

# A Novel Method of Assessing Cervical Collagen Integrity Utilizing Image Segmentation Analysis

Sean M. Keeler, MD<sup>1,3</sup>, Daniel Kiefer, MD<sup>2</sup>, Orion Rust, MD<sup>3</sup>, Eran Bornstein<sup>1</sup>, Matthew Sottile<sup>4</sup>, Carolyn Salafia<sup>5</sup>

<sup>1</sup>New York University, New York, New York; <sup>2</sup>Winthrop University Hospital, Mineola, New York; <sup>3</sup>Lehigh Valley Hospital, Allentown, Pennsylvania; <sup>4</sup>University of Oregon, Oregon; <sup>5</sup>Placental Analytics, LCC, Larchmont, New York

## Abstract

**OBJECTIVE:** Cervical shortening is a strong and early predictor of spontaneous preterm birth, and its pathogenesis and pathophysiology are poorly understood. Collagen histologic staining reflects collagen integrity. We describe a novel method of analyzing cervical collagen integrity using image segmentation analysis.

**STUDY DESIGN:** Asymptomatic women with a transvaginal cervical length  $\leq 25$  mm between 16-24 weeks underwent cervical *M. hominis* and *U. urealyticum* cultures and a micro-cervical biopsy at the 4 o'clock position on the ectocervix using The Bard® Monopty® Instrument (C.R. Bard, Inc., Murray Hill, N.J.). Cervical biopsies were stained with hematoxylin and eosin (H&E) and examined by a single reviewer blinded to clinical data. One 40X photomicrograph was taken of each biopsy. The R channel histogram was extracted and its mean, standard deviation, skew and kurtosis calculated. A single collagen staining factor score (CFS) was extracted from these data by principal components analysis. Dense collagen is represented by a high CFS and loose collagen by a low CFS. Spearman correlation was used to determine the relationship between cervical culture status and the CFS.

**RESULTS:** Eighty seven paired cervical cultures and cervical biopsy specimens were available for analysis. The collagen staining score was related to the mycoplasma and ureaplasma cervical culture status with a correlation score of 0.264 ( $p = 0.014$ ).

## CONCLUSION:

Image segmentation of cervical collagen on the R channel extracts structural features and informs on collagen integrity that is significantly related to cervical *M. hominis* and *U. urealyticum* culture status in patients with an ultrasonographic short cervix in the midtrimester.

## Background

- Inflammation plays a central role in initiating the cervical remodeling that ultimately leads to spontaneous preterm parturition.
  - Vaginal infection with *Ureaplasma urealyticum* has been associated with preterm delivery via induction of inflammatory responses.
  - *Ureaplasma urealyticum* have been found in amniotic fluid of patients with short cervix.
  - 20% of neonates between 23 - 32 weeks' gestation are born with bacteremia caused by genital *Mycoplasmas*.
- We describe a novel method of analyzing cervical collagen integrity using image segmentation analysis and examine the correlation of collagen integrity to cervical *Mycoplasma/Ureaplasma* culture .

## Materials and Methods

- All studies were performed under an Institutional Review Board approved protocol.
- Inclusion Criteria
  - All pregnancies between 16 - 24 weeks' gestation.
  - Ultrasound-diagnosed cervical length  $\leq 25$ mm.
  - Funneling of chorio-amniotic membranes  $\geq 25$  % into the endocervical canal.
- Exclusion Criteria
  - Any known fetal chromosomal or structural anomaly
  - Ruptured membranes
  - Vaginal bleeding
  - Prolapse of endocervical membranes beyond the external cervical os
  - Persistent uterine activity accompanied by cervical change
  - Obstetrically indicated delivery
- Patients had a cervical *Mycoplasma hominis* and *Ureaplasma urealyticum* cervical culture under sterile speculum examination.
- Patients were consented for a controlled fine needle biopsy (FNB) of the uterine cervix.
  - The Bard Monopty® biopsy instrument (C.R. Bard, Inc., Murray Hill, N.J.),
    - Automated spring-loaded instrument that extends an 18 gauge cutting needle to penetrate 11mm into the cervical stroma.
    - Obtains a 7mm x 0.003cm<sup>3</sup> controlled FNB.
- All patients had an ultrasound-guided transabdominal amniocentesis
  - Evaluation for intra-amniotic infection (low glucose, elevated WBC count, and aerobic/anaerobic culture)
- Cervical biopsy specimens were stained with hematoxylin and eosin (H&E)
  - Examined by a single reviewer blinded to clinical and assay data.
  - 40X photomicrograph was taken of each biopsy.
    - Collagen properties were characterized based on the distribution of intensities in the red color channel of the images.
    - The level of red was quantified by measuring the distribution of pixel intensities (zero to 255) isolated in the red channel.
    - R channel histogram of pixel intensities (which range from 0-255) was extracted.
    - Mean, standard deviation, skew and kurtosis calculated.
  - A single collagen staining factor score (CFS) was extracted from these data by principal components analysis.
    - High CFS represent poor collagen integrity.
      - The biopsy image is composed of loose, poorly interconnected collagen fibrils, thus has a lot of white space in the image.
    - Low CFS represent good collagen integrity.
      - The biopsy image is composed of dense, closely interconnected collagen fibrils, thus has little white space in the image.
  - Spearman's correlation was used to compare the CFS to genital *Mycoplasma* and *Ureaplasma* culture status.

