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S-relaxin as a predictor of preterm delivery in women with symptoms of preterm labour

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Objective To evaluate whether serum relaxin (S-relaxin) can predict spontaneous delivery before 34 weeks of gestation in high risk pregnancies.

Design A prospective cohort study.

Setting Calculated sample size was reached over a two-year period, during which 9507 women gave birth. Of these, 157 healthy women were eligible for the study as they were admitted with symptoms of delivery before 34 weeks of gestation. Ninety-three women were included. Overall participation rate was 59%.

Population Healthy women with singleton pregnancies with symptoms of delivery before 34 weeks of gestation.

Methods S-relaxin was measured using a standard sandwich ELISA.

Main outcome measures End points were preterm delivery before 34 weeks of gestation and delivery within three days from initiation of symptoms. The best possible prediction of preterm delivery was established using logistic regression for risk factors individually associated with preterm delivery before 34 weeks of gestation. S-relaxin was dichotomised to obtain best possible fit and then entered into the model. The same analyses were done for delivery within three days.

Results Median S-relaxin levels varied significantly in the women with preterm prelabour rupture of membranes (PPROM) (316 pg/mL), contractions (222 pg/mL) or ripe cervices (203 pg/mL) ($P < 0.05$). S-relaxin above the 80th centile ($\geq$300 pg/mL) was associated with an increased risk of preterm delivery [crude OR = 4.8; (95% CI: 1.9–12)]. Likelihood ratio of a positive test is 2.6 (1.5–4.9) and S-relaxin resulted in a post-test probability of preterm delivery of 0.72, compared with a pre-test probability of 0.49. S-relaxin contributed to the identification of delivery within three days [adj. OR = 11 (95% CI: 1.8–64)].

Conclusion S-relaxin may be a useful predictor in women with symptoms of delivery before 34 weeks of gestation.

INTRODUCTION

Preterm birth is the leading cause of neonatal morbidity and mortality. However, many women who develop symptoms of preterm labour [preterm contractions, preterm prelabour rupture of membranes (PPROM) or cervical ripening] do not deliver preterm. Clinically, it would be useful to be able to predict who will deliver preterm. Thereby treatment and care could be intensified in the high risk group, while the low risk group could be spared excessive treatment, long hospital stays and, not the least, the worry of delivering an immature baby.

Serum relaxin (S-relaxin) concentrations in normal pregnant women increase until 10–12 weeks of gestation, gradually decrease from the 12th to the 24th week and then remain constant for the remainder of the pregnancy[1]. S-relaxin shows no diurnal variation or prelabour surge[1]. Raised S-relaxin levels between the 18th and 30th week are useful in the prediction of preterm delivery in low risk pregnancies[2,3]. Further, raised first trimester S-relaxin concentrations in pregnant women following ovarian stimulation predict immaturity risk and preterm delivery[4].

In a number of species, relaxin has profound effects on the connective tissue in the reproductive tract, including induction of cervical softening and loosening of pelvic ligaments[5]. Furthermore, relaxin weakens human fetal membranes *in vitro*[6], and relaxin increases interstitial collagenase or matrix metalloproteinase-1 (MMP-1) in both fetal membranes[7] and the cervix[8]. Relaxin inhibits myometrial contractions in a number of species, but

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neither physiologic nor pharmacologic concentrations of relaxin influence contractions of the human myometrium[9,10]. It is possible that the changes in the human cervix and the fetal membranes throughout pregnancy could be part of a controlled ripening, preparing the genital tract for vaginal delivery. Thus, raised S-relaxin may thereby cause an accelerated ripening, resulting in preterm delivery.

Our hypothesis for this study was that S-relaxin might signal or cause preterm delivery in women with symptoms of delivery. We therefore conducted a prospective cohort study of women with symptoms of preterm delivery to evaluate S-relaxin as a predictor of preterm delivery and delivery within three days.

METHODS

The study took place at the Department of Obstetrics and Gynaecology, Aarhus University Hospital, Denmark, where every pregnant woman is asked to complete self-administered questionnaires containing basic demographic information and medical history. Further, the attending midwife at the time of delivery completes a brief form concerning the clinical aspects of the delivery and the course of the pregnancy.

