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Article

Histomorphometric Analysis of the Endometrium of Jennies and Mares: Anatomical Differences and Possible Reproductive Implications

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Simple Summary

The donkey population has significantly decreased over the last decades, and some breeds are now considered endangered. Assisted reproductive techniques (ARTs) play a key role in saving donkeys from extinction. However, the application of current ARTs often yields poorer fertility results in donkeys than in the species they are usually compared to, horses. It has been suggested that the key to this disparity might lie in the species-specific uterine response, but the anatomical and physiological differences between the uterus of these species still remain unknown. In this study, we compared the uterine characteristics of reproductively sound donkeys (jennies) and horses (mares) using histomorphometry, a method that allows objective and quantitative analysis of the endometrium, the inner layer of the uterus. The results of this study showed interspecific differences in both the size and density of the endometrial glands. Jennies displayed larger and more widely distributed glands than mares. Increased glandular tissue has previously been found in older mares with uterine disorders and reduced fertility. These findings describe for the first time the histomorphometric characteristics of the jenny endometrium and underline its differences with mares, highlighting the interspecific differences and the need for further research to optimize ARTs for donkeys.

Abstract

Assisted reproductive technologies (ARTs) are often extrapolated from horses to donkeys, despite poorer fertility outcomes in jennies, highlighting the need to identify species-specific traits to optimize ARTs in donkeys. This study compared, through histomorphometry, the endometrium of jennies and mares. Endometrial biopsies were taken from reproductively sound jennies ($n = 6$) and mares ($n = 6$) in estrus. Histomorphometric analysis evaluated luminal (LE, μm) and glandular epithelium height (GE, μm), glandular lumen diameter (LD, μm), glandular area (GA, μm^2), number of glands (#G), and glandular tissue percentage (GT, %), measured in the stratum compactum (SC) and spongiosum (SS). Results were statistically compared between species (jennies vs. mares), parity status (maiden vs. foaling), and stratum (SC vs. SS). Jennies exhibited higher ($P < 0.05$) values than mares for LE, LD-SC, GA-SC, and GT-SC. Greater glandular size and density in mares have been linked to susceptibility to endometritis, intrauterine fluid accumulation, aging, and poorer reproductive outcomes in mares. These findings suggest that the histomorphometric features observed in reproductively sound jennies reflect anatomical differences that might contribute to a

distinctive endometrial inflammatory response compared to mares. In conclusion, histomorphometry revealed significant endometrial differences between mares and jennies, the latter displaying larger luminal epithelium, greater glandular size, and density than mares.

Keywords: histomorphometry; endometrium; biopsy; equine; donkey

1. Introduction

Donkeys have lost their traditional role in industrialized communities, leading to a drastic reduction in their numbers, particularly in developed regions such as Europe, over the last two decades. As a result, most European donkey breeds have become endangered or extinct [1]. Nowadays, donkeys are assuming new roles in society, such as landscape maintenance, flock protection, donkey milk production and participation in social and leisure activities [2]. Their endangered status and increasing relevance in society makes it crucial to preserve and enhance their genetic pool [3].

Assisted reproductive technologies (ARTs) play a key role on the conservation of endangered species [4]. ARTs are often extrapolated from horses to donkeys, despite obtaining poorer fertility outcomes in this species [5–8]. Crossbreeding studies have suggested that the lower fertility in donkeys is related to an exacerbated inflammatory response of the endometrium of the jenny [7,9], which mechanism remains unclear [10–13]. A possible explanation could be the differences on the reproductive physiology and anatomy of jennies and mares. Jennies present proportionally larger internal genitalia, narrower and more tortuous cervix forming a deeper fornix, less endometrial edema in estrus, and more branched uterine glands [14]. This unique reproductive anatomy of the jennies might contribute to an exacerbated post-breeding endometrial inflammation [13]. However, to the best of our knowledge, no studies have objectively compared the uterine anatomical differences between jennies and mares.

Histomorphometry has been extensively used to objectively evaluate uterine features of several species including variations among individuals [15,16], breeds [17] and species [18]. In mares, histomorphometry has been used to evaluate the effect of the estrus cycle [19]; age and reproductive history [20]; susceptibility to endometritis [21]; intrauterine fluid accumulation [22]; and treatment responses [22,23] on the uterine anatomy. Nevertheless, there is limited research on this topic in jennies [14]. A comparative study could shed light on physiologic anatomical features predisposing to poorer reproductive outcomes in jennies. Thus, the objective of this study was to compare the histomorphometric characteristics of the endometrium of reproductively sound jennies and mares.

