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The anatomy of the sheep cervix and its influence on the transcervical passage of an inseminating pipette into the uterine lumen

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Abstract

The anatomy of the sheep cervix is highly variable between animals and may explain the differing success of transcervical AI between individuals. This study aims to quantify the variation in cervical morphology between ewes and investigate the relationship between cervical anatomy and cervical penetration.

Two series of reproductive tracts were collected. Series A: 132 adult anoestrous ewes, and series B: 165 cycling adult ewes and ewe lambs which were identified as luteal or non-luteal based on the presence of a corpus luteum. The morphology of the cervical external os was classified as slit, papilla, duckbill, flap or rose. An inseminating pipette was inserted into the lumen and the depth of penetration recorded. The cervix was opened longitudinally, its length recorded, the number of cervical rings counted and the arrangement of those rings graded.

The maximum depth of cervical penetration was affected by cervical grade (series A: $P = 0.021$; series B: $P = 0.037$) and the stage of the oestrous cycle ($P = 0.008$). Grade 1 cervices were more penetrable than grade 2, with grade 3 the least penetrable and non-luteal cervices could be penetrated further than luteal cervices. The distribution of os types differed with age, with rose types more common in adult ewes, and papilla os types more common in ewe lambs. These results indicate that the depth of cervical penetration is affected by the anatomy of the cervical lumen. Cervices with a less convoluted lumen (grade 1) were more penetrable. Non-luteal cervices are likely to have higher

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oestradiol concentrations than luteal, stimulating cervical relaxation and enabling deeper penetration. The difference in os types with age may be contributable to a morphological alteration at parturition.
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Keywords: Cervix; Anatomy; Penetration; Artificial insemination; Sheep

1. Introduction

Within the livestock industry, artificial insemination (AI) is used to enhance the production of improved offspring via the introduction of superior genotypes, maximise the use of superior rams and to control contagious disease within flocks [4]. These advantages are greatly facilitated by the ability to perform AI with frozen–thawed (F–T) semen. However, in sheep the conception rates following cervical AI with F–T semen are poor. For AI fertility and lambing rates with F–T semen in sheep to approach those achieved by natural service, intrauterine deposition of F–T semen is required [16]. Currently laparoscopy is the only technique used for commercial intrauterine insemination in sheep. However, there has not been widespread adoption of this procedure by the sheep industry because of welfare concerns, financial constraints and its inaccessibility to unskilled technicians. The alternative technique, transcervical AI (TCAI) is limited due to the anatomy of the ovine cervix, which generally prevents the passage of the AI pipette through to the body of the uterus [4].

The ovine cervix is a long, fibrous tubular organ composed predominantly of connective tissue with an outer serosal layer and inner luminal epithelium. The lumen is highly convoluted and tortuous due to the presence of 4–7 cervical rings that point caudally providing a physical barrier to external contaminants [3,6]. It is these cervical rings that present the major barrier to TCAI as they project into the lumen and the second and third rings are frequently out of alignment with the first, resulting in the inseminating pipette being misdirected away from the central lumen [14]. Furthermore, it is at the level of the first three rings that the lumen is at its narrowest (2–3 mm) [7,14] and consequently the inseminating pipette is rarely inserted more than 1 cm into the cervical canal [4].

Breed, age, parity and physiological state influence the length of the ovine cervix. The mean length of the cervical canal has been described as, 6.5, 5.5 and 6.7 cm [6,7,14] respectively and the length ranges from 5.7 to 10 cm [1] illustrating the high variability between individuals.

The morphology of the external cervical os also differs between animals. The os projects into the vagina and one or more folds of fibrous tissue surround, and in some cases completely obscure, the os. The arrangement of these folds is extremely variable, particularly in older ewes, which may be a consequence of parity [3]. Two classification systems have been developed to describe cervices in relation to the appearance of their external os [3,7]. These systems, although different, have recognised similar features and they have been used previously to investigate relationships between os type and cervical penetration in ewes [6]. This great variation in cervical anatomy between animals may explain the differing success of TCAI between individual ewes. Therefore, it is necessary to study the anatomical structure of the ovine cervix to gain an understanding of how a successful transcervical AI procedure in ewes may be developed. The aim of this study was to determine if any relationships exist between the morphology of the external os, the structure of the internal cervical canal, endocrine state, age, cervical length and depth of penetration of the cervix by an inseminating pipette.

