Multidimensional screening for a multifunctional cervix: Examining cervical gland area at cervical length screening to predict spontaneous preterm birth.

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Abstract

OBJECTIVE: To sonographically characterize the cervical gland area (CGA) and determine if its evaluation at the time of cervical length (CL) screening can be useful for preterm birth (PTB) prediction. **DESIGN:** Pilot retrospective cohort study. **SETTING:** Academic medical center (NYU Langone Health Tisch Hospital). **POPULATION:** Singleton gestations with universal CL screening performed between 18 $^{0/7}$ – 23 $^{6/7}$ weeks with subsequent live neonate delivery. **METHODS:** Transvaginal ultrasound (TVUS) cervical images and clinical data were reviewed, comparing sonographically present and absent CGA groups. **MAIN OUTCOME MEASURES:** Spontaneous PTB <37 weeks and quantitative CGA measurements. **RESULTS:** The cohort of 772 patients demonstrated similar characteristics when stratified by absent and present CGA. Rates of PTB and absent CGA were 2.6% and 2.3%, respectively. Absent CGA was significantly associated with delivery <37, <34, and <32 weeks ($p<0.001$), but gland measurements did not correlate with gestational age at delivery. There was good agreement between reviewers for qualitative CGA (PABAK 0.89). Multiple logistic regression modeling demonstrated better performance of CL screening for PTB prediction with the addition of qualitative CGA evaluation ($p<0.001$). **CONCLUSIONS:** Qualitative evaluation of the CGA on mid-gestation TVUS may improve CL screening for PTB. Given the biologic activity of the cervical glands, optimal screening in populations with various risk profiles may warrant a multimodal approach that evaluates the mechanical and biological functions of the cervix.

INTRODUCTION

Preterm birth (PTB) is a major cause of neonatal morbidity and mortality, affecting 10% of births worldwide [1, 2]. Long-term pulmonary or neurodevelopmental disabilities and the mortality risk of prematurity extend beyond the neonatal ICU, even into childhood and adolescence [3-5]. The majority of births before term are spontaneous due to preterm labor (PTL), preterm prelabor rupture of membranes (PPROM), and cervical insufficiency, but the etiology is often unknown [6]. Shortened cervical length (CL) is the target of several preventative interventions, such as progesterone supplementation and cervical cerclage, both of which have been shown to reduce PTB risk [7, 8]. However, sonographic CL has variable sensitivity for screening, and its predictive value in the general obstetric population remains suboptimal[7, 9, 10]. The character of the cervical canal itself, specifically the cervical gland area (CGA), has been proposed as another useful reflection of cervical function [11-13].

The CGA is a hyper- or hypoechoic zone lining the cervical canal, distinct in echotexture from the cervical stroma, and corresponds to the histologic endocervical crypts (Figure 1) [14]. This glandular tissue is responsible for maintaining the collagen, elastin, and proteoglycans that help impart the firm cervical consistency necessary to sustain a pregnancy, as well as producing endocervical mucus to help prevent ascending genital infections[15-17]. Normal and preterm cervical ripening is thought to be related to collagenolysis and changes in

cervical water content that disrupt the structural integrity of these glands, resulting in softening, effacement, and insufficiency. The collagenolysis involved in cervical ripening may correspond to loss of a sonographically detectable CGA [12, 18, 19]. However, CGA is not a widely known marker of cervical integrity in clinical practice. Exploring a relationship between the CGA, CL, and PTB could potentially facilitate beneficial interventions. The objective of our study is to sonographically characterize the CGA and determine if its evaluation at the time of routine CL screening can be useful for PTB prediction.

MATERIALS AND METHODS

This is a retrospective cohort pilot study of patients who underwent universal transvaginal ultrasound (TVUS) CL screening during routine second-trimester anatomy scan and subsequently delivered at a single academic institution in 2018. This study was approved by the institutional review board of New York University Langone Health (NYULH), as was a waiver of consent, given the retrospective observational nature of this research.

