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# Influence of experimental conditions on some in-vitro biomechanical properties of the sow's perineum

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**The aim of this work was to develop an experimental protocol that takes into account the influence of experimental conditions on these perineal tissues, before determining their mechanical properties. Samples of each perineal tissue layer were obtained from the skin, the vagina, the external anal sphincter (EAS), the internal anal sphincter (IAS) and anal mucosa of freshly dead sows. They were tested in quasi-static uniaxial tension using the Mach-1 testing machine. Stress-strain curves of each perineal tissue layer before the first damage for each sow were obtained and modeled by hyperelastic laws described by three coefficients: C1, C2, and C3 (Yeoh model). The influence of sample preparation conditions such as tissue freezing, hygrometry and sample orientation were evaluated, and the conditions under which the tests were performed such as the displacement velocity during testing were also evaluated by analysing C1-coefficient. This study suggested that sample preparation conditions such as tissue freezing for 24 h, storage in cellophane paper for two hours and the strain rate did not statistically affect the C1-hyperelastic coefficient for each perineal layer ($p > 0.05$). Samples should not be stored in saline for 2 h ($p < 0.05$). Sample orientation did not influence C1-hyperelastic coefficient ($p > 0.05$). This experimental protocol could be used to study in vitro biomechanical properties of perineal tissues in order to better understand perineal tears during delivery.**

**Keywords** Stress-strain curve, Perineum, Childbirth, Perineal tear, Biomechanical properties, Deformation

The majority of women who give birth vaginally will sustain some form of genital tract trauma[1]. The type of trauma can range from bruising of the perineal tissues, a spontaneous vaginal tear, a intentional surgical incision (episiotomy), or obstetric anal sphincter injuries (OASIS). OASIS are tears involving the anal sphincter and/or anal mucosa[2]. Perineal trauma affects millions of women each year. The prevalence of varies from 0.25 to 6% in the general population, 1.4 to 16% in primiparous patients, 0.4 to 2.7% in multiparous patients[3]. These lesions can lead to long-term morbidity such as anal incontinence, chronic perineal pain, dyspareunia, urinary symptoms or fistulas[4–6].

The perineum consists of all the soft tissues below the pelvic floor that close the pelvic cavity. Genital tract trauma following birth is described according to the RCOG classification[7]. First-degree tear includes injury to the perineal skin and/or vaginal mucosa. Second-degree tear gathers injury to the perineum involving the perineal muscles but not the anal sphincter. Third-degree tear involves the anal sphincter complex (external anal sphincter (EAS) only or both external anal sphincter and internal anal sphincter (IAS)). Fourth-degree tears include perineal injuries involving the anal sphincter complex (EAS and IAS) and anal mucosa.

Data on the mechanical properties of the perineum[8–17] and fetal stresses[18–23] during childbirth are very limited. These are essentially finite element numerical models of the the levator ani muscle distension, also known as the pelvic floor. Studying the mechanical properties of perineal tissues may help to develop a mechanical model of the perineum during childbirth. This could be a tool for understanding perineal tears and preventing perineal tears.

The perineum is a structure composed of several tissues (skin, vagina, muscles, anal sphincter and anal mucosa) whose behavior must be characterized in order to understand the tearing mechanism. The behavior

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of the different perineal tissues depends on environmental factors whose influence has never been assessed. Since in vivo experiments raise ethical issues, we decided to start our work by studying the biomechanical properties of porcine perineum in vitro. A porcine model was chosen because of its similar morphological and immunohistochemical properties, as well as the results of microindentation tests reported in the literature[24–26]. So, the aim of this work was to develop an experimental protocol that takes into account the influence of experimental conditions on these perineal tissues, before determining their mechanical properties.

## Materials and methods

### Experimental technique

We performed an experimental study on porcine perineal tissues. Ten samples of each perineal layer were analyzed. They were dissected from fresh dead sows provided by local slaughterhouse waste. No ethics application was required since the animals were bred and killed for food production and there was the possibility to use organs/tissues for research in accordance with French regulations. No animals were slaughtered for the study. The sow breed was the French pork butcher's pig. Sow were slaughtered 24–48 h before the experimentation. Sows were nulliparous. For each sow, two samples were collected from the perineal skin layer, the vaginal perineal layer, the internal anal sphincter (IAS) and the anal mucosa (Fig. 1), and only one sample was collected from the external anal sphincter (EAS) due to its small size.

A total of 45 sows were dissected. Perineal sows were refrigerated during the period prior to collection. In order to obtain samples in a reproducible manner and to preserve fiber integrity, a precise dissection method was implemented (Fig. 2). Dissection was performed by an urogynecologist with an expertise in perineal tear anatomy. Tensile tests were performed by a mechanical engineer. The instruments used were fine scissors and atraumatic forceps. No traction was applied to the tissues. A careful midline incision next to the vulvar area between the vaginal and anal openings was made. The EAS 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 access to the vaginal. One right and one left vaginal samples were dissected from the perineum on the side of the rectovaginal septum. Histologically, the vaginal sample included the vaginal wall composed of mucosa, lamina propria, muscularis and adventitia. The EAS was then carefully dissected and sectioned at 9 o'clock and 3 o'clock. Finally, the dorsal wall of the rectum and anus at 12 o'clock was incised. Two samples (right then left) of the anal mucosa and the IAS were obtained. The samples were oriented (dorso-ventral, left-right, cranio-caudal).

