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short communication

Biomechanics of fetal membranes – relation with newborn and maternal anthropometric data

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Abstract

Background and purpose: *Cyclic biaxial burst test is a novel approach in biomechanical testing of fetal membranes. The purpose of the study is to determine are there differences between cyclic biaxial burst test and standard burst test. Relations between newborn and maternal anthropometric data and membrane biomechanics were investigated.*

Materials and methods: *Thirty-nine fetal membranes were tested. Each membrane was cut in half acquiring one half closer to placenta and one closer to cervix. Samples for tensile, biaxial cyclic and standard tests were taken from each. Maternal and newborn anthropometric data was examined.*

Results: *Cyclic burst pressures were significantly higher than standard burst pressures in samples closer to placenta (83.8 ± 19.4 vs 69.3 ± 17.9 mmHg; $p<0.001$) and cervix (67.9 ± 23.4 vs 58.1 ± 15.8 mmHg; $p=0.013$). When compared according to type of delivery, cyclic pressures were higher than standard in c-section samples (80.6 ± 22.4 vs 59.1 ± 14.8 mmHg; $p<0.001$) and vaginally delivered samples (73.5 ± 22.7 vs 65.9 ± 18.6 mmHg; $p=0.017$). Positive correlation between pregnancy BMI and uniaxial test rupture force was established.*

Conclusions: *Cyclic biaxial test is closer to physiological conditions and tests membrane viscoelastic properties better than standard test. A positive correlation between maternal pregnancy BMI and uniaxial force-to-rupture may indicate a connection between maternal nutritional status and fetal membrane strength.*

INTRODUCTION

Fetal membrane is a structure enveloping fetus and amniotic fluid. It's structural integrity is vital for normal pregnancy. The intact fetal membrane is composed of two layers – amnion and choriodecidua, choriodecidua being thicker and cellular *(1)*. Although amnion layer accounts for only around 20% of fetal membrane thickness, said layer determines the biomechanical properties of the intact fetal membrane *(1)*. Biomechanical properties of fetal membranes have been previously investigated mostly in relation to their histological and biochemical properties *(2–6)*. Although such studies provided certain insight in the properties of fetal membranes, there is a limited number of published studies examining biomechanical properties of fetal membranes in relation to maternal and newborn anthropometric data *(7)*. Furthermore, our group has developed an apparatus for biaxial burst test which we are reporting.

MATERIALS AND METHODS

Samples

The samples were collected at the Gynecology and obstetrics clinic of the Osijek Clinical hospital center with the permission of the Institutional Ethics Board (IEB). Thirty-nine fetal membranes (13 caesarian sections and 26 vaginal deliveries) were obtained and were subjected to uniaxial tensile and biaxial rupture tests. Fetal membranes were collected from women after normal term (37–42 weeks) pregnancies. There were 20 male (51 %) and 19 female (49 %) babies. We also collected anthropometric data from women who delivered said membranes and newborns. Informed consent was obtained from all individual participants included in the study. The exclusion criteria were multiple gestation, signs of chorioamnionitis (maternal fever, uterine tenderness, purulent amniotic fluid or vaginal discharge, maternal leukocytosis), known pre-gestational diabetes, severe chronic disease (autoimmune, cardiac, renal, lung or gastrointestinal) and pregnancies with fetal malformations. There were no special conditions during pregnancy – food supplementation other than folic acid, mothers were neither addicts nor on medication. No prolonged periods of fasting were reported.

Membrane sampling protocol

All membranes were tested within 12 hours of delivery to allow them to recover. Initial processing for every membrane was carried out by a single gynecologist following written protocol. Every membrane was washed in cold saline and the clots removed. Each membrane was cut in half relative to the placental disc and each half was placed in a sample container with saline and kept at 4°C until the testing. That way we acquired two larger samples for each membrane, one closer to the placental disc and one overlying the cervical portion of the uterus. From each half two circular samples were taken for biaxial testing (70 mm in diameter) and one test strip for uniaxial test (10 x 30 mm). One sample underwent biaxial burst test which we call 'dynamic' because it consists of cyclic strains mimicking uterine contractions and the other underwent typical burst test which we call 'static' since there are no cycles. Said samples were taken using special knifes designed at the Faculty of mechanical engineering in Slavonski Brod. The samples that underwent biaxial testing were additionally cut to obtain strips (10x30 mm) for uniaxial testing after the biaxial tests. Combined chorion and amnion thickness and amnion thickness were measured for each half of the membrane. All samples were kept hydrated with saline during cutting.

Maternal and newborn data

Data on parity, gestation, height, weight gain and weight prior to delivery were collected. BMI was calculated from said data. Newborn birth weight and length was also measured.

Testing equipment

1.Uniaxial testing equipment

The uniaxial testing equipment was designed and manufactured in association with Faculty of mechanical engineering in Slavonski Brod (Fig. 1). The machine was equipped with specially constructed clamps for sample mounting that prevented the samples from slipping out during the test. The machine has a wide speed range (1 – 100 mm/min). Our samples were stretched at the speed of 25.4 mm/min which was also used in previous studies (8,9). The data from the machine was collected and analyzed via software that was provided with digital sensor. The machine was independently tested at the Laboratory for Testing Mechanical Properties and it complies to accuracy class 2 according to HRN EN ISO 7500-1:2007 standard. That way we ensured the accuracy and reproducibility of our results.

