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Marine Lallemant[1,2], Tiguida Kadiakhe[2] , Jeröme Chambert[2], Arnaud Lejeune[2], Rajeev Ramanah[1,3], Nicolas Mottet[1,3], Emmanuelle Jacquet[2]

[1] Department of Gynecologic Surgery, Besancon University Medical Centre, Besançon, France

[2] Department of Applied Mechanics, FEMTO-ST Institute, University of Franche-Comte, UMR 6174 CNRS, Besançon, France

[3] Nanomedicine Imaging and Therapeutics Laboratory, INSERM EA 4662, University of Franche-Comte, Besançon, France

Corresponding author

Marine Lallemant

Service de Gynécologie- Obstétrique, CHU Besancon, bd Alexandre Fleming, 25000 Besancon, France

Email : marine.lallemant@wanadoo.fr

Conflict of interest:

None

ABSTRACT

Introduction : Data concerning the mechanical properties of the perineum during delivery are very limited. In-vivo experimentations raise ethical issues. The aim of the study was to describe some of the biomechanical properties of each perineal tissue layer collected from sows in order to better understand perineal tears during childbirth.

Material and methods: Samples of each perineal tissue layers were obtained from the skin, the vagina, the external anal sphincter (EAS), the internal anal sphincter (IAS) and anal mucosa of fresh dead sows. They were tested in quasi-static uniaxial tension using the testing machine Mach-1®. The tests were performed at a displacement velocity of 0.1 $mm.s^{-1}$. Stress-strain curves of each perineal tissue layers before the first damage for each sow were obtained and modeled by hyperelastic Yeoh model described by three coefficients: C1, C2, and C3. Pearson correlation coefficient was calculated to measure the correlation between C1-coefficient and the duration between the first microfailure and the complete rupture for each perineal tissue layer. Pearson correlation was computed between C1-coefficient and the number of micro-failures before complete rupture for each tissue.

Results: Ten samples of each perineal tissue layer were analyzed. Mean values of C1-coefficient and corresponding standard deviations were 46 ± 15 kPa, 165 ± 60 kPa, 27 ± 10 kPa, 19 ± 13 kPa, 145 ± 28 kPa for the perineal skin, the vagina, the EAS, the IAS and the anal mucosa, respectively. According to this same sample order, the first microfailure in the population of 10 sows appeared at an average of 54%, 27%, 70%, 131% and 22% of strain. A correlation was found between C1 hyperelastic-coefficient and the duration between the first microfailure and the complete rupture ($r=0.7$, $p=0.02$) or the number of micro-failures before complete rupture only for the vagina ($r=0.7$, $p=0.02$).

Conclusions: In this population of fresh dead sow's perineum, the vagina and the anal mucosa were the stiffest tissue. The IAS and EAS were the more extensible and the less stiff. A significantly positive correlation was found between C1-coefficient and the duration between the first microfailure and the complete rupture of the vagina, and the duration between the first microfailure and the complete rupture of the vagina.

Key words

stress-strain curve, perineum, childbirth, perineal tear, biomechanical properties, rupture, deformation

Abbreviations

EAS: external anal sphincter,

IAS, internal anal sphincter

Key message

C1-hyperelastic coefficients of perineal tissues (fresh dead sow) were ranked in ascending order: IAS, EAS, skin, anal mucosa and vagina. Similar human results could maybe explain anal sphincter integrity and isolated rectal buttonhole perineal tears in childbirth.

INTRODUCTION

The perineum is a soft structure made up of skin and muscles closing the pelvic. It is located under the levator ani muscle. Its mechanical properties ensure normal pelvic stability. During childbirth, the morphological and dynamic adaptation of the perineum to the fetal presentation depends on its resistance to the stresses induced by the presentation. Under the compressive efforts induced by the presentation, the perineum becomes thinner until sometimes it tears. Sometimes, it leads to more or less severe perineal tears. Stages III and IV correspond to obstetrical lesions of the anal sphincter and/or anal mucosa. These perineal lacerations can impact women's quality of life with anal incontinence, perineal pain, dyspareunia, recto-vaginal fistula and depression [1,2]. The rate of obstetrical anal sphincter injuries varies between 0.25 and 6% depending on the study [3].