2. Materials and methods

2.1. Animals

Two series of sheep cervices were examined. The first was a series of 132 cervices from adult ewes obtained from an abattoir in Devon in May during the non-breeding season (series A). These cervices were collected on two occasions approximately 12 months apart: series A1 ($n = 78$) and A2 ($n = 54$), and were predominantly aged cull Mules (crossbreed unknown). The second was a series of 165 cervices obtained from ewes in January during the breeding season, from an abattoir in Bedfordshire (series B). These were predominantly hill breeds and were a mixture of ewe lambs and adult ewes. The tracts from series B were further subdivided into adult ewes and ewe lambs based on the morphology of the uterus and into luteal and non-luteal (presumably follicular) based on the morphology of the ovary. Ovaries with a corpus luteum were described as luteal and those without a corpus luteum were termed non-luteal.

2.2. Measurements

Following collection the tracts were transported on ice to the laboratory where they were examined within 2–6 h of slaughter. The tracts were prepared by excising the majority of ligamentous tissue and then separated cranially at the body of the uterus and caudally at the vestibule. The tissue of the anterior vagina was incised longitudinally to expose the cervical external os.

Following some preliminary observations a classification system for the external os, based on that developed by [7] was developed. Five types of external os were identified (Fig. 1):

1. The Duckbill: two opposing folds of cervical tissue protruding into the vagina with a central horizontal slit like external os.
2. The Slit: no protrusions into the anterior vagina with a slit like opening at the os of the cervix giving entry to the cervical canal.
3. The Rose: a cluster of cervical folds protruding into the anterior vagina obscuring the external os.
4. The Papilla: a papilla protruding into the anterior vagina with the external os at its apex
5. The Flap: one-fold of cervical tissue protruding into the anterior vagina and completely or partially overlaying the external os creating the appearance of a flap.

An ovine inseminating pipette (IMV technologies, 10, rue Clemenceau-BP81 61302 L'AIGLE, Cedex) was passed into the cervix to its maximum depth of penetration without

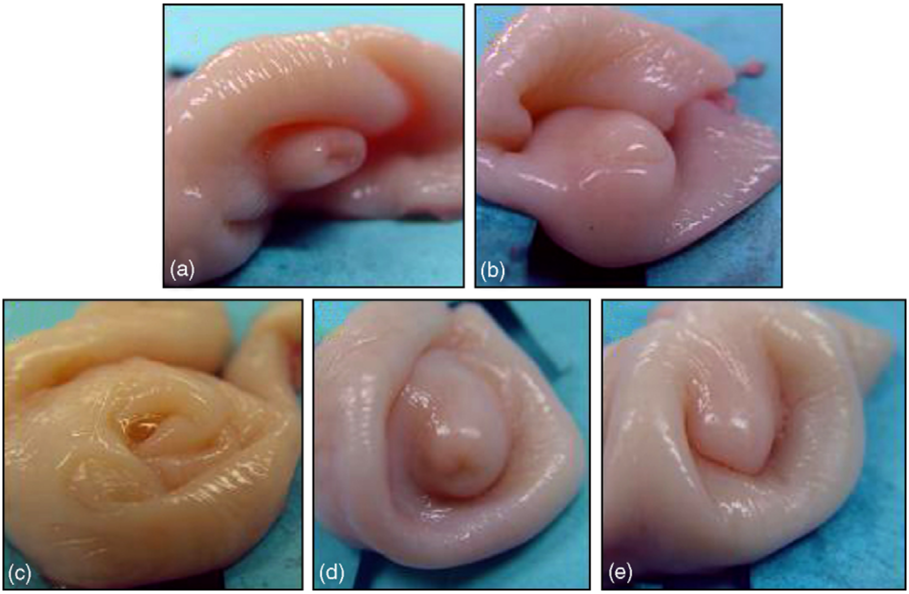

Fig. 1. The classification of the appearance of the external os of the ewe (a) duckbill, (b) slit, (c) rose, (d) papilla, and (e) flap.

the use of excessive force or manipulation of the cervix, and the distance from the tip of the pipette to the entrance of external os was recorded to the nearest mm.

