

Cervix Length and Relaxin as Predictors of Preterm Birth

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Abstract

Background: Measurement of mid-gestation cervix length has become a common screening tool for preterm birth. Our study was designed to assess the value of cervix length and serum relaxin in the prediction of spontaneous preterm birth at < 35 and < 37 weeks' gestation in a general obstetric population.

Methods: A prospective cohort of women with a singleton pregnancy had blood collected at 24 and 28 weeks' gestation for determination of serum relaxin immediately before transvaginal ultrasound measurement of cervical length. Patients and referring physicians were blinded to cervix length and relaxin levels to preclude changes in management. The primary outcomes were spontaneous delivery at < 35 and < 37 weeks' gestation.

Results: A total of 1004 women entered the study. Delivery data were not available for 20 women who delivered elsewhere. Twenty women were excluded because preterm delivery was undertaken because of pregnancy abnormalities. Of the 964 women with known gestational age at delivery, 46 (4.8%) delivered at < 37 weeks and 16 (1.7%) at < 35 weeks' gestation. Mean cervix length at 28 weeks (36.7 ± 7.3 mm) was significantly shorter than at 24 weeks (37.8 ± 7.1 mm) ($P < 0.001$). Cervix length at 24 and 28 weeks' gestation was equally predictive of preterm birth. A cervix length of ≤ 30 mm at 28 weeks had a sensitivity of 57.1%, a specificity of 81.8%, and a positive predictive value of 4.5% for birth at < 35 weeks. Serum relaxin levels were not correlated with cervix length at either 24 or 28 weeks. Serum relaxin at 24 and 28 weeks' gestation was not associated with preterm birth before or after controlling for patient characteristics and cervix length.

Conclusion: Serum relaxin levels at 24 and 28 weeks' gestation are not associated with preterm birth. Although cervix length is associated with preterm birth, its positive predictive value is low. Given the lack of proven therapies for those at risk, cervix length does not appear to be a useful screening tool for preterm delivery in the general obstetric population.

Résumé

Contexte : La mesure de la longueur cervicale à mi-gestation est devenu un outil de dépistage courant en ce qui concerne l'accouchement préterme. Notre étude a été conçue de façon à

évaluer la valeur de la longueur cervicale et de la relaxine sérique, pour ce qui est de la prévision d'un accouchement préterme spontané avant la 35^e semaine de gestation et avant la 37^e semaine de gestation au sein d'une population obstétricale générale.

Méthodes : Du sang a été prélevé entre la 24^e et la 28^e semaine de gestation, chez une cohorte prospective de femmes connaissant une grossesse monofoetale, aux fins de la détermination du taux sérique de relaxine immédiatement avant la mesure de la longueur cervicale par échographie transvaginale. La longueur cervicale et les taux de relaxine n'ont pas été révélés aux patientes et aux médecins traitants afin d'éviter les modifications de la prise en charge. L'accouchement spontané avant la 35^e semaine de gestation et l'accouchement spontané avant la 37^e semaine de gestation constituaient les critères d'évaluation principaux.

Résultats : Au total, 1 004 femmes ont participé à l'étude. Des données sur l'accouchement n'étaient pas disponibles dans le cas de 20 femmes ayant accouché ailleurs. Vingt femmes ont été exclues puisque, dans leur cas, l'accouchement préterme a été mis en œuvre en raison de la présence d'anomalies de grossesse. Parmi les 964 femmes dont l'âge gestationnel du fœtus était connu au moment de l'accouchement, 46 (4,8 %) ont accouché avant la 37^e semaine de gestation et 16 (1,7 %), avant la 35^e semaine de gestation. La longueur cervicale moyenne à 28 semaines ($36,7 \pm 7,3$ mm) était considérablement moins importante que celle qui a été constatée à 24 semaines ($37,8 \pm 7,1$ mm) ($P < 0,001$). La longueur cervicale à la 24^e semaine et la longueur cervicale à la 28^e semaine de gestation présentaient le même potentiel de prévision de l'accouchement préterme. Une longueur cervicale de ≤ 30 mm à 28 semaines présentait une sensibilité de 57,1 %, une spécificité de 81,8 % et un coefficient de prévision d'un test positif de 4,5 %, en ce qui concerne l'accouchement avant la 35^e semaine de gestation. Les taux sériques de relaxine n'étaient pas en corrélation avec la longueur cervicale, que ce soit à 24 semaines ou à 28 semaines. Les taux sériques de relaxine à 24 semaines et à 28 semaines n'étaient pas associés à l'accouchement préterme, que ce soit avant ou après avoir neutralisé l'effet de la longueur cervicale et des caractéristiques des patientes.