2. Materials and Methods

2.1. Animals and Breeding Soundness Evaluation

A total of twelve animals were included in this study: six mares (*Equus caballus*), three Purebred Spanish Horse and three Purebred Arabian Horse; and six Andalusian Donkey Breed jennies (*Equus asinus*). The age of the animals ranged from 3 to 16 years old. Prior to their inclusion in the study, a reproductive soundness evaluation was conducted. Parity status and reproductive history was recorded. Females were classified as maiden, if they had never been bred; or foaling, if they had foaled the year of the study [24].

The reproductive soundness evaluation consisted of the assessment of cyclic ovarian activity and the absence of pathological images in the reproductive system evaluated by transrectal ultrasonography (Logiq V2, GE Medical Systems, China), endometrial biopsy, and endometrial cytology. Cytology and biopsy samples were taken in estrus, as explained below. The animals were considered reproductively sound if the percentage of polymorphonuclear leukocytes (PMNs) in endometrial cytology smear was lower than 5% [25] and the biopsy was Category I or IIa according to Kenney and Doig (1986) endometrial biopsy grading system (KDGS).

2.2. Sample Collection

Ovarian cycle control was performed weekly by seriated ultrasound examinations. Endometrial samples were collected when behavioral estrus signs were displayed, in the presence of a dominant follicle > 30 mm in diameter, uterine edema > 2 (scale 0-3, being 0 absence of edema and 3 maximum edema), and soft, relaxed cervix on vaginal palpation.

After perineal disinfection [26,27], guarded endometrial biopsy was collected employing the double sleeve technique [28,29] using a biopsy forceps with a cutting surface of 4 x 15 mm (Equivet, Kruuse, Denmark). The tissues were immediately tapped onto a sterile slide for cytology and then transferred into a test tube containing 4% formaldehyde (Qemical, Quality Chemicals S.L., Spain) for fixation.

A total of 12 endometrial biopsies, 6 from jennies and 6 from mares, were routinely processed and sectioned into 4 µm tissue sections and stained with hematoxylin-eosin [21,30], for blind histopathological evaluation and later histomorphometric analyses (Figure 1A). The parameters luminal (LE) and glandular (GE) epithelium height, glandular lumen diameter (LD), and glandular area (GA) were measured at 400x magnification, whereas the parameters number of glands (#G), and glandular tissue percentage (GT) were measured at 100x magnification. Each parameter was recorded separately for SC and SS.

