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CERVICAL LENGTH AND QUANTITATIVE FETAL FIBRONECTIN IN THE PREDICTION OF SPONTANEOUS PRETERM BIRTH IN ASYMPTOMATIC WOMEN WITH CONGENITAL UTERINE ANOMALY

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Condensation: Predictive tests for preterm birth (cervical length and quantitative fetal fibronectin) do not have clinical utility in women with congenital uterine anomalies related to fusion defects.

Short Title: Preterm birth prediction by cervical length and quantitative fetal fibronectin in congenital uterine anomalies.

AJOG at a GLANCE:

A: Why was the study conducted?

- To assess the performance of current predictive markers of sPTB, quantitative fetal fibronectin (qfFN) and transvaginal cervical length (CL) measurement in asymptomatic high-risk women with Congenital Uterine Anomalies (CUA)
- To characterise rates of early delivery by type of CUA

B: What are the key findings?

- CUA, particularly fusion defects, are associated with high rates of late miscarriage and PTB
- CL and qfFN have utility in prediction of sPTB in women with resorption defects, however were no better than chance in women with fusion defects. This is contrary to other high-risk populations."

C: What does this study add to what is already known?

These findings need to be accounted for when planning antenatal care and have potential implications for the predictive tests used in sPTB surveillance and intervention.

Key Words

Bicornuate, Canalisation defects, Cervical length, Congenital uterine anomaly, Fetal fibronectin, Fusion defect, Unicornuate, Unification defects, Uterus didelphys, Preterm birth, Resorption defect

Abstract

Background: Congenital uterine anomalies (CUA) are associated with late miscarriage and spontaneous preterm birth (sPTB).

Objectives: Our aim was to 1) determine the rate of sPTB in each type of CUA and 2) assess the performance of quantitative fetal fibronectin (qfFN) and transvaginal cervical length (CL) measurement by ultrasound in asymptomatic women with CUA for the prediction of sPTB at <34 and <37 weeks of gestation.

Study design: This was a retrospective cohort of women with CUA asymptomatic for sPTB, from four UK tertiary referral centres (2001-2016). CUAs were categorised into fusion (unicornuate, didelphic and bicornuate uteri) or resorption defects (septate, with or without resection and arcuate uteri), based on pre-pregnancy diagnosis.

All women underwent serial transvaginal ultrasound CL assessment in the second trimester (16 to 24 weeks' gestation); a subgroup underwent qfFN testing from 18 weeks' gestation. We investigated the relationship between CUA and predictive test performance for sPTB before 34 and 37 weeks' gestation.

Results: Three hundred and nineteen women were identified as having CUA within our high-risk population. 7% (23/319) delivered spontaneously <34 weeks, and 18% (56/319) <37 weeks' gestation. Rates of sPTB by type were: 26% (7/27) for

unicornuate, 21% (7/34) for didelphic, 16% (31/189) for bicornuate, 13% (7/56) for septate and 31% (4/13) for arcuate.

80% (45/56) of women who had sPTB <37 weeks did not develop a short CL (<25 mm) during the surveillance period (16-24 weeks). The diagnostic accuracy of short CL had low sensitivity (20.3) for predicting sPTB <34 weeks.

Cervical Length had ROC AUC of 0.56 (95% CI 0.48 to 0.64) and 0.59 (95% CI 0.55 to 0.64) for prediction of sPTB <34 and 37 weeks' respectively.

The AUC for CL to predict sPTB <34 weeks was 0.48 for fusion defects (95% CI 0.39 to 0.57) but 0.78 (95% CI 0.66 to 0.91) for women with resorption defects.

Overall **quantitative fetal fibronectin** had a AUC of 0.63 (95% CI 0.49 to 0.77) and 0.58 (95% CI 0.49 to 0.68) for prediction of sPTB <34 and 37 weeks, respectively.

AUC for prediction of sPTB <37 weeks with qfFN for fusion defects was 0.52 (95% CI 0.41 to 0.63), but 0.79 (0.63 to 0.95) for women with resorption defects. Results were similar when women with intervention were excluded.

Conclusion: Commonly used markers CL and qfFN have utility in prediction of sPTB in resorption congenital uterine defects but not in fusion defects. This is contrary to other high-risk populations. These findings need to be accounted for when planning antenatal care and have potential implications for predictive tests used in sPTB surveillance and intervention.

Background

The presence of a congenital uterine anomaly (CUA) is a well-established cause of pregnancy complications, including infertility, recurrent first and second trimester miscarriages, preterm birth (PTB) with or without preterm pre-labour rupture of membranes (PPROM), as well as intra-uterine growth restriction, fetal malposition and caesarean section[1–4]. The types of CUA are individually associated with varying degrees of adverse outcomes.

Formation of the female reproductive tract involves a chain of complex steps, with differentiation, migration, unification and subsequent canalization of the Müllerian ducts [5]. A deviation anywhere along this stepwise development pathway will result in a CUA, from arcuate uterus, a subtle variation from normal anatomy, to complete failure of fusion of the Müllerian ducts, with two discrete cervical canals and uterine cavities (uterus didelphys). Recognition of CUA is often only noted in the presence of pathology, e.g. recurrent miscarriage or early delivery. However, in women with recurrent pregnancy loss, the rate can be as high as 10%[6,7].

While specific CUAs differ in rates of sPTB, and reliable control data to quantify this is lacking, all are associated with poor reproductive outcomes[2], emphasizing the clinical importance of antenatal surveillance for this group. Identifying those most at risk of sPTB is the strategy currently employed globally. The value of quantitative fFN and CL has been proven in large prospective cohorts however reports have concentrated on asymptomatic singletons with prior preterm birth, late miscarriage or

cervical surgery. There is limited evidence to support the use of predictive markers in women with CUAs.

We prospectively collected serial CL and qfFN data from a large cohort of high-risk women with congenital uterine anomalies who were asymptomatic for sPTB. Our aim was to determine the clinical utility of current used predictive markers of sPTB in this group.

Study Design

This is a retrospective cohort study of prospectively collected data from asymptomatic pregnant women with CUAs presenting to high-risk preterm surveillance clinics (PSC) at four tertiary referral hospitals in London (Queen Charlotte's and Chelsea Hospital, St Thomas' Hospital, Chelsea and Westminster Hospital and University College London Hospital), over a fifteen-year period (2001 to 2016). Women were included if the diagnosis of a CUA (unicornuate, didelphyic, bicornuate, septate or arcuate) was made prior to pregnancy by imaging or surgery, and classified according to the American Fertility Society classification (AFS) (1988) (currently the American Society of Reproductive Medicine). Surgical repair was recorded, as were any additional referral risk factors (one or more previous sPTB or PPROM), previous late miscarriage (14 to 23^{+6} weeks) or previous cervical surgery).

As part of routine clinical care within the preterm surveillance clinics, women underwent serial transvaginal ultrasound (TVUS) surveillance of CL between 16 and 24 weeks' (second trimester screening). Frequency of surveillance (TV USS and qfFN) varied between 2 and 4 weeks according to clinical need and continued until

24weeks, independent of prophylactic intervention (cerclage and/or progesterone). Elective cervical cerclage was offered as per contemporaneous clinical practice based on the woman's previous obstetric history or ultrasound indicated cerclage based on a short CL in the index pregnancy, defined as a CL <25 mm <24 weeks' gestation. In a subgroup of women, qfFN measurement was carried out at each visit just prior to ultrasound, between 18 and 24 weeks of gestation. FFN samples from women who reported sexual intercourse within 24 hours or with frank bleeding were excluded from the analysis according to manufacturer's instructions (Hologic Inc, USA).