Conclusion : Les taux sériques de relaxine à 24 semaines et à 28 semaines ne sont pas associés à l'accouchement préterme. Bien que la longueur cervicale soit associée à l'accouchement préterme, son coefficient de prévision d'un test positif est faible. Compte tenu de l'absence de traitements éprouvés pouvant être offerts aux femmes exposées au risque, la longueur cervicale ne semble pas être un outil de dépistage utile en ce qui concerne l'accouchement préterme au sein de la population obstétricale générale.

Key Words: Preterm birth, cervix, relaxin

Competing Interests: None declared.

Received on March 29, 2008

Accepted on June 23, 2008

J Obstet Gynaecol Can 2008;30(12):1124–1131

INTRODUCTION

The prediction of preterm birth has always been compromised by the lack of a reliable diagnostic test and of interventions proven to delay gestation, both of which would be required for valid screening.¹ Despite these limitations, screening tests for predicting preterm birth, such as measurement of cervix length, have been proposed in response to the significant personal, societal, and economic impact of preterm birth.² It has been our impression that some practitioners have misinterpreted cervix length measurement as a diagnostic tool, leading to interventions without proven benefit, such as cervical cerclage.³ We designed our study to assess the value of cervix length and serum relaxin level as screening tests for preterm birth in a general obstetric population.

METHODS

Between September 1999 and February 2002, women with a singleton pregnancy who planned to deliver at the study site were invited to participate in this prospective trial.

Exclusion criteria included the presence of cervical cerclage, placenta previa, or major fetal anomaly, being the recipient of oocyte donation, and having a multiple gestation, or a lack of previous fetal anatomic assessment. Gestational age was determined using previous ultrasound measurements or menstrual dates. If menstrual dates were unknown, or more than 10 days discordant with ultrasound dates at the 18–20 week anatomical ultrasound assessment, then ultrasound measurements were used.

Gestational age at the time of assessment was 24 ± 1 weeks and 28 ± 1 weeks. A blood sample was collected by venipuncture for serum relaxin determination immediately prior to transvaginal sonographic measurement of the cervix. Serum samples from each patient were stored at -70°C for determination in batch within the same assay. Cervix length was measured using the technique previously described by Iams et al.² Digital examination of the cervix was not performed. With the bladder empty, the 7 MHz 120° intracavitary transducer of a GE Logiq 700 (General Electric, Waukesha, WI) was placed against the cervix. The cervix was viewed along its sagittal axis, ensuring that the triangular area of echodensity at the external os, the endocervical canal, and the V-shaped notch of the internal os were all seen. To ensure no undue pressure against the cervix, the probe was withdrawn until the image blurred and then reapplied until the image was restored. The length of the cervix was measured from the notch of the external os to the notch of the internal os. The process was repeated three times. Ultrasound examinations were performed by RDMS certified sonographers who were trained in the

technique described above prior to patient recruitment. All images were reviewed by a single observer blinded to patient history and results of the serum relaxin assay. The shortest measurement of the cervix showing the described anatomic landmarks was recorded. Intraobserver and interobserver variations were not calculated.