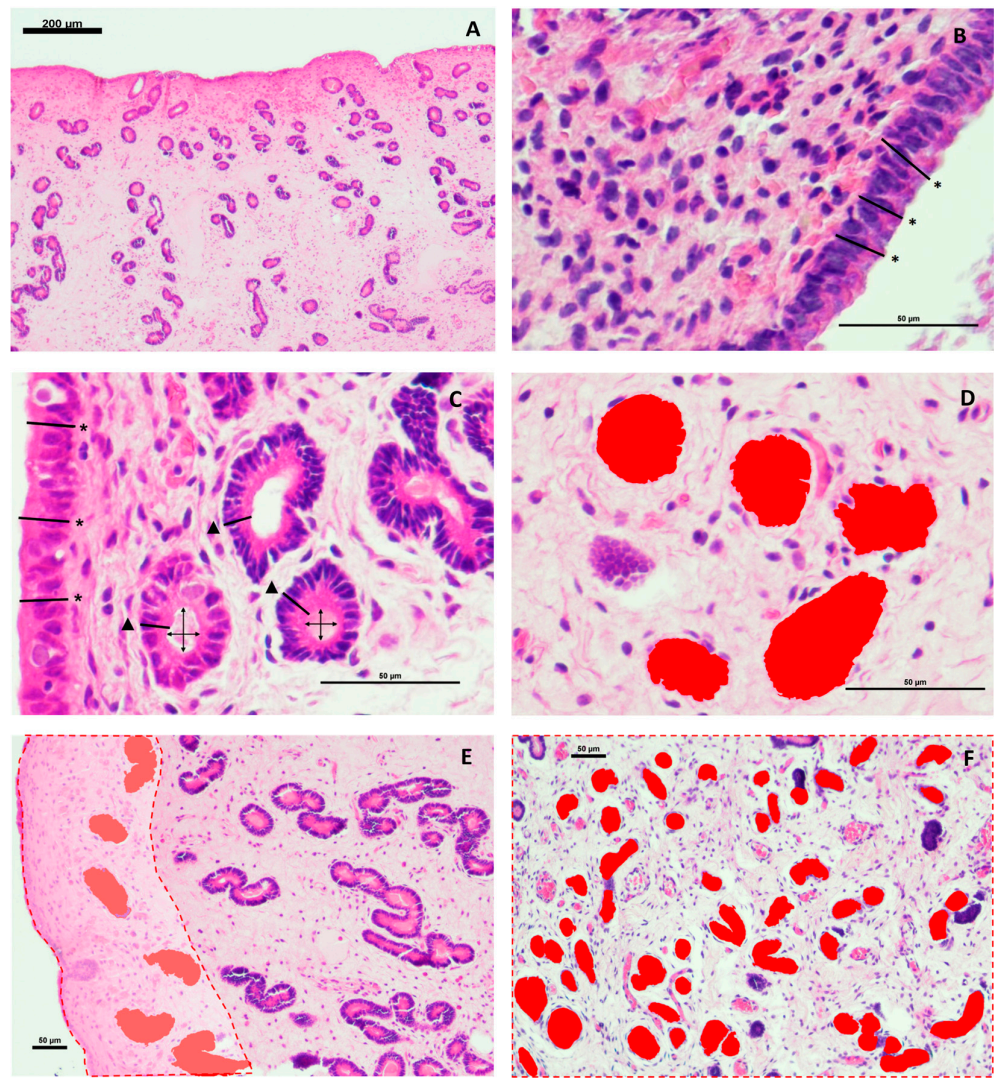


Figure 1. Endometrial histomorphometric evaluation. A. Histological section of the equine endometrium (jenny – J1) stained with hematoxylin and eosin at x40 magnification, scale bar = 200 µm. B. Example of three

measurements for LE height (asterisk) at x400 magnification. C. Example of three measures of LE height (asterisk), GE height (triangle), and perpendicular luminal diameters (arrows). D. Automated measurement of the glandular area coloured in red. E and F. Examples of SC (E) and SS (F) at 100x magnification for mean glandular area and glandular tissue percentage measure. Glandular area coloured in solid red and endometrial tissue area delimited by dashed red line. 400x and 100x. Scale bars = 50 μm .

2.3. Endometrial Histomorphometric Evaluation

The endometrial histomorphometry was evaluated using the NIS-Elements Basic Research software version 5.21.03 (Nikon, Tokyo, Japan) on images provided by a camera (DS-Fi3, Nikon, Tokyo, Japan) connected to an inverted microscope (ECLIPSE Ti1-U IVF, Nikon, Tokyo, Japan).

2.3.1. Measurement of Luminal Epithelium Height and Glandular Size Parameters

The height of the luminal epithelium (LE) was measured as the distance from the basement membrane to the apical edge of the epithelial cell [19], at 400x magnification following the methodology described elsewhere [19,20,22,31] with modifications (Figure 1B). The LE was calculated as the mean from 30 measures of each endometrium [21]. The results were expressed in micrometers.

The height of the glandular epithelium (GE) was also measured as the distance from the basement membrane to the apical border of the epithelial cell, at 400x magnification [19,20,22,31] (Figure 1C). For each stratum (glandular epithelium in the stratum compactum, GE-SC; and glandular epithelium in the stratum spongiosum, GE-SS) 30 glands were measured [21]. The results were expressed in micrometers.

Glandular lumen diameter (LD) was calculated as the mean of two perpendicular diameters in circular section glands randomly selected [20–23] from the SC (LD-SC) and from the SS (LD-SS). Measures were taken at 400x magnification. This parameter was measured in all the circular glands found in SC and SS. To standardize the gland selection for the measures, glands were considered circular when the parameter “circularity” was higher than 0.5. *Circularity* was measured by automatically detecting the object area by the NIS-Br software and calculated as: $\text{Circularity} = 4 \times \pi \times \text{Area} / \text{Perimeter}^2$. The tools *Measure Vertical* and *Measure Horizontal* were used to ensure exact 90-degree perpendicular measures (Figure 1C). The results were expressed in micrometers.