Data concerning the mechanical properties of the perineum and fetal stresses during delivery are very limited [4–10]. They result essentially from numerical models of the distension of the levator ani muscles. Only Zemčík et al., managed to quantify perineal strain during vaginal delivery using stereophotogrammetry [10]. But the perineum is a structure composed of different tissues whose behavior must be characterized in order to understand the tearing mechanism. Therefore, biomechanical parameters of the perineum are necessary to understand perineal tears. But, in-vivo experimentations raise ethical issues. To begin our work, we decided to study biomechanical properties of porcine perineum. A porcine model was chosen because of its similar morphological and immunohistochemical properties, and the results of microindentation tests as reported by the literature [11,12]. So the aim of our study was to describe some of the biomechanical properties of each perineal tissue layer collected from sows in order to better understand perineal tears during childbirth.

MATERIAL AND METHODS

We performed an experimental study on porcine perineal tissues. Ten samples of each perineal layer were analyzed. They were dissected from fresh dead sow provided by local slaughterhouse waste. The sow breed was the French pork butcher's pig. Sow were slaughtered 24-48 hours before the experimentation. Sows were not frozen. For each sow, one sample was

obtained from the skin perineal layer, the vagina perineal layer, the external anal sphincter (EAS), the internal anal sphincter (IAS) and anal mucosa perineal layer (Figures 1 and 2.).

Perineal sows were refrigerated during the time before collection. In order to obtain samples in a reproducible way and preserve fiber integrity, a precise dissection method has been implemented (Figure 2.). The dissection was performed by an urogynecologist expert in anatomy of perineal tears. The used instruments were fine scissors and atraumatic forceps. No traction on the tissues was performed. A careful midline incision next to the vulvar area between the vaginal and anal openings was made. The external anal sphincter was immediately identified and isolated. The perineal skin was dissected from the anus to the ventral extremity into two dorso-ventral samples (right and left). Next, the ventral part of the vagina was incised at 6 o'clock to facilitate vaginal access. A right and left vaginal samples from the perineum were dissected on the rectovaginal septum side. Histologically, the vaginal sample included the vaginal wall composed of mucosa, lamina propria, muscularis and adventitia. Then, the external anal sphincter was carefully dissected and sectioned at 9 o'clock and 3 o'clock. Finally, the dorsal wall of the rectum and anus was incised at 12 o'clock. Two samples (right then left) of the anal mucosa and the internal anal sphincter were obtained. Only the samples on the right side were analyzed for the perineal skin, the perineal vagina, the perineal anal mucosa and the IAS. The left samples of these sows were analyzed in another study with non-comparable experimental conditions. EAS was taken. The entire EAS was collected between the vagina and the anus. Due to its thinness, it was not possible to divide it into two samples like the other layers. The entire EAS was used for sampling, along the fibers, in this study. Samples were oriented (dorso-ventral, left-right, cranio-caudal).

To obtain uniform stresses at the center of the sample during tensile testing, standardized samples were cut for each perineal layer with a scalpel and a caliber. Samples were obtained using a rectangular pattern 30 mm long and 10 mm wide in the cranio-caudal direction for all tissues except the EAS, which was in the latero-lateral direction (direction shown in Figure 3.).

Each sample end was covered with instant glue (Loctite 401®) and held in paper. These ends were inserted into the jaw in such a way as not to over- or under-tighten (Figure 3.). If the sample slipped, it would not be taken into account in the post-processing analysis.

Uniaxial tensile tests were carried out using Mach-1® mechanical tester (Biomomentum Inc, Canada). Tensile forces, stretching forces acting on the tissue, were

133 measured with a 250 N cell. A preload of 0.3 N was applied during two minutes before each
134 test to compare sample results. Sample sizes were measured after the pre-stress. The initial
135 measurements of thickness (t_0), width (w_0) and length (l_0) measurements were obtain using a
136 digital caliper after preload in the middle of the sample to avoid compression tissue and
137 deformation near the jaws. The moving crosshead displacement (Δl) of the tensile-testing
138 machine was recorded to an accuracy of 0.5 µm in the direction of stress (traction), along with
139 force at a frequency of 100 Hz. The displacement was applied until failure. Tensile tests
140 stopped when the sample broke. The tests were performed at a displacement velocity of 0.1
141 $mm.s^{-1}$ and at a constant temperature of 21°C to avoid dehydration.

