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1 **In-vitro biomechanical properties of porcine perineal tissues to better**
2 **understand human perineal tears during delivery**

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19

20 **Conflict of interest:**

21 None

22

23

24 **ABSTRACT**

25

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

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

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

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

53

54

55 **Key words**

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

58

59 **Abbreviations**

60 EAS: external anal sphincter,

61 IAS, internal anal sphincter

62

63 **Key message**

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

67

68

69

70 **INTRODUCTION**

71

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

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

94

95 **MATERIAL AND METHODS**

96

97 We performed an experimental study on porcine perineal tissues. Ten samples of
98 each perineal layer were analyzed. They were dissected from fresh dead sow provided by local
99 slaughterhouse waste. The sow breed was the French pork butcher's pig. Sow were slaughtered
100 24-48 hours before the experimentation. Sows were not frozen. For each sow, one sample was

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

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

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

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

131 Uniaxial tensile tests were carried out using Mach-1® mechanical tester
132 (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 $\text{mm}\cdot\text{s}^{-1}$ 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: C_1 , C_2 , and C_3 . 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

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

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

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

175 Coefficients were expressed as mean \pm standard deviation. C1-hyperelastic
176 coefficients were compared according to the perineal layer using an ANOVA model. If a
177 statistically difference was found, pairwise comparisons using t-tests and including Bonferroni
178 correction were performed identify which perineal layers were different from the others. In
179 order to determine whether the C1 coefficient was predictive of tissue damage, Pearson
180 correlation coefficient was calculated to measure the correlation between C1-coefficient and the
181 duration between the first microfailure and the complete rupture for each tissue. Pearson
182 correlation was computed between C1-coefficient and the number of micro-failures before
183 complete rupture for each tissue. Statistical analysis was performed using R software (version
184 4.3.0). For all analyses, we considered a p-value less than 0.05 to be statistically significant.

185

186 **RESULTS**

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

189 ± 15 kPa, 165 ± 60 kPa, 27 ± 10 kPa, 19 ± 13 kPa, 145 ± 28 kPa for the perineal skin, the
190 vagina, the EAS, the IAS and the anal mucosa, respectively (Figure 4. and Table 2.). C1-
191 hyperelastic coefficients were statistically different between perineal layers ($p < 0.001$)
192 (Supporting Information Appendix S1). C1-hyperelastic coefficients of the anal mucosa were
193 statistically different to the C1-hyperelastic coefficients of the perineal skin, the EAS and IAS
194 ($p < 0.001$). C1-hyperelastic coefficients of the perineal vagina were statistically different to the
195 C1-hyperelastic coefficients of the perineal skin, the EAS and IAS ($p < 0.001$). The other
196 perineal layers had statistically comparable C1-hyperelastic coefficients.

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

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

206 The mean stress-strain curves and their standard deviation for each perineal tissue
207 before the first damage in this population were drawn (Figure 5.). The first microfailure in the
208 population of 10 sows appeared at least at 29%, 13%, 38%, 46% and 14% of strain for the
209 perineal skin, the vagina, the EAS, the IAS and the anal mucosa, respectively (Table 4.). The
210 first microfailure in the population of 10 sows appeared at an average of 54%, 27%, 70%,
211 131% and 22% of strain for the perineal skin, the vagina, the EAS, the IAS and the anal
212 mucosa, respectively. The external and internal anal sphincters were the most extensible and
213 less stiff tissue. The vagina was the stiffest tissue. The anal mucosa was the less extensible
214 tissue.

215 The duration between the first microfailure and the complete rupture of each tissue
216 was 79 ± 105 s, 69 ± 86 s, 146 ± 168 s, 151 ± 162 s, 166 ± 108 s for the perineal skin, the
217 vagina, the EAS, the IAS and the anal mucosa, respectively (Table 5.). The number of
218 microfailures before complete rupture of each tissue was 16 ± 14 , 6 ± 4 , 23 ± 19 , 18 ± 17 , $38 \pm$
219 23 for the perineal skin, the vagina, the EAS, the IAS and the anal mucosa, respectively (Table

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

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

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

231

232 **DISCUSSION**

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

236 In this population, the vagina and the anal mucosa were the stiffest. In other words,
237 these rigid tissues had smaller deformation in response to an applied force. C1-coefficients of
238 the vagina and the anal mucosa were higher than C1-coefficients of the perineal skin (165 and
239 145 versus 46 kPa). This difference was also described by Gabriel et al. who compared the
240 hyperelastic coefficient at low strain of the vagina and the abdominal skin of unfrozen cadavers
241 without relevant pelvic organ prolapse (0.35 versus 0.14 MPa, $p>0.05$) [16]. In the literature, no
242 study assessed biomechanical properties of the anal mucosa. Only one rectal tissue evaluation
243 was found [17]. Rubod et al. compared vaginal and rectal tissue on fresh female cadavers
244 without prolapse performing multiaxial tension tests [17]. They demonstrated that the vagina
245 was much more rigid and less extensible than the rectal tissue. In our study, the anal mucosa
246 and the vagina were the less extensible tissues. Their first microfailure appeared at a mean
247 strain of 22% and 27% respectively.

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

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

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

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

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

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

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

289 At our knowledge, our study is the first one in the literature to attempt to describe
290 biomechanical properties of each perineal layer in order to better understand perineal tears.
291 This study focused on deformation. It concerns only the different tissues of the perineum,
292 considered independently. This work is a preliminary step towards understanding the
293 mechanisms involved. The next steps are aimed at identifying predictive parameters for the risk
294 of perineal tears. These results could be used in simulation using Finite Element Model.
295 Structural tests on the whole perineum are currently underway, and will be used to validate the
296 model of the whole perineum with controlled boundary conditions. This ongoing study aims to
297 identify predictive parameters for the risk of failure associated with extreme loading conditions
298 (>250% deformation).

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

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

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

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

338

339 **CONCLUSION**

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

347

348 **Ethics statement**

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

353

354 **Author contributions:**

355 Marine Lallemand : conceptualization, data curation, formal analysis, methodology, writing-
356 original draft; Tiguida Kadiakhe : conceptualization, data curation, formal analysis,
357 methodology, writing-review; Jérôme Chambert : conceptualization, methodology, writing-
358 review ; Arnaud Lejeune : conceptualization, methodology, writing-review; Rajeev Ramanah:
359 writing-review ; Nicolas Mottet : writing-review; Emmanuelle Jacquet: conceptualization,
360 methodology, writing-review.

361

362

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- 474

475 **Legends**

476

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

478

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

480

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

484

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

487

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

490

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

493

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

496

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

498

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

500

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

503

504 **Supporting Information legend**

505

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

509

510