The body of the uterus was incised to enable the insertion of a pair of scissors into the internal os and the cervix was opened longitudinally from the cranial end. The length of the cervix (to the nearest mm) from the external os to the internal os was then measured. The cervical rings were counted and the degree of completeness and interdigitation of these rings was recorded as one of three grades (Fig. 2):

1. Grade 1 cervices had a series of complete aligned cervical rings lying across the opened lumen with no interdigitation of the cervical rings.
2. Grade 2 cervices had a mixture of complete rings (as in grade 1) and incomplete cervical rings that lay partially across the opened lumen and interdigitated with one another, obscuring the central lumen.
3. Grade 3 cervices had predominantly incomplete and interdigitating cervical rings that were not aligned.

2.3. Statistical analysis

Descriptive statistics on cervix length were derived from pooled data. For analytical statistics, the data from the two series of observations were individually analysed if there were any significant differences between the series. This was the case for (i) the length of cervix, (ii) the depth of penetration and (iii) the percentage depth of penetration. The data

Fig. 2. The classification of cervical grade in the ewe (a) grade 1, (b) grade 2, and (c) grade 3. Arrows illustrate the direction and maximum depth of penetration.

were analysed by univariate analysis of variance with post hoc tests where appropriate using the LSD test. Distributions were analysed by the Chi-square test. A regression analysis was used to examine the relationship between cervical length and the number of cervical rings.

3. Results

3.1. Between and within series differences

There were significant differences in cervical length ($P < 0.001$), the depth of penetration ($P < 0.001$) and percentage depth of cervical penetration ($P < 0.001$) between series A and B. Series A1 and A2 differed significantly from each other with respect to cervical length ($P < 0.001$), depth of penetration ($P = 0.002$) and percentage depth of penetration ($P = 0.04$).

3.2. Length of the cervix

The mean (±S.E.M.) length of the 297 cervices from both series of observations was 53.53 ± 0.991 mm and the range was 25–105 mm (Fig. 3). As the data were significantly skewed it was log-transformed to give a normal distribution, from which the reference

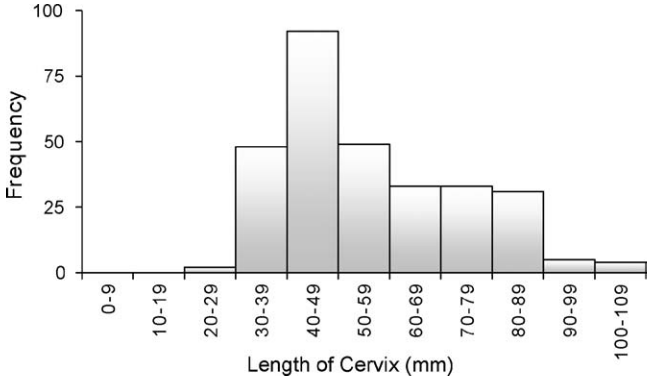

Fig. 3. The frequency distribution for the length of the cervix from a mixed population of 297 sheep.

range was derived. The normal reference range for cervical length of ewes was 27.6–94.4 mm.

For both series A ($P < 0.001$) and series B ($P = 0.002$) the longer the cervix the greater the average number of cervical rings (Fig. 4). In both series, the length of the cervix was not related to either the grade of the cervix or to the type of external os. Similarly, the length of the cervix was not related to either the stage of the oestrous cycle or the age of the animals.

3.3. Depth of penetration

The mean (±S.E.M.) depth of penetration for series A was 35.73 ± 1.76 and 12.77 ± 0.67 mm for series B. The mean (±S.E.M.) penetration as a percentage of cervical length was 52.0 ± 2.26% for series A and 30.2 ± 1.35% for series B. Penetration into the body of the uterus (expressed as the maximum depth of penetration as a proportion of cervical length) was achieved in 19/132 (14%) cervices in series A but in only 1/165 (<1%) of the cervices from series B.