To obtain uniform stresses in the center of the sample during tensile testing, standardized samples were cut with a scalpel and a caliber for each perineal layer. Samples were cut in a rectangular pattern 30 mm long and 10 mm wide in the cranio-caudal direction for all tissues except the EAS, which was cut in the latero-lateral direction (direction shown in Fig. 1).

Each sample end was covered with instant glue (Loctite 401) and held in paper. These ends were inserted into the jaw so as not to over- or under-tighten (Fig. 3). If the sample slipped, the case would be excluded from the results.

Uniaxial tensile tests were performed using Mach-1 mechanical tester (Biomomentum Inc, Canada). Tensile forces, which are the stretching forces applied to the tissue, were measured using a 250 N cell. A preload of 0.3 N was applied for two minutes prior to each test to compare sample results. The sample were measured after the preload. Initial measurements of thickness ($t_0$), width ($w_0$) and length ($l_0$) were obtain using a digital caliper after preloading in the center of the sample to avoid compression of tissue and deformation near the jaws. The displacement of the moving crosshead ($\Delta l$) of the tensile-testing machine was recorded with an accuracy of

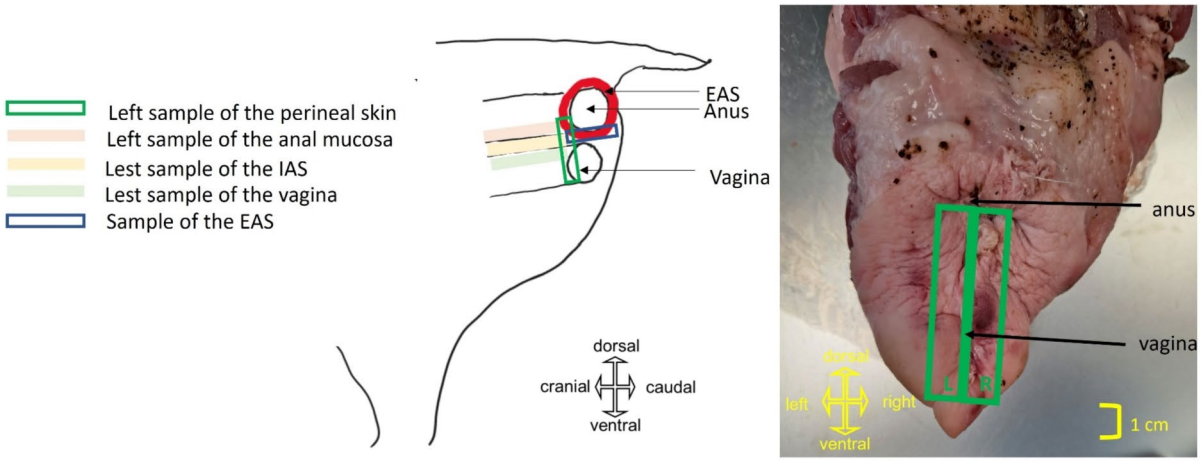


**Fig. 1**. Anatomy of the sow's perineum and sampling of perineal tissue layers. *L* left sample, *R* right sample, *EAS* external anal sphincter, *IAS* internal anal sphincter.

