

Anatomical and histological characterization of the cervix in Santa Inês hair ewes

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Abstract

The aim of the present study was to describe the morphology of the cervix in Santa Inês ewes. One hundred cervixes were collected from females of different ages (12 to 48 months). Five types of cervical os were observed: duck-bill, flap, rosette, papilla and spiral, with duck-bill being the most predominant type (51%) overall. The average cervix length was 4.68 cm (range of 3 to 7 cm). Shorter cervixes ($P < 0.05$) were observed in younger females (12-18 months). The number of rings was on average 5.68 (range 3 to 8). There was a positive correlation between cervical length and animal age ($r = 0.22$; $P < 0.0001$) and between cervical length and number of rings ($r = 0.21$; $P < 0.0001$). Cervical rings were generally funnel-shaped and the mean internal diameter of the rings was 2.98 mm. The average number of rings with fornix was 5.17. The presence of fornix in the first three rings was higher than in the subsequent rings, gradually decreasing until the seventh ring. The internal shape of the cervix resembled two inverted cones, being wider at both ends and narrower in the middle. Differences in the internal characteristics of the cervical canal (length, diameter, number of rings, and number of fornices) were observed only between some of the cervical os shapes, and the success of the cervical passage cannot be predicted by the determination of the cervical os shape. Some anatomical features of the cervical canal in Santa Inês ewes differ from those reported for other sheep breeds, showing that information obtained can be useful to define the characteristics of devices to be used for achieving successful cervical penetration during insemination of Santa Inês ewes.

Keywords: artificial insemination, cervical rings, morphometry.

Introduction

The main limitation for the application of reproductive technologies such as artificial insemination (AI) in sheep has been the low fertility rate obtained with fresh, chilled and frozen semen when using primarily vaginal and cervical insemination (Naqvi *et al.*, 1998; Ax *et al.*, 2000). In contrast, laparoscopic insemination gives better results with frozen semen, but requires the use of expensive equipment, a qualified veterinarian, and is generally expensive for the small farmer (Ax *et al.*, 2000).

In sheep, the inability to cross the long, rigid

and narrow cervix has led to the low use of intrauterine deposition of semen (Croy *et al.*, 1999). The transcervical technique, where a pipette is used to cross the cervix to deposit semen in the body of the uterus, may lead to an increase in the use of frozen semen. Nevertheless, the irregular and excentric distribution of folds in the cervical canal in sheep leads to a reduced lumen in the cervix to carry out transcervical insemination (Halbert *et al.*, 1990; Souza, 1993; Naqvi *et al.*, 1998) and lesions may occur if attempts are made to pass the pipette through it, which reduces the success of the technique (Campbell *et al.*, 1996).

For AI to be commercially viable in sheep it is necessary to develop an efficient transcervical technique, with low operating cost and little stress to the animals. For this, knowledge of cervical characteristics becomes essential to develop adequate equipment to better manipulate the cervical canal (Halbert *et al.*, 1990) in programs of transcervical artificial insemination (TCAI). The anatomical characterization of the ovine cervix was reported for some sheep breeds (Halbert *et al.*, 1990; Souza, 1993) but so far there are no scientific publications that describe the morphological characteristics of the cervix of hair sheep. Such breeds are commonly used in the tropics, and in Brazil the Santa Inês is the most popular (McManus *et al.*, 2010).

The present study aims to describe the anatomical and histological features of the cervix of Santa Inês sheep in order to obtain information for the development of instruments and methods of manipulation of the cervical canal during insemination.

Materials and Methods

One hundred cervixes from non-pregnant Santa Inês ewes collected in an abattoir were used in this study. They were divided into five groups according to the age of ewes: A (12 to 18 months), B (18 to 24 months), C (24 to 30 months), D (30 to 38 months) and E (38 to 48 months).

After cleaning, an anatomic and macroscopic morphometry of the cervix was carried out considering the following aspects:

1. Shape of the cervical os: the cervical os was classified as flap, duckbill, rosette, spiral or papilla (Fig. 1).
2. Number of rings and presence of fornix on the rings: the number of rings and fornix was recorded after the longitudinal opening of the cervix.

