# Vaginal changes after ovariectomy in ewes – a large animal model for Genitourinary Syndrome of Menopause

Eva Vodegel[1], Zeliha Guler[1], Lamees Ras[2], Katerina Mackova[3], Anne Groeneveld[1], Deon Bezuidenhout[2], Jan Deprest[4], Stephen Jeffrey[2], and Jan-Paul Roovers[1]

[1]Amsterdam UMC Location AMC
[2]University of Cape Town
[3]KU Leuven
[4]KU Leuven Department of Development and Regeneration

September 13, 2022

**Abstract**

Objective: To evaluate the effect of iatrogenic menopause on the physiology of the vagina of the ewe, and to evaluate if vaginal changes in ewes can be translated to women with the genitourinary syndrome of menopause (GSM). Design: Animal study, randomised design for ovariectomy or control, blinded for allocation and outcome assessment. Setting: University of Cape Town, South Africa. Population or Sample: Twenty-five Dohne Merino ewes. Methods: Iatrogenic menopause was induced in 20 animals by bilateral ovariectomy. Five animals served as a control group (no intervention). Differences between groups were determined by linear regression analyses (adjusted for baseline scores) at 5 months after ovariectomy. Main Outcome Measures: Vaginal epithelial thickness, pH, vaginal maturation value, vaginal maturation index, epithelial glycogen accumulation, content of elastin fibres, collagen, and vascularity. Results: Ovariectomised ewes showed epithelial thinning of the vaginal wall from 146 µm to 47 µm (mean, p <0.001 ). In addition, epithelial glycogen accumulation (43%) and the vascularity (23%) of the vaginal wall significantly decreased as compared to the control group. No differences were found for vaginal pH, vaginal cytology outcomes, elastin fibres and collagen content. Conclusions: This study established the ewe as a suitable large animal model for GSM. Furthermore, the similar relevant outcomes in humans and ewes hold great value for future translational research for the evaluation and optimisation of different treatment modalities for GSM. Funding: None. Keywords: Atrophy, ewe, genitourinary syndrome of menopause, model, ovariectomy.

## Vaginal changes after ovariectomy in ewes – a large animal model for Genitourinary Syndrome of Menopause

**Authors:** Eva V. VODEGEL (MD)[1,2], Zeliha GULER (PhD)[1,2], Lamees RAS (MD)[3], Katerina MACKOVA (MD, PhD)[4,5], Anne C.H.M. GROENEVELD[1,2], Deon BEZUIDENHOUT (PhD)[6], Jan DEPREST (MD, PhD)[4,7], Stephen T. JEFFERY (MD)[3], Jan-Paul W.R. ROOVERS (MD, PhD)[1,2]

1. Amsterdam UMC location University of Amsterdam, Dept. of Obstetrics and Gynaecology, Meibergdreef 9, Amsterdam, The Netherlands.
2. Amsterdam Reproduction and Development research institute, Amsterdam, the Netherlands.
3. Department of Obstetrics and Gynaecology, Groote Schuur Hospital, University of Cape Town, Cape Town, South Africa.
4. Department of Development and Regeneration, KU Leuven, Leuven, Belgium.
5. Institute for the Care of Mother and Child, Third Faculty of Medicine, Charles University, Prague, Czech Republic.

6. Cardiovascular Research Unit, Cape Hearth Centre, University of Cape Town, Cape Town, South Africa.
7. Pelvic Floor Unit, University Hospitals KU Leuven, Leuven, Belgium.

**Corresponding author:** Eva V. Vodegel, MD Amsterdam UMC, University of Amsterdam, location AMC Dept. of Obstetrics and Gynaecology, room H4-222 Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands

Telephone: +31(0)20-5666429

E-mail: e.v.vodegel@amsterdamumc.nl

**Running title:** Vaginal changes after ovariectomy in ewes

**Study conducted in:** South Africa

## ABSTRACT

**Objective:** To evaluate the effect of iatrogenic menopause on the physiology of the vagina of the ewe, and to evaluate if vaginal changes in ewes can be translated to women with the genitourinary syndrome of menopause (GSM).