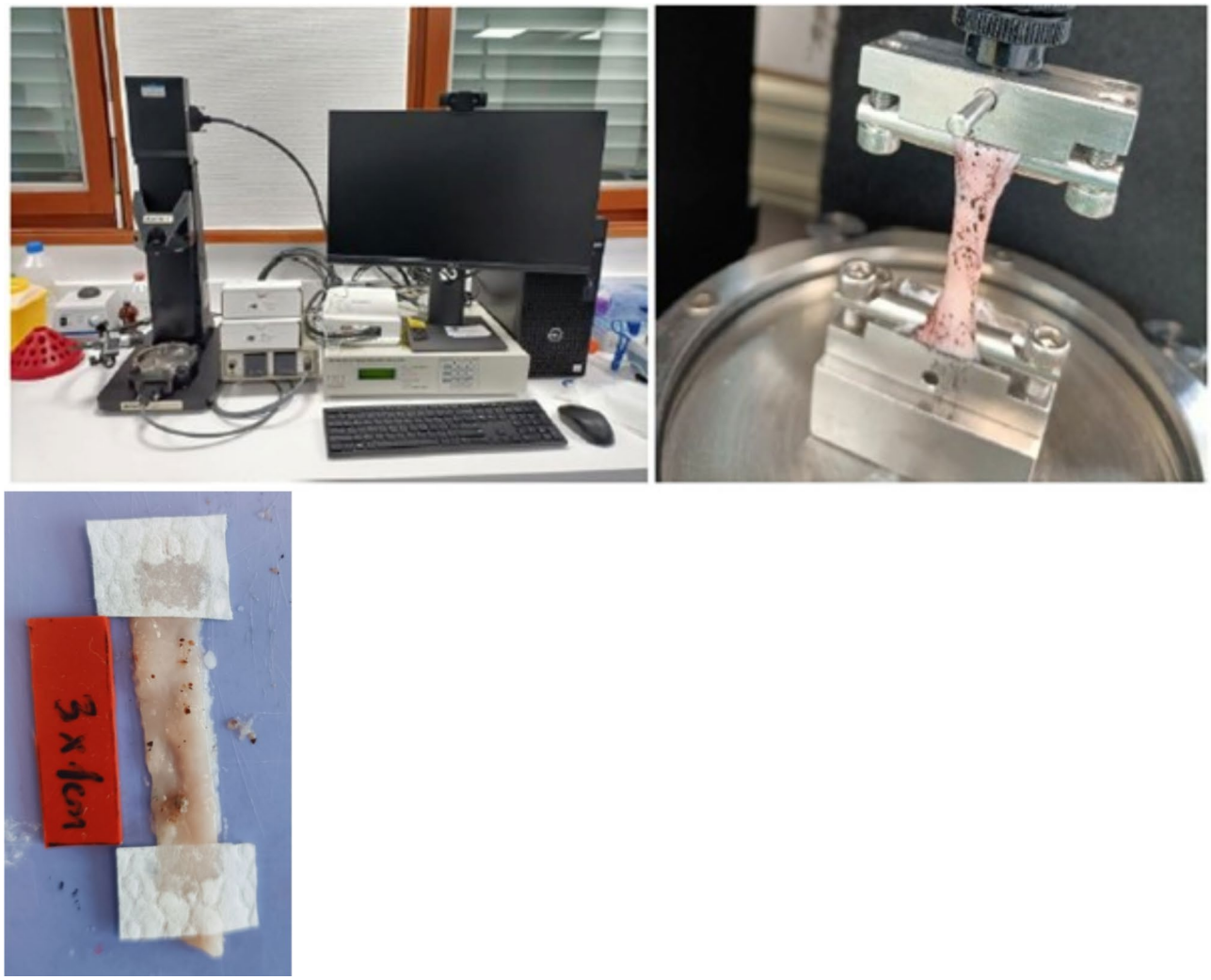


**Fig. 2**. Vaginal sampling and uniaxial tensile test using Mach-1® machine (Biomomentum Inc, Canada) and grips for soft tissues.

0.5 µm in the direction of stress (traction), together with the force at a frequency of 100 Hz. The displacement was applied until failure. The tensile tests were stopped when the sample broke. The tests were performed at a displacement velocity of 0.1 mm/s and at a constant temperature of 21 °C to avoid dehydration.

The uniaxial engineering stress $\sigma$ (kPa) in the loading direction was defined by Eq. (1).

$$\sigma = \frac{F}{A_0} = \frac{F}{t_0 \bullet w_0} \tag{1}$$

where $F$ is the measured force and $A_0$ is the initial cross-sectional area of the specimen.

The uniaxial engineering strain $\epsilon$ (%) in the loading direction is obtained by Eq. (2).

$$\epsilon = \frac{\Delta l}{l_0} \tag{2}$$

The stress-strain curves of each perineal tissue before the first damage for each sow and their mean were obtained. Unlike elastic materials, where stress varies linearly with respect to strain, soft tissues are hyperelastic. This means that these tissues exhibit non-linear elastic behavior[27]. Like elasticity, hyperelasticity models reversible behavior. The non-linear elastic behavior of these tissues was modeled by Yeoh's model[28] (Eq. 3).

$$\Psi = \sum {}_{i=1}^{3} C_i (I_1 - 3)^i \tag{3}$$

This hyperelastic law is described by three coefficients: C1, C2, and C3. These coefficients were identified by the method of least mean squares from experimental curves using the Levenberg-Marquardt algorithm before