The study started in April 1997 and closed in April 1999, as the calculated sample size was accomplished[3]. During these two years, 9507 women gave birth at the department. One hundred fifty-seven women with singleton pregnancies were eligible for the study as they had spontaneous contractions, PPROM or cervical ripening between 24 + 0 and 33 + 6 weeks of gestation. Of these, 93 women were included in the study, giving an overall participation rate of 59%. The remaining were not included, because they declined to participate ($n = 5$), delivered at home ($n = 1$), to quickly for sampling ($n = 1$) or inclusion of the patient into the project was forgotten by staff ($n = 57$). These women did not differ significantly from the participants in any respect examined [age, body mass index (BMI), previous pregnancies, parity, smoking, caesarean section rate, *in vitro* fertilisation rate, education or gestational age at delivery]. Thus, the study base consisted of 93 women with singleton pregnancies; 46 delivered before 34 + 0 weeks and 47 after 34 + 0 weeks. Women with elevated blood pressure, major medical disease, vaginal haemorrhage or multiple gestations were not included.

Serum samples were obtained at enrolment (range: 24 + 0 to 33 + 6 days). All women gave written informed consent as required by the Regional Scientific-Ethics Committee of Aarhus County. The Danish Data Surveillance Authority approved data collection, in accordance with their standards for multipurpose register studies.

Gestational age and estimated date of delivery were calculated from the last menstrual period adjusted to a cycle length of 28 days according to Naegele's rule, and verified by ultrasound scanning (fetal biparietal diameter) before 21 completed weeks of gestation.

A priori, preterm delivery was defined as delivery before 34 weeks of gestation. PPROM was defined as rupture of membranes before contractions before 34 weeks of gestation. Cervical ripening was defined as progression of cervical status between two digital examinations without PPROM or contractions before 34 weeks of gestation. Previous normal pregnancy and delivery was defined as a term delivery of a living child in a previous pregnancy. Low maternal weight was defined as a pre-pregnancy BMI below 19, normal weight as BMI from 19 to 25 and overweight as BMI above 25. All above definitions were defined before initiation of the study.

Duration from initiation of symptoms to delivery was analysed as a continuous variable, or dichotomised into delivery within or after three days. This cutoff was selected during data analysis because it could separate the deliveries, where induction of lung maturation had or had not been possible.

S-relaxin was dichotomised into raised ($\geq$300 pg/mL) or normal relaxin levels ($<$300 pg/mL), using receiver operating characteristic curves (ROC curves) to obtain the best fit. The area under the ROC curve was established using both the SPSS statistical analysis package and the method described by Hanley and McNeal[11].

S-relaxin levels were measured using a commercially available ELISA (Immundiagnostik, Bensheim, Germany), in which the primary antibody was polyclonal rabbit anti-human relaxin-2, and the secondary antibody was biotinylated rabbit anti-human relaxin-2, and relaxin was quantified by horseradish peroxidase conversion of $3,3',5,5'$ tetramethylbenzidine. The ELISA measured 2–250 pg/mL. The inter-assay coefficient of variation was 21% and 10% for 15 and 152 pg/mL relaxin, respectively, and the intra-assay coefficient of variation was 10% for 15 pg/mL relaxin. All samples were blinded for the analyst and measured simultaneously. Samples above 250 pg/mL were diluted and re-analysed. In-house data demonstrated linear dilution of samples with high concentrations of relaxin.

χ^2 and odds ratios were obtained for data in categories. Mann–Whitney or Kruskal–Wallis tests were used to compare means of continuous variables and correlation coefficients (Spearman) to describe relationships of continuous variables. P values below 0.05 were considered significant in two-tailed tests. S-relaxin followed a normal distribution only after performing logarithmic transformation, therefore the logarithmically transformed data were used for linear regression analysis.

In order to establish the best possible prediction of delivery before 34 weeks of gestation, a logistic regression model was employed for all risk factors that are individually associated with preterm delivery. The dichotomised S-relaxin was then entered into the model. The same procedure was followed for delivery within three days.