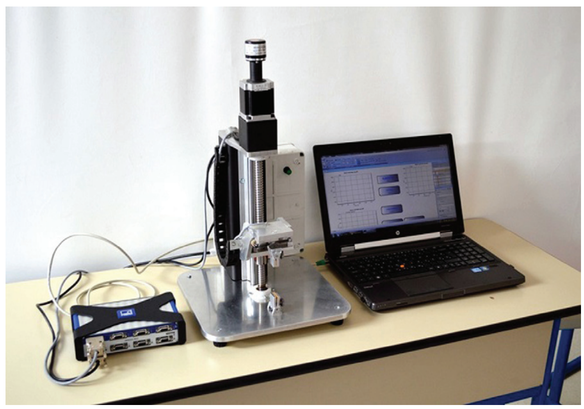

Figure 1 *Uniaxial testing equipment*

2. Biaxial testing equipment

The equipment for biaxial tests was also designed and manufactured in association with Faculty of mechanical engineering in Slavonski Brod (Fig. 2). The machine was equipped with a cylinder upon which circular samples were fitted using specially designed clamp. The burst test was performed using the water pressure created by a stepping motor. The pressure was measured via tube connecting manometer (Dongguan Xintai Instrument Co, mod. HT-1890, CE certified) with the tubes leading from the cylinder to one larger tube used as outlet. The stepping motor came with software that allowed us to control burst test speed and program the motor for cycles (10). Each sample for dynamic test was exposed to 7 simulated contractions after which the sample was inflated until burst.

Sample mounting and handling

As is previously described, the handling of the membranes due to their properties is a big issue. The membranes tend to twist and break quite easily. For uniaxial test,

Figure 2 *Biaxial burst test equipment*

we wrapped the strip's ends with filter paper to prevent the strip from twisting (4,9,11). Furthermore, such handling alleviated the problems with sample fixation between clamps. For the biaxial tests we transferred the samples from the rubber on which they were cut to a flexible plastic from which the samples were mounted on the test cylinder by letting them slide since the samples were well hydrated. That way we prevented the samples from twisting and minimized the chances of breaking.

Statistical analysis

Values are presented as mean (SD). Normality of distribution was tested with Shapiro – Wilks test. Biaxial dynamic and static rupture pressures were compared using one sample t test. Correlation analysis were done using Pearson's correlations. Forward stepwise regression models for prediction of biaxial dynamic and static rupture pressures were created using localization, thickness of chorion and amnion, number of deliveries and abortions, length of gestation and anthropometric measures as predictor variables. A two-sided $p < 0.05$ was considered significant. IBM SPSS Statistics v15.0 was used for statistical analyses.

RESULTS

Our results show moderate positive correlation between BMI and uniaxial force needed to rupture the samples ($r = 0.396$, $p=0.01$) (Fig. 3).

We have also observed weak positive correlation between newborn length at birth and burst pressures in standard biaxial test ($r = 0.253$, $p=0.031$).

Naturally delivered samples closer to placenta had significantly higher biaxial static burst pressures than c-section delivered samples closer to placenta (60.3 vs 73.8; $p=0.024$).

Biaxial dynamic burst pressures in placental region were significantly higher than biaxial static burst pressures (83.8 vs. 69.3; $p<0.001$). Similarly, biaxial dynamic burst pressures of samples overlying the cervix were significantly higher than biaxial static burst pressures (67.9 vs. 58.1; $p=0.013$). When compared according to type of delivery, c-section (80.6 vs. 59.1; $p<0.001$) and vaginally (73.5 vs 65.9; $p=0.017$) delivered samples had significantly higher biaxial dynamic burst pressures than biaxial static burst pressures (Table 1).

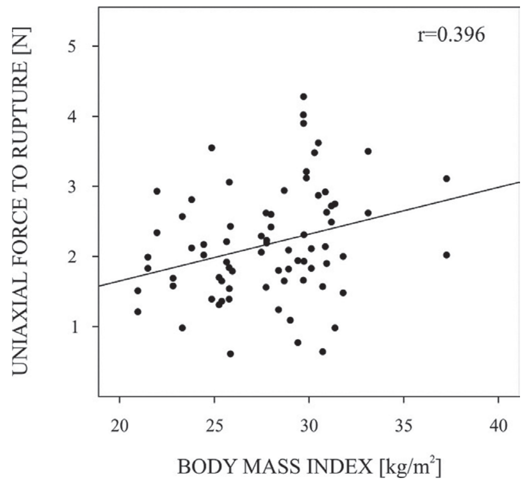

Figure 3 *Correlation between tensile rupture force and BMI*

Table 1 *Dynamic burst pressures vs static burst press ures*

Table 1	Dynamic test (mmHg)	Static test (mmHg)	Significance
Placental region	83.8 (19.4)	69.3 (17.9)	p<0.001
Cervical region	67.9 (23.4)	58.1 (15.8)	p=0.013
C-section delivery	80.6 (22.4)	59.1 (14.8)	p<0.001
Vaginal delivery	73.5 (22.7)	65.9 (18.6)	p=0.017

Regression model for biaxial dynamic pressures was able to explain 21.8% of variance, the only significant predictor variables that were included in the final model were sample location (closer to placenta or cervix), gestatation and number of deliveries. Placental sample localization was shown to increase biaxial dynamic pressures and it was the most important predictor variable, while gestation and number of deliveries decreased biaxial dynamic pressures (Table 2).