Study population

Study participants were identified using obstetrical delivery ICD10 encounter codes and sonogram CPT codes. Patients were included in the study if meeting the following screening TVUS and delivery criteria: age between 18 to 50 years, underwent TVUS CL screening at NYULH Maternal Fetal Care Center (MFCC) between 18 weeks 0 days – 23 weeks 6 days gestation during a routine anatomic survey, and delivered a live neonate at NYULH Tisch Hospital in the year 2018. Screened patients with multiple gestation pregnancy, medically indicated preterm birth, uterine anomalies, or cervical cerclage *in situ* were excluded. Enrolled patients were also excluded if TVUS cervical images were not electronically available from the medical record to review or if available images were suboptimal for assessment of the cervix. Demographics, clinical characteristics, and obstetrical outcomes were manually collected for all patients; short interpregnancy interval was defined as <18 months. As several of the predetermined exclusion criteria are known risk factors for PTB, we anticipated a relatively low-risk final study population; to determine the overall PTB rate at our institution, a post-hoc evaluation of all excluded patients was performed for gestational age (GA) at delivery to identify the number of PTBs inadvertently excluded from final analysis of the study cohort.

Image acquisition and review

TVUS cervical images were obtained using standardized CL Education and Review (CLER) criteria by accredited sonographers with supervision by maternal fetal medicine physicians at the NYU MFCC. Ultrasound units with 4- to 9-MHz IC5-9D (Voluson e8; GE Healthcare, Milwaukee, WI) or 4- to 8-MHz C8-4v (IU22; Phillips Healthcare, Andover, MD) wide-view transducers were used to obtain all images [20]. Stored CL images were evaluated for this study by a single reviewer, who was blinded to delivery outcome. Of the patients included in final analysis, 25% were randomly selected for a second review of stored CL images by another study team member to confirm reliability of interpretation and to calculate scores of interobserver variability. Cervical images were assessed for quality, reported CL, and visibility of the CGA. Optimal cervical imaging was defined as a sagittal view of the cervix with clearly visible internal and external ostia, approximately equal thickness anterior and posterior cervical lips, complete (or near complete) visualization of the cervical canal, and minimal urine in the maternal bladder [21].

Outcome measures

Data were stratified by CGA presence or absence on TVUS. The primary outcome was spontaneous PTB <37 weeks gestation. Secondary outcomes included the prediction of PTB by quantifiable measures of CGA when present, and the impact of CGA assessment on CL screening for the prediction of the primary outcome. Present CGA was defined as a well-demarcated hyper- or hypoechoic region lining the cervical canal, distinct in echotexture from the peripheral cervical stroma. To attempt to quantify the extent of the CGA if present, the visualized CGA was measured for visualized length (calipers parallel to the cervical canal), as well as widest and narrowest widths (calipers perpendicular to the cervical canal). Average widths were calculated from the widest and narrowest width measurements. Approximate areas of the CGA were

calculated by multiplying the measured parameters, estimating an approximately rectangular CGA overlying a linear cervical canal in the sagittal view.

Statistical analysis

Statistical analysis was performed with R Studio software (Version 1.3.959). Categorical variables were compared using χ^2or Fisher's exact test where appropriate, and continuous variables were compared using T-test. The Mann Whitney U Test was used to compare quantitative CGA measurements and their associations with the primary outcome. Further, the analogous continuous outcome of GA at delivery was assessed relative to the quantitative CGA measurements using Spearman's correlations. The relationship between PTB, CL, and CGA absence was modeled using multivariate logistic regression via a stepwise backward elimination approach, controlling for variables identified as significant in univariate analysis or that were deemed clinically relevant for inclusion in the model. In constructing the model, Spearman's correlation testing was used to make pair-wise comparisons between predictor variables to ensure absence of multicollinearity. Full and reduced versions of the final model, which differed only by inclusion of CGA evaluation with TVUS CL screening, were compared using the likelihood ratio test. Receiver operating curves (ROCs) were constructed to evaluate performance of the nested models (with and without evaluation of CGA at TVUS CL screening) for predicting PTB <37 weeks, and the areas under the curve (AUC) were determined. Statistical significance was defined as $p<0.05$.

