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ORIGINAL ARTICLE

First delivery and ovariectomy affect biomechanical and structural properties of the vagina in the ovine model

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Abstract

Introduction and hypothesis Animal models are useful for investigating the genesis of pelvic floor dysfunction and for developing novel therapies for its treatment. There is a need for an alternative large-animal model to the nonhuman primate. Therefore we studied the effects of the first vaginal delivery, ovariectomy and systemic hormonal replacement therapy (HRT) on the biomechanical and structural properties of the ovine vagina.

Methods We examined the gross anatomical properties of nulliparous, primiparous, ovariectomized multiparous, and ovariectomized hormone-replaced multiparous sheep (six animals per group). We also harvested mid-vaginal and distal vaginal tissue to determine smooth muscle contractility and passive biomechanical properties, for morphometric assessment of the vaginal wall layers, to determine collagen and elastin content, and for immunostaining for α-smooth muscle actin and estrogen receptor-α.

Results There were no regional differences in the nulliparous vagina. One year after the first vaginal delivery, stiffness and contractility of the distal vagina were decreased, whereas the elastin content increased. The mid-vagina of ovariectomized sheep was stiff, and its epithelium was thin and lacked glycogen. HRT decreased the stiffness of the mid-vagina by 45% but had no measurable effect on contractility or elastin content, and increased epithelial thickness and glycogen content. HRT also increased the epithelial thickness and glycogen content of the distal vagina. At this location, there were no changes in morphology or stiffness.

Conclusion In sheep, life events including delivery and ovariectomy affect the biomechanical properties of the vagina in a region-specific way. Vaginal delivery mainly affects the distal region by decreasing stiffness and contractility. HRT can reverse the increase in stiffness of the mid-vagina observed after surgical induction of menopause. These observations are in line with scanty biomechanical measurements in comparable clinical specimens.

Keywords Animal model · Biomechanics · Contractility · Sheep vagina · Vaginal delivery

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Introduction

Pelvic floor dysfunction (PFD) is a multifactorial disease, with vaginal delivery, age and menopausal status as important causal factors [1]. Recent data from the PROLONG study show that 12 years after vaginal delivery, 54% of women have measurable pelvic organ prolapse (POP) which is symptomatic in with half of them [2]. Along the same lines, urinary and fecal incontinence occur at rates as high as 53% and 13%, respectively. PFD impairs the quality of life of women as much as stroke and dementia. The longer life expectancy and increased physical activity in the elderly have raised the need for therapy [3]. Surgery remains the mainstay of therapy, with a variety of surgical procedures using either native tissues or implants. Each has its limitations, including recurrence and local complications. Given the prevalence and the impact of PFD, and the limitations of current therapeutic solutions, there is a need for preclinical research to investigate the pathophysiology of PFD, more efficient or less invasive therapies and potentially preventive strategies [4].

One way to meet this need is to use appropriate animal models [5] which should mimic the anatomical and biological vaginal environment. Ideally, in these models those life events considered as risk factors in women should result in comparable structural and functional changes in the pelvic floor [1]. The leading factors for PFD are vaginal birth and age, and menopausal status is considered an additional trigger [1]. Several groups consider the sheep as such an animal model because of the similarities in pelvic anatomy and the reproductive tract between sheep and humans [6–8]. The model permits vaginal surgery and mesh insertion, as first described by de Tayrac et al. [7]. Moreover, sheep frequently have complicated vaginal deliveries, and around 15% have antepartum POP. Similar to women, the risk factors for PFD in sheep include multiparity, previous history of POP, increased intra-abdominal pressure, or intake of (phyto)estrogens [5]. In Europe, sheep have become an alternative large-animal model, as research using nonhuman primates has become nearly impossible. Most experiments in sheep have so far been to study therapeutic interventions, and there is only limited research available on the causal factors for PFD. The primary aim of this study was to describe the biomechanical effects in two specific conditions: (1) the medium-term effects of the first vaginal delivery, and (2) the effects of hormone replacement therapy (HRT) in previously ovariectomized sheep.

Materials and methods

This study included six nulliparous, six primiparous, and 12 multiparous reproductive Swifter sheep, which were obtained from the Zoötechnical Institute of the KU Leuven. The primiparous and multiparous sheep were the same age and 1 year after their last lambing, and hence no longer lactating. The ovarian cycles of the nulliparous and primiparous sheep were synchronized by means of a medroxyprogesterone acetate vaginal sponge (Veramix; Pfizer, IJssel, The Netherlands). Five days after removal of the sponge these animals were euthanized. Multiparous sheep underwent bilateral ovariectomy under a previously described operative and anesthetic protocol [9]. After 60 days half of the sheep were killed and the remaining animals received HRT for another 60 days [10, 11]. Sustained release estradiol was used for HRT, as described by Brasted et al. [12]. 17-β-Estradiol (Estradiol, Sigma-Aldrich, Diegem, Belgium) powder (10 mg) was placed in a cylindrical container (30 × 1.6 mm; Dow Corning, Seneffe, Belgium), and was implanted subcutaneously under local anesthesia with the animal in a restriction cage. Prior to implantation, the containers were incubated overnight at 37 °C in phosphate-buffered saline (PBS) containing 5% fetal calf serum. In each multiparous sheep group (the ovariectomy group and the HRT group), two had two previous vaginal deliveries and four had three previous deliveries.