- Length of the cervix and diameter of the rings: the length of the cervical canal was taken from the external opening to the union with the uterine body. With the aid of a caliper, the perimeter (P) of each cervical ring was measured and the diameter (D) was calculated using the formula $D = P/\pi$.

From each group, five cervixes were randomly selected to be analyzed histologically. Three portions (approximately 2 cm each) of cervical tissue were collected from the following anatomic regions: caudal portion (entry by the external cervical os), median portion and cranial portion (next to the uterine body). Each tissue portion was fixed in 10% buffered formalin solution (formaldehyde 40% - 100 ml; monobasic sodium phosphate - 4g; dibasic sodium phosphate anhydrous - 6.5g, and distilled water - 900 ml) for 24 h before being put in 70% ethanol.

After dehydration, clarification and infiltration with paraffin wax, 4 μ m sections were obtained with a semi-automatic microtome (Leica RM 2145[®]), and were mounted on glass microscope slides. The mounted sections were dried (58°C, for 12 h) and stained with the hematoxylin and eosin technique (Ostör, 1993).

The epithelium was classified according to the number of layers in simple, stratified or pseudostratified. According to the shape of cells, the epithelium was classified as cuboidal, columnar or pavementous. The presence of villi, cilia and keratinization was also investigated. Connective tissue was classified as loose or dense connective tissue (regular or irregular).

The area occupied by each type of connective tissue was semi-quantified in a scale varying from 1 to 5, where 1 was attributed to the smaller area and 5 to the greater area. The presence of glands, blood vessels or nerves in the connective tissue was also evaluated.

Statistical analyses were performed with the Statistical Analysis System software (SAS[®] version 9.2). Cervix length, number of cervical rings, ring diameter and number of fornices were analyzed through analysis of variance using the GLM procedure, considering age groups, cervical os shape or ring as fixed factors. Means were compared using the Tukey test

at a 5% significance level. Correlations between traits were estimated using the CORR procedure. Percentages of cervical os shape and percentages of fornix presence were compared with the Chi-Square test.

Results

Anatomical findings

Five types of cervical os were observed: duckbill, spiral, rosette, flap and papilla (Fig. 1), with no differences ($P > 0.05$) in the frequency between age groups (Table 1). Overall, duckbill was the most predominant shape (51%) in Santa Inês ewes. The average length of the cervix was 4.7 cm (range of 3-7 cm), and significant differences ($P < 0.05$) were observed among age groups, with shorter cervixes being observed in younger females (Table 1). The number of rings was not significantly different between age groups (Table 1) with an average of 5.68 rings (range of 3-8 rings). There was a positive correlation between cervical length and animal age ($r = 0.22$; $P < 0.0001$) and between cervical length and the number of rings ($r = 0.21$; $P < 0.0001$). There was also a positive correlation between cervix length and ring diameter ($r = 0.27$; $P < 0.001$). Cervical ring diameter was on average 2.98 ± 1.21 mm. Average diameters were larger in the cranial and caudal portions, with a narrowing in the middle portion (Table 2). Rings were generally funnel-shaped and centripetal (Fig. 2A), with some cases of centrifugal rings (Fig. 2B). The average number of fornices was 5.17, being similar between age groups ($P > 0.05$). There were females showing fornix up to the eighth ring. The presence of fornix in the first three rings was higher than in the subsequent rings and gradually decreased until the seventh ring (Table 2). The number of cervical rings was higher ($P < 0.05$) in spiral and flap os types than in duckbill, papilla and rosette types (Table 3). The length of cervix was higher ($P < 0.05$) in flap type compared to spiral, rosette and papilla types. The highest number of fornices was observed in the spiral cervical os shape ($P < 0.05$).