RESULTS

Table 1 shows some of the characteristics of the 93 patients with spontaneous symptoms of delivery: 46 patients had contractions, 35 had PPROM and 12 had cervical ripening diagnosed by digital palpation. A higher proportion of women with cervical ripening, compared with the other groups, had previously had a late miscarriage (χ^2; $P < 0.01$). There were no differences between groups in the gestational age at initiation of symptoms, but there were differences in gestational age at delivery; 63% of the group with contractions delivered at term, whereas none from the PPROM group delivered at term (≥37 weeks). The median level of S-relaxin varied significantly (Kruskal–Wallis; $P < 0.05$) in women with PPROM (316 pg/mL), contractions (222 pg/mL) or ripe cervices (203 pg/mL).

S-relaxin was significantly higher in women delivering before 34 weeks of gestation when compared with deliveries after 34 weeks of gestation (Kruskal–Wallis; $P < 0.01$). The 80th centile (≥300 pg/mL) of S-relaxin of the women delivering at term was established as the optimal cutoff level for predicting preterm delivery before the 34th gestational week using ROC curves. The area under the ROC curve predicting delivery before 34 weeks of gestation was 69% (95% CI: 57–79). When dichotomised, raised S-relaxin (≥300 pg/mL) predicted preterm delivery (Table 2) with a crude odds ratio of 4.8 (95% CI: 1.9–12). Further, the association remained unchanged after stratifying for symptoms initiating delivery (Mantel–Haenszel; OR = 4.8, 95% CI: 1.4–16.2). When including only the non-induced deliveries (n = 60, evenly distributed among groups), the association between raised S-relaxin and preterm delivery increased (OR = 8.6, 95% CI: 1.3–59).

Table 2. S-relaxin (≥300 pg/mL) as a single predictor of spontaneous preterm delivery before 34 weeks of gestation (n = 93). LR = likelihood ratio (sensitivity/1−specificity); aetiological fraction = population attributable risk percentage = excess fraction.

	Raised S-relaxin (95% CI)
Crude odds ratio	4.8 (1.9–12)
Sensitivity (%)	58 (43–79)
Specificity (%)	78 (68–90)
Positive predictive value (%)	72 (68–90)
Negative predictive value (%)	65 (51–81)
LR of positive test	2.6 (1.5–4.9)
LR of negative test	0.5 (0.4–0.8)
Aetiological fraction (%)	30

Twenty out of 57 (35%) women with normal S-relaxin delivered preterm, whereas 26 out of 36 (72%) with raised S-relaxin levels delivered preterm as shown in Fig. 1a. Fig. 1b shows that in the women with contractions, four out of 32 (13%) women with normal S-relaxin delivered preterm, whereas six out of 14 (43%) women with raised S-relaxin levels delivered preterm.

The authors first established the best possible model for predicting delivery based on the available risk factors (BMI, smoking, previous preterm delivery, parity, not having had a previous normal pregnancy and delivery, abortions, miscarriage, vaginal bleeding, pelvic pain, pelvic instability, social economic status, educational level, work hours, years of infertility, treatment for infertility, interval between pregnancies). Using a logistic regression model, we found that the following were predictors of preterm delivery before 34 weeks of gestation: symptoms initiating delivery (PPROM, contractions or ripe cervices), overweight (BMI >25) and not having had a previous normal

Table 1. Characteristics of the women with preterm contractions, PPROM or preterm cervical ripening. Values are median (range) or count [percentages]. GA = gestational age in days; PPROM = preterm prelabour rupture of membranes (<34 weeks).