Outcome measures

Animals were killed by intravenous injection of 100 mg/kg phenobarbital (Release; Ecuphar). First, the vagina and surrounding tissue were removed *en bloc* and the vagina was opened longitudinally along the urethra. Length and width were measured using a micrometer (Horex; Helios Preisser, Gammertingen, Germany). Vaginal length was defined as the distance between the cervix and the hymen and the width was measured at the narrowest part of the vagina. The posterior vaginal wall was divided into thirds. The aim was to test the biomechanics with the plunger placed 1 cm cranially to the hymenal ring (distal vagina). A sample suitable for biomechanics (30 × 30 mm) was obtained, and then another 30 × 30-mm sample was obtained with the area of interest in the middle third (Fig. 1).

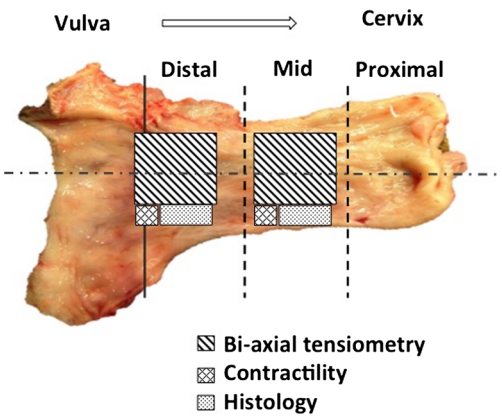

Fig. 1 Location of the explanted specimens used for histological, biomechanical and biochemical analysis

Biomechanics

We have previously described our testing protocol in detail [13]. Briefly, we used a ball burst test on a 500 N Zwick tensiometer (Zwick GmbH&Co. KG, Ulm, Germany) with a spherical plunger (diameter 11.5 mm) and a 20-mm aperture. Specimens were trimmed to 30 × 30 mm and stored in saline-soaked gauzes at −20 °C until testing. Prior to testing each specimen was thawed at room temperature for 6 h and then clamped with the epithelial side facing upward. The plunger was centered over the aperture and the specimen was preloaded to 0.1 N at a rate of 5 mm/min. The specimen was then loaded at a rate of 10 mm/min until disruption or until the maximal cell load of 200 N had been reached. Load–elongation curves were analyzed using Excel (Microsoft Office, Redmond, WA, USA) and divided into a comfort zone and a stress zone, as described previously [14].

The method for measuring contractility is reported in detail elsewhere [13]. Briefly, strips from the distal vagina and mid-vagina (about 10 × 8 mm) oriented along the circumferential axis of the vagina, were freshly harvested and placed into a physiological Krebs solution at 37 °C. Samples were preloaded (0.1 mN) and allowed to equilibrate for 1 h and then immersed in 80 mM KCl. The length, width and thickness of each sample were measured using a micrometer to estimate the volume of each specimen. The contractile force measurements were normalized to tissue volume (millinewtons per millimeter squared).

Histology and immunohistochemistry

Tissue specimens were fixed in 4% paraformaldehyde, embedded in paraffin, cut into 6-μm sections and stained with hematoxylin and eosin (H&E), Masson trichrome, Miller's pentachrome and periodic acid-Schiff (PAS), and immunohistochemically for α-smooth muscle actin (α-SMA-clone 1A4, 1:200 dilution; DAKO). The thickness of the epithelium, lamina propria and muscularis were measured with ZEN2 lite software (Carl Zeiss Microscopy GmbH) on an Axioplan 40 microscope (Zeiss, Oberkochen, Germany). PAS-stained sections of the vagina were evaluated qualitatively for the presence of glycogen in the epithelium.

Image evaluation

Virtual images were first acquired with the fully automated digital microscopy system dotSlide (Olympus, BX51TF, Aartselaar, Belgium) coupled with a Peltier-cooled high-resolution digital color camera (1,376 × 1,032 pixels; XC10, Olympus). Digital images of the whole tissue sections were digitized at high magnification (×100), producing virtual images in which the pixel size was 0.65 μm.

Image processing and measurements were performed using the image analysis toolbox of MATLAB R2016a (9.0.0.341360; Mathworks, Inc., Natick, MA, USA) according to a previously described method [15, 16]. Figure 2 shows original stained images and the corresponding binary images in which the detected αSMA, collagen and elastin regions are white (pixel value equal to 1) and the background is black (pixel value equal to 0). On these binary images, elastin density (area occupied by elastin fibers per unit surface), total collagen density (area occupied by the collagen fibers per unit surface) and density of smooth muscle cells were measured in the whole tissue section. Densities were first calculated for each animal at each location considered. Mean densities were determined for each location and expressed as means ± standard error of the mean (SEM).