Serum relaxin levels were measured using an enzyme-linked immunosorbent assay. Human recombinant relaxin, primary antibody, and detection antibody were provided by Connetic Corporation (Palo Alto, CA). Serum was diluted five-fold with PBS containing 0.5% bovine serum albumin, 0.05% Tween 20, 0.01% thimersal, and 0.02% goat IgG (Sigma-Aldrich, St. Louis, MO). Human recombinant relaxin (750 pg/mL) was serially diluted in the same buffer with the equivalent of 20 μL male serum/100 μL to generate standards from 750 pg/mL to 11.72 pg/mL. Unknowns and standards were pipetted in triplicate in volumes of 100 μL to 96 well microliter plates (Nunc Maxisorp, Naperville, IL) coated with an affinity purified goat anti-human relaxin antibody and incubated overnight at 4°C . Plates were washed before adding the detection antibody (rabbit anti-human relaxin conjugated to horseradish peroxidase) and incubated at room temperature for four hours. Following washing, TMB microwell peroxidase substrate (Kirkegaard and Perry Labs, Gaithersburg, MD) was added and colour allowed to develop for 10 minutes before the reaction was stopped with 1.0M phosphoric acid. Absorbance was measured at a wavelength of 450 nm with a reference wavelength of 630 nm using a spectrophotometer (Diagnostic Product Corporation, Los Angeles, CA). Unknowns were calculated using Softmax (San Diego, CA). Intra-assay and interassay coefficient of variation was 6.1% and 9.4% respectively.

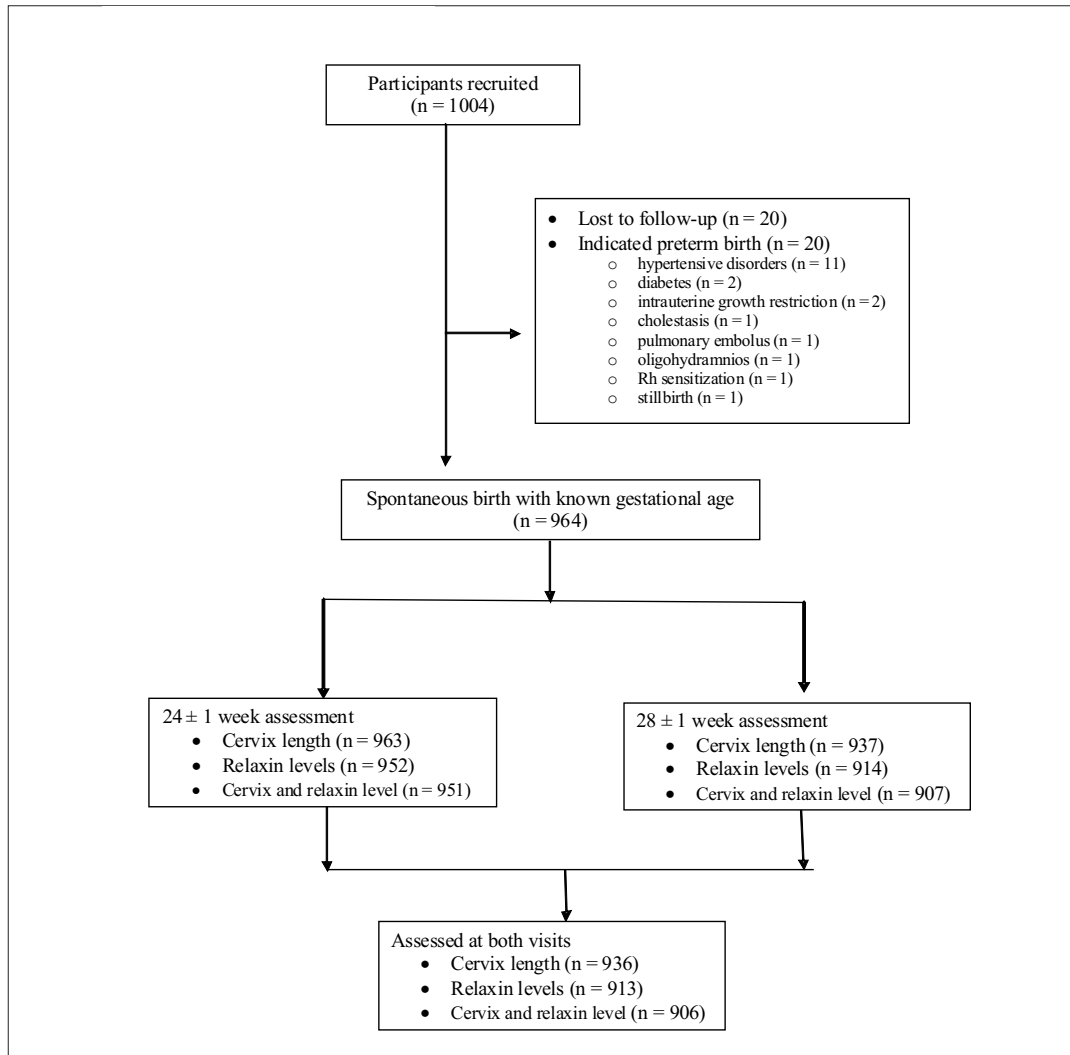
Relaxin samples were assayed as triplicates in batch after all patients were recruited. A standard curve ranging from 12 to 750 pg/mL was constructed using recombinant human relaxin. Assay sensitivity was 5 pg/mL.