RESULTS

A total of 1000 patients were screened for study eligibility and a cohort of 772 were included in final analysis (Figure S1). Demographic and clinical characteristics were compared between patients with sonographically visualizable (present) and non-visualizable (absent) CGA (Table 1). The majority of patients were White (68%) with an average maternal age of 33 years, and an average CL on screening TVUS of four centimeters. Patients with no visualizable CGA were more likely to be parous (prior term births 1.2 vs 0.6, p=0.07), but this was not a statistically significant difference. The CGA absent group was more likely to have had progesterone supplementation during pregnancy (17% vs 4%, p=0.04); this composite includes use of vaginal or intramuscular progesterone formulations, which did not differ by CGA group when examined individually (vaginal n(%): 6 (0.8) vs 1 (5.6) among CGA present vs absent, p=0.397) (intramuscular n(%): 24 (3.2) vs 2 (11.1) among CGA present vs absent, p=0.237). Otherwise, the sonographically present and absent CGA groups were overall similar. Notably, there was no difference in history of prior spontaneous PTB, in GA at time of TVUS, or in reported CL.

Of the patients included in final analysis, 18 (2.3%) were found to have sonographically absent CGA, and the overall rate of spontaneous PTB in the cohort was 2.6% (Table 2). Compared to those with a visualizable CGA, patients with absent CGA were significantly more likely to have a spontaneous PTB <37 weeks (2% vs 28%, p<0.001), a difference that persisted for PTB <34 and <32 weeks (p<0.001). Second-trimester CGA visibility rates were 98% and 75% for patients that delivered at term and preterm, respectively (p<0.001). Neonates born to mothers with visually absent CGA were more likely to have a lower birthweight (3124 vs 3358g, p=0.03), but did not vary by mode of delivery or by postnatal disposition to the newborn nursery or a neonatal ICU. Of note, a post-hoc evaluation of GA at delivery among the screened patients who were ultimately excluded from the final study analysis revealed an additional 53 births <37 weeks, for a collective overall PTB rate of 7.1% among the 1000 patients initially screened.

The best performing and most clinically relevant model for prediction of PTB <37 weeks was a multivariable model with five variables: total parity, GA at TVUS, CL at TVUS, any supplementation with progesterone, and absence of CGA on TVUS. Multicollinearity between the included predictor variables was found to be absent using Spearman's correlation, as all rho coefficients were very low and none statistically significant. Performance of the final models were illustrated via ROCs, calculating odds ratios and the AUC for each (Figure 2). The full model (inclusive of both CL and CGA evaluation) demonstrated a greater AUC compared to the reduced model (inclusive of CL evaluation only) (AUC 0.83 vs 0.742, respectively)[22]. Further, the likelihood ratio test demonstrated improved model fit for the full model compared to the reduced model (p

<0.001) [23].

Agreement between reviewers for qualitative evaluation of CGA (visually absent or present glands at screening TVUS) was assessed via Cohen's Kappa statistic, which was adjusted for the low prevalence of CGA absence and the rare outcome of PTB. The prevalence-adjusted bias-adjusted kappa (PABAK) was 0.89 (bias index 0.055, prevalence index 0.905), reflecting strong agreement between reviewers.