Maternal demographic data, serial CL and qfFN measurements, and maternal and neonatal outcome details were analysed. Women were considered to have had a spontaneous preterm birth if they had spontaneous onset of labour, or experienced preterm rupture of membranes and delivered prematurely, regardless of mode of delivery. Women with iatrogenic delivery before the gestational time point of interest, twin pregnancies, and those with incomplete outcome data were excluded from the analysis. We repeated the analysis excluding women with intervention in situ.

This study was exempt from requiring ethical approval under the UK Health and Social Care Act 2012, which states that research involving anonymised routinely collected clinical data is excluded from research ethics committee review.

Technique of qfFN measurement

During speculum examination, a polyester swab was inserted into the posterior fornix of the vagina (10 seconds) to collect a sample of cervicovaginal fluid. The swab was

placed into the test buffer solution and analyzed immediately. An aliquot (200 microliters) of the sample was analyzed using the quantitative Rapid fFN 10Q analyzer according to manufacturer' s instructions. All clinicians received appropriate training to use the analyzers.

Thresholds of 10 (lower limit of test), 50 (previous standard), and 200 ng/mL (based on existing literature) were predefined. Quantitative fFN assay results are reported in units of ng/mL and the result was standardized using purified fetal fibronectin and A128 measurement with an extinction coefficient = 1.28. The reliability of the Rapid 10Q analyzer has previously been reported. For the 10Q Assay the intra-assay CV is 5.7% - 7.3% and the intra-assay CV is 5.9% - 7.5%. Experiments that were performed during product development confirmed a good correlation between ELISA and 10Q tests (slope = 0.97; r^2 = 0.82) [Personal communication with Jerome Lapointe, Hologic].

Technique of cervical length assessment

Serial CL assessment was undertaken in accordance with standardized guidelines by trained operators.[11,12] In summary, the woman was asked to empty her bladder and then the TVUS probe was inserted into the anterior fornix of the vagina to obtain a sagittal long axis view of the echogenic endocervical mucosa along the length of the cervical canal, allowing identification of both the internal and external os. Without causing undue pressure on the cervix with the probe to avoid falsely elongating it, the linear distance between the external and internal os was recorded three times in millimeters over a minimum of three minutes using optimal magnification and zoom settings and the shortest CL was recorded. Transfundal pressure was exerted for 15

seconds and subsequent demonstration of a cervical funnel was noted if present. The shortest total closed CL of three measurements was considered the length for analysis, with “short” CL defined as less than 25mm.

Statistical analysis

Descriptive statistics were used to depict the study population. Predictive statistics were carried out to determine if predictive tests (CL and qfFN) accurately predicted sPTB <34 and 37weeks’ gestation. Statistical analysis was performed using Stata 14.0. Receiver operating characteristic (ROC) curves were generated and compared. Data from repeated sampling of the same individuals was analysed. Therefore clustered bootstrapping with bias correction was used to calculate confidence intervals for ROC curves (Ng, Grieve & Carpenter, 2013)[13]. Quantitative fFN analysis was carried out for a subgroup of women. Due to sample size, descriptive data alone were generated for this group.

Results

Four hundred and twenty-nine women with congenital uterine anomalies were identified in the four high-risk preterm surveillance clinics. One hundred and ten women were subsequently excluded from analysis as a result of missing outcome data/uterine anomaly classification (n=91), multiple pregnancy (n=9) and incomplete qfFN or CL data (n=10).

Of the women included in the analysis (n=319), 9% (27) had unicornuate, 11% (34) didelphic, 59% (189) bicornuate, 18% (56) septate and 4% (13) arcuate uteri. The rate of sPTB <37 weeks according to the type of CUA was 26% (7/27) of women with unicornuate, 21% (7/34) with didelphic, 16% (31/189) with bicornuate, 13% (7/56)

with septate and 31% (4/13) with arcuate uteri. Overall, the sPTB rate was 7% (23/319) at <34 weeks and 18% (56/319) at <37 weeks' gestation.

Two hundred and fifty-seven women (81%, 257/319) had CUA as their sole risk factor (ie. no additional history of sPTB/late miscarriage or cervical surgery). Rates of sPTB <37 weeks for this group were as follows: 27% (7/26) for unicornuate, 20% (6/30) for didelphic, 9% (13/143) for bicornuate, 13% (6/48) for septate and 10% (1/10) for women with an arcuate uterus (Table 1).

Women with septate uteri had a high rate of previous 1st trimester miscarriage (42%, 15/36). One fifth (21%, 36/173) of women with bicornuate uteri had a previous history of sPTB. Over 20% (2/9) of the cohort with arcuate uteri had a history of ≥1 previous late miscarriage. Maternal characteristics relevant to risk of sPTB are shown in Table 2.

The incidence of sPTB <34 and 37 weeks was 7% (23/319) and 18% (56/319), although when categorised by anomaly type, this increased to 26% (7/27) for unicornuate and 31% (4/13) for women with an arcuate uterus <37 weeks (Table 1).

Cervical length assessment

Three hundred and nineteen women received a total of 955 TVUSS CL measurements. On average, each women had 2.2 measurements per pregnancy (range 1 to 6). Twenty-nine women in this high-risk population (9%) were found to have a short CL (<25 mm), of whom 48% (14/29) delivered <37 weeks.

CL was a poor predictor of sPTB <34 and 37 weeks' gestation when the cohort was analysed as a whole (AUC 0.56 (95% CI 0.48 to 0.64) and 0.59 (95% CI 0.55 to

0.64) respectively) (Table 3), with a low diagnostic sensitivity when a cutoff of <25 mm was used (20.3 and 15.2 for sPTB < 34 and 37 weeks' respectively).

However, when the cohort was grouped according to fusion or resorption defects, CL behaved predictably for sPTB <34 weeks in women with resorption (AUC 0.78, 95% CI 0.66 to 0.91) but not fusion defects (AUC 0.48, 95% CI 0.39 to 0.57) (Figure 1).

CL was predictive for sPTB <34 weeks in women with septate uteri (AUC 0.80, 95% CI 0.62 to 0.97) (Figure 2) (CL <25 mm: sensitivity 50.0), and in the arcuate group for delivery <34 and 37 weeks (AUC 0.83, 95% CI 0.51 to 0.98, sensitivity 30.0). Results did not change after exclusion of women with intervention [septate excluding cervical cerclage: AUC 0.85 (95% CI 0.79 to 0.91].

Prediction of sPTB at <34 and 37 weeks was poor in women with fusion defects (AUC 0.48 (95% CI 0.39 to 0.57) and AUC 0.60 (95% CI 0.55 to 0.65). Figure 1. For specific fusion defects, CL was also not predictive of sPTB <37 weeks (unicornuate 0.48 (95% CI 0.34 to 0.62), didelphic 0.55 (95% CI 0.42 to 0.68) and 0.62 (95% CI 0.56 to 0.69) for bicornuate uteri). Diagnostic accuracy for individual CUA defects can be seen in Table 4.

