and ethnic groups: **Yes**
- Positively associated with sexual function: **Yes, mostly**
Summary:

• Maturation value is an integrative biomeasure of estrogenization of women, useful for analyses of health and sexuality

• In contrast to 1960s benchmark clinical data, current population estimates of vaginal estrogenization are higher and do not exhibit a decline with age.

• Differences may be explained in part by studying different populations:
  • Women who come to a clinic
  • Representative sample of community-dwelling older women in the US.
Study limitations

Selection bias: women who provided a vaginal specimen were, on average:

- younger
- more educated
- more likely to have urinary problems
- more likely to use HT
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Additional information can be found at the CCBAR website

http://biomarkers.uchicago.edu/
Chicago Core on Biomarkers in Population-Based Aging Research

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