	Contractions	PPROM	Ripe cervix
Number	46 [49]	35 [38]	12 [13]
GA at symptoms	217 (73)	214 (69)	207 (62)
S-relaxin (pg/ml)‡	222 (884)	316 (1285)	203 (943)
Age	29 (18)	30 (25)	31.5 (10)
Primigravida	18 [39]	11 [31]	2 [17]
Parity	1 (4)	2 (3)	1 (1)
Pregnancies achieved after *in vitro* fertilisation	3 [7]	2 [6]	0
Previous preterm delivery	3 [7]	3 [9]	1 [8]
Previous late miscarriage*	4 [9]	2 [6]	5 [42]
Smoking in pregnancy (any)	18 [39]	16 [46]	1 [8]
GA at delivery‡‡	269.5 (111)	222 (63)	260.5 (116)
Time to delivery (days)**	46 (117)	4 (67)	40.5 (119)
Delivery <28 weeks	3 [6]	6 [17]	3 [25]
Delivery <32 weeks**	6 [13]	20 [57]	4 [33]
Delivery <34 weeks**	10 [22]	32 [91]	4 [33]
Delivery <37 weeks**	17 [37]	35 [100]	6 [50]

* $P < 0.01$, χ^2.
** $P < 0.001$, χ^2.
‡ $P < 0.05$, Kruskal–Wallis.
‡‡ $P < 0.001$, Kruskal–Wallis.

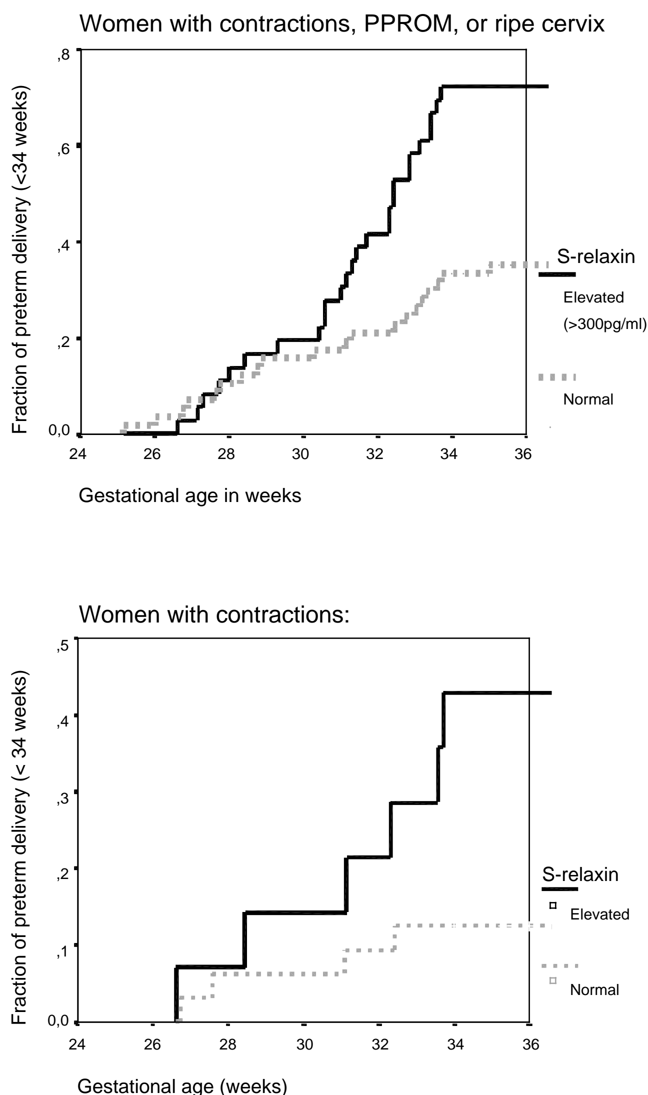

Fig. 1. (a) The fraction of spontaneous preterm delivery (<34 weeks) by raised ($\geq$300 pg/mL) and normal levels of S-relaxin in women with spontaneous symptoms of delivery (n = 93, log rank, P < 0.001). Black full line: raised S-relaxin (n = 36); Gray dotted line: normal S-relaxin (n = 57). (b) The women with contractions (n = 46) separated by S-relaxin (log rank, P < 0.05). Black full line: raised S-relaxin (n = 14); Gray dotted line: normal S-relaxin (n = 32).

pregnancy and delivery (Table 3). These three risk factors could predict 36 out of 46 preterm deliveries (78%). When S-relaxin was entered into the model, 40 out of 46 preterm deliveries could be predicted (87%). The dichotomised S-relaxin level increased the predictive abilities of the test (P = 0.02) significantly, and raised S-relaxin gave an adjusted odds ratio in this model of 5.4 (1.4–21.5).

