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Biomechanical and morphological properties of the multiparous ovine vagina and effect of subsequent pregnancy

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ABSTRACT

Pelvic floor soft tissues undergo changes during the pregnancy. However, the degree and nature of this process is not completely characterized. This study investigates the effect of subsequent pregnancy on biomechanical and structural properties of ovine vagina. Vaginal wall from virgin, pregnant (in their third pregnancy) and parous (one year after third vaginal delivery) Swifter sheep ($n = 5$ each) was harvested. Samples for biomechanics and histology, were cut in longitudinal axis (proximal and distal regions). Outcome measurements describing Young's modulus, ultimate stress and elongation were obtained from stress-strain curves. For histology samples were stained with Miller's Elastica staining. Collagen, elastin and muscle cells and myofibroblasts contents were estimated, using image processing techniques. Statistical analyses were performed in order to determine significant differences among experimental groups. Significant regional differences were identified. The proximal vagina was stiffer than distal, irrespective the reproductive status. During the pregnancy proximal vagina become more compliant than in parous (+47.45%) or virgin sheep (+64.35%). This coincided with lower collagen (−15 to −21%), higher elastin (+30 to +60%), and more smooth muscle cells (+17 to +37%). Vaginal tissue from parous ewes was weaker than of virgins, coinciding with lower collagen (−10%), higher elastin (+50%), more smooth muscle cells (+20%). It could be proposed that after pregnancy biomechanical properties of vagina do not recover to those of virgins. Since elastin has a significant influence on the compliance of soft tissues and collagen is the main "actor" regarding strength, histological analysis performed in this study justifies the mechanical behavior observed.

1. Introduction

Pelvic floor soft tissues undergo changes during pregnancy, labour and delivery, and may be affected by age and hormonal status (Lukacz et al., 2006; Ashton-Miller and Delancey, 2007; Bump et al., 1996).

During the physiological processes, such as pregnancy and parturition, the vaginal wall and pelvic floor organs undergo adaptation. After delivery, these structures should return to a prepregnant-like state. Epidemiologic studies suggest that many women fail to recover completely from this event; indeed, vaginal distention trauma appears to play an important role as a cause of pelvic organ prolapse (POP) (Patel et al., 2006; Hendrix et al., 2002). Prolapse of internal genital organs, is a condition in which the pelvic organs (bladder, cervix, vaginal and rectal wall) form a hernia into the vaginal lumen.

A detailed biomechanical assessment of vaginal tissue structures can improve the understanding of how subsequent pregnancies may affect the tissue properties and composition. Several studies have been conducted that evaluate the biomechanical properties of human vaginal tissues (Goh, 2002, 2003; Lei et al., 2007; Rubod et al., 2008; Pena et al., 2010; Martins et al., 2013; Chantereau et al., 2014; Goh, 2003), yet access to fresh samples is limited, due to restrictions on the dimensions of specimens, due to ethical concerns. Animal models, such as mice, rabbits, dogs

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(domestic mammals) or non-human primates are used instead to evaluate biomechanical properties of relevant tissues (Rahn et al., 2008; Miesner and Anderson, 2008; Otto et al., 2002; Mattson et al., 2005; Ulrich et al., 2014; Rubod et al., 2007). To define changes in vaginal distensibility induced by pregnancy and vaginal delivery, some studies were already carried out on rats (Feola et al., 2011; Tokar et al., 2010). In the work reported a sheep animal model will be used since its pelvic floor tissue anatomy is structurally similar (dimensions and organization) to that of humans (Abramowitch et al., 2009; Ulrich et al., 2014). Moreover PFD (Pelvic Floor Disorders) risk factors such as increased intraabdominal pressure, parity or obesity, are comparable to that in humans (Abramowitch et al., 2009; Patnaik et al., 2012). Sheep may also spontaneously develop utero-vaginal prolapse during pregnancy (Shepherd, 1992; Couri et al., 2012). Some studies on sheep vaginal tissue properties have been conducted already. One study compared ovine and human posterior vaginal tissue in terms of histological composition (Ulrich et al., 2014). In another, ovine proximal vaginal tissue biochemical composition and biomechanical properties at different reproductive stages were determined (Ulrich et al., 2014; Rynkevic et al., 2016). Several papers/reports have been published on vaginal tissue composition and mechanical properties, however they used different methods, different animal models, different testing and sample collections protocols, which makes the comparison of results difficult and sometimes conflicting.

The objective of this study was to identify pregnancy-induced changes and effect of vaginal deliveries on the biomechanical and morphological properties of vaginal wall. The virgin sheep was used as baseline model for this study. To investigate the link between biomechanical tissue properties and tissue morphology, all the layers of the vaginal tissue were carefully analyzed and evaluated. It was conducted a detailed morphological comparison between proximal and distal regions of sheep vaginal wall. Thickness and contents (collagen, elastin and smooth muscle) of each region were investigated using image analysis.