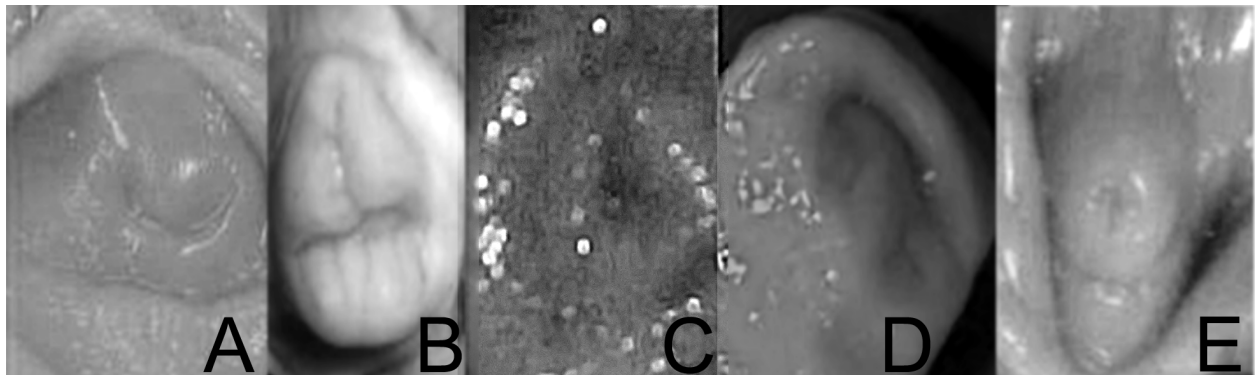


Figure 1. Shape of the cervical os in Santa Inês sheep: Flap (A) - one-fold of cervical tissue protruding into the anterior vagina; Duckbill (B) - two opposing folds of cervical tissue; Rosette (C) - a cluster of cervical folds; Spiral (D) - spiral-shaped cervical tissue; Papilla (E) - a papilla protruding into the anterior vagina with the external os at its apex.

Table 1. Length, diameter, number of rings, and frequency of cervical os shape in Santa Inês ewes.

Group of age (months)	N	Length (cm)	Diameter (mm)	Number of rings	Shape of the cervical os (%)				
					FL	DB	RS	SP	PAP
A (12-18)	20	4.12 ± 1.09 ^c	2.77 ± 1.28 ^b	5.80 ± 1.29	10	45	15	20	10
B (18-24)	20	5.05 ± 0.96 ^a	3.23 ± 1.15 ^a	5.55 ± 1.33	0	55	15	15	15
C (24-30)	20	4.50 ± 1.03 ^b	2.83 ± 1.06 ^b	5.75 ± 1.05	5	55	25	10	5
D (30-38)	20	4.53 ± 0.77 ^b	3.26 ± 1.46 ^a	5.75 ± 1.18	15	55	10	15	5
E (38-48)	20	5.20 ± 0.89 ^a	2.79 ± 0.97 ^b	5.55 ± 1.12	20	45	5	15	15

^{a,b,c}Different superscripts in the column indicate significant differences ($P < 0.05$) by the Tukey test. FL= flap; DB= duckbill; RS= rosette; SP= spiral and PAP= papilla.

Table 2. Diameter (mean ±SD) of the cervical rings and frequency distribution of fornices on each cervical ring in Santa Inês ewes.

Ring	Diameter (mm)	Presence of fornix n/n (%)
1°	3.54 ± 1.62 ^{ab}	100/100 (100 ^a)
2°	2.85 ± 1.26 ^b	100/100 (100 ^a)
3°	2.77 ± 1.01 ^b	99/100 (99 ^a)
4°	2.72 ± 1.02 ^b	89/98 (91 ^b)
5°	2.90 ± 1.02 ^b	72/83 (87 ^b)
6°	2.91 ± 1.00 ^b	36/54 (67 ^c)
7°	3.20 ± 0.84 ^{ab}	16/25 (64 ^c)
8°	3.77 ± 1.08 ^a	6/7 (86 ^{abc})

^{a,b,c}Different superscripts in the column indicate significant differences ($P < 0.05$) by the Tukey test.