Estrogen receptor-α immunohistochemistry and evaluation

On deparaffinized vaginal wall slices endogenous peroxidase activity was blocked with 0,5% H_2O_2 in methanol for 30 min at room temperature. Sections were then heated at 78 °C for 2 h in Tris-HCl buffer (0.01 M, pH 9.0) with 1 mM EDTA to enhance antigen retrieval. Nonspecific binding was minimized by incubating sections in 2% bovine serum albumin and 1% milk in PBS 0.1% Tween 80 for 30 min. Sections were incubated overnight at 4 °C with the primary monoclonal antibody against estrogen receptor-α (ER-α) at 1:25 dilution (α-ER clone 1D5, 166 μg/mL; DAKO, Glostrup, Denmark). Specific secondary antibodies were used. The color reaction was developed with 3,3′-diaminobenzidine (Sigma). For ER-α analysis, images were captured at ×400 magnification at the epithelium, lamina propria and muscularis in five randomly chosen fields [13]. The total number of positively stained cells (brown reaction product) were measured with the particle analysis tool in ImageJ [17], and expressed as a ratio in relation to the total number of cell nuclei (brown reaction product + blue hematoxylin).

Statistics and Ethics Committee approval

The normality of distributions was tested with the Kolmogorov–Smirnov test. The presence of outliers was tested using Grubbs' test and where appropriate outliers were excluded. Parametric data are reported as means ± standard deviation (SD) and nonparametric data are reported as medians and interquartile ranges (IQR).

To compare samples obtained from the distal vagina and mid-vagina within a single group, a paired *t* test or Mann-Whitney test was used as appropriate. The following comparisons were made: vaginas from primiparous and nulliparous animals were compared, and vaginas from animals on HRT and ovariectomized animals were compared (unpaired *t* test or Mann-Whitney test). Analyses were performed with Prism 5

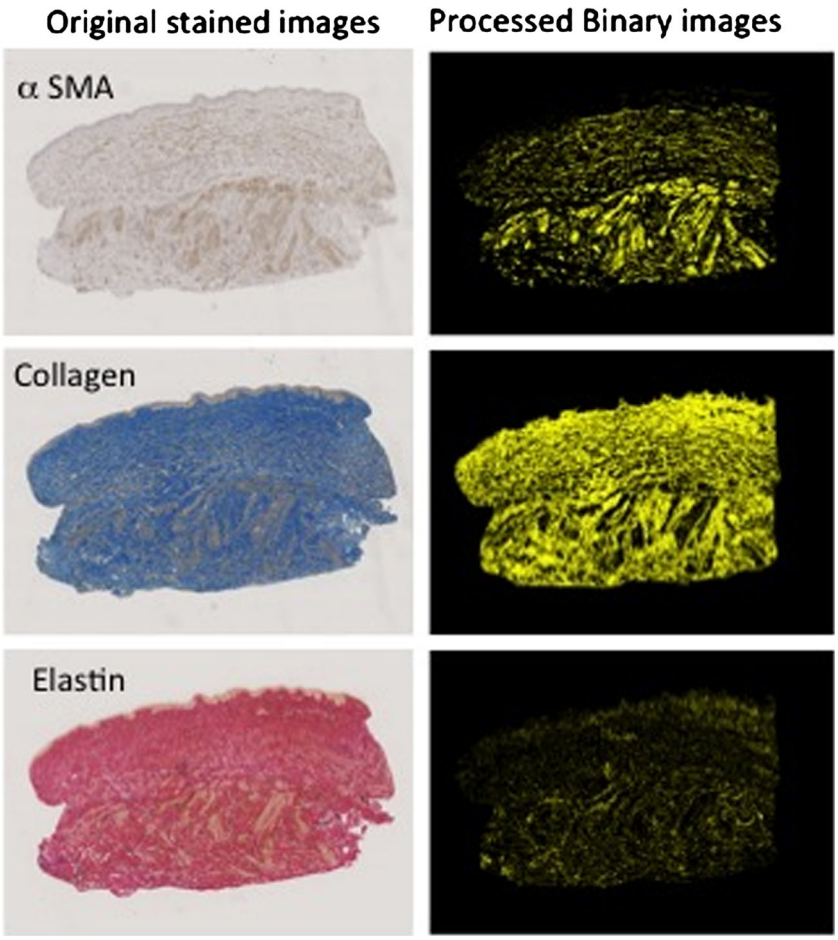

Fig. 2 Image processing for density evaluation. Original stained images are converted to binary (black and yellow) images. The density is determined as the area occupied by the designated tissue component (yellow product)

(GraphPad Software, Inc., La Jolla, CA, USA). The results were considered significant for p values <0.05. Figures display individual data points. Animals were treated in accordance with current national guidelines on animal welfare. This study was approved by the Ethics Committee for Animal Experimentation of the Faculty of Medicine of the KU Leuven (P005-2013).