Results were similar after excluding women with intervention (cerclage and/or progesterone) [unicornuate 0.55 (95% 0.39 to 0.74, didelphic 0.55, 95% CI 0.34 to 0.70 and 0.62 (95% CI 0.51 to 0.72) for bicornuate uteri].

Quantitative fetal fibronectin

One hundred and fifty five women underwent 793 cervicovaginal qfFN protein analysis. Overall qfFN had a ROC AUC of 0.63 (95% CI 0.49 to 0.77) and 0.58 (95% CI 0.49 to 0.68) for prediction of sPTB <34 and 37 weeks, respectively.

We found qfFN to be an accurate test of sPTB <34 and 37 weeks in women with resorption defects (AUC 0.83 (95% CI 0.62 to 1.00) and AUC 0.79 (95% CI 0.63 to 0.95) respectively) (Figure 3). This did not hold true for fusion defects (AUC for sPTB <37 weeks 0.52 (95% CI 0.41 to 0.63)).

Management

Over half of the women in our cohort delivered by caesarean section (56%, 124/221), with the highest number in those with didelphic (77%, 17/22) and unicornuate uteri (73%, 16/22). Sixty per cent (9/15) of women with uterus didelphys had a fetal malposition at time of delivery (Table 5). In total, 11% (35/319) of women had a cervical cerclage during their pregnancy. 51% (18/35) were ultrasound indicated, based on a CL <25mm at gestation <24 weeks. 11% of women were prescribed progesterone during their pregnancy, although we only have data on progesterone prescribing practices for 138/319 women (Table 6). 80% (45/56) of women who delivered spontaneously <37 weeks' did not develop a short CL during our surveillance period (16 to 24 weeks').

Comment

Principle Findings:

Commonly used markers, CL and qfFN, have utility in prediction of sPTB in resorption congenital uterine defects but not in fusion defects. This is contrary to other high-risk populations. 80% (45/56) of women who went into spontaneous labour preterm did not develop a short CL during the antenatal surveillance period.

In our cohort, 21% (7/34) women with a didelphic uterus (a fusion defect) delivered <37 weeks' gestation, and 8% (3/34) <34 weeks' gestation. Early pregnancy CL measurement was no better than chance at predicting delivery <37 weeks, with poor AUC, sensitivity and negative predictive value.

Asymptomatic qfFN screening in our whole cohort was a poor predictor of delivery at <34 weeks' gestation. This was confirmed for fusion defects (<34 weeks AUC 0.55, 95% CI 0.39 to 0.70, <37 weeks AUC 0.52, 95% CI 0.41 to 0.63). This is contrary to other cohorts at high risk of sPTB (e.g. history of late miscarriage) and therefore it is important that clinicians are aware of this when planning antenatal surveillance and choosing predictive tests for sPTB.

Clinical Implications:

Whilst women with CUA are considered to be at high-risk of sPTB, data correlating individual congenital uterine anomaly and outcome is limited. The existing strategy used for prediction of sPTB in women at high-risk for other reasons is recognised to be inadequate. An understanding of the increased risk posed to women with each type of anomaly will help to determine their subsequent antenatal management pathways, and the appropriate diagnostic tests. In this study we report the accuracy of predictive markers of sPTB in asymptomatic high-risk women with CUA, correlating both CL and qfFN with individual defect types and categorised according to resorption or fusion defects.

The pathophysiological processes underlying early delivery in CUA cases remain uncertain. Deficiency in the endometrium overlying any anatomical variation, for example the septum, may provide a suboptimal site for implantation, disorderly and decreased blood supply insufficient to support placentation[14] and embryonic growth. Other potential hypothesized mechanisms include abnormal myometrial architecture producing uncoordinated uterine contractions[15] or reduced uterine capacity,[16] affecting stretch. The structure of the cervix is integral to the maintenance of pregnancy;[17] disruption in cervical architecture, particularly the internal cervical os may account for increased rates of sPTB.

The difference in predictive test performance between fusion and resorption groups may be related to the underlying mechanism of preterm birth. In women with resorption defects (septate and arcuate uterus), predictive markers performed as seen in other high-risk populations; both CL and qfFN were useful predictors of sPTB

<34 and 37 weeks' gestation. Resorption defects have relatively normal uterine architecture. By definition an arcuate uterus has an intrauterine indentation of less than 1cm and therefore it is plausible that it does not impact on either the cause of preterm delivery or the mechanism by which markers CL and qfFN predict delivery.

For more severe structural anomalies, such as unicornuate or uterus didelphys, the converse is likely to be true, and poor pregnancy outcome is hypothesized to be related to stretch effects secondary to altered uterine architecture, decreased muscle mass and abnormal cervical architecture, with or without abnormal uterine vasculature[18]. If the cervix plays no part in the aetiology of labour onset, it may not predict delivery in this group. Further research needs to focus on novel predictive markers in this high-risk group.

Late miscarriage and preterm birth are frequently thought to be associated with inflammation and infection. Recent literature has linked true positive fFN results with placental inflammation, hypothesised to disturb the decidua-chorionic interface, threatening the integrity of the maternal-fetal interface and leading to the release of fFN into the cervico-vaginal secretions where it is detected[19]. Quantitative fFN is a leading predictor of sPTB and its value as a screening tool for high-risk asymptomatic women is increasingly recognised[8]. However, abnormal myometrium and stretch effects may not cause this same release of fFN, which may account for its poor predictive value in fusion defects.

Strengths and Weaknesses:

Three previous studies reported the use of CL measurement in women with CUA[20–22], and one has evaluated the addition of qualitative fFN[23]. Consensus concluded that short CL on TVUS correlates with increased risk of sPTB in women with CUA. However these studies do not comment on the differences between types of CUA. They are small (the largest 120 women[23] compared to 319 reported here) and therefore do not have sufficient power for this analysis. Increased sample size allowed our analysis to discern a difference in predictive tests, qfFN and CL, between fusion and resorption defects, rather than examining the cohort as one heterogeneous group.

Consistent with our findings, Airoldi et al (2005) highlighted no cervical shortening in the two women with didelphic uteri (n=2/11) who went on to deliver preterm (n=11)[20]. The two studies describing CL measurement both extended their sampling windows up to 30[21] and 32[23] weeks respectively, and developed a new cut off of 30mm, based on their individual data set (n=52)[21]. With this increased sampling window Crane et al report 100% sensitivity for a CL cut off of 30mm. As this was only 3 out of 3 events identified and both studies were sampling outside of current clinical guidelines, we believe our data supersedes this.

It is important to acknowledge the limitations of our study. Women and healthcare providers were not blinded to CL and qfFN assessments. The study population included women who were referred to a preterm birth surveillance clinics for high-risk monitoring. We do not know the number of women with a uterine anomaly who were not referred for asymptomatic screening. Also while this larger cohort allows us to

draw some conclusions about individual subgroups, we recognise we do not have adequate power to undertake further analysis investigating the additive value of qfFN and CL. Future research in women with resorption defects would help understand the synergies between predictive tests, as well as seeking the ideal surveillance window and CL and qfFN cut offs for this population.