Glandular area (GA) was calculated as the mean area of the glands measured separately for each stratum (glandular area in the stratum compactum, GA-SC; and glandular area in the stratum spongiosum, GA-SS), at 400x magnification. GA was individually quantified using the feature *Automated Measurement > Auto detect*, highlighting the selected areas with *Object Colors* as shown in Figure 1D. This tool is based on traditional computer vision algorithms, analyzing pixel intensity levels and variations in adjacent pixels to detect cell or gland boundaries. The results were expressed as μm^2 .

2.3.2. Measurement of Glandular Density Parameters

Number of glands (#G) is a glandular density parameter that refers to the number of glands present in a microscopic field. It was measured separately for the SC and the SS (#G-SC, #G-SS), calculated as the mean of the number of glands in 10 fields per stratum at 100x magnification. Glands were quantified as described in previous studies [17,19,31], with some modifications; using the *Object Count* tool of NIS-BR software, disregarding the glands that were touching the image borders. Glands were recognized as individual structures when, regardless of their shape, their structure was delimited by glandular epithelium throughout their perimeter, and there was identifiable stromal tissue around them. The results were expressed as number of glands per field.

Glandular tissue percentage (GT) is a glandular density parameter representing the percentage of the endometrial tissue surface covered by glands. In this study, this parameter was evaluated using the methodology described in cattle by Wang et al. [16] with some modifications. In brief, the area of

the glands in the SC and SS was measured separately in 10 microscopic fields, and the corresponding endometrial tissue area was measured using the manual area tool (dashed lines in Figure 1 E and F). GT was recorded for each microscopic field, stratum, and sample (GT-SC, GT-SS).

$$\text{Glandular tissue percentage} = \frac{\sum \text{gland area } (\mu\text{m}^2)}{\text{endometrial tissue area } (\mu\text{m}^2)} \times 100$$

2.4. Statistical Analysis

All analyses were performed using the statistical package SPSS v29.0.1.0 (IBM Spain, Madrid, Spain). All histomorphometric parameters were compared between species (jenny vs. mare), parity status (maiden vs. foaling), and strata (SC vs. SS). Data distribution normality was assessed using the Kolmogorov–Smirnov test. The results were compared by one-way ANOVA test followed by post-hoc analysis using the Tukey test. Results were presented as mean ± SEM. The level of significance was set at *P* < 0.05.

3. Results

3.1. Breeding Soundness Evaluation

The results of the individual breeding soundness evaluation of the animals included in the study are displayed in Table 1.

Table 1. Results of the breeding soundness evaluation of the animals included in the study (jennies, n=6; mares, n=6).

Parameters	Jennies						Mares					
	J1	J2	J3	J4	J5	J6	M1	M2	M3	M4	M5	M6
Parity status ¹	Maiden	Maiden	Foaling	Foaling	Foaling	Foaling	Maiden	Maiden	Foaling	Foaling	Foaling	Foaling
Age (years) ²	3	5	10	10	11	12	3	3	12	13	14	16
PMNs (%) ³	1.62	0.50	0.17	2.61	3.71	0.66	0.66	0.33	0.50	0.00	0.33	0.00
KDGS ⁴	I	IIa	I	IIa	I	IIa	I	IIa	IIa	IIa	IIa	I

¹ Parity status: Maiden: they have not been bred; Foaling, they had foaled the year of the study. ² Age of the animals at the moment of the study. ³ Percentage of polymorphonuclear leukocytes (PMS, %) in endometrial cytology. ⁴ Categories according to Kenney and Doig’s endometrial biopsy grading system (I, IIa, IIb, III).

3.2. Histological Characteristics of the Endometrial Tissue

The histological characteristics of the endometrial tissue of mares and jennies are illustrated in Figure 2. The luminal epithelium was observed under bright field microscopy (Figure 2A and D), displaying simple cuboidal to columnar epithelium with varying pseudostratification in both species. The lamina propria exhibited estrus-induced stromal edema, and uterine glands showed variable tortuosity (Figure 2B, C, E, F), apparently higher in jennies. Mild subepithelial inflammatory infiltrate was present, with a more pronounced proportion of eosinophilic infiltrates in jennies. These findings align with previous literature descriptions [19,21,23,32–34].