142 The uniaxial engineering stress σ (kPa) in the loading direction was defined by:

$$\sigma = \frac{F}{A_0} = \frac{F}{t_0 \cdot w_0}$$

143 where F is the measured force, A_0 is the initial cross-sectional area of the specimen.

144 The uniaxial engineering strain ε (%) in the loading direction is obtained by:

$$\varepsilon = \frac{\Delta l}{l_0}$$

145

146 Stress-strain curves of each perineal tissue before the first damage for each sow and
147 their mean were obtained. Unlike elastic materials where stress varies linearly with respect to
148 strain, soft tissues are hyperelastic. It means that these tissues have a non-linear elastic behavior
149 [13]. Like elasticity, hyperelasticity models reversible behavior. Non-linearity allows to take
150 account large deformations. Non-linear elastic behavior of these tissues were observed and
151 modeled by Yeoh model [14]:

$$\Psi = \sum_{i=1}^{3} C_i (I_1 - 3)^i$$

152 This hyperelastic law is described by three coefficients: C1, C2, and C3. These
153 coefficients were identified by the method of least mean squares from experimental curves
154 using the Levenberg-Marquardt algorithm before the initial sign of damage appeared in each
155 tissue sample [15]. The damage, also called microfailure, is due to local and microscale effects.
156 Occurrence of damage results in the weakening of the tissue and makes it more likely to rupture
157 and tear. A microfailure is associated with the inflexion point on the experimental curve and is

identified by locating the maximum on the derivative of the curve. The inflexion of the curve shows a reduction in the tissue's ability to withstand mechanical stress, which is interpreted by the appearance of fiber rupture or delaminations in the tissue. This inflection generally precedes macroscopic tissue rupture.

Only C1-coefficients were analyzed because of its meaning: initial slope of stress-strain curve at low strains (less than 5%). A high C1 hyperelastic coefficient means that the tissue is stiff (rigid) with a small deformation in response to an applied force. On the contrary, a low C1 hyperelastic coefficient means that the tissue is easy to deform even in response to a low applied force.

Tangent modulus (kPa) E0 and E1 were calculated during low and high strain respectively. For each tissue, the mean stress and the mean strain were calculated up to the first rupture among all curves for the same tissue. Low strain was defined at the beginning of the master (mean) curve and used to define E0, i.e. at 0.4% deformation on average. E1 was defined as the tangent modulus of the curve at the end of the mean curve, i.e. at 59% deformation on average. Minimal E0 and E1 were calculated from the lowest curve for each layer of perineal tissue. Maximal E0 and E1 were calculated from the highest curve for each layer of perineal tissue.

Coefficients were expressed as mean ± standard deviation. C1-hyperelastic coefficients were compared according to the perineal layer using an ANOVA model. If a statistically difference was found, pairwise comparisons using t-tests and including Bonferroni correction were performed identify which perineal layers were different from the others. In order to determine whether the C1 coefficient was predictive of tissue damage, Pearson correlation coefficient was calculated to measure the correlation between C1-coefficient and the duration between the first microfailure and the complete rupture for each tissue. Pearson correlation was computed between C1-coefficient and the number of micro-failures before complete rupture for each tissue. Statistical analysis was performed using R software (version 4.3.0). For all analyses, we considered a p-value less than 0.05 to be statistically significant.

RESULTS

Ten samples of each layer were analyzed. Samples were described in Table 1. No sample slipped. Mean values of C1-coefficient and corresponding standard deviations were 46

± 15 kPa, 165 ± 60 kPa, 27 ± 10 kPa, 19 ± 13 kPa, 145 ± 28 kPa for the perineal skin, the vagina, the EAS, the IAS and the anal mucosa, respectively (Figure 4. and Table 2.). C1-hyperelastic coefficients were statistically different between perineal layers (p<0.001) (Supporting Information Appendix S1). C1-hyperelastic coefficients of the anal mucosa were statistically different to the C1-hyperelastic coefficients of the perineal skin, the EAS and IAS (p<0.001). C1-hyperelastic coefficients of the perineal vagina were statistically different to the C1-hyperelastic coefficients of the perineal skin, the EAS and IAS (p<0.001). The other perineal layers had statistically comparable C1-hyperelastic coefficients.