A further limitation was that septate uteri were a small group in this study. The data did not lend itself to biological plausibility with regard to separating the groups into those who had had surgical removal of their septum, and those who had not, and therefore we highlight this as an area that would benefit from future research. Arcuate uteri also appeared particularly high-risk in our cohort, however the numbers were small and in this group all but one case had additional risk factors. Therefore CUA may have been an incidental finding and a significant proportion of preterm deliveries may be due to aetiology unrelated to CUA, for example infection and inflammation.

If a short cervix (CL <25mm) was detected within the surveillance period, an ultrasound-indicated cerclage may have been carried out, depending on local hospital clinical practice. Repeat analysis excluding women with intervention (cerclage and/or progesterone) confirmed predictive markers were no better than chance in women with fusion defects but have clinical utility in women with resorption defects. The literature confirms the continued value of CL measurement as a reliable predictor of sPTB with cerclage in situ, and 80% of women who delivered preterm spontaneous did not develop a short CL during the surveillance period. Only 6% (18/319) of our total cohort had an ultrasound-indicated cerclage.

Conclusions and future research implications

Our findings suggest different aetiological contributions to the pathophysiology of sPTB in CUA, which do not follow the predictable pattern of cervical shortening and dilatation seen in women who deliver early due to inflammation and infection. This needs to be accounted for when planning antenatal care, with potential implications for sPTB surveillance and intervention.

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435 References:

436 1. Hua M, Odibo AO, Longman RE, MacOnes GA, Roehl KA, Cahill AG. Congenital uterine
437 anomalies and adverse pregnancy outcomes. Am J Obstet Gynecol [Internet].
438 2011;205(6):558.e1-558.e5. Available from: http://dx.doi.org/10.1016/j.ajog.2011.07.022

439 2. Chan YY, Jayaprakasan K, Tan A, Thornton JG, Coomarasamy A, Raine-Fenning NJ.
440 Reproductive outcomes in women with congenital uterine anomalies: A systematic review.
441 Ultrasound Obstet Gynecol. 2011;38(4):371–82.

442 3. Venetis CA, Papadopoulos SP, Campo R, Gordts S, Tarlatzis BC, Grimbizis GF. Clinical
443 implications of congenital uterine anomalies: A meta-analysis of comparative studies. Reprod
444 Biomed Online [Internet]. 2014;29(6):665–83. Available from:
445 http://dx.doi.org/10.1016/j.rbmo.2014.09.006

446 4. Chan YY, Jayaprakasan K, Zamora J, Thornton JG, Raine-Fenning N, Coomarasamy A. The
447 prevalence of congenital uterine anomalies in unselected and high-risk populations: A
448 systematic review. Hum Reprod Update. 2011;17(6):761–71.

449 5. Acién P, Acién MI. The history of female genital tract malformation classifications and proposal
450 of an updated system. Hum Reprod Update. 2011;17(5):693–705.

451 6. Acien P. Reproductive performance of women with uterine anomalies. Acta Obs Gynecol
452 Scand. 1982;61(l):157–62.

453 7. Raga F, Bauset C, Remohi J, Bonilla-Musoles F, Simón C, Pellicer A. Reproductive impact of
454 congenital Mü llerian anomalies. Hum Reprod. 1997;12(10):2277–81.

455 8. Abbott DS, Hezelgrave NL, Seed PT, Norman JE, David AL, Bennett PR, et al. Quantitative
456 fetal fibronectin to predict preterm birth in asymptomatic women at high risk. Obstet Gynecol.
457 2015;125(5):1168–76.

458 9. Min J, Watson HA, Hezelgrave NL, Seed PT, Shennan AH. Ability of a preterm surveillance
459 clinic to triage risk of preterm birth: a prospective cohort study. Ultrasound Obstet Gynecol.
460 2016;

461 10. Kuhrt K, Seed P, Smout E, Hezelgrave N, Shennan A. Development and validation of a
462 predictive tool for spontaneous preterm birth incorporating cervical length and quantitative fetal
463 fibronectin in asymptomatic high risk women. BJOG An Int J Obstet Gynaecol. 2014;

464 11. Iams JD, Goldenberg RL, Meis PJ, Mercer BM, Moawad A, Das A, et al. The Length of the
465 Cervix and the Risk of Spontaneous Premature Delivery. N Engl J Med [Internet].
466 1996;334(9):567–73. Available from:
467 http://www.nejm.org/doi/abs/10.1056/NEJM199602293340904

468 12. Berghella V, Talucci M, Desai A. Does transvaginal sonographic measurement of cervical
469 length before 14 weeks predict preterm delivery in high-risk pregnancies? Ultrasound Obstet
470 Gynecol. 2003;21(2):140–4.

471 13. Ng ESW, Grieve R, Carpenter JR. Two-stage nonparametric bootstrap sampling with
472 shrinkage correction for clustered data. Stata J. 2013;

473 14. Leible S, Munoz H, Walton R, Sabaj V, Cumsille F, Sepulveda W. Uterine artery blood flow
474 velocity waveforms in pregnant women with mullerian duct anomaly: a biologic model for
475 uteroplacental insufficiency. Am J Obstet Gynecol. 1998;178(5):1048–53.

476 15. Kupesic S. Clinical implications of sonographic detection of uterine anomalies for reproductive
477 outcome. Ultrasound Obstet Gynecol. 2001;18(4):387–400.

478 16. Simon C, Martinez L, Pardo F, Tortajada M, Pellicer A. Mullerian defects in women with normal
479 reproductive outcome. Fertil Steril. 1991;56(6):1192–3.

17. Nott JP, Bonney EA, Pickering JD, Simpson NAB. The structure and function of the cervix during pregnancy. Transl Res Anat. 2016;2:1–7.

18. Akar ME, Bayar D, Yildiz S, Ozel M, Yilmaz Z. Reproductive outcome of women with unicornuate uterus. Aust New Zeal J Obstet Gynaecol. 2005;45(2):148–50.

19. van der Krogt L, Ridout AE, Seed PT, Shennan AH. Placental inflammation and its relationship to cervicovaginal fetal fibronectin in preterm birth. Eur J Obstet Gynecol Reprod Biol [Internet]. 2017;214:173–7. Available from: http://dx.doi.org/10.1016/j.ejogrb.2017.05.001

20. Airoldi J. Transvaginal Ultrasonography of the Cervix to Predict Preterm Birth in Women With Uterine Anomalies. 2005;106(3):553–6.

21. Crane J, Scott H, Stewart A, Chandra S, Whittle W, Hutchens D. Transvaginal ultrasonography to predict preterm birth in women with bicornuate or didelphus uterus. J Matern Neonatal Med. 2012;25(10):1960–4.

22. Fox NS. Gestational age at cervical length measurement and incidence of preterm birth. Obs Gynecol [Internet]. 2007;110(6):1427; author reply 1427. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18055747

23. Fox NS, Saltzman DH, Gerber RS, Stern E, Klauser CK, Rebarber A. Prediction of spontaneous preterm birth in patients with congenital uterine anomalies using combined fetal fibronectin and cervical length. 2013;1(1):47–52.