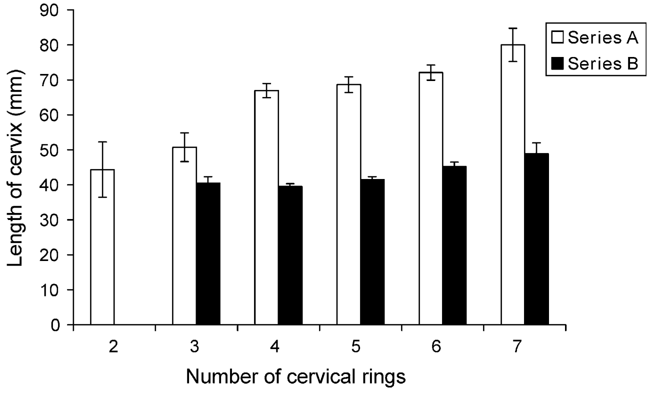

Fig. 4. The relationship between the mean (±S.E.M.) length of the cervix (mm) and the number of cervical rings from series A and B.

Table 1
The relationship between cervical grade and the mean (±S.E.M.) depth of penetration of the ovine cervix

Cervical grade	Maximum depth of penetration from the external os (mm)		Maximum depth of penetration from the external os (%)	
	Series A	Series B	Series A	Series B
1	38.7 ± 3.18 a	15.7 ± 2.06 a	53.3 ± 4.13 a	34.3 ± 3.26
2	38.4 ± 2.84 a	12.6 ± 0.68 b	55.1 ± 3.52 a	30.4 ± 1.67
3	27.4 ± 2.57 b	10.2 ± 1.01 b	45.4 ± 3.94 b	25.7 ± 2.72

Within columns values with different letters (a, b) differ significantly.

For both series A and B the maximum depth of penetration was not related to the number of cervical rings or the type of external os. However, the maximum depth of penetration was related to cervical grade (series A: $P = 0.021$ and series B: $P = 0.037$). In series A, grade 3 cervices were less penetrable than either grade 1 ($P = 0.012$) or grade 2 ($P = 0.014$) cervices with no difference between grades 1 and 2. Similarly in series B, grade 3 ($P = 0.003$) and grade 2 ($P = 0.042$) cervices were less penetrable than grade 1 cervices. When the depth of maximum cervical penetration was expressed as a percentage of cervical length, the effect of grade was significant for series A ($P = 0.029$) but not for series B ($P = 0.087$) although the same trends were observed (Table 1).

The depth of maximum cervical penetration was significantly related to the stage of the oestrous cycle both as an absolute measurement ($P = 0.008$) and as a percentage of maximum cervical penetration ($P = 0.010$). The maximum depth of penetration of the cervix was reduced in luteal ewes compared to non-luteal ewes (Table 2). Neither measure of cervical penetration was affected by the age of the ewes.

3.4. Cervical rings and grade

The mean number of cervical rings (±S.E.M.) was 4.89 ± 0.058 with a range of 2–7 rings per cervix. The number of cervical rings was not related to either os type or stage of the oestrous cycle. The number of cervical rings was significantly related to the grade of the cervix (Table 3) and ewes with grade 1 cervices had significantly more ($P < 0.001$) cervical rings than those ewes with grade 2 or 3 cervices. There were 88/297 (29.6%) grade 1, 137/297 (46.1%) grade 2 and 72/297 (24.2%) grade 3 cervices. The number of cervices in each grade was not related to the type of external os.

Table 2
The effect of age and stage of cycle on the mean (±S.E.M.) depth of penetration of the ovine cervix

Parity	Stage of cycle (*n*)	Maximum depth of penetration from the external os (mm)	Maximum penetration from the external os (%)
Ewe lambs	Luteal (14)	10.50 ± 1.582 a	25.7 ± 3.74 a
Ewe lambs	Non-luteal (23)	15.74 ± 1.406 b	36.2 ± 3.20 b
Adult ewes	Luteal (106)	11.78 ± 0.56 a	28.4 ± 1.62 a
Adult ewes	Non-luteal (22)	15.86 ± 3.353 b	36.06 ± 4.69 b

Within columns values with different letters (a, b) differ significantly. Numbers within parentheses represent the total number of animals in that group.