The best model for prediction of delivery from initiation of symptoms within three days ($\leq$3 days) consisted of symptoms initiating delivery (PPROM, contractions or ripe cervices) and BMI (low, normal, overweight) (Table 3). When S-relaxin was entered into the model, the odds of delivery within three days increased 11 times (95% CI: 1.8–64). Again the association between S-relaxin and the risk of delivery within three days was more pronounced in the non-induced deliveries (OR = 22.7, 95% CI: 2.1–243). S-relaxin was only related to the time to delivery in women with contractions, and changed the prediction of deliveries within three days from four out of 10 based on risk factors alone to five out of 10 on risk factors with S-relaxin. Curiously, S-relaxin alone identified six out of 10 deliveries within three days in women with contractions.

In order to investigate whether relaxin exerted its effects via cervical maturation, the authors studied the cervical status in the women with contractions. Information was only available for 36 out of 46 women, and the cervical status was assessed only on a three-point scale by the attending midwife at admission: no maturation, slight maturation, ripe cervix. We found a correlation between raised relaxin and the cervical maturation (Spearman; P < 0.05). Median S-relaxin levels were 247, 289 and 476 pg/mL in the women with contractions with no maturation (n = 23), slight maturation (n = 11) or ripe cervices (n = 2), respectively (Kruskal–Wallis; NS, P = 0.19).

S-relaxin was raised in 40% of the total number of women, but only in one out of six (17%) of the women with previous preterm delivery (χ^2; NS), suggesting that S-relaxin may not be associated with recurrence of preterm delivery.

The authors found no association between smoking in pregnancy and S-relaxin. S-relaxin tended to be associated with BMI (Spearman; $r = -0.21$, $P = 0.051$), and there was a tendency for raised median S-relaxin levels in pregnancies achieved after *in vitro* fertilisation (397 *vs* 320 pg/mL, Mann–Whitney, P = 0.2). There was a negative association between S-relaxin and previous pregnancies (Spearman; $r = -0.31$, $P < 0.01$) and parity ($r = -0.28$, $P < 0.05$).

Women with raised S-relaxin had fewer birth canal tears (perineum, vagina, labia, cervix and sphincter ani) than women with normal S-relaxin in non-instrumented deliveries (3/33 *vs* 17/53, Pearson: $P < 0.05$; OR = 0.23, 95% CI: 0.06–0.88). This association was confirmed by a logistic regression model adjusting for either gestational age at delivery ($r = 0.15$, $P < 0.05$, adjusted OR = 0.1, 95% CI: 0.01–0.88) or birthweight of the child ($r = 0.14$, $P < 0.05$, adjusted OR = 0.1, 95% CI: 0.01–0.97). In line with these findings, S-relaxin was negatively associated with blood loss estimated by attending midwifes immediately after delivery. Linear regression demonstrated a significantly negative association between S-relaxin and the estimated total blood loss when correcting for differences in gestational age ($r = -0.22$, $P < 0.05$).

DISCUSSION

The authors describe an association between raised S-relaxin levels and preterm delivery in women with contractions, PPROM or ripe cervices (OR = 4.8, 95%

Table 3. Logistic regression model for prediction of spontaneous preterm delivery (<34 weeks) among women with symptoms ($n = 93$). In order to establish the best possible prediction of preterm delivery, a forward logistic regression model was employed for all risk factors that are individually associated with preterm delivery. The dichotomised S-relaxin was then entered into the model.

	Adjusted OR (95% CI)	
	Without S-relaxin	With S-relaxin
PPROM/contractions†	67 (14–336)	70 (13–380)
Ripe cervix/contractions†	1.6 (0.4–7.6)	1.8 (0.3–9.6)
Overweight/not overweight†	6.0 (1.3–28)	8.4 (1.6–44)
No previous normal pregnancy/previous normal pregnancy†	3.9 (1.0–15)	2.6 (0.6–10)
High/normal S-relaxin†	–	5.4 (1.4–21)
The same procedure was followed for delivery within three days		
PPROM/contractions†	5 (1.6–14)	13 (2.9–57)
Ripe cervix/contractions†	0.3 (0.0–2.6)	0.6 (0.1–7.2)
Low weight/normal weight†	0.3 (0.1–1.3)	0.3 (0.1–1.0)
Overweight/normal weight†	5.7 (1.5–21)	8.0 (1.8–36)
High/normal S-relaxin among women in labour†	–	11 (1.8–64)

† Referent category.