**Design:** Animal study, randomised design for ovariectomy or control, blinded for allocation and outcome assessment.

**Setting:** University of Cape Town, South Africa.

**Population or Sample:** Twenty-five Dohne Merino ewes.

**Methods:** Iatrogenic menopause was induced in 20 animals by bilateral ovariectomy. Five animals served as a control group (no intervention). Differences between groups were determined by linear regression analyses (adjusted for baseline scores) at 5 months after ovariectomy.

**Main Outcome Measures:** Vaginal epithelial thickness, pH, vaginal maturation value, vaginal maturation index, epithelial glycogen accumulation, content of elastin fibres, collagen, and vascularity.

**Results:** Ovariectomised ewes showed epithelial thinning of the vaginal wall from 146 μm to 47 μm (mean, $p$ <0.001 ). In addition, epithelial glycogen accumulation (43%) and the vascularity (23%) of the vaginal wall significantly decreased as compared to the control group. No differences were found for vaginal pH, vaginal cytology outcomes, elastin fibres and collagen content.

**Conclusions:** This study established the ewe as a suitable large animal model for GSM. Furthermore, the similar relevant outcomes in humans and ewes hold great value for future translational research for the evaluation and optimisation of different treatment modalities for GSM.**Funding:** None.

**Keywords:** Atrophy, ewe, genitourinary syndrome of menopause, model, ovariectomy.

**Tweetable abstract:** Iatrogenic menopause in ewes mimic the physiologic vaginal changes in post-menopausal women encountering genitourinary syndrome of menopause (GSM). This animal model is vital to improve treatment for GSM.

## 1 | INTRODUCTION

The genitourinary syndrome of menopause (GSM), formerly known as vulvovaginal atrophy, represents a variety of genitourinary symptoms and signs associated with menopause and is directly related to decreased circulating oestrogen levels.[1] Clinical symptoms include vaginal dryness, pruritus, burning, irritation, dyspareunia and urinary symptoms of urgency, dysuria and recurrent urinary tract infections. Physiologic changes result in epithelial thinning, increased vaginal pH, and reduced vaginal blood flow, collagen content, vaginal secretions, and elasticity.[2-4]

GSM has a negative impact on women's quality of life and their sexual health.[3] Although the true prevalence of GSM is difficult to determine, it is estimated that up to 50% of postmenopausal women are affected.[5] These symptoms can be chronic and progressive.

With the increasing life expectancy, women will spend one-third of their life in the postmenopausal period.[6] This emphasises the great extent of the problem and growing interest in the development and improvement of existing and future therapies for GSM. Current treatments are vaginal moisturizers, lubricants and vaginal oestrogen therapy. Alternative treatments could be vaginal dehydroepiandrosterone (DHEA), ospemifene or vaginal laser therapy. However, more data is needed to attain proof of concept if new therapies are safe and efficacious in the treatment of GSM. For approval for clinical use, the Food and Drug Administration (FDA) requires preclinical evidence of safety and efficacy of any novel treatments. It is for this reason that the establishment of a suitable animal model is crucial. The preferential use of an animal model over in-human studies is critical to initially establish safety and efficacy. Some of the reasons for this are the potential safety concerns when evaluating treatment dosing and timing (for example with vaginal laser therapy settings), concerns with the invasiveness of multiple vaginal biopsies in women when these measurements are required, and the high cost of clinical trials.

Thus far, the ovariectomised ewe has been used in a few studies as a large animal model for GSM.[12-14] However, the validity of these studies is questionable as there were a few flaws in the study design. These include a lack of premenopausal control groups, baseline measurements, long term follow-up, comparative analyses between the study groups at the same point in time and methods of statistical analyses. Hence, this study is needed to objectively examine the effect of (iatrogenic) menopause on the physiologic changes in the ewe's vagina using specific points of measurement that are affected in women with GSM, thereby validating the animal model for GSM. Such a large animal model is vital for future development and optimisation of new treatment interventions for women suffering with GSM.