In order not to alter clinical management, patients and their physicians were not informed of the cervix length measurements or relaxin assay results.

Sample size calculation identified a requirement for 998 women in order to demonstrate a 10% change in the positive predictive value of cervix length using two marker screening (cervix length and relaxin) versus cervix length alone, assuming an 80% power and alpha of 0.05.

Cervix length was described by means with standard deviations. Due to its strong positive skew, relaxin was described by medians with interquartile ranges. The statistical significance of within subject changes from week 24 to week 28 was assessed by the Wilcoxon signed-rank sum test.

Figure 1. Flow of study participants



Between-group differences were assessed by the Wilcoxon-Mann-Whitney test. The strength of linear associations was assessed using Spearman's correlation.

The primary endpoints of this study were spontaneous preterm birth at < 35 and < 37 weeks' gestation. The relative risk estimate with Fisher exact test was used to compare risk between dichotomous groups. The diagnostic potential of cervix length and relaxin levels was assessed using receiver operating characteristic (ROC) curves. It was decided a priori that the diagnostic accuracy of screening tests based on cervix length < 30 mm, < 25 mm, < 20 mm and relaxin levels above the 90th percentile would be assessed. Logistic regression was used to reassess the multivariate association of preterm birth with cervix length and relaxin after controlling for significant demographic or obstetric history variables.

The study protocol was approved by the Queen's University Health Sciences Research Ethics Board.

RESULTS

A total of 1004 women agreed to participate in the study. Of these, 1003 women underwent examination at 24 weeks' gestation, and 971 women underwent examination at 28 weeks' gestation. The flow of participants is presented in Figure 1. Twenty women did not deliver at the study site and were lost to follow-up. The demographics and obstetric outcome of the participating women are shown in Table 1. One woman who had relaxin and cervix length assessment at 24 weeks underwent cervical cerclage at 25 weeks' gestation when she presented with clinical features of incompetent cervix.

Of the 984 women with known gestational age at delivery, 66 (6.7%) delivered at less than 37 weeks' gestation.

Table 1. Patient demographics and obstetric outcome

	Mean or percent	Range
Age (SD)	28.8 ± 5.3	15–43
Gravida	2.4 ± 1.5	1–18
Parity	0.8 ± 0.9	0–7
Nulliparous	42.8%	
Previous SAB	28.9%	
Previous TAB	15%	
Previous PTB	13.8%	
Previous SPTB	9.2%	
Vaginal bleeding < 12 wks	20.3%	
Vaginal bleeding 12-23 wks	8.3%	
Corticosteroids	1.2%	
Tocolysis	0.2%	
Cervical cerclage	0.1%	

SAB: spontaneous abortion; TAB: therapeutic abortion; PTB: preterm birth; SPTB: spontaneous preterm birth

Thirty-four had spontaneous onset of labour, and 12 had preterm premature rupture of membranes. Twenty women were excluded from the analysis because of planned preterm delivery for the following reasons: hypertensive disorders (11), diabetes (2), intrauterine growth restriction (2), pulmonary embolus (1), Rh sensitization (1), cholestasis (1), oligohydramnios (1) and stillbirth (1). Twenty-three births (2.3%) occurred at less than 35 weeks' gestation; 12 had spontaneous labour and four had preterm premature rupture of membranes.