Results

The demographic data are displayed in Table 1. The effects of the studied life events are first described in terms of regional differences within each group. Comparisons between nulliparous and primiparous animals, and between ovariectomized and hormone-replaced animals follow.

Nulliparous animals and effect of first pregnancy and vaginal birth

The median length of nulliparous vaginas was 9.5 cm (IQR 1.2 cm). The narrowest region was the distal third which had a circumference of 4.3 cm (IQR 0.5 cm; Table 1). The vaginal epithelium was multilayered and rich in glycogen. The lamina propria was globally very thin. In the distal vagina, the lamina muscularis was not very well organized; muscular bundles varied in dimensions and orientation. In the mid-vagina, the lamina muscularis was organized into an inner longitudinal and an outer circular layer. There were no regional differences (distal vagina vs. mid-vagina) in vaginal wall stiffness, contractility or histological measurements (Table 2; Figs. 3 and 4). Primiparous distal vagina was 52% less stiff than mid-vagina ($p = 0.007$), and had a 63% lower contractility ($p = 0.074$, not significant). The distal vaginal epithelium was 20% thicker and contained 85% more elastin than the mid-vagina ($p = 0.006$ and $p = 0.015$, respectively).

Primiparous vagina was 25% wider at the narrowest part and 15% longer than nulliparous vagina ($p = 0.0097$ and $p = 0.04$; respectively). The distal vagina was most affected by the first delivery. Primiparous vagina was 61% less stiff ($p = 0.014$) and had a 32% longer comfort zone ($p = 0.002$) than nulliparous vagina. Smooth muscle activity in primiparous vagina generated 64% less force ($p = 0.008$). Morphologically the lamina propria of primiparous distal

Table 1 Biometry and gross anatomical findings

	Nulliparous vs. primiparous		Multiparous, ovariectomy vs. HRT	
	Nulliparous ($n = 6$)	Primiparous ($n = 6$)	Ovariectomy ($n = 6$)	HRT ($n = 6$)
Biometry				
Weight (kg)	49.0 (43.7–56.2)	53.5 (44.3–73.5)	68.1 (58.8–72.6)	62.2 (53.8–66.3)
Age (months)	12.6 (10.5–14.6)[a]	34.2 (33.4–43.0)[a]	36.2 (35.6–44.5)	42.5 (34.5–43.0)
Macroscopy				
Vaginal length (cm)	9.4 (8.4–10.7)[b]	11.0 (10.3–11.6)[b]	8.0 (7.2–10.2)	8.2 (7.9–9.0)
Vaginal circumference (cm)	4.5 (3.9–4.7)[c]	6.0 (5.2–6.1)[c]	3.3 (3.5–4.1)[d]	5.5 (4.4–6.0)[d]

Data are displayed as medians (IQR)

P values: [a] = <0.0001, [b] $p = 0.04$, [c] $p = 0.002$, [d] $p = 0.006$

vagina was 31% thinner ($p = 0.004$), and primiparous distal vagina contained 79% more elastin and 29% less collagen ($p = 0.015$, $p = 0.041$, respectively). The proportion of ER-α-positive nuclei was much lower in the epithelium of the distal vagina 1 year after the first delivery compared with that in nulliparous vagina. The mid-vagina underwent more limited change. The only change in primiparous mid-vagina was thinning of the lamina propria; the other variables were not different from those in nulliparous vagina.

Effect of HRT on the vagina of ovariectomized sheep

In the ovariectomized sheep, the vaginal epithelium was thin and lacked PAS positivity (Fig. 4). In half of the menopausal sheep it was difficult to identify the muscularis in the distal vagina, which showed sporadic α-SMA-positive cells spread throughout the tissue. In the other menopausal sheep the muscularis was qualitatively comparable to that of nulliparous sheep. Their mid-vagina was 47.5% stiffer than the distal vagina ($p = 0.0004$). There were no other regional differences in elastin, collagen or α-SMA density (Figs. 3 and 4). Animals on HRT displayed regional differences in vaginal tissue. Contractility of the mid-vagina was 91% higher than that of the distal vagina ($p = 0.045$). The mid vagina contained 56% less elastin ($p = 0.065$). Similar to the ovariectomized group, no muscularis could be identified in the distal vagina in four of the six animals.

Sheep on HRT had a 32% greater vaginal circumference than ovariectomized sheep ($p = 0.006$), but there was no difference in vaginal length (Table 1). The biological effect of HRT was clear in all animals with widespread PAS positivity of the epithelium, together with a higher number of ER-α-positive cells mainly in the mid-vagina (Table 2, Fig. 4). There were no detectable histomorphological changes in the distal vagina. Sheep on HRT had a 45% lower mid-vaginal stiffness than ovariectomized sheep ($p = 0.005$), but there was no difference in contractility. The other variables were not affected by HRT.