The vagina and the anal mucosa had the highest C1 hyperelastic coefficients. The mean ratio of C1 hyperelastic coefficients of the vagina to the anus was 0.3 (standard deviation of 0.1). The anal sphincter (external and internal) had the lowest hyperelastic coefficients. Tensile tests lasted less than 15 minutes for the IAS and less than 10 minutes for the other perineal layers.

Tangent modulus during low and high strain were presented Table 3. The E1 modulus derived from the mean of the curves tended to triple compared with the E0 modulus for the skin and the vagina. Modulus E1 increased by a factor of 1.3 for SAE and SAI. Anal mucosa increased by a factor of 1.2.

The mean stress-strain curves and their standard deviation for each perineal tissue before the first damage in this population were drawn (Figure 5.). The first microfailure in the population of 10 sows appeared at least at 29%, 13%, 38%, 46% and 14% of strain for the perineal skin, the vagina, the EAS, the IAS and the anal mucosa, respectively (Table 4.). The first microfailure in the population of 10 sows appeared at an average of 54%, 27%, 70%, 131% and 22% of strain for the perineal skin, the vagina, the EAS, the IAS and the anal mucosa, respectively. The external and internal anal sphincters were the most extensible and less stiff tissue. The vagina was the stiffest tissue. The anal mucosa was the less extensible tissue.

The duration between the first microfailure and the complete rupture of each tissue was 79 ± 105 s, 69 ± 86 s, 146 ± 168 s, 151 ± 162 s, 166 ± 108 s for the perineal skin, the vagina, the EAS, the IAS and the anal mucosa, respectively (Table 5.). The number of microfailures before complete rupture of each tissue was 16 ± 14, 6 ± 4, 23 ± 19, 18 ± 17, 38 ± 23 for the perineal skin, the vagina, the EAS, the IAS and the anal mucosa, respectively (Table

5.). The mean ratio of vaginal to anal microfailures before complete rupture was 0.2 (standard deviation 0.2)

A significantly positive correlation was found between C1-coefficient and the duration between the first microfailure and the complete rupture of the vagina (r=0.7 p=0.02) (Table 4.). Also, a significantly positive correlation was found between C1-coefficient and the duration between the first microfailure and the complete rupture of the vagina (r=0.7 p=0.02) (Table 5.).

For the others tissue, no correlation was found between C1-coefficient and the duration between the first microfailure and the complete rupture of each tissue (p>0.05) (Table 4.). Also, no correlation between C1-hyperelastic coefficient and the number of micro-failures before complete rupture of each tissue except for vagina (p>0.05) (Table-5).

DISCUSSION

In this study, some biomechanical properties of each perineal layer of the sow has been obtained. C1-coefficients for perineal skin, the vagina, the EAS, the IAS and the anal mucosa of fresh dead sow were measured.

In this population, the vagina and the anal mucosa were the stiffest. In other words, these rigid tissues had smaller deformation in response to an applied force. C1-coefficients of the vagina and the anal mucosa were higher than C1-coefficients of the perineal skin (165 and 145 versus 46 kPa). This difference was also described by Gabriel et al. who compared the hyperelastic coefficient at low strain of the vagina and the abdominal skin of unfrozen cadavers without relevant pelvic organ prolapse (0.35 versus 0.14 MPa, p>0.05) [16]. In the literature, no study assessed biomechanical properties of the anal mucosa. Only one rectal tissue evaluation was found [17]. Rubod et al. compared vaginal and rectal tissue on fresh female cadavers without prolapse performing multiaxial tension tests [17]. They demonstrated that the vagina was much more rigid and less extensible than the rectal tissue. In our study, the anal mucosa and the vagina were the less extensible tissues. Their first microfailure appeared at a mean strain of 22% and 27% respectively.

The IAS and EAS were the more extensible and the less stiff. The EAS is a skeletal muscle. Passive extensibility of skeletal muscles is an important component of total muscle function. It is well described in the literature [18]. Studies with animal muscles have shown that

passive extensibility is influenced by the size (mass) and length of muscle fibers, and the amount and arrangement of the connective tissues of the muscle belly. The resistance to passive lengthening is influenced by the readily adaptable amount of muscle tissue, including the contractile proteins and the non-contractile proteins of the sarcomere cytoskeletons.