Cervix length at 28 weeks' gestation (36.7 ± 7.3 mm, range 6–62 mm) was significantly shorter than at 24 weeks (37.8 ± 7.1 mm, range 16–59 mm) ($P < 0.001$). Although women with a cervix length ≤ 30 mm at 24 and 28 weeks' gestation had an increased risk of delivery at < 35 and < 37 weeks' gestation (all $P < 0.05$, except cervix length at 28 weeks' gestation predicting delivery at < 37 weeks' gestation, $P = 0.069$), the diagnostic accuracy of the tests based on cervix length was poor. As shown in Tables 2 and 3, only cervix length measurements ≤ 20 mm are associated with a positive predictive value greater than 20% for preterm birth at < 37 weeks and < 35 weeks, respectively. Cervix length measurements ≤ 20 mm were found in only 0.5% and 1.4% of the study population at 24 and 28 weeks, respectively. Receiver operator curves using cervix length at 24 and 28 weeks' gestation to predict preterm birth at < 35 and < 37 weeks' gestation demonstrate that no cervix length threshold simultaneously provides high sensitivity and specificity (Figure 2). Cervix length decreased more from 24

to 28 weeks' gestation in women who delivered at < 37 weeks than in those who delivered at term, although this trend did not reach statistical significance (-2.7 ± 5.9 vs. -1.1 ± 6.4 mm, $P = 0.12$). Five women delivered at < 32 weeks' gestation. The cervix lengths for these women at 24 weeks' gestation were 16 mm, 24 mm, 44 mm, 46 mm, and 49 mm.

Median (interquartile ranges) serum relaxin levels at 24 weeks' gestation were greater than those at 28 weeks' gestation, (658 [range 398–952] vs. 600 [range 386–895] pmol/L, $P < 0.001$). Relaxin levels did not correlate with cervix length at 24 or 28 weeks' gestation, ($r = -0.04$, $P = 0.22$; $r = -0.01$, $P = 0.79$), and there was no correlation observed between the change in relaxin levels and cervix lengths from 24 to 28 week, ($r = -0.01$, $P = 0.73$). Receiver operator characteristic curves demonstrated that relaxin levels at either gestation did not predict preterm birth (Figure 3). Subjects with relaxin levels above the 90th percentile at 24 and 28 weeks did not have a significantly higher risk of birth at < 37 weeks (RR 0.87 at 24 weeks, 95% CI 0.32–2.4; and RR 1.4 at 28 weeks, 95% CI 0.62–3.3).

A history of preterm birth was associated with spontaneous birth before 37 weeks' gestation. Patients with a history of preterm birth had a 3.1-fold higher risk of having a spontaneous birth before 37 weeks. No other variable listed in Table 1 independently predicted preterm birth after controlling for a history of preterm birth. The association between cervix length at 24 and 28 weeks and birth before 37 weeks did not change after controlling for a history of

Table 2. Prediction of birth at < 37 weeks using cervix length

	Cervix length at 24 weeks			Cervix length at 28 weeks		
	≤ 20 mm	≤ 25 mm	≤ 30 mm	≤ 20 mm	≤ 25 mm	≤ 30 mm
n (%)	5 (0.5)	31 (3.2)	142 (14.7)	13 (1.4)	46 (4.9)	176 (18.8)
Sensitivity	2.2	13.0	26.1	7.0	16.3	30.2
Specificity	99.6	97.3	85.8	98.9	95.6	81.8
PPV	20.0	19.4	8.5	23.1	15.2	7.4
NPV	95.3	95.7	95.9	95.7	96.0	96.1
LRP	5.0	4.8	1.8	6.2	3.7	1.7
LRN	0.98	0.89	0.86	0.94	0.88	0.85
RR	4.3	4.5	2.0	5.3	3.8	1.9

PPV positive predictive value; NPV negative predictive value; LRP likelihood ratio positive; LRN likelihood ratio negative; RR relative risk

Table 3. Prediction of birth at < 35 weeks using cervix length

	Cervix length at 24 weeks			Cervix length at 28 weeks		
	≤ 20 mm	≤ 25 mm	≤ 30 mm	≤ 20 mm	≤ 25 mm	≤ 30 mm
n (%)	5 (0.5)	30 (3.2)	142 (14.7)	13 (1.4)	46 (4.9)	176 (18.8)
Sensitivity	6.3	25.0	50.0	7.1	21.4	57.1
Specificity	99.6	97.1	85.9	98.7	95.3	81.8
PPV	20.0	12.9	5.6	7.7	6.5	4.5
NPV	98.4	98.7	99.0	98.6	98.8	99.2
LRP	14.8	8.8	3.5	5.5	4.6	3.1
LRN	0.94	0.77	0.58	0.94	0.82	0.52
RR	12.8	10.0	5.8	5.5	5.3	5.8