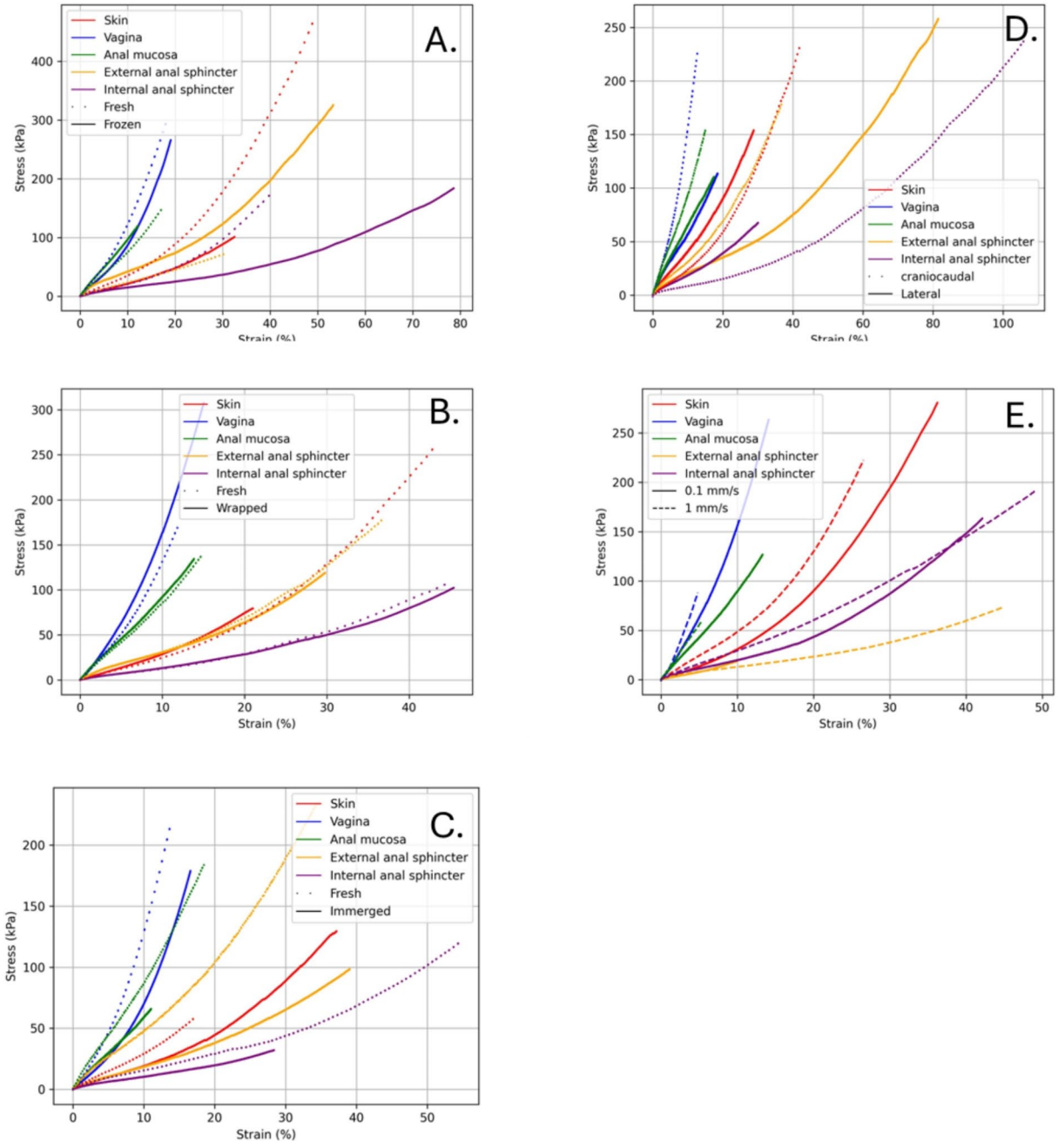


**Fig. 3**. Influence of experimental conditions on mean stress-strain curves for each sow's perineal tissue before first damage. (**A**) Influence of freezing samples. (**B**) Influence of storing samples in cellophane paper for 2 h. (**C**) Influence of storing samples in saline solution for 2 h. (**D**) Influence of sampling orientation. (**E**) Influence of displacement velocity.

the first sign of damage appeared in each tissue sample[29]. The damage, also called microfailure, is due to local and microscale effects. The occurrence of damage results in the weakening of the tissue, making it more likely to rupture and tear. A microfailure is associated with the inflection point on the experimental curve and is identified by locating the maximum on the derivative of the curve. The inflection of the curve indicates a reduction in the tissue's ability to withstand mechanical stress, which is interpreted by the appearance of fiber rupture or delamination in the tissue. This inflection generally precedes macroscopic tissue rupture.

Only the C1-coefficients was analyzed because of its significance: initial slope of the stress-strain curve at low strains (less than 5%)[30]. A high C1 hyperelastic coefficient means that, at low strain, 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 easily deformed even in response to a small applied force at low strain.

In the context of the Yeoh model for hyperelastic materials, the parameters C2 and C3 play a crucial role in describing the mechanical response of materials under varying degrees of strain. They capture the nonlinear material behavior at higher degrees of deformation. The C2 coefficient describes the hardening of the material with increasing strain. This means that at moderate levels of strain, the material becomes more resistant to further deformation. The effect of the C3 coefficient is a more complex and depends on its sign. If the C3 coefficient is positive, it means that the tissue becomes progressively harder as the deformation increases significantly. If the C3 coefficient is negative, it can introduce a softening behavior at high strain, which can be useful for modeling phenomena such as the Mullins effect (where the material becomes softer after repeated deformation cycles) or other non-linear behaviors where the material deteriorates or loses stiffness under high stresses[31].

Hyperelastic coefficients were expressed as mean ± standard deviation. C1 hyperelastic coefficients were compared according to the studied condition using the non-parametric Wilcoxon test. Statistical analysis was performed with R software (version 4.3.0) https://posit.co/. For all analyses, a p-value less than 0.05 was considered statistically significant.

## Test conditions

Tensile tests were performed under several predefined experimental conditions in order to create an experimental protocol that would control for factors that could alter the results on perineal tissue. We evaluated the influence of sample preparation conditions such as tissue freezing, hygrometry and sample orientation. We also evaluated the conditions under which the tests were performed such as the displacement velocity during the test. The influence of each of these factors was studied separately for each perineal layer such as the perineal skin, the vagina, the EAS, the IAS and the anal mucosa. Each factor was studied in 10 sows.

Except for the tested parameter, the other parameters were kept constant during the given test. Each of the perineal layers was divided into two samples, except for the EAS. For the EAS, it was not possible to create two samples due to their small size. Each parameter was tested on 10 samples except for the orientation factor. Indeed, only five samples with a horizontal orientation were created because of their small sizes. For each tissue except the EAS, the right samples were arbitrarily considered as the baseline samples (sample no. 1, also called E1). The left samples (sample no. 2, also called E2) were compared to the right samples. For the EAS, the baseline reference samples were arbitrarily defined as the five first samples. The baseline samples were subjected to tensile tests at a displacement velocity of 0.1 mm/s and at a constant temperature of 21 °C. The room hygrometry was kept constant at 45 ± 12%. The samples were tested in ambient air. No sample slipped during the tensile test.

## Influence of sample preparation conditions

*Influence of freezing*

Measurements were performed on each perineal layer immediately after cutting for sample no. 1 and after freezing at − 20 °C for more than 24 h for sample no. 2. When a sample was frozen, it was defrosted in a fridge at 5 °C for 9 h before tensile tests were performed. Tensile tests were performed at a constant displacement velocity of 0.1 mm/s and at a constant room temperature of 21 °C.

*Influence of hygrometry: cellophane wrapping paper*

Two different approaches were used to prepare perineal tissue samples for biomechanical testing. The first set of samples was processed immediately after dissection (sample no. 1). The second set was wrapped in cellophane and left at room temperature for two hours prior to testing (sample no. 2). Tensile tests were performed at a constant displacement velocity of 0.1 mm/s and at a constant room temperature of 21 °C.