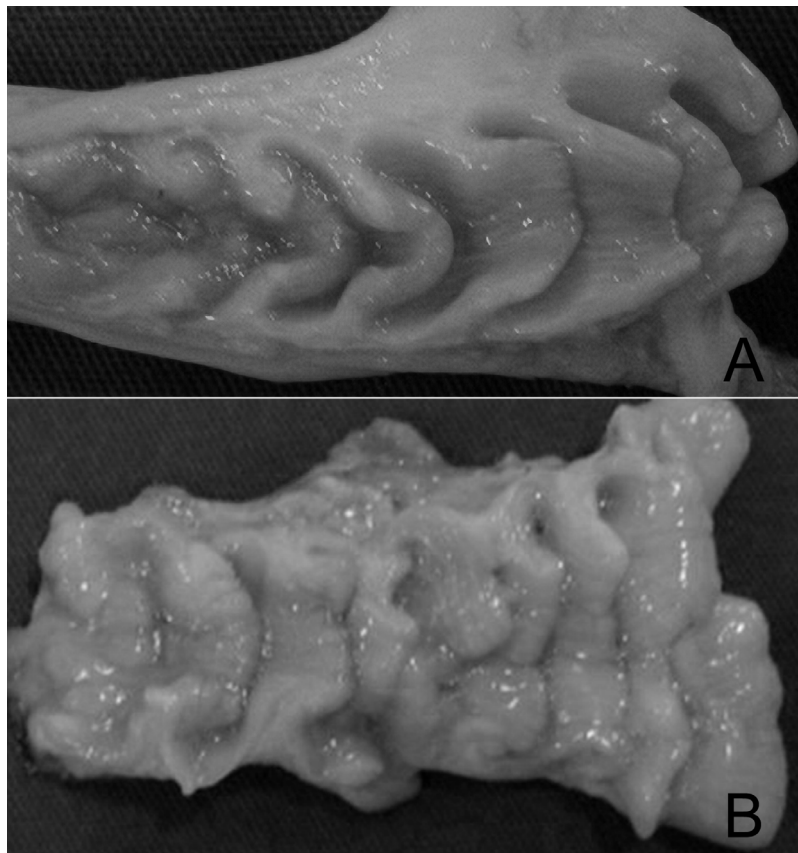


Figure 2. Longitudinally sectioned cervixes of Santa Inês sheep showing centripetal (A) and centrifugal (B) rings.



Table 3. Number of rings, length, diameter, and number of fornices (means ± standard deviation) according to the cervical os shape in Santa Inês ewes.

Shape of the cervical os (%)	Number of rings	Length (mm)	Diameter (mm)	Number of fornices
Spiral	6.27 ± 0.86 ^a	4.50 ± 1.02 ^{bc}	2.59 ± 0.93 ^b	5.66 ± 1.25 ^a
Flat	6.00 ± 1.19 ^a	5.00 ± 1.27 ^a	2.91 ± 1.55 ^{ab}	5.10 ± 1.45 ^b
Duck-bill	5.53 ± 1.32 ^b	4.75 ± 1.06 ^{ab}	3.06 ± 1.15 ^{ab}	5.10 ± 1.35 ^b
Papilla	5.30 ± 0.79 ^b	4.60 ± 0.77 ^{bc}	3.17 ± 1.39 ^a	5.00 ± 1.10 ^b
Rosette	5.64 ± 1.05 ^b	4.43 ± 0.80 ^c	3.05 ± 1.26 ^{ab}	5.07 ± 1.17 ^b
Mimimum	3	3.00	1.00	3.00
Maximum	8	7.00	6.00	8.00

^{a,b,c}Different superscripts in the column indicate significant differences (P < 0.05) by the Tukey test.

Histological findings

Table 4 presents the frequency of epithelium type according to age and cervical portions of Santa Inês ewes. In the cranial (close to the uterus) and central portion of the cervix, 100% of the ewes showed simple

ciliated columnar epithelium. In the caudal portion (close to the vagina) all the females in group E showed simple ciliated columnar epithelium whereas in the other groups the presence of stratified pavementous epithelium was also observed. No significant glandular structures were observed in the animals in the present study.