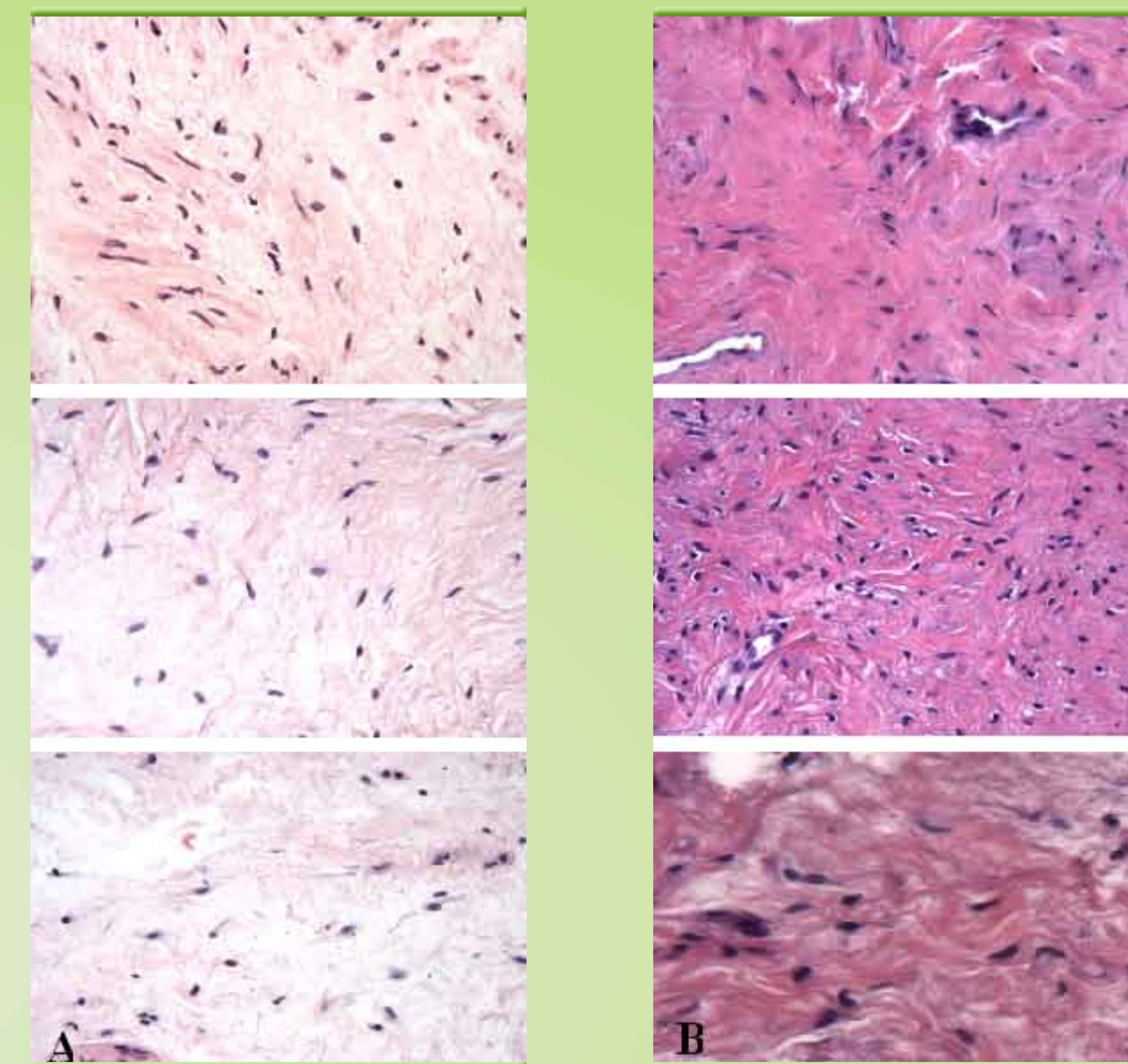
## Results

Characteristic	N = 87
Age (years)	28.1 $\pm$ 7.0
Race	
Caucasian	36 (41.4)
Hispanic	29 (33.3)
African American	20 (22.9)
Asian	2 (2.4)
Multiparity	54 (62.1)
Singleton gestation	78 (89.7)
Previous spontaneous preterm birth	39 (44.8)
Cervical length at enrollment (mm)	16.5 $\pm$ 6.3

- Eighty seven paired cervical cultures and cervical biopsy specimens were available for analysis. The collagen staining score was related to the mycoplasma and ureaplasma cervical culture status with a correlation score of 0.264 ( $p = 0.014$ ).

## Conclusion

- Image segmentation of cervical collagen on the R channel extracts structural features and informs on collagen integrity that is significantly related to cervical *M. hominis* and *U. urealyticum* culture status in patients with an ultrasonographic short cervix in the midtrimester.



**Histologic comparison of high (A) vs. low (B) cervical collagen staining score**