CI: 1.9–12). We found that 0.35 of the women with normal S-relaxin delivered before 34 weeks compared with 0.72 of the women with raised S-relaxin. Moreover, after having established the best possible prediction model with the use of risk factors, S-relaxin contributed further to identify the women delivering before 34 weeks of gestation. S-relaxin also improved the identification of the women with contractions who would deliver within three days.

When evaluating S-relaxin as a clinical test[12,13], S-relaxin in this study produced likelihood ratios [2.6 (1.5–4.9)] comparable with fetal fibronectin in vaginal swabs [2.6 (1.8–3.7), pooled data][14]. This study demonstrates S-relaxin to cause a high post-test probability of preterm delivery of 0.72, however, the pre-test probability of preterm delivery in this high risk population was 0.49. So far there is no quick test available for S-relaxin, which clinically is a major drawback in comparison with fetal fibronectin. Moreover, the S-relaxin test also has a high frequency of false positive and negative test results, complicating the result interpretation for the clinician—as seen in other tests[14–16]. In conclusion, the optimal predictor of preterm delivery is not yet found, but S-relaxin may contribute in the diagnosis of preterm delivery in symptomatic women. We find the results on S-relaxin in preterm labour promising enough to warrant larger scale prospective studies to confirm current findings and to examine the ability of S-relaxin to predict preterm delivery in combination with other tests.

S-relaxin was significantly higher among women with PPROM than in women with contractions, which is in accordance with the findings of Petersen *et al.*[3] Moreover, incubation with relaxin decreases the strength of the human fetal membranes[6]. We find no association between raised S-relaxin and delivery within three days in women with PPROM, however, numbers are low ($n = 18$). It is plausible that relaxin could induce PPROM. However, the time to delivery after having developed PPROM is probably heavily linked to the occurrence of infection. Thus, digital vaginal examination significantly decreases the time to delivery when compared with the use of a sterile speculum by increasing the risk of infection[17]. Further, the use of antibiotics can increase the time to delivery in women with PPROM[18]. Earlier examination of S-relaxin could potentially have identified the women at risk of PPROM[3].

In the women with contractions, raised S-relaxin was associated with increased risk of preterm delivery and delivery within three days. In order to investigate whether relaxin exerted its effects via cervical maturation[19–21], we studied the cervical status in the women with contractions. We found a correlation between raised S-relaxin and the degree of cervical maturation. From this, we hypothesise that in pregnant women with raised S-relaxin, contractions can be secondary to a relaxin-induced cervical ripening.

The aetiologic fraction (or population attributable risk percentage, Table 2) was 30%. This calculation is based on two assumptions, that S-relaxin levels of the term deliveries describe a normal range in spite of the episode of symptoms, and that relaxin is a causal factor of preterm delivery.

It is possible that relaxin is just an inert marker, but several findings indicate that it is involved not only in the pathologic process leading to preterm delivery, but also in the physiology of delivery. In several species, relaxin has been able to initiate the softening of the cervix and the pelvis needed for normal vaginal delivery[5]. Relaxin-induced maturation could result from stimulation of collagen breakdown, a decrease in collagen cross links, and a change in synthesis from the collagen-stabilising proteoglycans to the collagen-destabilising proteoglycans.

Incubation with relaxin decreases the strength of the human fetal membranes[6]. The changes in the cervix and fetal membranes throughout pregnancy could be part of a controlled maturation process preparing the genital tract for vaginal delivery, and the imbalance of relaxin may cause accelerated maturation leading to preterm delivery. The hypothesis of relaxin's involvement in the controlled maturation of the genital tract needed for vaginal delivery is supported by the finding of fewer tears and a lesser amount of bleeding in the women with high relaxin levels.

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