To quantify sonographically visible CGA when present, gland length and width were measured on an optimal sagittal cervical image. The visualized width of the CGA ranged from 0.12 cm at narrowest to 2.5 cm at widest, with an average width of 0.9 cm. Visualized CGA length was 3.4 cm on average. Mathematically estimated area of the CGA was calculated by multiplying the measured CGA length and widths. Unlike the dichotomous finding of a sonographically present or absent CGA, quantitative measures of CGA did not significantly differ between the patients who delivered at term and those who delivered preterm <37 weeks (Figure S2), nor did they differ significantly compared to those who delivered at <34 weeks or <32 weeks. Quantitative CGA measures also did not demonstrate clinically significant association with the continuous outcome of GA at delivery as correlation coefficients were very low (approximately zero), though statistical significance was achieved for CGA length and narrowest width (p=0.018 and p=0.025, respectively).

DISCUSSION

Main Findings

In this retrospective cohort pilot study, we demonstrated that patients with sonographically absent CGA at the time of second-trimester CL screening were more likely to spontaneously deliver preterm. CGA assessment was readily reproducible, and the addition of this simple qualitative factor to CL screening improved PTB prediction. Among patients with visualizable CGA, quantitative measurements did not exhibit the same predictive ability for PTB.

Interpretation

Universal CL screening meets World Health Organization criteria for an effective screening test [24]. CL is inversely related to PTB risk, but its predictive value is variable as short cervix (CL <2.5cm) is rare and not ubiquitous to all PTB cases[6, 25-28]. At our institution, CL screening is performed at the time of the second-trimester anatomy scan. Our study suggests that qualitative CGA evaluation may be useful in building upon a pre-existing, well-accepted screening modality with negligible impact on patient or provider performance burden.

Wide variation in CGA detection rates in the literature is likely due to differences in study design, CGA density, and subjectivity of TVUS interpretation. Our study demonstrates good reproducibility for CGA detection when prevalence-adjusted for our low-risk population. Several studies have investigated using CGA to triage high-risk patients, such as those with threatened PTL or with prior spontaneous PTB, quoting rates of sonographic CGA absence as high as 56-69% [19, 29]. One longitudinal study used serial TVUS and digital cervical exams to chart the natural course of CL, CGA, and Bishop score with advancing GA in low-risk patients delivered at term; they found increasing rates of CGA absence from 31 weeks to term, correlating with a rising cervical maturation index [13]. Conversely, persistent visibility of CGA at term has been associated with increased need for late-term labor induction and increased rates of failed induction[30, 31]. Loss of a sonographically detectable CGA consistently correlates with cervical maturation, supporting the biologic plausibility of a connection between this ultrasound finding and increased PTB risk from premature cervical ripening.

The biologically active endocervical glands play a crucial role in the balance of collagen and other proteins that contribute to the biomechanical strength of the cervix, as well as the cervical mucous required to maintain a barrier between the vagina and an intrauterine gestation [15, 17, 32, 33]. Sonographic CGA evaluation may lend itself to an improved functional appraisal of PTB risk as the loss of visualizable CGA coincides with underlying cervical ripening[13, 19, 29-31]. This connection may be applicable across several disciplines of obstetrics and gynecology, such as for birth outcomes after infertility or oncologic care. For instance,

cervical mucus collected from patients following intrauterine insemination or in vitro fertilization features unique proteome profiles[34]. The composition of cervical mucus has proven predictive of PTB when it features abnormal proportions of inflammatory mediators, such as in the presence of dysbiotic vaginal flora[35]. The known role of progesterone in PTB prevention is related to maintenance of cervical collagen organization and inflammatory mediation; thus, it is not surprising that progesterone supplementation after embryo transfer results in higher live birth rates[17, 33, 36, 37]. Further, history of excisional treatment for cervical intraepithelial neoplasia is a known risk factor for second-trimester pregnancy loss and PTB [38]. A study found that cumulative excision length significantly predicted PTB, even when second-trimester CL did not vary by gestational age at delivery [39]. Higher grade cervical lesions are more likely to involve endocervical glands, requiring deeper excisions and imparting greater PTB risk, perhaps due to more substantial loss of glandular activity [40]. Incorporation of CGA evaluation at CL screening, may better assess for dysfunction in the mechanical and biological activities of the glands, both of which are integral to cervical sufficiency.