Table 1: Pregnancy outcome in women with congenital uterine anomaly

Pregnancy Outcome	Cohort (n=319)	Unicornuate (n=27)	Didelphys (n=34)	Bicornuate (n=189)	Septate (n=56)	Arcuate (n=13)
sPTB <37 weeks	17.6% (56)	25.9% (7)	20.6% (7)	16.4% (31)	12.5% (7)	30.8% (4)
sPTB < 34 weeks	7.2% (23)	3.7% (1)	8.8% (3)	6.3% (12)	5.4% (3)	30.8% (4)
sPTB < 37 weeks when CUA is the sole risk factor	12.8% (33/257)	26.9% (7/26)	20.0% (6/30)	9.1% (13/143)	12.5% (6/48)	10% (1/10)

551 **Table 2: Maternal Characteristics of women with congenital uterine anomaly**

Maternal Characteristic (n, %)	Cohort (n=319)	Unicornuate (27, 8.5%)	Didelphys (34, 10.7%)	Bicornuate (189, 59.3%)	Septate (56, 17.6%)	Arcuate (13, 4%)
Primiparous	55.2% (176)	66.7% (18)	67.6% (23)	47.6% (90)	66.1% (37)	61.5% (8)
Multiparous	44.8% (143)	33.3% (9)	32.4% (11)	52.4% (99)	33.9% (19)	38.5% (5)
Previous term delivery	35.0% (50/143)	22.2% (2/9)	36.4% (4/11)	38.4% (38/99)	26.3% (5/19)	20% (1/5)
Previous first trimester miscarriage	31.9% (61/191)	30.8% (4/13)	30.4% (7/23)	29.9% (35/117)	41.7% (15/36)	0% (0/2)
Previous sPTB < 37 weeks	15.9% (45/283)	0% (0/22)	12.5% (4/32)	20.8% (36/173)	8.5% (4/47)	11.1% (1/9)
Previous mid-trimester loss	9.2% (26/283)	4.5% (1/22)	3.1% (1/32)	10.4% (18/173)	8.5% (4/47)	22.2% (2/9)
Previous cervical surgery	13.1% (37/283)	9.1% (2/22)	3.1% (1/32)	14.5% (25/173)	14.9% (7/47)	22.2% (2/9)
Ethnicity **1- White** **2- Asian** **3- Black** **4- Unknown**	 48.6% (155) 3.4% (11) 5.3% (17) 42.6% (136)	 8.4% (13) 18.1% (2) 0 8.8% (12)	 11.6% (18) 18.1% (2) 0 10.3% (14)	 58.1% (90) 36.3% (4) 82.4% (14) 60.0% (81)	 17.4% (27) 27.3% (3) 5.9% (1) 18.4% (25)	 5.0% (7) 0 11.8% (2) 2.9% (4)
BMI (median, IQR)	23.1 21.0 – 39.0	23.5 22.3 – 30.0	24.0 22.4– 33.8	23.0 20.9 – 39.0	23.0 20.6-36.8	23.9 21.0 – 36.7

552 Results given as % (n) or median [interquartile range]

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560 **Table 3: Accuracy of qfFN and CL for the prediction of sPTB**

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Type of anomaly	CL prediction		qfFN prediction	
	ROC AUC	95% confidence intervals	ROC AUC	95% confidence intervals
Whole cohort (n=319)				
sPTB<34weeks	0.56	0.48 to 0.64	0.63	0.49 to 0.77
sPTB<37weeks	0.59	0.55 to 0.64	0.58	0.49 to 0.68
Fusion defects				
sPTB<34weeks	0.48	0.39 to 0.57	0.55	0.39 to 0.70
sPTB<37weeks	0.60	0.55 to 0.65	0.52	0.41 to 0.63
Resorption defects				
sPTB<34weeks	0.78	0.66 to 0.91	0.83	0.62 to 1.00
sPTB<37weeks	0.66	0.55 to 0.78	0.79	0.63 to 0.95

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Table 4: Accuracy of CL for the prediction of sPTB in subgroups

Type of anomaly	ROC AUC 95% confidence intervals	
Unicornuate (n=27)		
sPTB<34weeks	0.56	0.32 to 0.80
sPTB<37weeks	0.48	0.34 to 0.62
Didelphys (n=34)		
sPTB<34weeks	0.50	0.31 to 0.70
sPTB<37weeks	0.55	0.42 to 0.68
Bicornuate (n=189)		
sPTB<34weeks	0.46	0.35 to 0.56
sPTB<37weeks	0.62	0.56 to 0.69
Septate (n=56)		
sPTB<34weeks	0.80	0.62 to 0.97
sPTB<37weeks	0.61	0.47 to 0.76
Arcuate (n=13)		
sPTB<34weeks	0.79	0.51 to 0.98
sPTB<37weeks	0.79	0.51 to 0.98

Table 5: Pregnancy outcome in women with congenital uterine anomaly

Pregnancy Outcome	Cohort (n=319)	Unicornuate (n=27)	Didelphys (n=34)	Bicornuate (n=189)	Septate (n=56)	Arcuate (n=13)
Primiparous women with sPTB <37 weeks	13% (22)	17% (3)	26% (6)	8% (7)	14% (5)	13% (1)
Multiparous women with sPTB <37 weeks	23% (33)	44% (4)	0% (0)	27% (24)	11% (2)	60% (3)
Rate of caesarean section	56% (124/221)	72.7% (16/22)	77.3% (17/22)	55.6% (70/126)	42.1% (16/38)	38.5% (5/13)
Fetal malposition	32% (39/121)	30.8% (4/13)	60% (9/15)	30.8% (16/52)	35.7% (10/28)	0% (0/13)
NICU admissions	16% (20/123)	25% (1/4)	0% (0/12)	15.6% (12/77)	20% (4/20)	30% (3/10)

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614 **Table 6: Antenatal management in asymptomatic women with CUA**

Pregnancy Outcome	Cohort (n=319)	Unicornuate (n=27)	Didelphys (n=34)	Bicornuate (n=189)	Septate (n=56)	Arcuate (n=13)
Cerclage	11.0% (35/319)	11.1% (3/27)	14.7% (5/34)	10.1% (19/189)	12.5% (7/56)	7.7% (1/13)
Ultrasound indicated	51.4% (18/35)	7.4% (2/27)	5.8% (2/34)	5.8% (11/189)	3.6% (2/56)	7.7% (1/13)
sPTB <37/40	*23.5% (5/18)*	*0% (0/2)*	*50% (1/2)*	*(5/11)*	*50% (1/2)*	*100% (1/1)*
sPTB <34/40	*23.5% (5/18)*	*50% (1/2)*	*50% (1/2)*	*(1/11)*	*50% (1/2)*	*100% (1/1)*
History indicated	48.6% (17/35)	3.7% (1/27)	8.8% (3/34)	4.2% (8/189)	8.9% (5/56)	0% (0/13)
sPTB <37/40	*23.5% (4/17)*	*0% (0/1)*	*33.3% (1/3)*	*25% (2/8)*	*20% (1/5)*	*0% (0/13)*
sPTB <34/40	*17.6% (3/17)*	*0% (0/1)*	*33.3% (1/3)*	*12.5% (1/8)*	*20% (1/5)*	*0% (0/13)*
sPTB without short CL	80.4% (45/56)	85.7% (6/7)	85.7% (6/7)	90.3% (28/31)	57.1% (4/7)	25% (1/4)
sPTB <37/40	*18% (56/319)*	*25.9% (7/27)*	*20.8% (7/34)*	*16.4% (31/189)*	*12.5% (7/56)*	*30.7% (4/13)*
Progesterone	10.8% (15/138)	30.8% (4/13)	7.7% (1/13)	7.9% (6/76)	13.8% (4/29)	0% (0/6)