Concerning IAS's biomechanical properties, no study was found in the literature. But a corroboration with vaginal smooth muscle cells could be done. Smooth muscle cells contribute to soft tissues' quasistatic and viscoelastic mechanical behavior [19–21]. According to Clark-Patterson et al., smooth muscle cells provide mobility by allowing the vagina to stretch under sustained pressures. By the same way, IAS could ensure mobility by allowing the anal mucosa to stretch under sustained pressures [22].

According to the tangent modulus, the skin and vagina seemed more resistant to deformation for high strain (much higher modulus). The stress applied to the tissues increased very quickly in the high strain zone which tends to show that these tissues will tear first during childbirth because the deformations of the perineal tissues are imposed by the passage of the fetus. The great ability of SAE and SAI to deformation (38 and 46%) is highlighted by lower values of E1 modulus for these tissues. The stresses applied was lower for the same level of deformation than those observed for the skin and the vagina. These data are compatible with what is observed in maternity wards. The skin and the vagina tend to tear first before the anal sphincter and the anal mucosa during childbirth.

In our study, the sow's anal sphincter was more extensible and less stiff than the vagina or the perineal skin. It could explain why severe perineal tears are rare in sows. If the same results are confirmed in humans, that could also maybe explain why the anal sphincter is not always torn during childbirth. In the same way, the sow's anal mucosa and its vagina were the less extensible tissues. If these biomechanical properties are also found in humans, it could maybe explain isolated rectal buttonhole perineal tear in obstetrics [23]. It is an isolated tear of the anal epithelium or rectal mucosa and vagina but without involving the anal sphincter. This kind of laceration is rare in human and only described in equine case report [24–26].

Concerning the experimental methodology, No failure or tears near the grip have been noticed. Operators trained before starting the study to avoid the learning phase and reduce these kinds of failure. Perineal stresses during delivery are not unidirectional. However, due to the size of the samples, only unidirectional traction tests at constant speed were performed. Yeoh model has been chosen because it described accurately the behavior of all the different

perineal tissues. A comparison of Yeoh, Mooney-Rivlin and Ogden models on pig skin (belly and back) confirmed that Yeoh model captures accurately the behavior of the pig skin [27]. It also highlighted the stability issues that arise when using the Ogden model as well as the failure of the Mooney-Rivlin model to properly describe the behavior of the tissues. Moreover, it requires a small number of parameters, its first parameter being interpreted as half the initial shear modulus.

At our knowledge, our study is the first one in the literature to attempt to describe biomechanical properties of each perineal layer in order to better understand perineal tears. This study focused on deformation. It concerns only the different tissues of the perineum, considered independently. This work is a preliminary step towards understanding the mechanisms involved. The next steps are aimed at identifying predictive parameters for the risk of perineal tears. These results could be used in simulation using Finite Element Model. Structural tests on the whole perineum are currently underway, and will be used to validate the model of the whole perineum with controlled boundary conditions. This ongoing study aims to identify predictive parameters for the risk of failure associated with extreme loading conditions (>250% deformation).

According to Rosenberg and Trevathan, non-human primates have the most similar pelvic anatomy to humans [28]. But its availability and its cost has led us seek another animal model. Ewes could have been used also but their availability remains difficult [29,30]. Broodmares with a perineal body and who are also subject to obstetrical anal sphincter injury could also be an animal model [31]. However, the model is difficult to obtain. The simple, reproducible, low cost and most frequently animal model for pelvic floor research is the sow. It allows comparing measurement of mechanical properties with the literature. Plus, porcine models are usually used to educate physicians in sphincter injury repair [32–34]. Similarly to humans, utero-vaginal prolapse are common in sows [35,36]. In a recent industry study, 21% of sow deaths in the USA could be attributed to POP [37].