## 2 | METHODS 2.1 | Animals

Twenty six (26) multiparous Dohne Merino ewes (6 years old) of similar origin were recruited for this study. However, the study was performed in 25 ewes as one animal died due to age related health issues before the study started. During the study the flock was held on the field with free access to food and water (ad libitum). The animals were acclimatised one week before the study started. During the study, the animals were monitored daily and weighed weekly, conforming with the care and husbandry guidelines of the National Health and Medical Research Council of South Africa for animals in research. This study was approved by the Animal Ethics Committee of the University of Cape Town (Protocol number: 018_005).

### 2.2 | Study design & procedures

#### *2.2.1 | Cycle synchronisation*

Ewes have cyclical changes in the vaginal epithelium with epithelial thickening during oestrus and a decrease in thickness during the pro-oestrus and dioestrus period.[15, 16] Cycle synchronisation was performed in all animals using progestogen impregnated sponges (flugestone acetate, 40mg/sponge, Ovakron®, Ani Pharm, Bloemfontein, South Africa). A sponge was inserted intravaginally for 14 days. In ewes, oestrus occurs approximately 48 hours after sponge removal.[17]

#### *2.2.2 | Allocation, randomisation and blinding*

After acclimatisation and cycle synchronisation, all the ewes were allocated with a unique study number using an ear clip. Twenty animals were randomly allocated to the (iatrogenic) menopause intervention arm, and had bilateral ovariectomy (OVX) performed on them. The remaining five ewes were allocated to the premenopausal control arm and therefore recieved no intervention (see Supplementary material Appendix S1 for the power calculation, including an explanatory note on the difference in group size). During the study, researchers and caregivers were blinded for allocation (including the identifying code), except at time of the ovariectomy when the animals were clipped, and the abdominal scar was still visible. All animals were operated and examined in a random order during the entire study. Also, the histology and cytology images

were blinded for allocation and time points (using Blind Analysis tools, ImageJ; 1.50i), and were assessed in a random order by two independent researchers.

*2.2.3 | Bilateral ovariectomy – study intervention*

48-72 hours after sponge removal, bilateral ovariectomy was performed via a small laparotomy (6-8 cm midline incision) under general anaesthesia. Surgery details are presented in the Supplementary material, Appendix S2. For the study design, including the intervention and assessments, see Table S1 of the Supplementary material.

### 2.3 | Outcome measures

*2.3.1 | Vaginal pH & cytology*

The vaginal pH was assessed monthly using litmus strips (VWR International bv, Leuven, Belgium, range 2-9; resolution 0.5), which was placed in the vagina for 4 seconds.

Vaginal cytology assessment was performed monthly using a cytology brush, which was placed in liquid-based fixative after swabbing the vagina. A Papanicolaou (PAP) test was performed (SA Path - Cape Town Laboratory) and slides were digitalised (bright field digital slide scanner; NOCTN44, Philips). The percentage of superficial, intermediate and parabasal cells were counted out of 100 cells using a Cell counter Plugin tool (ImageJ, 1.5i). The vaginal maturation value (VMV) was calculated (1 × superficial cells + 0.6 × intermediate cells + 0.2 × parabasal cells).[18]

*2.3.2 | Biopsies*

Monthly biopsies were taken in all ewes at the distal vaginal anterior wall 6 consecutive times (see also the Supplementary material, Table S1). Biopsies were performed under sedation, (ketamine, 5-10mg/kg, intramuscular) with the animal being restrained in a dorsal recumbent position on a surgical trolley. Biopsy size was ± 5 mm and they were taken at various locations of the distal anterior wall, 1 cm proximal from the hymen (at 11, 3, 9, 2, 10, and 1 o'clock respectively – where the anterior midline is assumed to be 12 o'clock), to prevent involvement of scar tissue.

*2.3.3 | Histology and histological examination*

All details about histology sample preparation and staining procedures can be found in the Supplementary material, Appendix S3. Vaginal biopsies were stained with haematoxylin and eosin (H&E) to evaluate the epithelial thickness. The presence of epithelial glycogen accumulation was demonstrated by Periodic acid-Schiff (PAS) staining. Van Gieson's and Masson's Trichrome stains were used to detect elastin fibres and to quantify collagen and vascularity in the specimens respectively.