Table 4. Frequency of epithelium type according to age and cervical portions of Santa Inês ewes.

Age group (months)	Portion	Type of epithelium	
		Simple ciliated columnar (%)	Pavimentous stratified (%)
A (12-18)	Cranial	100	0
	Central	100	0
	Caudal	20	80
B (18-24)	Cranial	100	0
	Central	100	0
	Caudal	60	40
C (24-30)	Cranial	100	0
	Central	100	0
	Caudal	40	60
D (30-38)	Cranial	100	0
	Central	100	0
	Caudal	80	20
E (38-48)	Cranial	100	0
	Central	100	0
	Caudal	100	0

In Table 5 are the results of the frequency of each type of connective tissue present in different portions of the cervix. The results revealed a tissue with large quantities of collagen fibers (dense modeled connective tissue) in group A animals, while there was a reduction in the area of dense modeled connective tissue in animals from group E. In contrast, the irregular dense connective tissue was present in a smaller area in group A than in group E ewes. There was a large area (level 5)

of loose connective tissue in group A ewes contrasting with a small area occupied by this type of connective tissue (level 3) in group E ewes. Blood vessels were not observed in the regular dense connective tissue, being restricted to the irregular dense connective tissue, and in a greater extent in the loose connective tissue, in all groups and cervical portions. Nerves were observed in loose connective tissue, less in the cranial portion when compared to middle and caudal portions.



Table 5. Percentage of different types of connective tissue according to age, cervical portions and levels of occupied area in Santa Inês ewes.

Group	Portion	Type of connective tissue											
		Dense									Loose (%)		
		Regular (%)					Irregular (%)				3	4	5
		1	2	3	4	5	3	4	5	3	4	5	
A	Cranial	0	0	0	0	100	100	0	0	0	0	100	
	Central	0	0	0	0	100	100	0	0	0	0	100	
	Caudal	0	0	0	0	100	100	0	0	0	0	100	
B	Cranial	0	100	0	0	0	100	0	0	0	80	20	
	Central	0	0	60	40	0	60	0	0	0	0	100	
	Caudal	0	20	0	80	0	60	0	0	0	0	100	
C	Cranial	0	100	0	0	0	80	0	20	0	80	0	
	Central	0	0	60	40	0	60	0	0	0	0	100	
	Caudal	0	20	0	80	0	40	0	20	0	0	80	
D	Cranial	80	20	0	0	0	60	40	0	60	40	0	
	Central	80	20	0	0	0	80	0	20	80	0	20	
	Caudal	60	20	0	0	0	40	0	60	40	0	60	
E	Cranial	100	0	0	0	0	0	0	100	100	0	0	
	Central	100	0	0	0	0	0	0	100	100	0	0	
	Caudal	100	0	0	0	0	0	0	100	100	0	0	

Group A - 12-18 months; B - 18-24 months; C - 24-30 months; D - 30-38 months, and E - 38-48 months. The area occupied by connective tissues was classified on a semi-quantitative scale from 1 to 5, where 1 was attributed to the smaller area and 5 to the greater area.

Discussion

The most predominant shape of cervical os in Santa Inês ewes was duck-bill, contrasting with observations in other sheep breeds, where the most predominant shapes were flap and rosette (Halbert *et al.*, 1990), flap (Souza, 1993; Kershaw *et al.*, 2005) or spiral (Naqvi *et al.*, 2005). The average length of the cervix observed in the present study was shorter than that reported for other sheep breeds (Halbert *et al.*, 1990; Souza, 1993; Kershaw *et al.*, 2005; Naqvi *et al.*, 2005). The differences observed in the length of cervix among age groups were also observed between ewe lambs and adult ewes by Navqi *et al.* (2005) and Kaabi *et al.* (2006). In other sheep breeds, less cervical rings were observed with means varying from 3.4 to 4.9 rings (Halbert *et al.*, 1990; Souza, 1993; Kershaw *et al.*, 2005; Naqvi *et al.*, 2005) but with a maximum of 7 rings, while here 8 were found. Cervical ring diameter was lower than that reported by Souza (1993) for Corriedale and Ideal sheep.