Discussion

The principal outcome measures in this study were active and passive vaginal biomechanical properties. The effects of life events such as delivery and menopause on passive biomechanics are regionally different, as previously observed in other species [1, 6, 18]. Compared with that in nulliparous animals, first vaginal delivery decreased distal vaginal stiffness by 62%, but did not affect the mid-vagina. Active contractility was also decreased in the distal vagina, but not in the mid-vagina. This was paralleled by an increased elastin content and a decreased collagen content in the distal vagina. Estrogen exposure decreased the stiffness of the mid-vagina compared with that in ovariectomized animals, but did not affect contractility. This did not coincide with morphometric elastin or collagen changes.

To compare these changes to clinical data is challenging. First, one can look at data from post-mortem studies, but obviously data on young nulliparous or primiparous women are scarce, and data from older women may be affected by several confounding factors. Second, few data are available on regional differences as the acquisition of such data requires collection of large specimens. Another source of information could be specimens taken during surgery. Those taken during hysterectomy are, however, usually taken from the apex of the vagina. Specimens from the lower vagina would be taken during prolapse surgery, and hence clearly pathological. Surgical specimens obviously may not be large enough for appropriate biomechanical testing. Despite all these shortcomings, the current literature suggests that menopausal women indeed have stiffer vaginal tissue than younger women [19, 20]. This is in line with our observations, but in biologically younger surgically ovariectomized animals. To our knowledge no clinical data are available which could be used to benchmark our findings in young primiparous women. The data we obtained in sheep are in line with the trends observed in other species including rats and nonhuman primates [21, 22]. More recently, other studies in sheep have become

Table 2 Active and passive biomechanical properties of the ovine distal vagina and mid-vagina and lower abdominal wall. Thickness and distribution of ER-α positive nuclei in vaginal epithelium, subepithelium and muscularis. Data are displayed as median (IQR) or as mean ± standard deviation according to their distribution

	Nulliparous		Primiparous				Multiparous, ovariectomized		Multiparous, HRT			
	Distal vagina	Mid-vagina	Distal vagina	*p* value vs. nulliparous	Mid-vagina	*p* value vs. nulliparous	Distal vagina	Mid-vagina	Distal vagina	*p* value vs. ovariectomized	Mid-vagina	*p* value vs. ovariectomized
Passive biomechanical properties of the vagina, median (IQR)[a]												
Comfort zone stiffness (N/mm^2)	0.28 (0.14–0.35)	0.22 (0.20–0.31)	0.11 (0.09–0.18)*	0.04	0.23 (0.17–0.32)*	–	0.21 (0.15–0.28)*	0.40 (0.29–0.43)*	0.18 (0.12–0.23)	–	0.22 (0.19–0.26)	0.01
Comfort zone length (mm)	3.78 (3.55–4.23)	4.84 (3.96–5.61)	5.55 (4.71–6.05)	0.002	4.47 (4.20–4.51)	–	4.62 (3.93–5.24)	4.53 (4.28–5.03)	3.39 (3.16–4.52)	–	5.50 (4.43–6.53)	–
Contractility of vaginal strips (mN/mm^3), median (IQR)	0.167 (0.115–0.189)	0.185 (0.141–0.254)	0.065 (0.035–0.084)	0.008	0.209 (0.119–0.435)	–	0.008 (0.001–0.058)	0.093 (0.038–0.169)	0.017 (0.141–0.254)	–	0.202 (0.050–0.376)*	–
Vaginal wall thickness (μm), median (IQR)[a]												
Epithelium	46.5 (36.9–51.6)	38.7 (34.2–54.5)	35.8 30.0–54.7)*	–	28.1 (24.8–43.1)*	–	14.8 (13.5–23.9)	18.7 (14.6–24.8)	30.0 (24.5–33.8)	0.017	32.6 (28.2–37.9)	0.004
Lamina propria	1,408.0 (1,396.0–1,553.0)	1,312.0 (1,090.0–1,420.0)	1,020.0 (880.4–1,085.0)	0.017	953.0 (680.5–1,137.0)	0.023	1,575.0 (804.6–1,805.0)	1,666.0 (1,437.0–1,7-30.0)	1,062.4 (746.0–1,481.0)	–	1,355.0 (1,121.0–1,4-41.0)	–
Muscularis	1,421.0 (1,038.0–2,0-85.0)	1,321.0 (1,103.0–1,8-68.0)	1,077.0 (624.7–1,30-0.0)	–	1,261.0 (1,137.0–1,297-.0)	–	1,679.0 (989.0–1,881.0)[c]	1,012.0 (708.6–2,327.0)	1,513,7 (1,260.0–1,767.0)[d]	–	1,333.8 (1,169.0–1,7-97.0)	–
Estrogen receptor-α distribution (%), mean ± SD[b]												
Epithelial	20.7 ± 10.8	11.0 ± 8.3	4.3 ± 4.0	0.043	4.3 ± 3.9	–	14.3 ± 9.0	9.6 ± 6.6	35.2 ± 8.0	0.008	48.7 ± 16.9	0.005
Lamina propria	47.7 ± 11.0	46.1 ± 8.1	42.4 ± 21.9	–	49.0 ± 14.3	–	32.8 ± 14.7	41.2 ± 16	43.2 ± 7.6*	–	72.4 ± 6.4*	0.02
Muscularis	56.3 ± 18.9*	30.4 ± 10.6*	32.7 ± 18.2	–	24.9 ± 10.1	–	33.4 ± 14.2	39.2 ± 5.5	36.6 ± 25.9	–	65.7 ± 14.8	0.007