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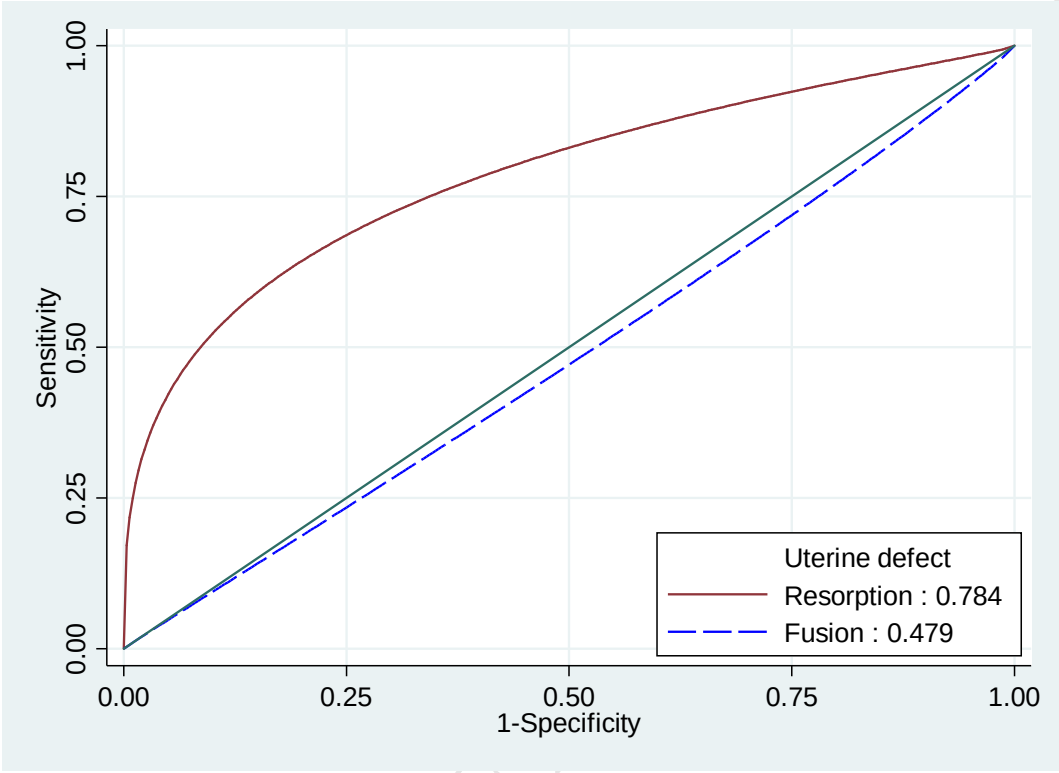
Figure 1: TVUSS CL to predict sPTB <34weeks in CUA grouped by fusion or resorption defect



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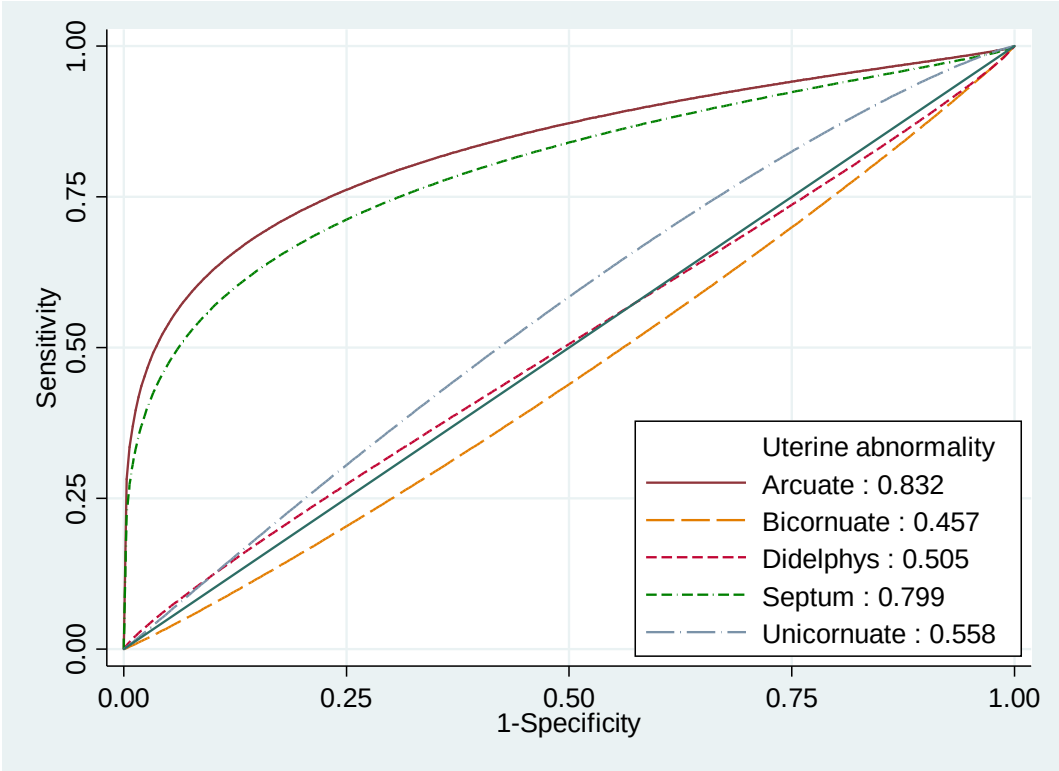
635 **Figure 2: TVUSS CL to predict sPTB <34 weeks by type of CUA defect**



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637 **using binomial modeling*

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646 **Figure 3: Quantitative fetal fibronectin to predict sPTB <37 weeks grouped by**