Table 3
The relationship between cervical grade and the mean (±S.E.M.) number of cervical rings from pooled data

Cervical grade	Number of cervical rings
1 (88)	5.27 ± 0.90 a
2 (137)	4.8 ± 0.88 b
3 (72)	4.6 ± 0.12 b

Within columns values with different letters (a, b) differ significantly. The number in parentheses is the number of cervices in each category

3.5. *Type of external os*

The most frequent external os type was flap 107/297 (36.0%) followed by duckbill 77/297 (25.9%) and rose 56/297 (18.9%). The remaining two os types, papilla (37/297; 12.5%) and slit (20/297; 6.7%) were the least common.

The distribution of external os type was affected by age ($\chi^2 = 11.34$; $P = 0.023$) but not by the stage of the oestrous cycle. In adult ewes the rose os (21.9%) was observed more frequently than in ewe lambs (5.4%), while the reverse was true for the papilla os type (9.4% in adult ewes versus 21.6% in ewe lambs). The remaining three os types had similar frequencies in both age categories.

4. Discussion

The variations encountered between series and within series is likely to be an effect of breed, physiological state and anatomical state of the specimens collected from the differing abattoirs. The proportions of specimens within one anatomical factor (i.e. cervical grade) were not evenly distributed between or within series, because of variation in the population. Consequently no two series were directly comparable, and therefore the differences observed are not surprising. The present study did not consider the effect of breed on cervical length or depth of penetration although it was noted at the time of collection that larger breeds tended to have larger tracts and longer cervices. It has been illustrated that pregnancy [10], and fertilisation rates [5] from cervical AI of F–T semen differ significantly between breeds. Furthermore, the differences in fertilisation rate encountered using cervical AI are not present when laparoscopic AI is used [5]. As pregnancy [9] and lambing rates [16] improve as the depth of semen deposition in the cervix increases, it is possible that the difference in fertilisation rates between breeds with cervical insemination [5], is caused by the ability to penetrate the cervix further in one breed of ewe compared to another.

The main findings of the present study were that the depth of cervical penetration in ewes is influenced by the grade of the cervical lumen and the stage of the oestrous cycle.

Previous studies of cervical morphology have not comprehensively investigated the alignment and interdigitation of the cervical rings along the lumen and this is the first report regarding the effect of the arrangement of these rings on cervical penetration. Cervices were graded according to the complexity of the convolutions of their canals with grade 1 being the least convoluted and grade 3 the most. The average depth of penetration of grade

1 cervices was greater than grade 3 and the depth of penetration of grade 2 cervices was intermediate.

It has been reported that the second or third cervical ring is out of alignment with the first [7,14], and furthermore the mean distance of the second ring from the external os is 26.0 ± 0.01 mm in ewe lambs and 41.0 ± 0.01 mm in adult ewes [15]. The mean depth of penetration in the present study was 12.8 ± 0.67 and 35.7 ± 1.76 mm in ewe lambs and adult ewes, respectively, which is approximately the same as the reported distance of the second cervical ring from the external os. This suggests that the alignment of the second or third cervical ring, in relation to the first ring is the principal determinant of depth of penetration and the major impediment to TCAI. Inseminating pipettes designed to overcome the convoluted lumen common to sheep are required to enable intrauterine AI. Pipettes that enable transcervical AI have been developed [8,20] although these are not able to consistently penetrate the cervix and have detrimental effects on lambing and fertility rates [21] which make them unsuitable for commercial sheep breeders. Furthermore, it has been illustrated that the success of cervical penetration using these pipettes also relies on other factors including the position of the ewe and possibly technical ability [8,21]. The grade of the cervical lumen was also related to the number of cervical rings. This has not been reported previously and it is possible that this may be an artefact produced during the counting process. As the rings became more interdigitated and incomplete they became progressively more difficult to count and therefore it may be that the number of rings in the higher grade cervices were underestimated.