$p < 0.05$, between vaginal regions within one group (paired *t* test or Mann-Whitney test) are marked with "*"

[a] Tested with the unpaired Mann-Whitney test

[b] Tested with the unpaired *t* test

[c] Muscularis was identifiable in only three sheep

[d] Muscularis was identifiable in only four sheep

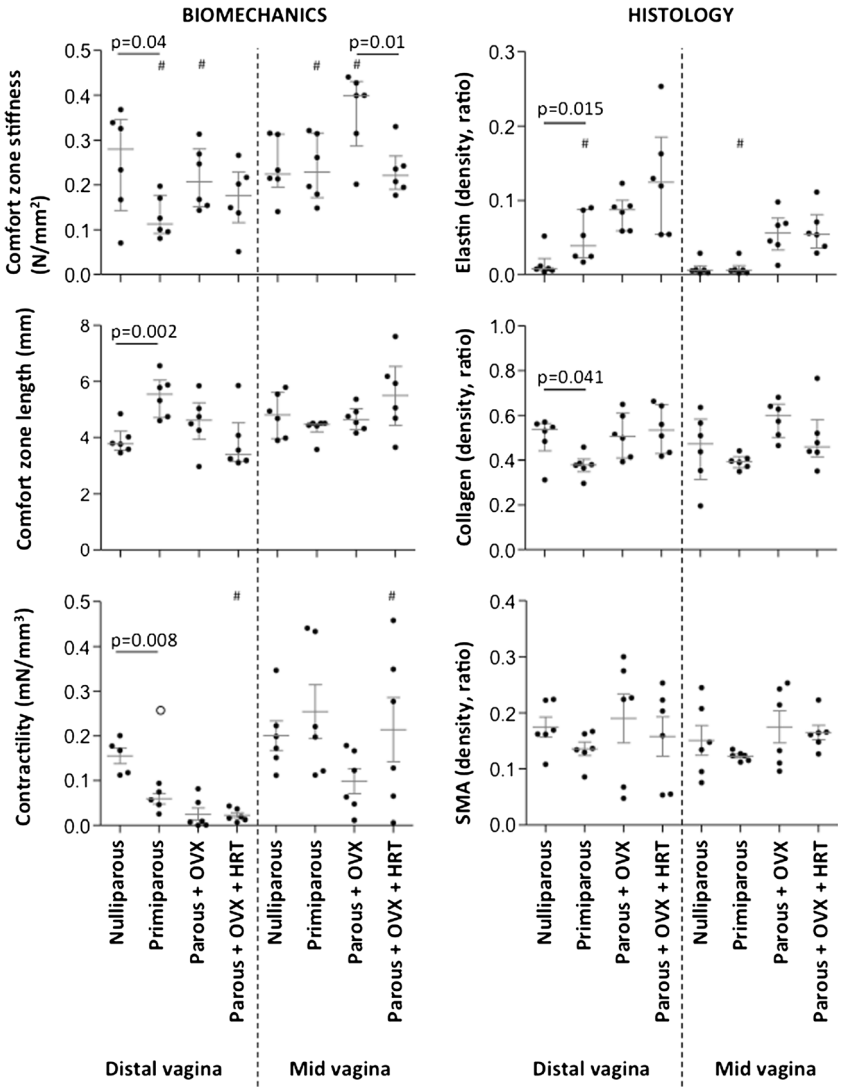

Fig. 3 Biomechanics (left column) including comfort zone stiffness, comfort zone length and contractility, and histology (right column) including the density of elastin, collagen and α-smooth muscle actin (αSMA), of the distal vagina and mid-vagina in nulliparous, primiparous and multiparous sheep following ovariectomy (Parous + OVX) and estrogen replacement therapy (Parous + OVX + HRT). The vertical line in each plot divides the distal vagina (left) and mid-vagina (right) data points. Data are displayed as individual values, and the box plots represent the medians and interquartile ranges. One outlier is displayed as an open circle. The hash symbols indicate significant differences between vaginal regions within one group. Histological outcomes were compared using the Mann-Whitney test and are represented by the ratios of positive and negative pixels

available [6, 23]. Our results parallel these trends, although the exact values are different, most probably because different testing methodologies and regions were used [6].