For the primary outcome epithelial thickness, a representative part of the biopsy was selected by two blinded researchers (at 100× magnification). The average epithelial thickness was assessed using a computer-assisted measurement program (ImagePro, v.10), taking the variability of the dermal papillae into consideration.[19] The upper border of the epithelium and the wave-like border of the epithelial basal layer were marked with a minimum length of 200 µm and a maximum length of 3000 µm. The epithelial thickness between these two lines was automatically calculated on a resolution of 1 µm and was averaged to determine a representative and standardised epithelial thickness of each specimen. In case of unresolved discrepancies or a questionable location of the basal membrane, another researcher was consulted.

For the assessment of the epithelial glycogen accumulation, elastin fibres, collagen, and vascularity; five images were randomly taken (at 400× magnification) from each specimen by a blinded researcher. Each image was scored for abundance on a 4-point grading scale, from 0 till 3, by two other independent blinded researchers, providing an average score (between 0-3) calculated from 10 scores per biopsy. Details are given in the Supplementary material Appendix S4. In case of discrepancies, the scoring was adjudicated by a third reviewer.

### 2.4 | Data analyses

Data was analysed using computer software IBM SPSS Statistics for Windows (Version 28.0. Armonk, NY: IBM Corp). Data and residuals from models were assessed for normality by histograms and Q-Q plots. At 5 months after OVX, differences between groups (OVX and non-OVX) were determined by linear regression analyses, adjusting for baseline scores. Data is reported as mean ± standard deviation (SD). Data analyses was performed in consultation with a statistician – Dr. van Eekelen.

## 3 | RESULTS

At baseline (before OVX), 25 Dohne Merino ewes were all aged six years old with a mean weight of 68.0 kg (SD 7.5), and all being multiparous (all ewes had a parity of four). At baseline, the two groups were similar for all outcomes (Table 1).

### 3.1 | Vaginal pH & cytology

At five months after OVX, the vaginal pH levels were not different between the OVX and the control group (OVX 8.1 ±0.31 SD; control 8.0 ±0.35 SD). For both the VMV (OVX 57 ±8 SD; control 62 ±13 SD) and the percentage of superficial cells (OVX 7% ±8 SD; control 32% ±25 SD) there were lower values in the OVX group when compared to the premenopausal control group, but these were not statistically significant. See also Table 1 and Figure 1.

### 3.2 | Histology

At five months after OVX, there is a statistically significant ($p<0.001$) decrease in epithelial thickness of in the OVX group (47 µm ±34 SD) when compared to the premenopausal control group (146 µm ±53 SD). Further analyses showed statistically significant ($p =0.02$) lower levels of glycogen accumulation in the OVX group (score 1.36 ±0.77 SD) when compared to control (score 2.40 ±0.60 SD). There was also a statically significant lower amount of blood vessels ($p <0.01$) in the OVX group (score 2.07 ±0.49 SD) when compared to control (score 2.68 ±0.28 SD). No differences were found for elastin fibres and collagen content when the two groups were compared at five months after OVX (See also Table 1, Figure 1, 2 and 3).

## 4 | DISCUSSION

### 4.1 | Main findings

This study shows that ovariectomy in ewes induces epithelial thinning of the vaginal wall, and decreases the glycogen accumulation and the vascularity in the vaginal wall. The effects of ovariectomy in ewes seem to reflect the physiologic changes of the vagina in women encountering GSM.

### 4.2 | Strengths and limitations

Our findings are obtained from a well-designed and conducted study including randomisation, appropriate sample size, similar group characteristics at baseline, allocation concealment, blind outcome assessment, and the longitudinal design including repeated measurements.[20, 21] Also, the use of a premenopausal control group made it possible to perform between-group analyses and therefore eliminate confounders due to time-related and environmental effects. This includes factors like fluctuating seasonal oestrogen levels (the ewe is a short-day breeder), or seasonal related intake of red clover (containing high levels of phyto-oestrogen). However, a limitation of between-group analyses is the individual difference variable. To minimise this confounder, we adjusted for baseline scores when performing linear regression analyses.