2. Methods

2.1. Animals

Ewes were either young post-menarchal virgins ($n = 5$; avg. weight = 45 kg), near term ($n = 5$; 140 days; term = 145 d; avg. weight = 65 kg) after two prior vaginal deliveries, further referred to as gravida-3, and at least one year after three vaginal deliveries ($n = 5$; avg. weight = 60 kg), referred to as para-3. Virgin sheep were nine-months-old, while the gravida-3 and para-3 sheep were 3 and 4 years old, respectively. Swifter sheep typically have two to three lambs per litter; which was also the case in the present study. Ewes were obtained from the Zoötechnical Institute of the Katholieke Universiteit (KU) Leuven. Animals were treated according to an experimental protocol approved by the Ethics Committee for Animal Experimentation of the Faculty of Medicine of KU Leuven. They were euthanized by intravenous injection of 10 mL of a mixture of embutramide 200 mg, mebezonium 50 mg and tetracaine hydrochloride 5 mg (T61; Hoechst Marion Roussel, Brussels, Belgium). Animals were placed in the dorsal recumbent position and pelvic floor organs were completely excised.

2.2. Sample preparation

The tissue was divided into samples for biomechanical testing and for histological analyses according to a standardized topographic protocol (Fig. 1a). Standardized samples for biomechanics were punched using a dog bone shape cutting form (2 mm × 25 mm) along the longitudinal axis (Fig. 1b). Testing was done within two hours after prelevation; prior to measurements tissues were kept in a wet saline soaked gauze at room temperature (~20 °C).

2.3. Uniaxial tensile testing protocol

Uniaxial testing was performed using a Zwick tensiometer with a 200 N load cell (Zwick GmbH & Co. KG, Ulm, Germany). The orientation of the set up was vertical. One clamp was attached to the base of the testing device, the other was attached to the crosshead of the device via a load cell. By using a specific algorithm, the load cell transforms resistance variations into force values.

Tissue specimens were fixed using small grips which in turn are inserted into the pneumatic clamps. The purposely built small grips allowed to reduce the stress concentration effect on the tissues and avoid slippage (Rubod et al., 2008). The tensile test was carried out by applying longitudinal axial load. Samples were preloaded till 0.1 N to remove slack from the tissue using a constant elongation of 5 mm/min, and then the clamp-to-clamp distance was measured. This point was defined as elongation zero. The width and thickness were measured using callipers, with an accuracy of 0.05 mm. Measurements were taken at three locations along

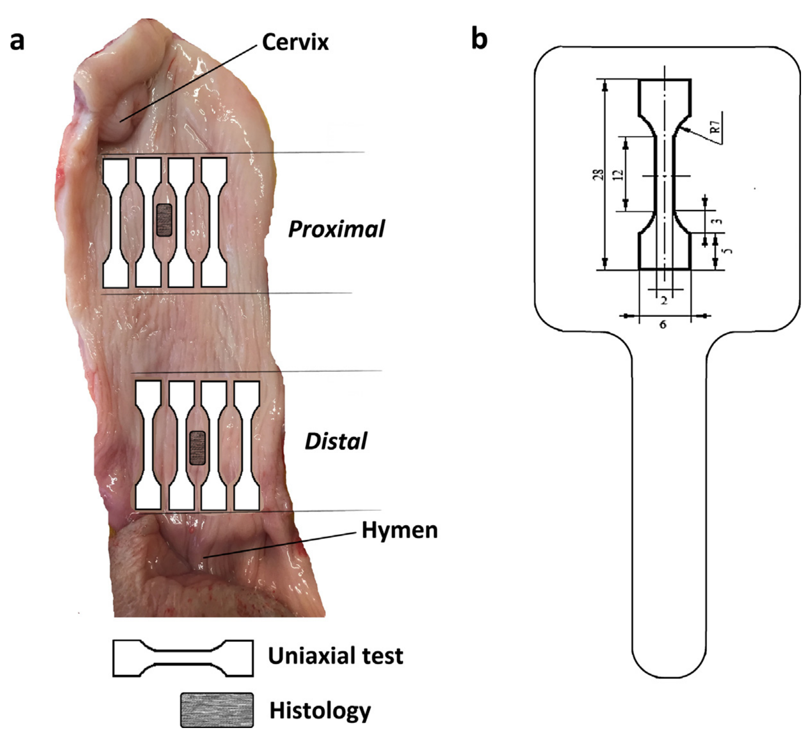

Fig. 1. **a** - Schematic division of the tissue into samples: dog bone shape samples for biomechanical testing and for histology, **b** - dog bone shape sample cutting form blade (with dimensions) mounted in a transparent acrylic frame.