In terms of the effect of ovariectomy, there is one previous study in ovariectomized sheep in which the biochemical composition and ultrastructure were compared with those in menopausal women [24]. Of relevance to our study, it included biomechanical testing, but by uniaxial tensiometry. Ulrich et al. did not identify differences in the passive biomechanical properties between the distal vagina and the mid-vagina. Since the vagina is anisotropic, this may well have been found in our samples as well if they had been tested using the same method [25]. In both the previous study and our study, no regional differences in composition were found, but again different methodologies were used. One caveat is that in both studies, relatively young sheep that had been ovariectomized were used. Older sheep are more difficult to obtain as sheep are usually kept for only a limited time (<7–8 years, depending on the breed) in breeding programs. The effects may have been different if the same experiment had been done in older sheep around menopausal age. Indeed, in rats, the biomechanical properties of the pelvic supportive structures following surgical induction of menopause were different in younger animals (4 months of age) than in older animals (9 months of age) [26]. Also other investigators have found that age has an additional effect to menopause [27]. Clinically, the functional effects on the vagina of surgically induced menopause in young women are also more obvious than in older women with menopause of natural onset [28].

We also performed a morphological study, under the assumption that structural changes would in some way reflect the biomechanical measurements. The relationship was, however, unclear. For instance, we observed higher elastin levels in the stiff vaginas of ovariectomized sheep, which may seem paradoxical. However, a higher elastin content on light microscopy observed in specimens from older women may also represent elevated elastin degradation and matrix turnover, which can coincide with higher tissue stiffness [29]. This