Furthermore, our research group has extensive experience with the evaluation of physiologic changes in the vagina and pelvic floor, including a variety of outcome measures and innovative technologies.[22-27] Also for this study, we devised an innovative way to determine the mean epithelial thickness of the vaginal wall, which provided a solid and representative value for our primary outcome. In addition, the 4-point grading scale, used for histology assessment, was determined from the data available from this study. As a result, the full range of abundancy was optimally used which enabled us to capture the effect of ovariectomy on these outcome measures the best possible. Lastly, the longitudinal design allowed us to see when a plateau phase of epithelial thinning was reached.

We are aware that our research has some limitations as well. The quality of the PAP stained cytology slides varied from poor to excellent, leading to difficulties in reading and interpretating with subsequent missing data. Therefore, analysis was done in a smaller sample size than originally planned. Furthermore, due to the small introitus of the ewe, we decided to not include gynaecological examination to evaluate the clinical signs of GSM. According to the principles of humane animal research (replacement, reduction and refinement), we aimed to minimise pain and distress. Last, due to an unexpected loss of one animal just before the study started, the control group consisted of five instead of six animals. However, the statistical power was not affected since a 10% loss of animals was taken into account.

### 4.3 | Interpretation

GSM comprises a variety of changes in the genitourinary tissues in response to the loss of oestrogen with menopause. In this study we obtained iatrogenic menopause in ewes by performing bilateral ovariectomy. As a result, there was a significant decrease in epithelial thickness, glycogen accumulation and vascularity of the vaginal wall. Similar changes are seen in women affected by GSM following menopause. When taking a closer look to the epithelial thickness as the primary outcome measure for GSM, an approximately 3 fold decrease after menopause in both women (220 μm to 80-125 μm.[27, 28]) and ewes (146 μm to 47 μm) were seen, even though women have a thicker vaginal wall than ewes.

Epithelial thinning results in less exfoliation of cells into the vagina, meaning less available glycogen, which is broken down into lactic acid by the action of lactobacilli. Therefore, vaginal pH rises after menopause and is associated with overgrowth of anaerobic bacteria causing opportunistic vaginal infections in women.[2, 29] Although we observed a significant decrease of epithelial glycogen accumulation in ewes following ovariectomy, interestingly the vaginal pH remained unchanged. This is explained by Miller et al. by the fact that lactobacilli dominance appears to be unique to the human vaginal microbiome, where the abundance of lactobacilli is >70%, compared to <1% in other mammals.[29]

Beyond outcome measures as epithelial thickness, glycogen levels, vaginal pH and vascularity, it is also recommended to evaluate vaginal cytology for objective assessment of GSM in women.[18]In this study, although not statistically significant, we observed lower levels of the VMV and the VMI following ovariectomy in ewes. On the other hand, it could be hypothesised that the distribution of superficial, intermediate and parabasal cells is less pronounced due to the fact that ewes have thinner vaginal walls.

Furthermore, a hypo-oestrogenic state results in loss of dermal collagen, elastin fibres, and blood vessels in the lamina propria.[30] Although a significant decrease in vascularity was observed, no significant difference in elastin and collagen content between ovariectomised ewes and the control group was found. Elastin is a very stable protein, where the metabolic turnover of mature elastin fibres is relatively slow.[31, 32]Although elastin likely turns over in the vulvovaginal tissues after menopause, the exact mechanism remains still unknown. It could be hypothesised that the fragmentation of elastin fibres in ewes was not yet completed at 5 months after ovariectomy. Regarding collagen content, the vaginal collagen levels might already be affected by the reproductive state. For instance, Ulrich et al. showed that the total collagen levels in parous ewes are different from both virgin and pregnant ewes.[33] The unchanged collagen levels after ovariectomy have been described previously.[13] In addition, there are studies reporting decreased collagen I/III ratio in women with GSM, whereas this has not been observed in ewes.[33, 34]

## 5 | CONCLUSION

Previously, adequate evaluation of physiologic vaginal changes in ewes following ovariectomy was lacking. This study used rigorous and novel assessment tools to evaluate the effect of iatrogenic menopause in ewes on multiple outcome measures for GSM. These effects seem to reflect the physiologic changes of the vagina in women encountering GSM. In addition, this study shows that relevant outcome measures used in women, are also applicable in the evaluation of GSM in ewes. Hence, this large animal model is of great value for future translational research for the evaluation and optimisation of different treatment modalities for GSM. In a successive study, we evaluated different interventions in the treatment of GSM in the ovariectomised ewe.