PPV positive predictive value; NPV negative predictive value; LRP likelihood ratio positive; LRN likelihood ratio negative; RR relative risk

preterm birth. Serum relaxin at 24 and 28 weeks was modelled as both a binary predictor dichotomized at the 90th percentile and a continuous predictor. No model identified a significant association between relaxin and spontaneous birth at < 37 weeks before or after controlling for a history of preterm birth and/or cervix length. Logistic regression was not performed on birth at < 35 weeks because of its low incidence.

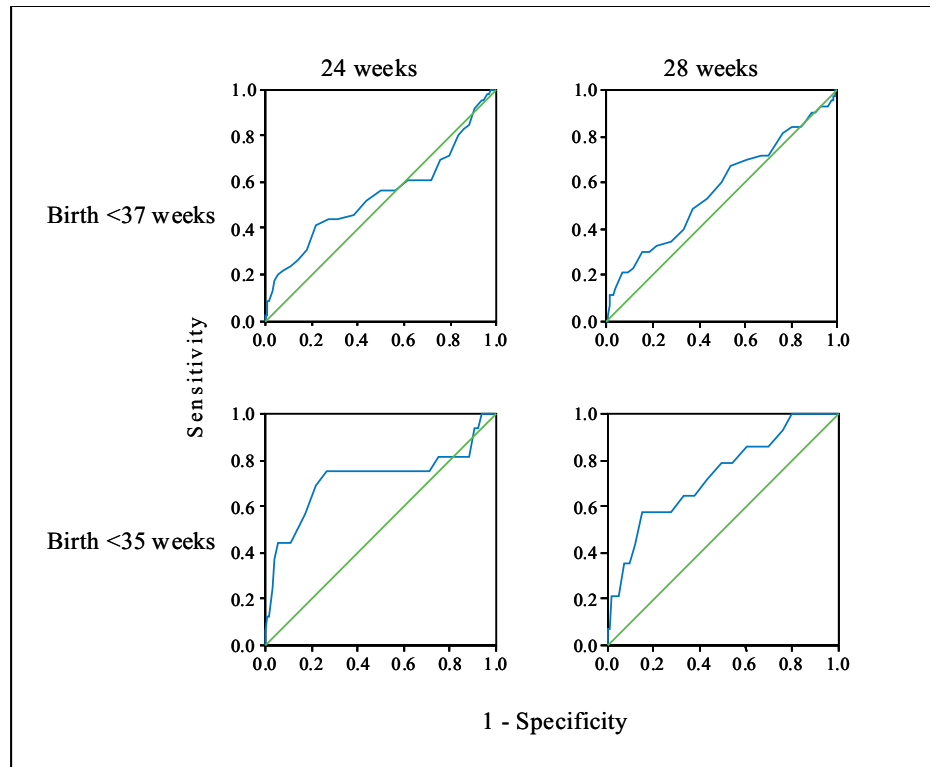
DISCUSSION

Preterm birth after spontaneous labour is a leading cause of perinatal morbidity and mortality associated with significant personal and societal costs. The current rate of preterm birth in Canada is 7.5% (2002),⁴ and in the United States it is 12.3% (2003).⁵ Efforts to reduce the incidence of preterm birth have focused on the identification of risk factors, the creation of screening tests to identify those at highest risk, and the development of therapies for prevention or treatment. The screening tests used for predicting preterm birth

are limited by the absence of a confirmatory diagnostic test, tempting clinicians to implement therapies for all women who screen positive. Therapies such as prolonged bed rest, chronic tocolysis, and cervical cerclage have been used proactively to prevent preterm labour. However, all of these therapies carry risks for both mother and fetus, and none have been shown to reduce the incidence of preterm birth.⁶⁻⁸ The results of recent investigations with chronic progesterone therapy appear encouraging but remain to be confirmed in large populations.⁹ In the absence of a diagnostic test or effective preventative treatment for preterm labour, only the most robust screening tests should be used when considering who should receive therapy.