647 **fusion or resorption defect**

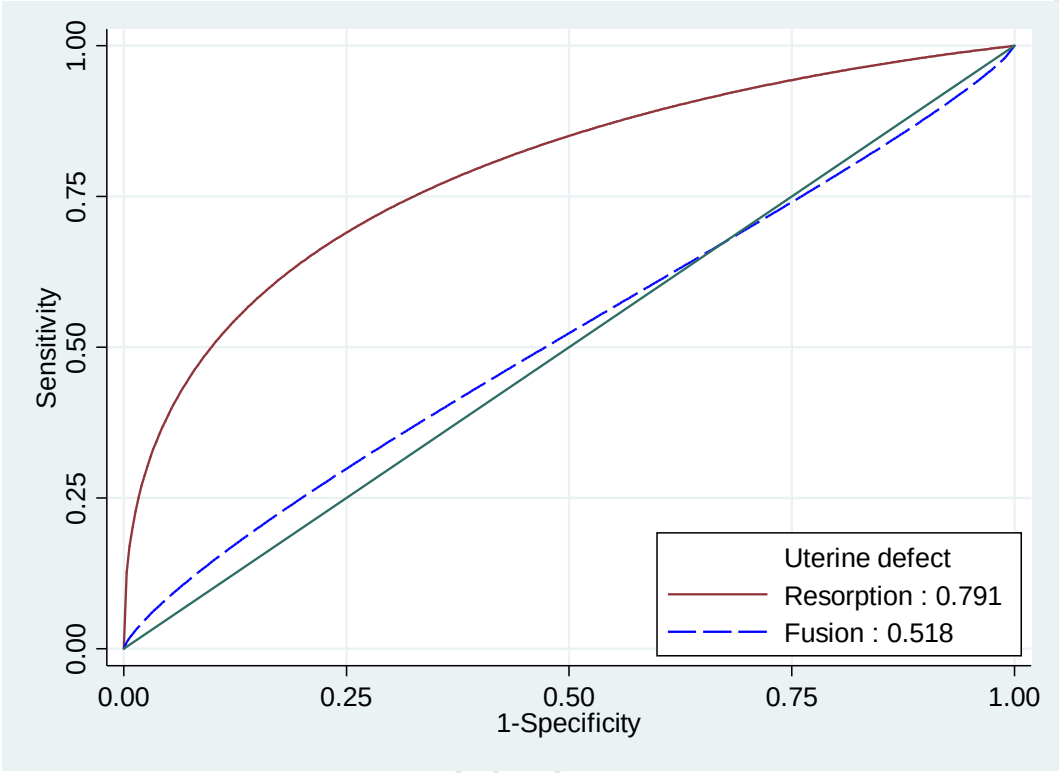

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Table 1: Pregnancy outcome in women with congenital uterine anomaly

Pregnancy Outcome	Cohort (n=319)	Unicornuate (n=27)	Didelphys (n=34)	Bicornuate (n=189)	Septate (n=56)	Arcuate (n=13)
sPTB <37 weeks	17.6% (56)	25.9% (7)	20.6% (7)	16.4% (31)	12.5% (7)	30.8% (4)
sPTB < 34 weeks	7.2% (23)	3.7% (1)	8.8% (3)	6.3% (12)	5.4% (3)	30.8% (4)
sPTB < 37 weeks when CUA the sole risk factor	12.8% (33/257)	26.9% (7/26)	20.0% (6/30)	9.1% (13/143)	12.5% (6/48)	10% (1/10)

Table 2: Maternal Characteristics of women with congenital uterine anomaly

Maternal Characteristic (n, %)	Cohort (n=319)	Unicornuate (27, 8.5%)	Didelphys (34, 10.7%)	Bicornuate (189, 59.3%)	Septate (56, 17.6%)	Arcuate (13, 4%)
Primiparous	55.2% (176)	66.7% (18)	67.6% (23)	47.6% (90)	66.1% (37)	61.5% (8)
Multiparous	44.8% (143)	33.3% (9)	32.4% (11)	52.4% (99)	33.9% (19)	38.5% (5)
Previous term delivery	35.0% (50/143)	22.2% (2/9)	36.4% (4/11)	38.4% (38/99)	26.3% (5/19)	20% (1/5)
Previous first trimester miscarriage	31.9% (61/191)	30.8% (4/13)	30.4% (7/23)	29.9% (35/117)	41.7% (15/36)	0% (0/2)
Previous sPTB < 37 weeks	15.9% (45/283)	0% (0/22)	12.5% (4/32)	20.8% (36/173)	8.5% (4/47)	11.1% (1/9)
Previous mid-trimester loss	9.2% (26/283)	4.5% (1/22)	3.1% (1/32)	10.4% (18/173)	8.5% (4/47)	22.2% (2/9)
Previous cervical surgery	13.1% (37/283)	9.1% (2/22)	3.1% (1/32)	14.5% (25/173)	14.9% (7/47)	22.2% (2/9)
Ethnicity **1- White** **2- Asian** **3- Black** **4- Unknown**	 48.6% (155) 3.4% (11) 5.3% (17) 42.6% (136)	 8.4% (13) 18.1% (2) 0 8.8% (12)	 11.6% (18) 18.1% (2) 0 10.3% (14)	 58.1% (90) 36.3% (4) 82.4% (14) 60.0% (81)	 17.4% (27) 27.3% (3) 5.9% (1) 18.4% (25)	 5.0% (7) 0 11.8% (2) 2.9% (4)
BMI (median, IQR)	23.1 21.0 – 39.0	23.5 22.3 – 30.0	24.0 22.4– 33.8	23.0 20.9 – 39.0	23.0 20.6-36.8	23.9 21.0 – 36.7

Results given as % (n) or median [interquartile range]

Table 3: Accuracy of qfFN and CL for the prediction of sPTB

Type of anomaly	CL prediction		qfFN prediction	
	ROC AUC	95% confidence intervals	ROC AUC	95% confidence intervals
Whole cohort (n=319)				
sPTB<34weeks	0.56	0.48 to 0.64	0.63	0.49 to 0.77
sPTB<37weeks	0.59	0.55 to 0.64	0.58	0.49 to 0.68
Fusion defects				
sPTB<34weeks	0.48	0.39 to 0.57	0.55	0.39 to 0.70
sPTB<37weeks	0.60	0.55 to 0.65	0.52	0.41 to 0.63
Resorption defects				
sPTB<34weeks	0.78	0.66 to 0.91	0.83	0.62 to 1.00
sPTB<37weeks	0.66	0.55 to 0.78	0.79	0.63 to 0.95

Table 4: Accuracy of CL for the prediction of sPTB in subgroups

Type of anomaly	ROC AUC 95% confidence intervals	
Unicornuate (n=27)		
sPTB<34weeks	0.56	0.32 to 0.80
sPTB<37weeks	0.48	0.34 to 0.62
Didelphys (n=34)		
sPTB<34weeks	0.50	0.31 to 0.70
sPTB<37weeks	0.55	0.42 to 0.68
Bicornuate (n=189)		
sPTB<34weeks	0.46	0.35 to 0.56
sPTB<37weeks	0.62	0.56 to 0.69
Septate (n=56)		
sPTB<34weeks	0.80	0.62 to 0.97
sPTB<37weeks	0.61	0.47 to 0.76
Arcuate (n=13)		
sPTB<34weeks	0.79	0.51 to 0.98
sPTB<37weeks	0.79	0.51 to 0.98

Table 5: Pregnancy outcome in women with congenital uterine anomaly

Pregnancy Outcome	Cohort (n=319)	Unicornuate (n=27)	Didelphys (n=34)	Bicornuate (n=189)	Septate (n=56)	Arcuate (n=13)
Primiparous women with sPTB <37 weeks	13% (22)	17% (3)	26% (6)	8% (7)	14% (5)	13% (1)
Multiparous women with sPTB <37 weeks	23% (33)	44% (4)	0% (0)	27% (24)	11% (2)	60% (3)
Rate of caesarean section	56% (124/221)	72.7% (16/22)	77.3% (17/22)	55.6% (70/126)	42.1% (16/38)	38.5% (5/13)
Fetal malposition	32% (39/121)	30.8% (4/13)	60% (9/15)	30.8% (16/52)	35.7% (10/28)	0% (0/13)
NICU admissions	16% (20/123)	25% (1/4)	0% (0/12)	15.6% (12/77)	20% (4/20)	30% (3/10)