**Abbreviations**

DHEA Dehydroepiandrosterone

FDA Food and Drug Administration

GSM Genitourinary syndrome of menopause

H&E Haematoxylin and eosin

OVX Ovariectomy

PAP Papanicolaou

PAS Periodic acid-Schiff

SD Standard deviation

VMI Vaginal maturation index

VMV Vaginal maturation value

**Declarations**

**Acknowledgement**

We like to thank Mr. Woudstra, Mr. Morris and the animal caretakers for the use of the research facility and taking proper care of the animals. We like to thank Mr. Mogotlane (MD), Ms. van Wilsem and Mr. Atigan (MD) for their contributions in the tissue processing. We also like to thank statistician Dr. van Eekelen for statistical advice and support.

**Disclosure of interests**

The authors report no conflict of interest.

**Contributions to authorship**

JPR, ZG, EVV and LR were responsible for the conception and design of this study. EVV, ZG, LR, ACHMG and STJ were responsible for the data collection and data management. EVV was responsible for the conduct analyses. All authors were involved in the interpretation of the data. EVV drafted the initial manuscript, which was reviewed and critically revised by all authors. All authors approved the final manuscript as submitted.

**Details of ethics approval**

This study was approved by de Animal Ethics Committee of the University of Cape Town (Protocol number: 018_005).

**Funding**

No funding is available for this study.

**SUPPLEMENTARY MATERIAL**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

- Supplementary Table S1. Study design: interventions and assessments
- Supplementary Table S2. Regression table
- Supplementary Appendix S1. Power calculation
- Supplementary Appendix S2. Bilateral ovariectomy
- Supplementary Appendix S3. Histology and immunohistochemistry
- Supplementary Appendix S4. Histological examination

## REFERENCES

1. Portman DJ, Gass ML. Genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the International Society for the Study of Women's Sexual Health and the North American Menopause Society. Menopause. 2014;21(10):1063-8.

2. Mac Bride MB, Rhodes DJ, Shuster LT. Vulvovaginal Atrophy. Mayo Clin Proc. 2010;85(1):87-94.

3. Nappi RE, Palacios S. Impact of vulvovaginal atrophy on sexual health and quality of life at postmenopause. Climacteric. 2014;17(1):3-9.

4. Tan O, Bradshaw K, Carr BR. Management of vulvovaginal atrophy-related sexual dysfunction in postmenopausal women: an up-to-date review. Menopause. 2012;19(1):109-17.

5. Santoro N, Komi J. Prevalence and impact of vaginal symptoms among postmenopausal women. J Sex Med. 2009;6(8):2133-42.

6. Palacios S, Henderson VW, Siseles N, Tan D, Villaseca P. Age of menopause and impact of climacteric symptoms by geographical region. Climacteric. 2010;13(5):419-28.

7. Turner AS. The sheep as a model for osteoporosis in humans. Vet J. 2002;163(3):232-9.

8. Egermann M, Goldhahn J, Schneider E. Animal models for fracture treatment in osteoporosis. Osteoporos Int. 2005;16 Suppl 2:S129-38.

9. Albertson AJ, Skinner DC. A novel animal model to study hot flashes: no effect of gonadotropin-releasing hormone. Menopause (New York, NY). 2009;16(5):1030-6.

10. Gaynor JS, Monnet E, Selzman C, Parker D, Kaufman L, Bryant HU, et al. The effect of raloxifene on coronary arteries in aged ovariectomized ewes. J Vet Pharmacol Ther. 2000;23(3):175-9.