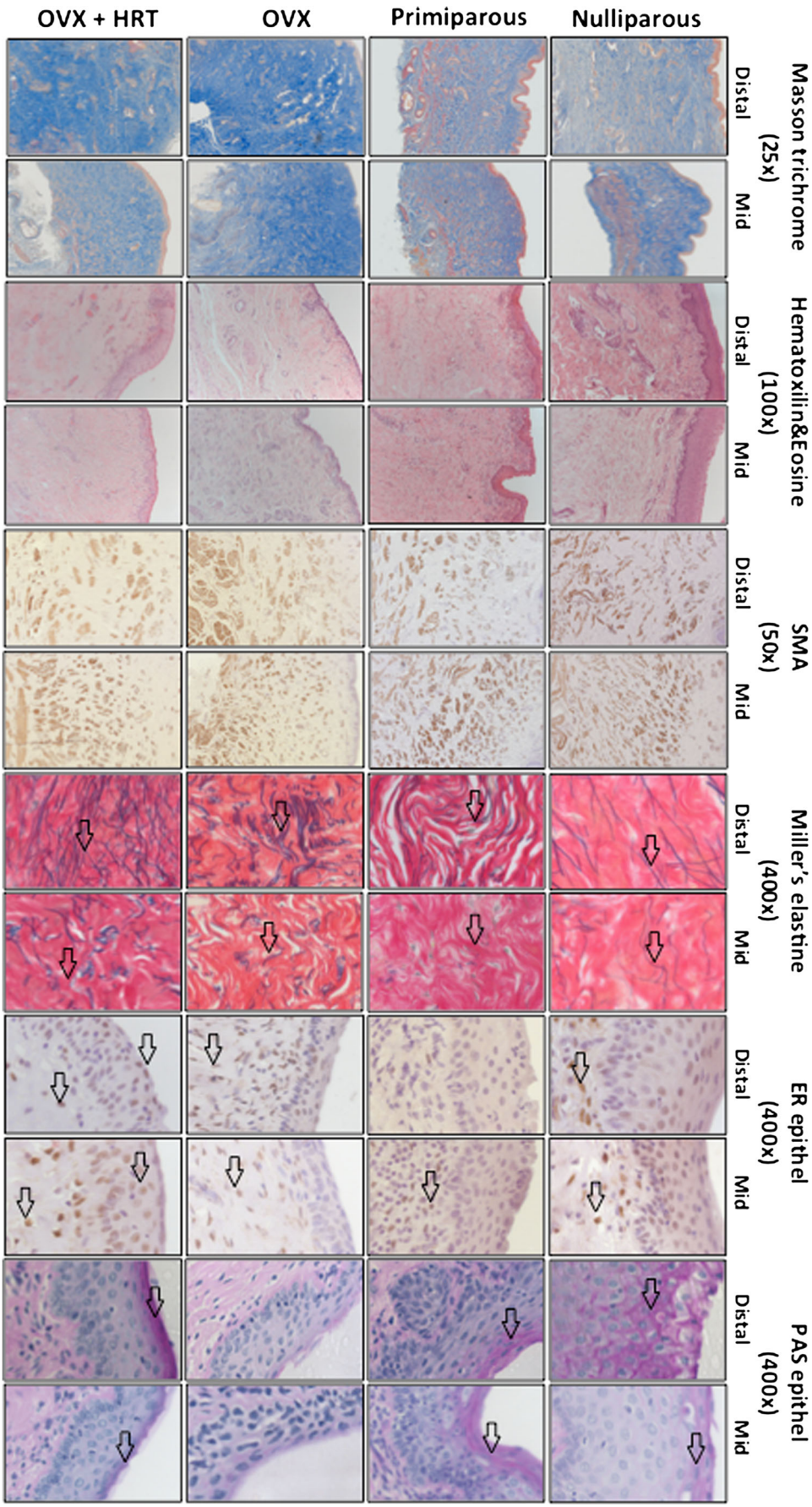

Fig. 4 Representative histological images showing collagen content, smooth muscle, elastin and ER-α in the distal vagina and mid-vagina, and glycogen in the mid-vagina in nulliparous, primiparous and multiparous sheep following ovariectomy (OVX) and estrogen replacement therapy (OVX + HRT). Elastin fibers, ER-α-positive nuclei and glycogen are indicated by arrows. Vaginal epithelium is always at the top of the images

demonstrates that one must be careful in extrapolating morphological to biomechanical findings. We also observed a lower density of elastin mainly in nulliparous and primiparous animals than in women. Elastin was measured with the same technique [16]. Interestingly, our results also show a much lower density of elastin determined morphologically than determined biochemically in sheep [6, 24]. This difference may be explained by changes in the appearance of elastin on light microscopy, as mentioned above (Fig. 4.). The morphological methods used are not truly quantitative and cannot differentiate between relevant subtypes and organization of extracellular matrix proteins. Unfortunately, we did not use biochemical or other more quantitative methods for the quantification of, for example, collagen subtypes and/or elastin [6]. Such methods may help better understand the relationship between morphology and biomechanics.

The other principal outcome measure was vaginal contractility. Contractility data are scarce in the translational research literature, with to our knowledge none available in sheep. In 6-month-old rats, ovariectomy led to a reduction in vaginal contractility [30]. Another study investigating the impact of vaginal delivery in 3-month-old rats showed an increased sensitivity to KCl without change in vaginal contractility [22]. However, in that study longitudinal strips of the entire vagina were examined. This is different from the circumferential strips we used, and we also investigated regional differences. We also investigated the potential relationship between active biomechanical properties and morphology, but found no true parallel pattern. Only in menopausal distal vaginal tissue did we find a paucity of muscular tissue that coincided with poor contractility.

Our experiments had several limitations. The most obvious limitation is that we were limited to a cross-sectional study design, and hence did not collect samples in a longitudinal fashion through the life phases. Further, due to the poor availability of similarly aged ewes, we were not able to standardize completely the number of deliveries in the ovariectomized groups. Last, it could be suggested that we should have measured estrogen levels in hormone-replaced animals. We did not do so since this had been well documented previously for the given HRT scheme [10, 11]. However, we did document the biological end-organ effects (which was one of the objectives of this study), including atrophic epithelial changes, glycogen absence and changes in ER-α expression [31]. Another criticism may be that we performed the experiments only 60 days after ovariectomy, as described previously [10, 11]. In smaller species, tissue changes are detected as early as 2 weeks after ovariectomy [32]. Clinically many tissue changes occur over the course of years. Although we documented measurable changes after 60 days, it would have been interesting to have performed the experiments later after ovariectomy to potentially observe more pronounced changes. Our study, despite its limitations, provides new data on the impact of two major female life events which in women contribute to the pathogenesis of PFD. To our knowledge, these are the first ovine biomechanical data on the effect of artificially induced menopause and HRT. These data might prove useful as there is now renewed interest in the local treatment of changes in PFD [33]. The study further expands our knowledge of the ovine model, which is increasingly being used for both pathophysiological and surgical studies [9, 23].

Conclusion

In sheep, vaginal delivery and simulated menopause affect the biomechanical properties of the vagina in a region-specific way. The first vaginal delivery mainly affects the distal vagina, decreasing its stiffness and contractility. This coincides with an increase in elastin and a decrease in collagen. HRT reverses the effects of ovariectomy on mid-vaginal stiffness with a trend for increased contractility. The distal vagina undergoes no changes following HRT. The few available biomechanical findings in clinical specimens are in line with these observations in sheep, supporting its role as a large-animal model.

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Compliance with ethical standards

Conflicts of interest None.

Ethical approval The study was approved by the Ethics Committee for Animal Experimentation of the Faculty of Medicine of the K.U. Leuven. All applicable international, national and institutional guidelines for the housing, care and use of animals were followed. Procedures performed in this study were in accordance with the ethical standards of the institution at which they were conducted.

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