*Influence of hygrometry: saline solution*

Measurements were made for each perineal layer immediately after cutting for sample no. 1 and after leaving it in a saline solution at room temperature for 2 h for sample no. 2. The tests were performed at a displacement velocity of 0.1 mm/s and at a constant room temperature of 21 °C.

*Influence of the sample orientation*

Two sets of measurements were performed to study the directional behavior of the vagina, the skin, the IAS and the anal mucosa. The vertical orientation of the EAS could not be studied due to its small width. A first series of samples was cut longitudinally (cranio-caudal axis) and the second series was cut transversely (right-left axis). The tests were performed immediately after cutting at a displacement velocity of 0.1 mm/s and at a constant room temperature of 21 °C.

## Influence of tensile test conditions: influence of the displacement velocity during testing

Measurements were performed for each perineal layer immediately after cutting, under identical conditions, except for the tensile displacement velocity. Each perineal layer was loaded at a displacement velocity of 0.1 mm/s for sample no. 1 and 1 mm/s for sample no. 2 at a constant room temperature of 21 °C.

# Results

The samples under baseline conditions are described in Table 1. For sample no. 1, the mean C1-coefficient values and corresponding standard deviations were 39 ± 27 kPa, 159 ± 69 kPa, 51 ± 48 kPa, 18 ± 15 kPa, 148 ± 54 kPa for the perineal skin, the vagina, the EAS, the IAS, and the anal mucosa, respectively. The vagina and the anal mucosa had the highest C1 hyperelastic coefficients. The internal anal sphincter had the lowest hyperelastic coefficients. Tensile tests lasted less than 15 min for the IAS and less than 10 min for the other perineal layers.

| Perineal tissue | Sample length (mm) | Sample width (mm) | Sample mean thickness (mm) | Room temperature (°C) | Room hygrometry (%) | Duration between dissection and tensile test (min) | C1 (kPa) | C2 (kPa) | C3 (kPa) |
|---|---|---|---|---|---|---|---|---|---|
| Skin | 37 ± 3 | 8 ± 1 | 8 ± 2 | 21 ± 0.2 | 45 ± 12 | 17 ± 8 | 39 ± 27 | 111 ± 91 | − 8 ± 52 |
| Vagina | 36 ± 3 | 8 ± 1 | 5 ± 2 | 21 ± 0.3 | 44 ± 11 | 20 ± 7 | 159 ± 69 | 1442 ± 1710 | − 3246 ± 6010 |
| EAS | 34 ± 4 | 8 ± 4 | 7 ± 2 | 21 ± 0.3 | 44 ± 12 | 18 ± 8 | 51 ± 48 | 114 ± 195 | 114 ± 195 |
| IAS | 37 ± 4 | 10 ± 2 | 8 ± 3 | 21 ± 0.2 | 44 ± 12 | 27 ± 15 | 18 ± 15 | 32 ± 36 | 3 ± 16 |
| Anal mucosa | 36 ± 3 | 7 ± 1 | 4 ± 1 | 21 ± 0.2 | 44 ± 11 | 27 ± 16 | 148 ± 54 | 376 ± 405 | − 1179 ± 1840 |

**Table 1**. Description of samples no. 1 and hyperelastics coefficients for each sow perineal tissues at baseline conditions. Results are expressed as mean ± standard deviation.

## Sample preparation conditions

*Influence of freezing (Fig. 3A)*

There was no statistically significant difference in terms of C1-hyperelastic coefficient between the frozen samples and the non-frozen samples ($p > 0.05$) (Table 2.). A significant variability of C1-hyperelastic coefficients between sows was observed for each perineal layer.

*Influence of hygrometry: cellophane wrapping paper (Fig. 3B)*

There was no statistically significant difference in terms of C1-hyperelastic coefficient between the samples stored in cellophane paper for 2 h (E1) and unstored samples (E2) ($p > 0.05$) (Table 2). Considering the standard deviation of C1-hyperelastic coefficients, a significant variability of C1-hyperelastic coefficients between sows could be observed for each perineal layer.

*Influence of hygrometry: saline solution (Fig. 3C)*

The C1-hyperelastic coefficient was statistically lower in skin (48 vs. 31 kPa, $p = 0.02$) and anal mucosa (152 vs. 91 kPa, $p = 0.03$) samples stored in saline solution for 2 h. The C1 hyperelastic coefficient tended to be lower in vaginal (139 vs. 73 kPa, $p = 0.09$) and external anal sphincter (80 vs. 31 kPa, $p = 0.09$) samples preserved in saline solution for 2 h, but without statistical significance (Table 2). A significant variability of C1-hyperelastic coefficients between sows was be observed for each perineal layer.

*Influence of the sample orientation (Fig. 3D)*

There was no statistically significant difference in terms of C1-hyperelastic coefficient between the samples with a vertical orientation (E1) and those with transverse orientation (E2) for each sow's perineal tissue ($p > 0.05$) (Table 2). A significant variability of C1-hyperelastic coefficients between sows could be observed for each perineal layer.

## Influence of tensile test conditions: influence of the displacement velocity during testing (Fig. 3E)

There was no statistically significant difference in the C1-hyperelastic coefficient between the samples loaded at a displacement velocity of 0.1 mm/s (E1) and those loaded at a displacement velocity of 1 mm/s (E2) for the perineal tissues of each sow ($p > 0.05$) (Table 2). A significant variability of C1-hyperelastic coefficients between sows could be observed for each perineal layer.