Such activity was the rationale behind our study's aim to quantify the CGA visualized on TVUS and to determine if variations in CGA size might reflect underlying physiologic changes that translate to differences in birth outcomes. However, our study found no appreciable association between birth timing and quantitative CGA measurements – though this may reflect insufficient power given our cohort's low PTB rate. Larger studies with higher PTB prevalence may be necessary to adequately explore the use of CGA measurements in PTB prediction.

Strengths and Limitations

There are several strengths of our study. We evaluated a large cohort of patients using CGA assessment as an adjunct screening tool for the general population undergoing universal CL screening. Novel to our study is quantification of the visualizable size of the CGA and evaluation of these metrics for PTB prediction. Further strengthening our analysis, all cervical images were obtained at a single institution using a standardized protocol for CL imaging and subsequently assessed for CGA visualization by a reviewer blinded to birth outcome.

Our study also has several limitations. The single-institution, retrospective design is subject to the limitations of preexisting medical record data and may impact the generalizability of our findings. The power of our analysis may also be limited by examination of a rare outcome and rare finding. Further, inclusion of patients with progesterone exposure may confound PTB rates, though progesterone use is not limited to cases of prior PTB or short cervix – for instance, in conceptions via assisted reproductive technologies [37]. Progesterone use was controlled for in our regression models, and most progesterone users (81%) were receiving the intramuscular formulation, which has uncertain efficacy for affecting PTB risk[41].

Further, our study population was relatively low-risk. There were low rates of clinical comorbidities, and our predetermined exclusion criteria, several of which are known risk factors for PTB, likely contributed to the low-risk nature of our cohort. Our PTB rate was 2.6% and reflects only spontaneous PTBs, but the overall rate rose to 7.1% when calculated for the entire screened population, reflecting a selection bias for low-risk pregnancies. Further, while the overall national PTB rate is 10%, the national rate of spontaneous PTB is lower (4.5%) and more comparable to our cohort [1, 42]. Thus, it is not surprising our cohort had a low rate of absent CGA (2.3%). Given the retrospective nature of our study, it was not possible to ensure all cervical images were optimized. Several patients were excluded for unavailable or suboptimal imaging and this could have inadvertently excluded patients with underlying CGA absence[21]. However, our CGA detection rate is comparable to that of prior studies with a similar population [11, 14, 43].

CONCLUSION

Our pilot study demonstrated that CGA absence was predictive of PTB and may be a useful adjunct to CL screening for improving PTB prediction. As the etiology of spontaneous PTB and cervical insufficiency is ill-defined and likely multifactorial, multimodal screening tools may be necessary for optimal prediction [44]. We hope this inspires future research exploring CGA screening, such as via three-dimensional renderings or elastography, in populations with different risk profiles, in the context of various progesterone regimens, and

in combination with cervical biomarkers or clinical phenotyping.

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DISCLOSURES OF INTERESTS, FUNDING, AND DATA AVAILABILITY

The authors have no conflicts of interest or funding sources to declare. The data underlying this article will be shared upon reasonable request to the corresponding author.

CONTRIBUTION TO AUTHORSHIP

J.A.M. designed the study protocol, acquired/analyzed all data, and drafted/edited the manuscript. M.L. and S.M-L. assisted in conceptual study design, contributed to data acquisition/analysis, and provided review of the manuscript. S.G.B. and A.S.R. contributed to study design and manuscript editing. All authors approved the final version of the manuscript and accept accountability for the integrity of this work.

DETAILS OF ETHICS APPROVAL

This study was approved by the institutional review board of New York University Langone Health (NYULH) on June 11, 2019 (# s19-00638). Given the retrospective observational nature of this research, a waiver of consent was also obtained from the review board.

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Table 1: Demographic and clinical characteristics of the study population

Data are n (%) or mean ±SD. Abbreviations: CGA (cervical gland area), TVUS (transvaginal ultrasound), PTB (preterm birth), GA (gestational age), CL (cervical length).