In 1996, Iams et al. published the first large cohort describing the use of transvaginal cervix length sonography as a screening tool for predicting preterm birth.² They also chose to study a general (mixed risk) population. Their results advanced the concept that the competence of the cervix is on a continuum, rather than being an absolute. Our

Figure 2. ROC curves of cervix length at 24 and 28 weeks' gestation to screen for birth at < 37 and < 35 weeks' gestation



results are consistent with those of Iams et al., and show an increasing risk of preterm birth as the cervix shortens. The statistically significant difference in mean cervix length we noted between 24 and 28 weeks' gestation (1.1 mm) is not expected to be of clinical significance.

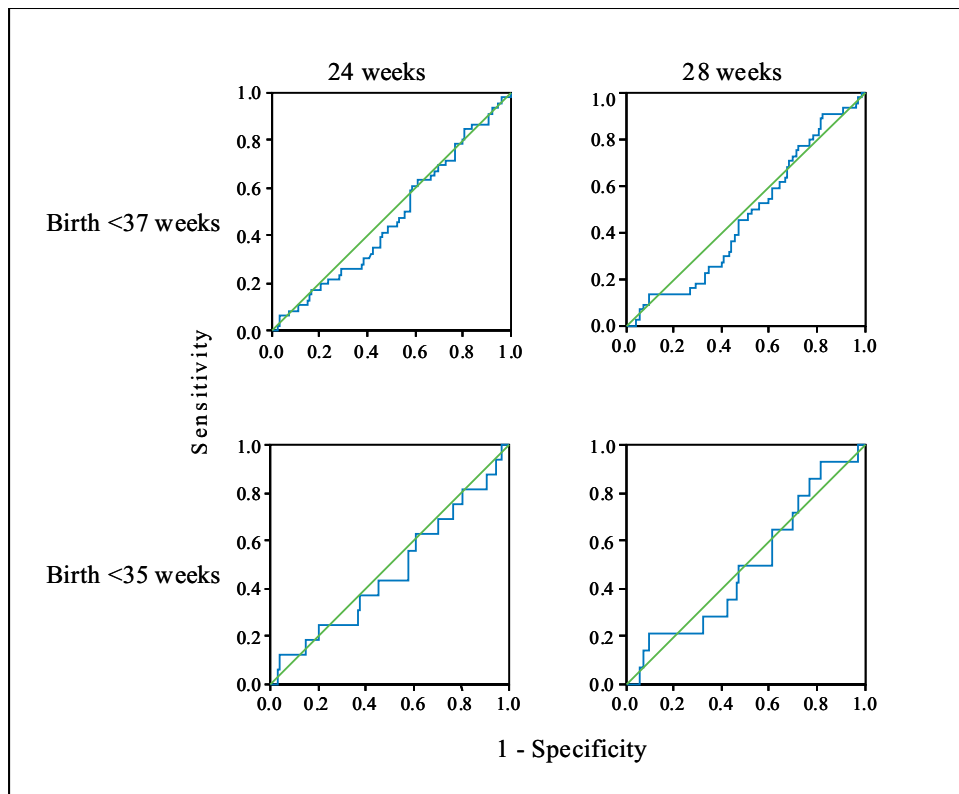
Like ours, the results published by Iams et al. showed that transvaginal cervix length has a low positive predictive value.² Both studies showed that the group of women at highest risk of preterm birth is patients with a cervix length ≤ 20 mm at 24 weeks' gestation. In our study, the positive predictive value for this group of subjects was 20%, and the relative risk for preterm birth < 35 weeks' gestation was 12.8. Although sonographic assessment of cervical length in an unselected population is associated with a less than ideal positive predictive value, it appears to be better than the alternative of digital examination.¹⁰ The population identified sonographically as having a shortened cervix may be best managed with more frequent monitoring to determine if further intervention is required.¹¹ Before considering initiating therapy, clinicians must be aware that 80% of the women in this at-risk group will actually deliver at term.