## Discussion

In this study, some biomechanical properties of each perineal layer of the sow were obtained. C1-coefficients were measured for the perineal skin, the vagina, the EAS, the IAS, and the anal mucosa of freshly dead sows. The vagina and the anal mucosa were the stiffest tissues. In other words, these rigid tissues had less deformation in response to an applied force. The internal anal sphincter was the more extensible and the less stiff tissue.

In this population, the vagina and the anal mucosa were the stiffest. The C1-coefficients of the vagina and the anal mucosa were higher than those of the perineal skin (159 and 148 kPa versus 39 kPa). This difference was also described by Gabriel et al. who compared the C1-coefficient of the vagina and the abdominal skin of unfrozen cadavers without relevant pelvic organ prolapse (0.35 versus 0.14 MPa, $p > 0.05$)[32]. In the literature, no study assessed the biomechanical properties of the anal mucosa. Only one evaluation of rectal tissue was found[33]. Rubod et al. compared vaginal and rectal tissues in fresh female cadavers without prolapse using multiaxial tensile tests[33]. 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.

No study was found in the literature regarding the biomechanical properties of the IAS. However, a corroboration with vaginal smooth muscle cells could be done. Smooth muscle cells contribute to the quasistatic and viscoelastic mechanical behavior of soft tissues[34–36]. According to Clark-Patterson et al., smooth muscle cells provide mobility by allowing the vagina to stretch under sustained pressure. Similarly, IAS may provide mobility by allowing the anal mucosa to stretch under sustained pressure[37].

According to the database, the C2-coefficient of these perineal tissues demonstrated their ability to stiffen under increased strain, which is a key characteristic of hyperelastic materials modeled by the Yeoh model[30]. The

| (A) C1-hyperelastic coefficients for each perineal tissue | No freezing (E1) | Freezing (E2) | $C_{E1}$–$C_{E2}$ | *p*-value |
|---|---|---|---|---|
| C1_Skin (kPa) | 53 ± 31 | 34 ± 26 | 19 ± 28 | 0.1 |
| C1_Vagina (kPa) | 164 ± 101 | 117 ± 96 | 46 ± 133 | 0.3 |
| C1_EAS (kPa) | 41 ± 23 | 63 ± 28 | − 22 ± 50 | 0.2 |
| C1_IAS (kPa) | 26 ± 16 | 18 ± 11 | 8 ± 15 | 0.3 |
| C1_Anal mucosa (kPa) | 133 ± 76 | 183 ± 123 | − 59 ± 144 | 0.3 |
| **(B) C1-hyperelastic coefficients for each perineal tissue** | **Open-air storage (E1)** | **Cellophane paper storage (E2)** | **$C1_{E1}$–$C1_{E2}$** | ***p*-value** |
| C1_Skin (kPa) | 39 ± 27 | 44 ± 26 | − 5 ± 16 | 0.4 |
| C1_Vagina (kPa) | 159 ± 67 | 184 ± 133 | − 24 ± 118 | 1 |
| C1_EAS (kPa) | 51 ± 48 | 46 ± 29 | 3 ± 68 | 0.8 |
| C1_IAS (kPa) | 18 ± 15 | 21 ± 14 | − 3 ± 10 | 0.6 |
| C1_Anal mucosa (kPa) | 148 ± 54 | 154 ± 59 | − 6 ± 80 | 0.5 |
| **(C) C1-hyperelastic coefficients for each perineal tissue** | **Open-air storage** | **Saline solution storage** | **$C_{E1}$–$C_{E2}$** | ***p*-value** |
| C1_Skin (kPa) | 48 ± 18 | 31 ± 11 | 17 ± 20 | **0.02** |
| C1_Vagina (kPa) | 139 ± 83 | 73 ± 39 | 67 ± 66 | 0.09 |
| C1_EAS (kPa) | 80 ± 81 | 31 ± 20 | 47 ± 63 | 0.09 |
| C1_IAS (kPa) | 21 ± 11 | 13 ± 7 | 8 ± 15 | 0.1 |
| C1_Anal mucosa (kPa) | 152 ± 65 | 91 ± 43 | 61 ± 64 | **0.03** |
| **(D) C1-hyperelastic coefficients for each perineal tissue** | **Vertical orientation (E1)** | **Transversal orientation (E2)** | **$C1_{E1}$–$C1_{E2}$** | ***p*-value** |
| C1_Skin (kPa) | 48 ± 18 | 60 ± 12 | − 10 ± 26 | 0.2 |
| C1_Vagina (kPa) | 196 ± 76 | 137 ± 85 | 83 ± 52 | 0.3 |
| C1_EAS (kPa) | – | 22 ± 9 | – | / |
| C1_IAS (kPa) | 48 ± 44 | 34 ± 10 | 21 ± 52 | 0.8 |
| C1_Anal mucosa (kPa) | 157 ± 52 | 162 ± 74 | − 2 ± 51 | 0.9 |
| **(E) C1-hyperelastic coefficients for each perineal tissue** | **0.1 mm/s (E1)** | **1 mm/s (E2)** | **$C_{E1}$–$C_{E2}$** | ***p*-value** |
| C1_Skin (kPa) | 48 ± 18 | 65 ± 26 | − 16 ± 34 | 0.1 |
| C1_Vagina (kPa) | 196 ± 76 | 253 ± 172 | − 82 ± 164 | 0.7 |
| C1_EAS (kPa) | 22 ± 9 | 20 ± 6 | 1 ± 8 | 0.7 |
| C1_IAS (kPa) | 48 ± 44 | 47 ± 25 | − 11 ± 41 | 0.5 |
| C1_Anal mucosa (kPa) | 157 ± 52 | 173 ± 83 | − 17 ± 77 | 0.8 |