Table 2 *Regression model for biaxial dynamic pressures*

Table 2	β	p	Importance
Localization (placenta/cervix)	15.35	0.001	0.451
Gestation	–5.05	0.001	0.277
Number of Deliveries	–12.08	0.011	0.272

With same predictor variables, regression model for biaxial static pressures was able to explain only 8.8% of variance. The only included predictor variable in the final model was sample localization (coefficient 11.18, p<0.001).

Biaxial static burst pressures between placental and cervical localizations in vaginal deliveries were significantly higher (73.87 ± 15.94 vs 58.3 ± 18.14, p= 0.002).

Regression model for uniaxial force-to-rupture was able to explain 24.4% of variance, significant predictor variables were BMI, gestation and body weight before pregnancy, while weight gain, delivery, number of deliveries, newborn birth weight and abortions were not included into the final model. Uniaxial force increased with increasing BMI, while gestation and body weight before pregnancy deacreased uniaxial force (Table 3).

Table 3 *Regression model for uniaxial force-to-rupture*

Table 3	β	p	Importance
BMI	0.123	<0.001	0.578
Gestation	–0.196	0.006	0.257
Body weight before pregnancy	–0.022	0.025	0.164

DISCUSSION

Biaxial tests in most previous studies used simple burst techniques, meaning straining samples continuously until rupture (12–14). A newer version of biaxial test, the puncture study, was introduced due to difficulties with biaxial burst tests – the equipment is large and requires fairly large samples (4,15). Puncture test also produces biaxial strain on the membrane and since Schober correlated the results of traditional burst test with puncture test, the puncture test became acceptable (16). In our study we used cyclic biaxial straining of the membranes and compared it to standard biaxial burst test. As our results show, biaxial dynamic tests provide significantly higher burst pressures than biaxial static tests. The fact that static burst pressures in vaginal deliveries are higher than those in c-section group could be attributed to membrane ´priming´. Vaginally delivered membranes were already subjected to strain caused by labor therefore priming the collagen fibers that are essential for biomechanical properties of the membranes. Priming would be gradual recruitment of collagen fibers in amnion and their straightening as described by Joyce et al. thus enabling vaginally delivered membranes to withstand higher pressures (17). Furthermore, our results indicate that the chorion and amnion thickness, number of deliveries, abortions and anthropometric measures are much better predictors of dynamic than static burst pressures. The fact that dynamic biaxial test shows higher burst pressures is in accordance with established viscoelastic properties of the membranes. Furthermore, regression model for biaxial dynamic test was able to explain variance significantly better than the model for biaxial static test using the same predictor variables. The fact that we confirmed that fetal membranes from term vaginal deliveries are weaker in the cervical area than those closer to the placenta, which is in accordance with previous biomechanical studies (18–20), indicates that biaxial dynamic test performed on our apparatus can successfully reproduce already established conclusions.

Our results show moderate positive correlation between maternal pregnancy BMI and uniaxial test rupture force. In a recent study Roland et al. found a positive effect of maternal BMI and placental weight (21) while Hasegawa found that gestational weight loss had an adverse effect on placental weight (22). Since the placenta is tied to the development of fetal membranes, such correlation could mean that maternal nutritional status might have an effect on fetal membrane biomechanics. A weak positive correlation between newborn birth length and burst pressures in standard biaxial test was also observed. Such correlation could support the connection between maternal nutritional status and fetal membrane biomechanics. Although newborn birth weight was not included in the final regression model for uniaxial force-to-rupture as predictor variable, further research is required to establish or refute such connection. There are certain limitations to our study of which we are very well aware of. Firstly, the number of c-section membranes is somewhat low. Secondly, we were able to take only 2 samples for biaxial testing from each half of the membrane. As has been stated previously, there are biomechanical differences even between samples taken from one region (20). The level of variance explained by the regression models is somewhat low which can be attributed to inherent lack of uniformitiy in biomechanical properties of fetal membranes. Although we have succeeded in taking 4 samples per placenta, future studies using this model should focus on further decreasing the necessary size of the samples thus achieving even more uniform results for interpretation. Preparations for further investigation of maternal nutrition and adiposity effects on biomechanical properties of fetal membranes are underway in our laboratory. Correlation between biaxial puncture and biaxial dynamic test should also be investigated in the future.

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Disclosure of interest: *The authors declare that they have no conflict of interest.*

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