[a] Mixed and Unknown Race categories not shown.

[b] Prenatal Genitourinary Infection includes sexually transmitted infections, bacterial vaginosis, urinary tract infections, and chorioamnionitis. Prior Uterine Surgeries includes dilation and curettage/evacuation, myomectomy, cesarean, and hysteroscopy. Prior Cervical Excision includes loop electrocautery procedure and cold knife cone. Progesterone Supplementation includes vaginal and/or intramuscular routes. Short Cervix is defined as CL <2.5 cm.

Table 2: Delivery characteristics of the study population

Data are n (%) or mean ±SD. Abbreviations: CGA (cervical gland area), TVUS (transvaginal ultrasound), NICU (neonatal intensive care unit), GA (gestational age).

Figure 1: Representative sagittal-view transvaginal ultrasound cervical images with (A) visualizable "present" and (B) non-visualizable "absent" cervical gland area

Calipers measuring cervical length in image (B).

Figure 2: Comparison of AUC for regression models with (full model) and without (reduced model) incorporation of qualitative GA evaluation during TVUS CL

screening

Full model (solid line). Reduced model (dashed line). Abbreviations: AUC (area under the receiver operating curve), CGA (cervical gland area), TVUS (transvaginal ultrasound), CL (cervical length), OR (odds ratio), aOR (adjusted odds ratio; adjusted for all listed predictor variables). *p<0.001

Figure S1: Study flow diagram

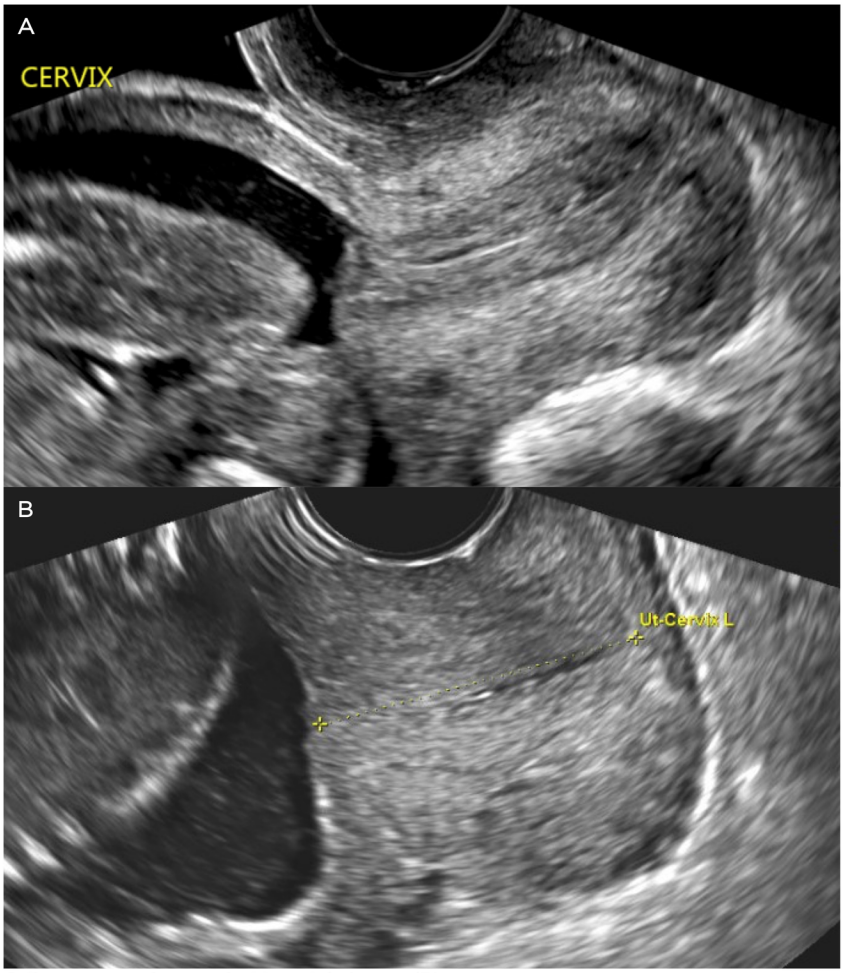
Abbreviations: TVUS (transvaginal ultrasound)