**Table 2**. Comparison of C1-hyperelastic coefficients between. A. Frozen samples (E1) and non-frozen samples (E2) for each perineal tissue before first damage. B. Samples stored in cellophane paper for 2 h (E1) and unstored samples (E2) for each perineal tissue. C. Samples stored in saline solution for 2 h (E1) and unstored samples (E2) for each sow's perineal tissue. D. Samples with a vertical orientation (E2) and those with a transversal orientation (E2) for each sow's perineal tissue. E. 0.1 mm/s displacement velocity (E1) and 1 mm/s displacement velocity (E2) for each perineal tissue. Results are expressed as mean ± standard deviation.

higher C2-coefficient concerned the vaginal tissues. It indicated a pronounced non-linear stiffening behavior under strain. The IAS had the lower C2-coefficient compared to other tissues, suggesting less pronounced stiffening. The lower variability might indicate more uniform properties across samples. High standard deviation were found for all perineal tissues except for IAS. This could reflect intrinsic material heterogeneity. The C3-coefficient provided insight into how each tissue type might behave under extreme deformations. At high strain, the vagina and the anal mucosa underwent a strong softening behavior. The near-zero mean value of the C3-coefficient for the skin, combined with a large standard deviation, indicated that the effect of the C3-coefficient on the mechanical behavior of the skin was minimal and highly variable. The negative sign indicated a potential for slight material softening at very high strains, although the small magnitude and variability made this effect uncertain. The IAS exhibited a very low positive C3-coefficient value, indicating minimal additional stiffening at high strains. This behavior suggested a more consistent and predictable response at extreme stretching, likely contributing to its functional stability. The positive C3-coefficient for the EAS, similar to its C2-coefficients value, suggested that the tissue continued to stiffen with increasing strain, enhancing its structural integrity under extreme deformations. The variability shown by the standard deviation indicated to some inconsistency in how this behavior manifested itself in different samples.

Due to complex testing protocols, it is important to assess the storage of sow perineal samples to ensure the reliability and reproducibility of the testing protocol aimed at determining the biomechanical properties of the sow perineum. Our study contributes to the development of a robust testing protocol by specifically investigating

the effect of storage solutions on these properties. We found storage of sow perineal samples in saline solution significantly affected the C1 hyperelastic coefficient ($p > 0.05$), suggesting alterations in the biomechanical properties of the tissue. This effect of saline solution on biological tissues can be attributed to several factors. First, saline may induce changes in tissue hydration and electrolyte balance, which could alter the mechanical properties by affecting the viscoelastic behavior of the collagen matrix within the tissue. Furthermore, prolonged exposure to saline might lead to leaching of cellular components and degradation of structural proteins such as collagen and elastin, which are critical for maintaining tissue integrity and mechanical strength. Similar observations were reported by Caro-Bretelle et al., who studied the effect of preservation conditions on the mechanical behavior of porcine skin. Their research demonstrated that saline immersion significantly altered the global mechanical behavior[38]. Caro-Bretelle et al. found that saline preservation tend to soften the tissues by increasing their water content, thereby enhancing the mobility of collagen and elastin fibers within the extracellular matrix. This is called the plasticizing effect of water: water molecules increase the chain mobility within the tissue[39]. This increased mobility could reduce the stiffness of the tissue, a phenomenon also observed in our study with a decrease in the C1-coefficient.

An alternative way to prevent dehydration could be to wrap samples in cellophane paper. In our study, storing perineal samples in cellophane paper for two hours did not statistically change the C1-hyperelastic coefficients. This kind of storage has not been described in the literature.

Sample preparation conditions such as tissue freezing for 24 h, storage in cellophane paper for two hours and sample orientation, as well as testing conditions such as the displacement velocity during testing, did not appear to statistically affect the C1-hyperelastic coefficient.

To simplify experiments and save time, samples are often frozen for storage and then defrosted prior to testing. Simple cryopreservation techniques, such as freezing tissue at -20 °C, are commonly used to preserve samples in both medical and research settings due to their ease of access. Nevertheless, research indicated that such freezing can cause the formation of ice crystals within the extracellular matrix (ECM), potentially leading to cellular dehydration and intracellular ice formation[40,41]. Observations include reductions in tissue size and weight after freezing, suggesting a phenomenon of bulk water movement during the process[42,43]. These alterations could compromise critical structural components such as smooth muscle cell viability and collagen and elastin fiber integrity, thereby impacting the mechanical properties of the tissue[42,44]. Although these microstructural changes are well documented. The consensus on their effects on the overall mechanical functionality of tissues remains controversial with studies showing mixed results. In our study, freezing did not affect the C1-hyperelastic coefficients for any of the perineal tissue. Rubod et al. also showed that freezing had negligible effects on the mechanical properties of ewe vaginas[45]. But, Caro et al. showed that a simple storage of porcine perineal skin at -20 °C altered the mechanical behavior of the tissue except for its elastic response[38]. For this team, cryopreservation was the only way to preserve the mechanical behavior of fresh tissues (even delaying the onset of damage phenomena). However, histological and collagen level analyses of the effects of cryopreservation on collagen integrity have not yet been performed. In our study, the freezing time was 24 h. According to Calvo-Gallago, freezing for more than 30 days significantly altered the viscoelastic properties of the tissues as shown by compressive stress relaxation tests compared to one day, one week, one month and 3 months[46].