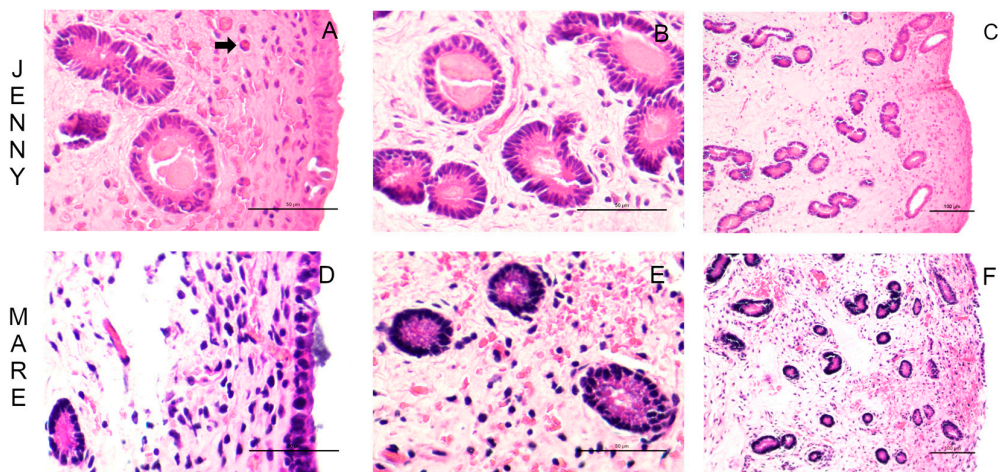


Figure 2. Histological characteristics of the endometrial tissue of maiden jenny (A-C) and maiden mare (D-F). A, B, D, E: at 400x magnification, scale bar = 50 μm . C, F: at 100x magnification, scale bar = 100 μm . A: Eosinophil ($\rightarrow$, arrow).

3.3. Endometrial Histomorphometric Evaluation

3.3.1. Results of Luminal Epithelium and Endometrial Glandular Size Parameters

The height of the luminal epithelium (LE), glandular lumen diameter (LD) and glandular area (GA) showed significantly higher values in the stratum compactum (SC) in jennies than in mares ($P < 0.05$, $P < 0.001$ and $P < 0.05$, respectively) (Figure 3). Significant intraspecies differences ($P < 0.01$) between *strata* (SC vs. SS) were observed in certain parameters: in jennies, GE-SC and LD-SC were larger than their equivalents in SS; whereas in mares GA-SC was smaller than GA-SS.

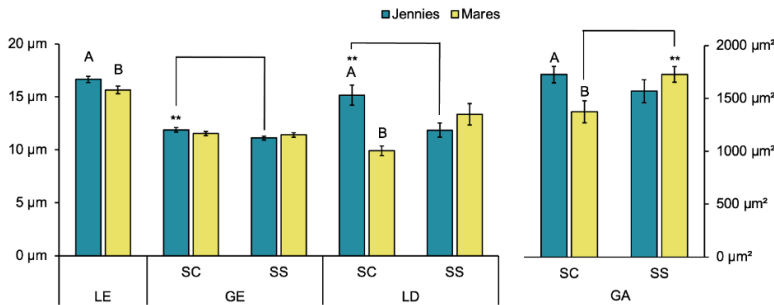


Figure 3. Results of luminal epithelium height and endometrial glandular size parameters (jennies vs. mares). LE = Luminal epithelium height (μm); GE = glandular epithelium height (μm); LD = glandular lumen diameter (μm); GA = glandular area (μm^2); SC = stratum compactum; SS = stratum spongiosum. Different superscripts in capital letters indicate significant differences between jennies and mares for each stratum (A, B , $P < 0.05$). Asterisks indicate significant differences between *strata* within each species (SC vs. SS): ** $P < 0.01$.

The differences among the glandular size parameters grouped according to species and parity status were also compared (Table 2). In both species, foaling animals presented higher values ($P < 0.001$) for LE, GE and GA-SC than maiden. Maiden and foaling jennies exhibited wider ($P < 0.001$) LD-SC than mares. There were also significant differences ($P < 0.01$) between *strata* (SC vs. SS) for some parameters: in maiden jennies, GE-SC was taller than GE-SS; in foaling jennies, LD-SC was wider than LD-SS. Foaling mares exhibited narrower LD-SC than LD-SS; maiden mares had smaller GA-SC than GA-SS.