The structure similar to the trunks of two cones with their smaller bases turned one to the other and pointing to the central portion differs from that reported for other sheep breeds (Halbert *et al.*, 1990; Souza, 1993; Navqi *et al.*, 2005) in which the largest rings were found in the caudal half of the canal, one or more of them were eccentrically aligned to the rest of the canal,

and the cranial section had smaller and poorly defined folds (Navqi *et al.*, 2005).

By making silicone molds, Naqvi *et al.* (2005) reported that the folds within the cervix were not truly rings but funnel like structures, with the narrow opening pointed caudally. In this study, rings were generally funnel-shaped and centripetal, with some cases of centrifugal rings. Ewes have rings that adapt to each other, safely occluding the cervix (Hafez, 1995), hence obstructing the communication between the uterus and vagina to prevent infection (Evans and Maxwell, 1990). Moreover, the presence of fornix was observed, especially in the first three rings. The fornices may be barriers which impede the progress of the insemination pipettes to the uterus. The knowledge of the presence of this structure is important for the inseminator so his manipulation of the pipette within the cervix during transcervical insemination is guided by the need to pass the rings, especially the first three.

In this study, internal characteristics of the cervical canal did not always reflect differences observed in the cervical os shape, which corroborates with Halbert *et al.* (1990), showing that the success of cervical passage cannot be predicted by the determination of the cervical os shape. This reinforces the assumption that an inseminator cannot use information about the cervical os shape to predict differences in length, number of rings or the narrowest



point of the cervical canal in ewes to be inseminated.

According to Halbert *et al.* (1990), the location in the canal of the most narrow and eccentric ring and the average length and number of rings are important for developing a method of manipulating instruments through the cervical canal. The instrumentation design, as well as the technique for transcervical passage must overcome these difficulties. The narrowest point of the canal will limit the diameter of the instruments that can be used. Due to the larger diameters in the caudal and cranial ends of the cervix, the entrance and exit of the AI device may not be the only points of concern for artificial insemination, but the reduction in diameter (bottleneck) in the center also becomes a complicating factor in cervical transposition.

The cervix is predominantly composed of connective tissue with little muscular tissue (Evans and Maxwell, 1990; Hafez, 1995). In the present study it was observed that young animals presented cervixes with more dense and organized connective tissue, while older animals had more disorganized connective tissue on their cervixes. Because the properties of the connective tissue depend on the type, concentration and interaction of molecules composing the extracellular matrix, the functional characteristics of the cervix are also affected by modifications in these elements (Hafez, 1995). It is well known that the cervix changes size and softness during late pregnancy and labour, mostly due to relaxin discharge. Relaxin promotes softening of the cervix and alters its histological characteristics by reducing the organization and density of cervical collagen fibers (Winn *et al.*, 1994). After delivery, the connective tissue returns to its previous composition, but not completely. So a possible cause for animals in Group E to present a higher degree of disorganization of connective tissue on their cervixes than Group A is that being older those animals probably have gone through more pregnancies and labours.

In conclusion, this is the first report describing morphological and histological features of the cervical canal in Santa Inês ewes. The anatomical features of the cervical canal of Santa Inês ewes differ from those reported for other sheep breeds, showing a predominance of duck-billed cervical os, shorter cervical length, higher number of cervical rings and rings that are larger in the cranial and caudal portions, with a narrowing in the medial portion.

The knowledge of anatomical variability in the cervical canal of different breeds is important to define the length, shape and gauge of the catheter for achieving successful transcervical passage, thereby improving the lambing rate following transcervical AI with frozen semen. For the development of a TCAI pipette, which improves AI success in Santa Inês of all ages, the length of the pipette must be at least 7 cm plus the length of the vagina (maximum value observed at the cervical length) and the narrowest point of the channel diameter of the fourth loop should be considered as the limit for the

diameter of the pipette. Thus, costs will be reduced, with no need for specific types of pipettes for each age group. For the choice of material, anatomical barriers and tissue limitations should be observed.

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