11. Fang M, Liroff KG, Turner AS, Les CM, Orr BG, Holl MM. Estrogen depletion results in nanoscale morphology changes in dermal collagen. J Invest Dermatol. 2012;132(7):1791-7.

12. Mackova K, Mazzer AM, Mori Da Cunha M, Hajkova Hympanova L, Urbankova I, Kastelein AW, et al. Vaginal Er:YAG laser application in the menopausal ewe model: a randomised estrogen and sham-controlled trial. BJOG. 2021;128(6):1087-96.

13. Hympanova L, Rynkevic R, Mori Da Cunha M, Diedrich CM, Blacher S, De Landsheere L, et al. The ewe as an animal model of vaginal atrophy and vaginal Er:YAG laser application. Menopause. 2020;28(2):198-206.

14. Urbankova I, Callewaert G, Blacher S, Deprest D, Hympanova L, Feola A, et al. First delivery and ovariectomy affect biomechanical and structural properties of the vagina in the ovine model. Int Urogynecol J. 2019;30(3):455-64.

15. Richardson C. Diagnosis of pregnancy in the ewe by vaginal biopsy. Br Vet J. 1972;128(6):316-30.

16. Restall BJ. Histological observations on the reproductive tract of the ewe. Aust J Biol Sci. 1966;19(4):673-86.

17. Abecia JA, Forcada F, González-Bulnes A. Hormonal control of reproduction in small ruminants. Anim Reprod Sci. 2012;130(3-4):173-9.

18. Weber MA, Limpens J, Roovers J. Assessment of vaginal atrophy: a review. International Urogynecology Journal. 2015;26(1):15-28.

19. Blomgren B, Johannesson U, Bohm-Starke N, Falconer C, Hilliges M. A computerised, unbiased method for epithelial measurement. Micron. 2004;35(5):319-29.

20. Kilkenny C, Parsons N, Kadyszewski E, Festing MF, Cuthill IC, Fry D, et al. Survey of the quality of experimental design, statistical analysis and reporting of research using animals. PLoS One. 2009;4(11):e7824.

21. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 2014;14:43.

22. Diedrich CM, Guler Z, Hympanova L, Vodegel E, Zündel M, Mazza E, et al. Evaluation of the short-term host response and biomechanics of an absorbable poly-4-hydroxybutyrate scaffold in a sheep model following vaginal implantation. BJOG. 2022;129(7):1039-49.

23. Guler Z, Roovers JP. Role of Fibroblasts and Myofibroblasts on the Pathogenesis and Treatment of Pelvic Organ Prolapse. Biomolecules. 2022;12(1).

24. Verhorstert KWJ, Gudde AN, Kortz BS, Limpens J, Roovers JWR, Hooijmans CR, et al. Animal experimental research assessing urogynecologic surgical mesh implants: Outcome measures describing the host response, a systematic review and meta-analysis. Neurourol Urodyn. 2021;40(5):1107-19.

25. Vodegel EV, Kastelein AW, Jansen C, Limpens J, Zwolsman SE, Roovers JWR, et al. The effects of oestrogen on vaginal wound healing: A systematic review and meta-analysis. Neurourol Urodyn. 2022;41(1):115-26.

26. Kastelein AW, de Graaf BC, Latul YP, Verhorstert KWJ, Holthof J, Guler Z, et al. Ultra-high-frequency ultrasound: promising technique to visualize pelvic floor mesh in vivo. Ultrasound Obstet Gynecol. 2021;57(4):653-4.

27. Weber MA, Diedrich CM, Ince C, Roovers JP. Focal depth measurements of the vaginal wall: a new method to noninvasively quantify vaginal wall thickness in the diagnosis and treatment of vaginal atrophy. Menopause. 2016;23(8):833-8.

28. Kastelein AW, Diedrich CM, Jansen C, Zwolsman SE, Ince C, Roovers JWR. Validation of noninvasive focal depth measurements to determine epithelial thickness of the vaginal wall. Menopause. 2019;26(10):1160-5.

29. Miller EA, Beasley DE, Dunn RR, Archie EA. Lactobacilli Dominance and Vaginal pH: Why Is the Human Vaginal Microbiome Unique? Front Microbiol. 2016;7:1936.