Figure S2: Distribution of CGA measurements in study population and association

with birth <37 weeks

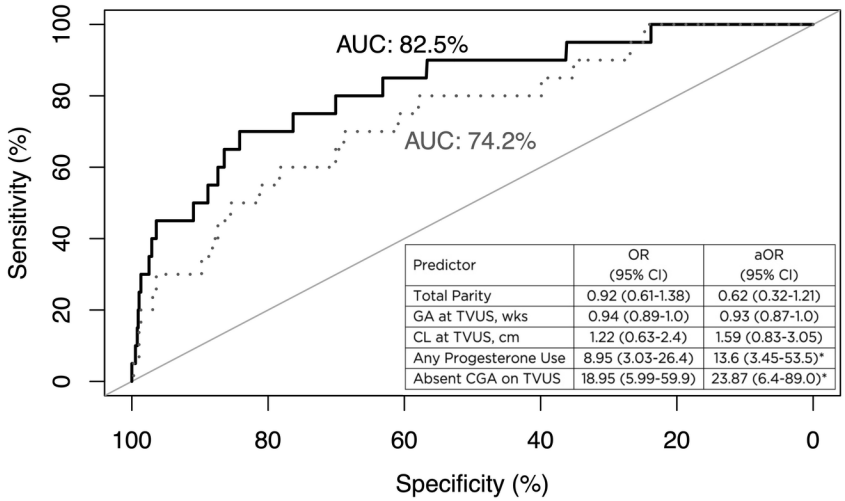
Cervical Gland Area (CGA) measurements – Length (visualized length), Width 1 (widest visualized width), Width 2 (narrowest visualized width), Width 3 (average visualized width), Area 1 (area estimated by Width 1 x Length), Area 2 (area estimated by Width 2 x Length), Area 3 (area estimated by Width 3 x Length); p-Values listed above associated paired brackets.

Figure 1: Representative sagittal-view transvaginal ultrasound cervical images with (A) visualizable “present” and (B) non-visualizable “absent” cervical gland area.



Calipers measuring cervical length in image (B).

Figure 2: Comparison of AUC for regression models with (full model) and without (reduced model) incorporation of qualitative CGA evaluation during TVUS CL screening.



Predictor	OR (95% CI)	aOR (95% CI)
Total Parity	0.92 (0.61-1.38)	0.62 (0.32-1.21)
GA at TVUS, wks	0.94 (0.89-1.0)	0.93 (0.87-1.0)
CL at TVUS, cm	1.22 (0.63-2.4)	1.59 (0.83-3.05)
Any Progesterone Use	8.95 (3.03-26.4)	13.6 (3.45-53.5)*
Absent CGA on TVUS	18.95 (5.99-59.9)	23.87 (6.4-89.0)*

Full model (solid line). Reduced model (dashed line). Abbreviations: AUC (area under the receiver operating curve), CGA (cervical gland area), TVUS (transvaginal ultrasound), CL (cervical length), OR (odds ratio), aOR (adjusted odds ratio; adjusted for all listed predictor variables). *p<0.001

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CGA_TABLE1.docx available at https://authorea.com/users/635858/articles/653053-multidimensional-screening-for-a-multifunctional-cervix-examining-cervical-gland-area-at-cervical-length-screening-to-predict-spontaneous-preterm-birth

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CGA_TABLE2.docx available at https://authorea.com/users/635858/articles/653053-multidimensional-screening-for-a-multifunctional-cervix-examining-cervical-gland-area-at-cervical-length-screening-to-predict-spontaneous-preterm-birth