Iams et al. suggested that sonography to measure transvaginal cervix length could be used to evaluate women with a historical or current risk factor, such as a previous

preterm delivery, to select candidates for clinical trials to evaluate cervical cerclage.² These clinical trials have now been performed and summarized in a meta-analysis.³ Unfortunately, using prophylactic cerclage in the at-risk populations identified by shortening of the cervix as seen on sonography has not reduced the incidence of spontaneous preterm birth.³ This may reflect the screening tools used or the treatment itself. To et al. have been able to improve the prediction of preterm birth by combining information about cervical length with a detailed obstetric history.¹² Future studies evaluating the use of other, more successful, therapies may help to clarify the true value of transvaginal cervix length sonography as a screening tool. The poor performance of transvaginal cervix length sonography in our general population should make clinicians cautious about routinely performing cervix length screening for fear of unnecessarily exposing women to the risks of therapies to prevent preterm birth.

Relaxin, an ovarian hormone, was first described some 70 years ago as causing separation and relaxation in the pregnant guinea pig pubic symphysis. This "relaxation" is actually the dissolution of the intrapubic ligament.^{13,14} For the first 60 years after relaxin was discovered, it could be measured only by biological assays. More recently, both a radioimmunoassay and an enzyme-linked immunosorbent

Figure 3. ROC curves of relaxin levels at 24 and 28 weeks' gestation to screen for birth at < 37 and < 35 weeks' gestation



assay have been developed for the measurement of relaxin.^{15,16} Circulating relaxin is solely a product of the corpus luteum.¹⁷ Relaxin can be detected in serum from the time of the first missed menses.¹⁵ Serum relaxin levels are highest in the first trimester, reaching a peak between the 8th and 12th week of pregnancy in the range of 1000–1200 pg/mL^{8,19} and gradually decreasing through the third trimester.^{15,20} Relaxin concentrations are stable throughout labour but decrease post partum to below the sensitivity of the assay in approximately three days.¹⁷

The role of relaxin in human pregnancy has not been well established. Unlike in many animal species, there is no clear pre-labour surge in humans.²¹ Diurnal variability in relaxin secretion has not been observed.²² In contrast, many rodent and other mammalian species experience a surge in serum relaxin approximately 24 hours before the onset of labour, resulting in shortening, softening, and opening of the cervix.²³ Pregnant rats that are deprived of their relaxin-secreting ovaries but given progesterone will labour but not deliver because of cervical rigidity.²⁴ Circulating relaxin is not essential for human pregnancy or delivery; women who become pregnant after egg donation have no detectable circulating relaxin, but they labour and deliver spontaneously.¹⁷ Although a antepartum rise in relaxin levels has not yet been reported in the human, it is postulated

that one of the main roles of relaxin is to facilitate connective tissue remodelling during gestation. In vitro studies on human fibroblasts exposed to relaxin have shown a decrease in collagen synthesis and an increase in collagen breakdown.^{25,26} Exogenous porcine relaxin has been used to enhance cervical ripening in humans.^{27,28}

To date, two conditions have been associated with increasing circulating relaxin levels: these are multiple gestation and ovarian hyperstimulation after ovulation induction.¹⁹ An association has been noted between risk for preterm delivery and relaxin concentrations between six and 12 weeks' gestation in women who have undergone ovarian stimulation for ovulation induction.²⁹ This risk increased with the serum level of relaxin and compounded the increased risk in cases of greater fetal number. In contrast to our findings in singleton pregnancies, Iams et al. found an association between relaxin levels at 24 weeks' gestation and risk of preterm birth at < 37 weeks, < 35 weeks, and < 32 weeks in twin gestations.³⁰ Consistent with our results, they found that relaxin levels did not correlate with transvaginal cervix length measurements. Our results confirm that assessments of relaxin levels should not be used as a screening tool for preterm birth in singleton gestations.

CONCLUSION

We have identified that serum relaxin levels at 24 and 28 weeks' gestation do not play a clinical role in the prediction of preterm birth. In our unselected population, the use of cervical length sonography to predict preterm birth was associated with a poor positive predictive value, although the likelihood of preterm birth is increased as cervical length shortens. The majority of women who deliver prematurely do not have a history of previous preterm birth. If progesterone therapy is proven to reduce preterm birth in women at risk, researchers should consider the role that cervical length measurement may play in identifying women from the general population who should be treated prophylactically with progesterone.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Physicians' Services Incorporated Foundation.

The authors wish to express their appreciation for the assistance of the sonographers and staff of the Kingston General Hospital Fetal Assessment Unit.

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