The perineal body is a fibromuscular structure located in the midline of the perineum and provides attachments of the perineal muscles in women [38]. Surprisingly, in our experience, sow’s perineum seemed to not have a perineal body. In the literature, Bassett et al. study is the only article describing the anatomy of sow’s perineum [39]. They did not report a perineal body. Plus, in the area between the vagina and the rectum of the sow, the only perineal muscle that was described is the anal sphincter. The retractor clitoridis muscle insertion is

located in the ventrolateral rectal wall. But, there is no perineal muscles other than the anal sphincter in this area. During our multiple dissections, the full area between the rectum and the vagina was excised, and the skin and the subcutaneous tissue removed such as described by Kochová et al. [40]. They called the resulting tissue the perineal body. But when we performed the same dissection as described by this research group, it seemed that they took the muscularis of the vagina, the adventitia of the vagina and fascia-like tissue. Kochová et al. has written in their work that they did not know the gross anatomy of the structures in the tissue samples. This discrepancy highlights the limits of the sow's model. The perineal body can by studied in humans using non-invasive technologies. Zemčik et al. studied the deformation of the perineum during normal delivery using stereophotogrammetry [10]. Chen et al. and Rostaminia et al. studied the perineal body stiffness in pregnant and on pregnant women [41,42].

In addition to a relatively small study group, the sow's parity was not known. These sows were nullipara. According to Rynkevic et al. and Drewes et al., biomechanical properties of the vaginal tissue after pregnancy would not recover to those of virgins [29,43]. These reasons could explain the interindividual variation among the sows. Furthermore, we did not study interactions between each layer of perineal layer. We investigated sow's cadaveric tissues. Postmortem changes must be considered. Indeed, stiffening of muscles appears 2 to 6 hours after death because of adenosine triphosphate (ATP) levels, which cause the binding of the muscle filaments of actin and myosin [44]. Even if the studied tissues cannot be considered to have intact chemomechanical properties, the results revealed significant differences in biomechanical behavior, providing new insights to better understand the mechanical responses of perineal structures to mechanical stress. In vivo experimentation is non-ethic. In vivo measurements using nondestructive tests should be performed to confirm these results.

CONCLUSION

In these perineal tissue samples of fresh dead sow, the vagina and the anal mucosa were the stiffest tissue. The IAS and EAS were the more extensible and the less stiff. A significantly positive correlation was found between C1-coefficient and the duration between the first microfailure and the complete rupture of the vagina and between C1-coefficient and the duration between the first microfailure and the complete rupture of the vagina. No correlation was found for the other perineal tissues. These results must be confirmed on humans using in vivo nondestructive tests.

Ethics statement

Sow's perineum were dissected from fresh dead sow provided by local slaughterhouse waste. Animals were not slaughtered for the study. No ethics application was needed since the animals were bred and killed for food production (French decree on the protection of animals used for scientific purposes).

Author contributions:

Marine Lallemant : conceptualization, data curation, formal analysis, methodology, writing-original draft; Tiguida Kadiakhe : conceptualization, data curation, formal analysis, methodology, writing-review; Jeröme Chambert : conceptualization, methodology, writing-review ; Arnaud Lejeune : conceptualization, methodology, writing-review; Rajeev Ramanah: writing-review ; Nicolas Mottet : writing-review; Emmanuelle Jacquet: conceptualization, methodology, writing-review.

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Legends

Table 1. Description of samples and tensile tests for each perineal tissues.

Table 2. Hyperelastic coefficients according to the perineal tissue (Yeoh model).

Table 3. Parameter values from experimental stress-strain curves according to the perineal tissue (Yeoh model): E0 and E1 tangent modulus, Stress (kPa) at the first microfailure, Strain (%) at the first microfailure (minimal value, average value)

Table 4. Correlation between C1 hyperelastic coefficient and the duration between the first microfailure and the complete rupture of each tissue (Yeoh model)

Table 5. Correlation between C1 hyperelastic coefficient and the number of micro-failures before complete rupture of each tissue (Yeoh model)

Figure 1. Anatomy of the sow's perineum. An incision of the perineal skin between the Vagina and the anus was performed to reveal the external anal sphincter (EAS).

Figure 2. Obtention of the skin, the vagina, the IAS, the EAS and the anal mucosa samples of the sow. IAS: internal anal sphincter, EAS: external anal sphincter.

Figure 3. Test machine Mach-1® (Biomomentum Inc, Canada) and grips for soft tissues.

Figure 4. Stress-strain curves of each perineal tissue before first damage for each sow.

Figure 5. Mean stress-strain curves and their standard deviation for each perineal tissue before the first damage in this population (n=10 for each perineal layers).

Supporting Information legend

Appendix S1. *P*-values of pairwise comparisons using t-tests and including Bonferroni correction to identify which perineal layers were different from the others according to C1-hyperelastic coefficient.