Table 6: Antenatal management in asymptomatic women with CUA

Pregnancy Outcome	Cohort (n=319)	Unicornuate (n=27)	Didelphys (n=34)	Bicornuate (n=189)	Septate (n=56)	Arcuate (n=13)
Cerclage	11.0% (35/319)	11.1% (3/27)	14.7% (5/34)	10.1% (19/189)	12.5% (7/56)	7.7% (1/13)
Ultrasound indicated	51.4% (18/35)	7.4% (2/27)	5.8% (2/34)	5.8% (11/189)	3.6% (2/56)	7.7% (1/13)
sPTB <37/40	*23.5% (5/18)*	*0% (0/2)*	*50% (1/2)*	*(5/11)*	*50% (1/2)*	*100% (1/1)*
sPTB <34/40	*23.5% (5/18)*	*50% (1/2)*	*50% (1/2)*	*(1/11)*	*50% (1/2)*	*100% (1/1)*
History indicated	48.6% (17/35)	3.7% (1/27)	8.8% (3/34)	4.2% (8/189)	8.9% (5/56)	0% (0/13)
sPTB <37/40	*23.5% (4/17)*	*0% (0/1)*	*33.3% (1/3)*	*25% (2/8)*	*20% (1/5)*	*0% (0/13)*
sPTB <34/40	*17.6% (3/17)*	*0% (0/1)*	*33.3% (1/3)*	*12.5% (1/8)*	*20% (1/5)*	*0% (0/13)*
sPTB without short CL	80.4% (45/56)	85.7% (6/7)	85.7% (6/7)	90.3% (28/31)	57.1% (4/7)	25% (1/4)
sPTB <37/40	*18% (56/319)*	*25.9% (7/27)*	*20.8% (7/34)*	*16.4% (31/189)*	*12.5% (7/56)*	*30.7% (4/13)*
Progesterone	10.8% (15/138)	30.8% (4/13)	7.7% (1/13)	7.9% (6/76)	13.8% (4/29)	0% (0/6)

Figure 1: TVUSS CL to predict sPTB <34weeks in CUA grouped by fusion or resorption defect

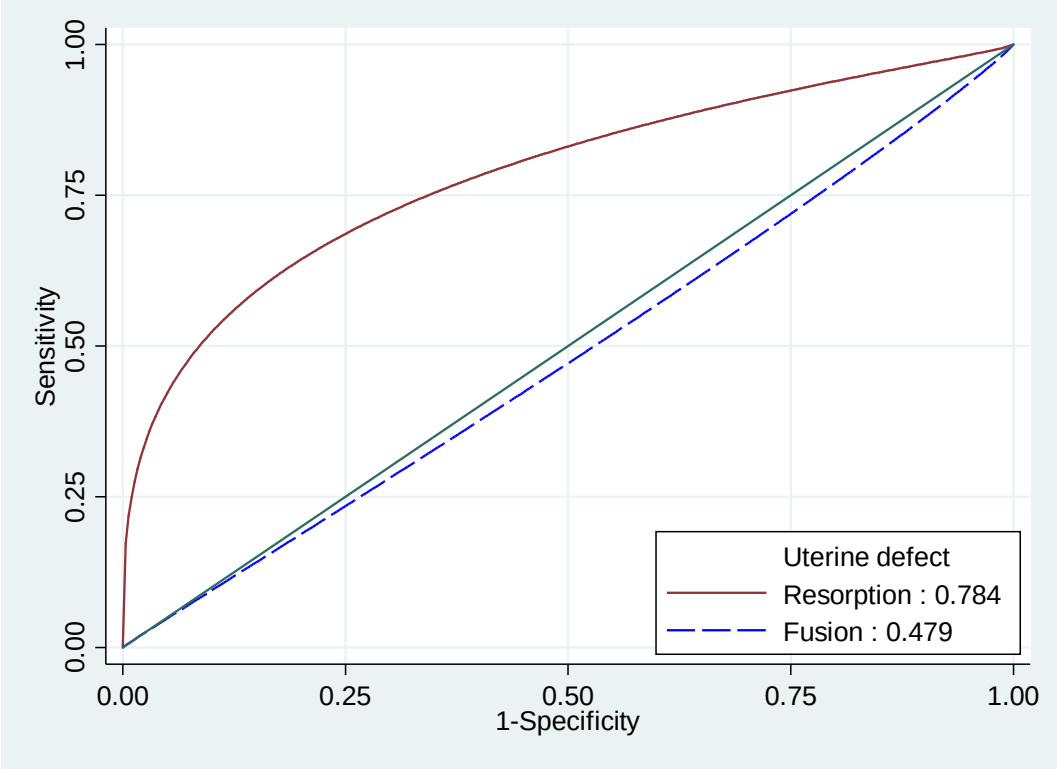
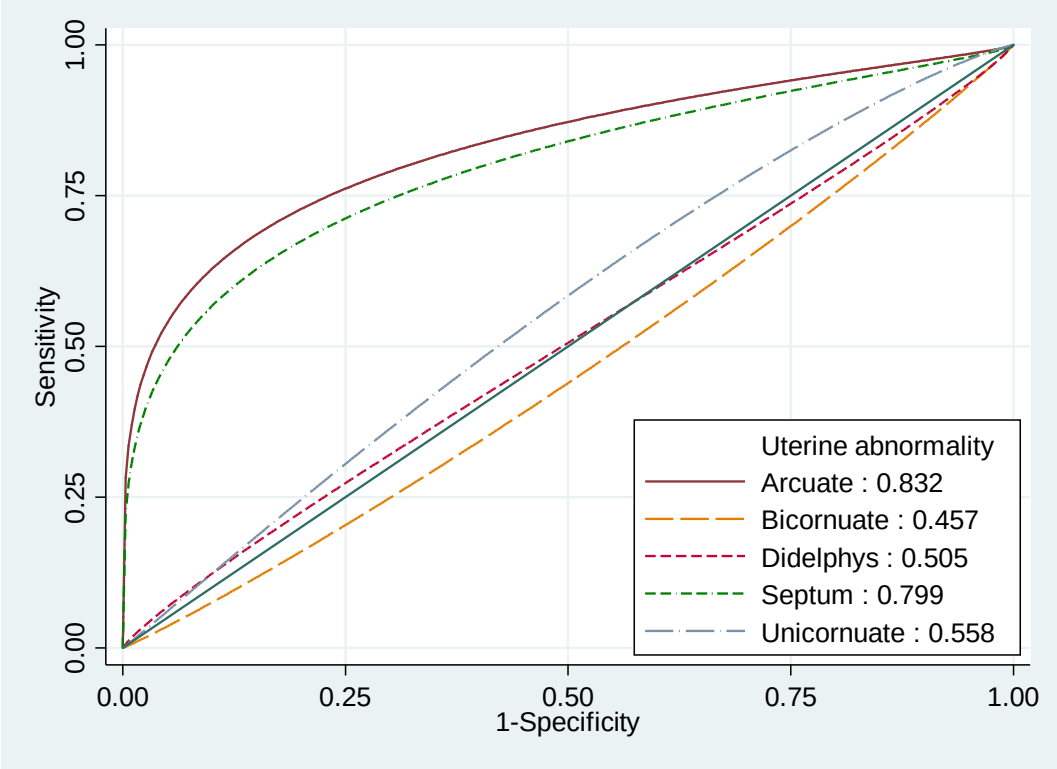


Figure 2: TVUSS CL to predict sPTB <34 weeks by type of CUA defect



**using binomial modeling*

Figure 3: Quantitative fetal fibronectin to predict sPTB <37 weeks grouped by fusion or resorption defect