30. Flores SA, Hall CA. Atrophic Vaginitis. StatPearls. Treasure Island (FL): StatPearls Publishing

Copyright © 2022, StatPearls Publishing LLC.; 2022.

31. Zong W, Stein SE, Starcher B, Meyn LA, Moalli PA. Alteration of vaginal elastin metabolism in women with pelvic organ prolapse. Obstet Gynecol. 2010;115(5):953-61.

32. Werb Z, Banda MJ, McKerrow JH, Sandhaus RA. Elastases and elastin degradation. J Invest Dermatol. 1982;79 Suppl 1:154s-9s.

33. Ulrich D, Edwards SL, Su K, White JF, Ramshaw JA, Jenkin G, et al. Influence of reproductive status on tissue composition and biomechanical properties of ovine vagina. PLoS One. 2014;9(4):e93172.

34. Moalli PA, Talarico LC, Sung VW, Klingensmith WL, Shand SH, Meyn LA, et al. Impact of menopause on collagen subtypes in the arcus tendineous fasciae pelvis. Am J Obstet Gynecol. 2004;190(3):620-7.

## FIGURE LEGENDS

**Figure 1:** Vaginal pH, VMV, VMI (% superficial cells), epithelial glycogen accumulation, elastin fibres, collagen, and vascularity in ewes. Blue represents the control (premenopausal) group, orange represents the OVX (postmenopausal) group. Measurements are taken five months after OVX. Data presented as mean ± SD, * = statistical significant difference between groups.

**Figure 2:** Longitudinal measurements of the vaginal epithelial thickness (μm) in ovariectomised ewes (orange) compared to the control group (blue); Data presented as mean ± SD. X-axis shown in months after

OVX.

**Figure 3:** Representative figures of vaginal changes before (A) and 5 months after (B) ovariectomy in ewes. **1** : Epithelial thickness; Haematoxylin and Eosin stain, magnification ×200. **2** : Glycogen accumulation (marked with yellow arrows); Periodic acid-Schiff stain, magnification ×200. **3** : Vascularity, (marked with yellow arrows); Masson's Trichrome stain, magnification ×100. **4** : Elastin fibres (black lines, marked with yellow arrows); Van Gieson's stain, magnification ×400. **5** : Collagen (stained blue); Masson's Trichrome stain, magnification ×400.

| Outcome | Baseline (before OVX) | Baseline (before OVX) | 5 months post OVX | 5 months post |
|---|---|---|---|---|
| | Premeno-pausal (n=5) | Postmeno-pausal (n=20) | Premeno-pausal (n=5) | Postmeno-pau |
| Epithelial thickness (µm) | 110 (45) | 116 (39) | 146 (53) | 47 (34) |
| Vaginal pH (2-9) | 8,0 (0,35) | 8,1 (0,46) | 8,0 (0,35) | 8,1 (0,31) |
| Vaginal cytology (VMV) | 57 (19) β1 | 52 (9) β2 | 65 (13) | 57 (8) β3 |
| Superficial cells (VMI %) | 24,0 (29,7) β1 | 12,7 (15,3) β2 | 32,4 (24,9) | 7,4 (7,9) β3 |
| Glycogen accumulation (0-3) | 2,56 (0,36) | 2,37 (0,47) | 2,40 (0,60) | 1,36 (0,77) |
| Elastin fibres (0-3) | 2,10 (0,83) | 1,27 (0,80) | 1,72 (0,70) | 1,63 (0,60) |
| Collagen (0-3) | 1,38 (0,37) | 1,53 (0,48) | 1,82 (0,46) | 1,76 (0,49) |
| Vascularity (0-3) | 2,12 (0,56) | 1,92 (0,57) | 2,68 (0,28) | 2,07 (0,49) |

**Table 1. Outcomes at baseline and 5 months post OVX.** Values presented as mean (SD). At baseline, there were no significant differences for all outcomes between groups. β1: n=4. β2: n=9. β3: n=5. * = significant difference between groups.