Table 2. Comparison of luminal epithelium height and glandular size parameters grouped according to species and parity status.

Parameters	Jennies		Mares	
	Maiden (n = 2)	Foaling (n = 4)	Maiden (n = 2)	Foaling (n = 4)
LE (µm)	14.19 ± 0.47 ^b	17.88 ± 0.33 ^a	10.93 ± 0.24 ^c	18.01 ± 0.37 ^a
GE-SC	11.23 ± 0.30 ^{b**}	12.21 ± 0.26 ^{ab}	9.75 ± 0.21 ^c	12.43 ± 0.25 ^a
GE-SS	10.10 ± 0.25 ^b	11.59 ± 0.22 ^a	9.39 ± 0.23 ^b	12.43 ± 0.25 ^a
LD-SC	15.25 ± 1.39 ^a	15.62 ± 1.27 ^{a**}	10.78 ± 0.75 ^b	9.37 ± 0.55 ^b
LD-SS	12.80 ± 1.45	11.47 ± 0.71	12.78 ± 1.47	13.71 ± 1.38 ^{**}
GA-SC	1322.03 ± 105.85 ^{bc}	1908.62 ± 148.64 ^a	1018.72 ± 74.21 ^c	1588.38 ± 103.27 ^{ab}
GA-SS	1501.21 ± 110.18	1599.21 ± 104.16	1485.8 ± 127.31 ^{**}	1835.28 ± 137.12

Parity status = (Maiden: they have not been bred; Foaling, they had foaled the year of the study). LE = Luminal epithelium height (µm); GE = glandular epithelium height (µm); LD = glandular luminal diameter (µm); GA = glandular area (µm²); SC = stratum compactum; SS = stratum spongiosum. Values are expressed as mean ± SEM. ^{a-d} Values within a row with different superscripts differ significantly at *P* < 0.05. Asterisks indicate significant differences between strata for each group (SC vs. SS): ** *P* < 0.01.

3.3.2. Results of Glandular Density Parameters

Glandular density parameters revealed further significant differences in glandular tissue distribution between species (Figure 4). The quantification of glands (#G) was similar in both species (*P* > 0.05), while glandular tissue percentage in the SC (GT-SC) was significantly higher in jennies than in mares (*P* < 0.01). In mares, GT-SS proportionally covered a greater area (*P* < 0.05) than GT-SC.

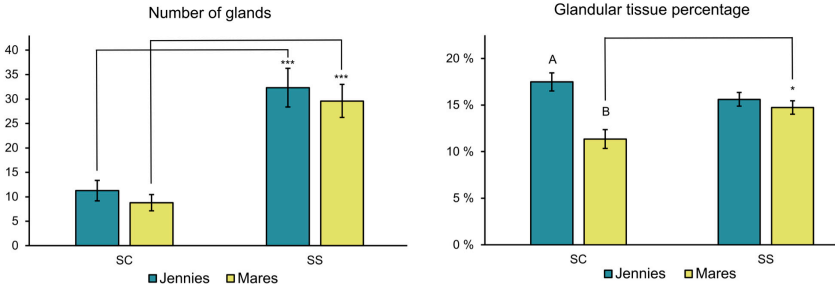


Figure 4. Results of glandular density parameters (jennies vs. mares). Different superscripts in capital letters indicate significant differences between total jennies and mares for each stratum (^{A,B} *P* < 0.05). Asterisks indicate difference between strata within species (SC vs. SS): * *P* < 0.05 and *** *P* < 0.001.

The comparison of density parameters grouped within species and parity status was also evaluated (Table 3). The number of glands (#G) did not significantly differ (*P* > 0.05) among species or parity status. In foaling jennies, GT-SC covered a larger (*P* < 0.001) area compared to mares (both foaling and maiden). Additionally, GT-SC in maiden jennies was significantly larger (*P* < 0.001) than in their mare counterparts. Regarding differences observed between *strata*, in both species, there were fewer #G (*P* < 0.001) in the SC. No differences between *strata* were observed for GT (*P* > 0.05).