The stage of cycle affected the penetrability of the cervix and the mean depth of penetration was 4.6 mm greater in non-luteal compared to luteal tracts. Increasing the depth of semen deposition into the cervix by just 10 mm increases lambing rates by approximately 10% [16] illustrating the significance of the greater depth of penetration seen in the non-luteal cervices. As these tracts were collected during the breeding season it is reasonable to believe that most of the non-luteal cervices would have been from animals in the follicular phase of the oestrous cycle. This is particularly true in the adult ewes as the proportion of luteal to non-luteal tracts is representative of cycling sheep (17% non-luteal versus 83% luteal). The proportion of luteal to non-luteal tracts in ewe lambs was not as expected with 62% of ewe lambs non-luteal. It is possible that some of the ewe lambs were not cycling and were instead pre-pubertal. These pre-pubertal ewe lambs were neither follicular nor luteal and were therefore termed non-luteal, as were other animals that did not have a corpus luteum. The greater depth of cervical penetration in non-luteal ewes suggests that there is a degree of natural relaxation of the cervix at oestrus, possibly as a consequence of the action of ovarian follicular steroids, which enables deeper penetration of the cervix. The stage of the cycle did not affect the grade of the cervix, implying that rather than altering the morphology of the cervix the peri-ovulatory changes in oestrogen and progesterone are reducing the resistance of the cervix to the AI pipette. We propose that the high oestradiol concentrations associated with oestrus and ovulation stimulate cervical oxytocin receptor (OTR) expression because in the sheep cervix OTRs are maximally expressed during oestrus [2], and because oestradiol increases cervical OTR expression in the rat [19]. Exogenous oxytocin induces cervical relaxation in sheep enabling deeper penetration of the cervix [12]. Furthermore, oxytocin stimulates the release of prostaglandin E_2 (PGE_2) from bovine cervical segments at oestrous via a local increase in cyclo-oxygenase-2 (COX-2) [17]. Indeed COX-2 mRNA expression is upregulated in the oestrous cervix of the ewe compared to luteal cervices [11] which we suggest will increase PGE_2 concentrations in the cervix and PGE_2 plays a key role in cervical relaxation and remodelling [13,18]. In conclusion, we suggest that the deeper penetration in non-luteal tracts is the result of natural cervical relaxation at oestrus caused by increased concentrations of oestradiol, oxytocin receptor, COX-2 and PGE_2. Current investigations are providing strong evidence in support of this hypothesis [11], Kershaw et al., unpublished data).

The present study also found that the distribution of external os types differed with age. In particular the rose type os was more common in adult ewes than ewes lambs and the reverse was true for the papilla type os. As the categorisation of os type is based on the arrangement of fibrous flaps that surround the opening to the cervix, it is possible that partial or complete prolapse of the first cervical ring may influence the classification of the os. The incidence of cervical prolapse is greater in parous ewes [3] and therefore the more complex os types such as rose, may be more common in older multiparous ewes. Similarly, the papilla type os, which was most common in ewe lambs, is likely to be a tightly constricted os that is most commonly seen in nulliparous ewes. It has been suggested that the classification of the cervical os may change at parturition [3], increasing in size and complexity. Our findings support this statement, and we propose that this alteration in morphology may be a consequence of prolapse of the first cervical ring in multiparous ewes. Alternatively, the more constricted os types such as papilla may become stretched and torn during parturition and subsequently alter their morphology during the healing process.

The external os type did not change with stage of the cycle, implying that the ovarian steroids, which may be responsible for relaxation of the cervix during the follicular phase, do not affect the appearance of the external os. It would be reasonable to suggest that the external os is determined genetically and influenced by mechanical factors such as parturition.

The mean cervical length in this study is similar to that reported previously [14,15]. Based upon the length of the cervix, the cervix length reference range was determined as 27.6–94.4 mm. This indicates that if an inseminating pipette is inserted to a depth of 94.4 mm, transcervical intrauterine insemination will have been achieved in 95% of ewes. This is more than adequate to ensure high fertility for TCAI using frozen thawed semen, as using this technique, lambing rates average at 73% [16].

In conclusion, the success of TCAI in ewes is highly dependent on the anatomy of the cervical lumen and the stage of the oestrous cycle. Further investigations are required to fully understand the mechanism of natural cervical relaxation that may occur during the follicular phase. Additional developments for a less traditional AI pipette that can overcome the tortuous cervical lumen may aid successful penetration of the cervix, thereby improving fertility rates following TCAI with FT semen.

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