The C1-hyperelastic coefficient was comparable for each perineal tissue at strain rates of 1 mm/s and 0.1 mm/s. This result is interesting because most tissues, and viscoelastic materials in general, demonstrate an increase in tangent modulus with increasing strain rate[47]. Rubod et al. studied experimental conditions on sheep vaginal tissue[45]. They also demonstrated that ovine vaginal tissue was minimally sensitive to strain rate. If the results are confirmed in vivo, it could be interesting. The tissues were not stiffer when the displacement velocity was higher. In other words, tissue elasticity could not be affected by high displacement rate at the onset of deformation.

Results from uniaxial tensile tests do not provide a precise description of how a tissue will behave in vivo under physiologic multiaxial loading. But these tests can help to assess the influence of factors on the mechanical properties. In our study, sample orientation (vertical or transversal) did not statistically alter the C1-hyperelastic coefficients for the skin, the vagina, the IAS and the anal mucosa. The EAS could not be studied because of its small width. Rubod et al. demonstrated that the ewe vagina was anisotropic[45]. Wong et al. also showed the anisotropy of the skin in the porcine model[48].

Regarding the experimental methodology, no failure or tears near the grip have been noticed. Operators were trained prior to the study to avoid the learning phase and reduce these kinds of failure. Perineal stress during delivery is not unidirectional. However, due to the size of the samples, only unidirectional traction tests at constant speed were performed. The Yeoh model was chosen because it describes accurately the behavior of all the different perineal tissues. A comparison of the Yeoh, Mooney-Rivlin, and Ogden models on porcine skin (abdomen and back) confirmed that the Yeoh model accurately captures the behavior of the porcine skin[49]. 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. It also requires a small number of parameters, with its first parameter being interpreted as half the initial shear modulus.

To our knowledge, this study was the first one to investigate the influence of experimental conditions on some biomechanical properties of perineal tissues. Our investigation highlighted the significant effect of storage conditions, particularly saline solution, on the biomechanical properties of sow perineal tissues. Understanding these effects is crucial for designing experimental protocols that closely mimic physiological conditions, which is especially relevant for studies of perineal tears during childbirth. By establishing that certain storage conditions, such as saline immersion, can alter tissue properties, we are paving the way for more accurate in vitro models. Our findings on the effects of saline immersion on perineal tissue properties may also provide insight into the clinical phenomenon of increased perineal tearing associated with prolonged labor. During prolonged labor, the tissues of the birth canal, including the perineum, are exposed to prolonged periods of stress and potential swelling

(edema), which can simulate the effects of saline immersion studied here. Edema increases tissue water content. This increased water content can alter the viscoelastic properties of the perineal tissues, making them more susceptible to tearing under stress. Our study shows that saline can decrease the mechanical strength of tissues, suggesting that edema could similarly weaken perineal tissues during labor. Understanding that edema can lead to tissue softening and increased tearing susceptibility provides a critical insight for obstetric management. This knowledge can guide interventions to minimize labor duration and proactively manage perineal integrity, such as the use of controlled pushing phases or local treatments to reduce swelling during labor. The application of cold compresses during labor could be explored as strategies to reduce perineal edema and preserve tissue integrity. Further research should investigate the specific mechanical thresholds at which edema leads to an increased risk of tearing. Experimental studies using biomechanical testing of edematous perineal tissues, along with computational modeling to simulate the dynamic of labor, would be valuable. Such studies could help refine our understanding of the effects of labor duration and tissue condition on the risk of perineal tears.

A weakness of our study was the sampling and trimming. It was performed by only one surgeon in a standardized manner on each sow. The vaginal wall and the IAS were excised with an amount of connective tissue that was not well defined. It was difficult to dissect and individualize these tissues. But, the inclusion of these tissues in the measurements was not biased, as they participate in the local mechanical reality. In addition, the age of the sows was not known. They were nulliparous. Furthermore, an important inter-individual variability was found. Finaly, vaginal delivery also causes damage to supporting structures, including ligaments and levator ani muscles. Our study focused on the biomechanical properties of the perineal soft tissues during childbirth to understand the direct causes of perineal tears, as they are directly involved in obstetric trauma. It could be interesting to study the ligaments and levator ani muscle in future research.

Subsequent research could involve the in vivo study of the mechanical properties of the human perineum. Non-invasive methods could be used during pregnancy and delivery such as elastography[11,50,51], stereophotogrammetry[10], vaginal elastometer[52] or using a tactile probe[8].

## Conclusion

This study suggested that the experimental protocol could include sample preparation conditions such as tissue freezing for 24 h, storage in cellophane paper for two hours, and 0.1 mm/s displacement velocity without statistically influencing C1-hyperelastic coefficient. Samples should not be stored in saline for 2 h. This experimental protocol could be used to study the biomechanical properties of perineal tissues in order to better understand perineal tears during childbirth.

## Data availability

Data available on request from the corresponding author.

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## Author contributions

ML: conceptualization, data curation, formal analysis, methodology, writing-original draft; TK: conceptualization, data curation, formal analysis, methodology, writing-review and editing; JC: conceptualization, methodology, writing-review and editing; AL: conceptualization, methodology, writing-review and editing; RR: writing-review and editing; NM: writing-review and editing; MC: writing-review and editing; EJ: conceptualization, methodology, writing-review and editing.

## Declarations

## Competing interests

M. Cosson has contracts from Ab medica, Promedon, Syliva, Proveday and receives consulting fees from Boston scientific and honoraria for educational events from Boston scientific and Promedon. He is also a founder of Digyne start-up. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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