Although #G did not show significant differences (*P* > 0.05) among species or parity status, GT differed between jennies and mares in the SC. Accordingly to glandular size parameters, the percentage of glandular tissue covered a greater area (*P* < 0.01) of the SC of the endometrium in jennies than in mares when comparing species within the same parity status.

Table 3. Comparison of glandular density parameters within species, grouped according to parity status.

Parameters	Jennies		Mares	
	Maiden (n = 2)	Foaling (n = 4)	Maiden (n = 2)	Foaling (n = 4)
#G-SC	11.68 ± 1.51	10.86 ± 1.20	8.70 ± 1.66	8.83 ± 1.22
#G-SS	26.25 ± 3.05***	35.53 ± 2.31***	31.90 ± 4.24***	25.91 ± 2.31***
GT-SC	15.67 ± 1.20 ^{ab}	18.31 ± 1.33 ^a	8.70 ± 1.52 ^c	12.67 ± 1.27 ^{bc}
GT-SS	14.37 ± 1.22	16.12 ± 0.91	12.87 ± 0.81*	15.41 ± 0.99

Parity status = (Maiden: they have not been bred; Foaling, they had a foal the year of the study). #G = number of glands; GT = glandular tissue percentage (%); SC = stratum compactum; SS = stratum spongiosum. Values are expressed as mean ± SEM. ^{a-d} Values within a row with different superscripts differ significantly at *P* < 0.05. Asterisks indicate significant differences between strata (SC vs. SS): * *P* < 0.05, *** *P* < 0.001.

4. Discussion

This study presents the first histomorphometric comparison between the endometrium of jennies and mares, providing objective differences among the endometrial features of these species. This novel characterization of the endometrial tissue of jennies provides valuable insights into their unique physiological traits that could play a role in the different response after artificial insemination in these species [7,9].

Our study revealed significant differences between jennies and mares glandular size parameters. During estrus, the luminal epithelium (LE) was significantly taller in jennies than in mares. The glandular epithelium (GE) of the glands in the stratum compactum (SC) was also taller in maiden jennies than maiden mares. The observed increase in epithelial height (luminal and glandular) in jennies compared to mares suggests a heightened level of glandular activity [32,35]. This fact was supported by the finding that LE and GE were also taller in animals that had foaled on that year than in maiden animals in both species. Glandular lumen diameter and area (LD-SC and GA-SC) were also larger in jennies compared to mares, reinforcing the idea of increased glandular activity, with the possible subsequent secretion accumulation in this species. Exuberant secretion may enlarge glands and impair their clearance, leading to glandular secretion accumulation. This build-up fluid could trap inflammatory debris, which would promote further mucus production and bacterial growth, creating a self-sustaining cycle of inflammation [36].

The presence of larger glands in jennies and foaling animals, as indicated by increased LD-SC and GA-SC, may result in an environment rich in uterine secretion and inflammatory mediators, which could facilitate bacterial colonization and increase the risk of uterine contamination [37]. Previous studies have shown that pathogenic microorganisms causing endometritis in mares are able to reside in the epithelia cells and within the endometrial glands [38–41]. Their presence in these locations has been proposed to reflect distinct survival mechanism: dormant bacteria inside the cells may evade detection until activated by specific triggers [38]; and within the glandular lumen, phagocytic binding may be impaired [41] while accessing the nutrient-rich glandular fluid [39]. Previous studies have shown that larger glandular structures are more prevalent in older mares with poor reproductive outcomes [20], and larger glands are also related to greater susceptibility to a more exacerbated post-breeding inflammation [21,22]. These findings agree with the results found in other species like the dog; bitches with cystic endometrial hyperplasia show glandular enlargement and mucus retention, creating favorable conditions for bacterial growth and predisposing these animals to suffer from pyometra [42]. According to these findings, wider glands (as those found in foaling females and jennies in particular) could be more predisposed to heightened inflammatory responses. This hypothesis would explain some reproductive differences found in jennies, such as higher basal polymorphonuclear leukocytes (PMNs) counts in endometrial samples [13,43,44], a more exacerbated post-breeding inflammatory response [12,45], and lower pregnancy rates after AI [7,9,46].

In addition to the size of luminal epithelium and glandular structures, glandular density across the stromal layers shows a wider picture of the endometrium. In our study, glandular density was

assessed by counting the number of glands per field (#G) and calculating the percentage of tissue covered by glands (GT). The latter incorporated the parameter gland area using a novel methodology for mares, adapted from what has been applied in other species [16]. This approach was chosen for its accuracy and ease of implementation in comparison to previous analogue approaches [17,19–21,31]. Our findings revealed that, in jennies, GT covered a larger area within the SC, while the #G did not differ significantly between species. The fact that GT covered a wider area in jennies and #G was similar for both species, stands by the finding that jennies display wider glands which accumulate more secretion in the glandular lumen, which may contribute to bacteria colonization and heightened inflammatory response, as previously discussed [36,38,41]. Previous studies conducted in mares found that an increased number of glands in mares is associated with a higher susceptibility to endometritis [21], while higher glandular density has been linked to intrauterine fluid accumulation [22]. These findings reaffirm that the higher amount of glandular tissue found in jennies might be linked to a more exacerbated post-breeding inflammation. In contrast, a study evaluating the histomorphometric features of older mares with poor reproductive outcomes reported a lower number of glands, concluding that insufficient functional tissue could lead to early embryo death [20]. However, this study did not take into consideration the percentage of glandular tissue these glands represented. These findings emphasize the need for future studies to further investigate glandular density and its potential implications in subfertile mares and jennies.

Remarkably, our results showed that significant differences in glandular size and density between jennies and mares were almost exclusively limited to the stratum compactum (SC), with minimal variations found in the deeper stratum spongiosum (SS). The SC seems to be more sensitive to environmental and physiological changes [47,48], and probably more noteworthy from a diagnostic point of view. These results highlight the importance of differentiating between *strata* when performing histomorphometric analyses, as significant differences may be overlooked without this separation.

In order to identify the physiological interspecific differences in the endometrium, this study only included reproductively healthy jennies and mares, meeting the criteria of i) no endometrial inflammation (PMNs < 5%) and ii) low degree of fibrotic endometrial degeneration with scores I or IIa according to the KDGS [33]. Future studies including samples showing signs of inflammation or fibrotic endometrial degeneration (KDGS IIb or III) could deepen our understanding on the reproductive adaptations of these closely related species and how endometrial status might affect reproductive outcomes.

5. Conclusions

In summary, the comparison of histomorphometry characteristics between reproductively sound jennies and mares revealed significant endometrial differences, with jennies displaying larger luminal epithelium, glandular size, and density compared to mares. These anatomical differences might play a role in the more exacerbated uterine response after AI in jennies. Further research is needed to understand the physiological mechanism underlying the uterine response in jennies.

Author Contributions: Conceptualization, P. Vallejo-Soto and I. Ortiz; methodology, P. Vallejo-Soto and I. Ortiz; formal analysis, P. Vallejo-Soto and I. Ortiz; investigation, P. Vallejo-Soto, I. Ortiz, R. Herrera-García, C. Álvarez-Delgado, J. Gómez-Laguna, A. de Santiago, M. Manrique, A. Gonzalez Ariza, and J.M. Leon Jurado; resources, J. Gómez-Laguna, A. de Santiago, M. Manrique, A. Gonzalez Ariza, J.M. Leon Jurado, M. Hidalgo, and J. Dorado; data curation, P. Vallejo-Soto and R. Herrera-García; writing—original draft preparation, P. Vallejo-Soto and I. Ortiz; writing—review and editing, P. Vallejo-Soto, I. Ortiz, C. Álvarez-Delgado, J. Gómez-Laguna, M. Hidalgo, and J. Dorado; visualization, P. Vallejo-Soto, I. Ortiz, M. Hidalgo, and J. Dorado; supervision, I. Ortiz, M. Hidalgo, and J. Dorado; project administration, M. Hidalgo and J. Dorado; funding acquisition, M. Hidalgo and J. Dorado. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

The following abbreviations are used in this manuscript:

#G	Number of glands
AI	Artificial Insemination
ARTs	Assisted reproductive technologies
GA	Glandular area
GE	Glandular epithelium
GT	Glandular tissue percentage
KDGS	Kenney and Doig biopsy grading system
LD	Lumen diameter
LE	Luminal epithelium
SC	Stratum compactum
SS	Stratum spongiosum

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