Short communication

Influence of age and cervical grade on anatomy, morphology and depth of cervical penetration in Sanjabi ewes

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Abstract The structure of the sheep cervix limits the application of reproductive biotechnologies such as embryo transfer and artificial insemination. The aim of this study was to determine differences in cervical anatomy and morphology between Sanjabi lambs and ewes, and the relationship between cervical anatomy and cervical penetration in ewes. Four hundred and sixty eight postmortum cervices belonging to six different age groups were used. The cervical external os was classified as slit, papilla, duckbill, flap or rose. Cervical folds were counted and the degree of completeness and interdigitations of the folds recorded as one of three grades 1, 2, and 3 cervices. There were significant differences ($P < 0.05$) in cervical length and number of folds amongst ewes of various ages. The cervix of the ewes at ages < 6 months and 6 -12 months, and grade 3 cervices were shorter and narrower, with more cervical folds. Cervical penetration differed according to the cervical grade, being deeper in grade 1 (simpler cervix) than in grade 3 cervices (more complex cervices). Cervical penetration was correlated positively with the length and width of the cervix, ($r = 0.93$ and $r = 0.88$; $P < 0.01$ respectively) and negatively with the number of cervical folds ($r = - 0.61$; $P < 0.01$). At ages < 6 and 6 - 12 months the distance between cervical folds was ($P < 0.05$) shorter than at another ages. At ages < 6 and 6 - 12 months the flap os, at ages 1 - 2 and 2 - 3 years the papilla os, at ages 3 - 4 years the duckbill os and at ages > 4 years the rose os were observed more frequently. These results showed that with advancing ewe age, the cervix tended to be larger and less complex, with a smaller number of cervical folds and this noticeably improved cervical penetration. Thus a practical point of view would favor the application of reproductive biotechnologies at ages > 4 years, even in 3-4 age years, because the cervix is more penetrable to catheter after 3 - 4 years of age.

Keywords: cervix, Sanjabi ewes, cervical penetration, cervical folds, age

Received: 26 Jan. 2015, *accepted:* 30 Aug. 2015, *published online:* 6 Dec. 2015

Introduction

During puberty, the morphometry of the ewe cervix shows marked breed and age-dependent variations. The folds change from a ring shape in young females to a flap shape in older ones (Kaabi et al., 2006). Also, the number of cervical folds decreases with advancing age, influencing cervical penetration of the catheter (Eppleston et al., 1994). The structure of the sheep cervix limits the use of non-surgical embryo collection and transfer making reproductive biotechnologies less readily available to the sheep industry (Halbert et al., 1990; Ayad et al., 2004). Anatomically, the ovine cervix is a long, fibrous, tubular convoluted organ with a number of features that effectively impedes transcervical artificial insemination (More, 1984; Croy et al., 1999). 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 (Fukui and Roberts, 1978). Naqvi et al. (1998) found that the low rate of cervical penetration in sheep as experienced for depth of penetration by Ritar and Salamon (1983) may be attributed to the differences in the structure of cervix. The average length of the cervical canal was 6.7 cm and contained about six ventral facing, funnel shaped annular folds that form crypts thought to hold ejaculated spermatozoa (More, 1984). In Merino breed, Eppleston et al. (1994) found an average cervix length of 4.87 cm and an average number of 3.97 cervical folds. These folds narrow the lumen and prevent the catheters from

reaching the uterus. The second-fold or vice versa generated the greatest obstacle to instrument insertion through the cervix (Naqvi et al. 2005). There are about 800,000 Sanjabi ewes in Kermanshah province and sheep production plays an important role in the regional economy. Sanjabi ewes, found predominantly in the west of Iran, are a potential source of meat as well producing milk of superior quality (Devendra and Mcleroy, 1982). The objective of the present study was to investigate the anatomy and morphology of the cervix of Sanjabi ewes at six different ages for better application of reproductive biotechnologies in this breed.

Materials and methods

Location of study and animals

This study was conducted from April to June 2008 at Razi University sheep farm and embryo transfer laboratory, Kermanshah province; located at longitude of 47° 3´E, latitude of 34° 18´ N and an altitude of 1410 m above mean sea level. During the period of approximately 3 months reproductive tracts were collected from 507 lambs and adult ewes slaughtered at Bisetoon and Mahidasht abattoirs. Overall, 7.7% of the tracts showed evidence of pathological changes. A total of 468 clinically healthy reproductive tracts were used (n = 78 for each age group) on several occasions. Prior to slaughter, the animals were marked on the basis of proficiency, dental configuration and milk teeth.

Collection of reproductive tracts

Reproductive tracts were excised with the aid of a scalpel and scissor within 5–10 min after slaughter, rinsed with sterile saline solution, and packed in ice cubes in a thermos flask containing 0.9% saline solution supplemented with 2 MIU of penicillin G and 2 g of Dihydrostreptomycin. Samples were transported to the laboratory within 30 min after slaughter. In the laboratory, the reproductive tracts were cleaned and dissected to remove the cervix from the rectum and the surrounding fat tissue. The samples were examined within 3 h of collection for pregnancy and any visible abnormalities. The tissue overlying the annular folds was carefully removed using a scalpel and forceps.

Measurements of reproductive tract

After recording the external measurements, the cervix was sectioned longitudinally and the internal measurements were taken. The anterior vagina was incised longitudinally to expose the cervical external os. The external os was classified into one of five types as described by Kershaw et al. (2005). Cervical penetration test was performed by using of a catheter. An ovine straight inseminating pipette (IMV technologies, 10, rue Clemenceau-BP81 61302 L'AIGLE, Cedex) was passed into the cervix to its maximum depth of penetration without 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. 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 vagina. The studied parameters were: cervical length, cervical width, number of cervical folds, cervical grade (classified into one of three types as described by Kershaw et al., 2005), type of fold (there were two different types, one ring shaped and another flap-shaped), cervical penetration, distance between the cervical folds and the external os (classified as slit, papilla, duckbill, flap and rose) in each case.

Statistical analysis

Data were analyzed using SAS™ software. Means were separated by using Duncan's multiple range test. The studied parameters of the cervix of sheep were analyzed using a general linear model (PROC GLM), where age (six classes) and cervical grade (Grade 1, 2 and 3 cervices) were the factors of variation. A Pearson correlation was used to examine the relationship between cervical penetration rates with the length and width of the cervix and the number of cervical folds.

Results

The mean (± S.D.) length, width and depth of penetration in 468 cervices were 5.54 ± 1.36 cm, 1.33 ± .18 cm and 2.81 ± 1.23 cm, respectively. There were significant differences ($P < 0.05$) in cervical length and width between ewes of various age groups (Table 1).

The mean number of cervical folds (± S.D.) was 4.4 ± 1.06 with a range of 3 – 7 folds per cervix. There were differences ($P < 0.05$) in the number of cervical folds between ages; < 6 months, 6 - 12 months and 1- 2 years with respect to ages; 2 - 3, 3 - 4 and > 4 years (Table 1). The ewes with grade 3 cervix had ($P < 0.05$) more cervical folds than those with grade 2 or 1 cervices (Table 2). The cervix at ages; < 6 months, and 6 - 12 months, and grade 3 cervices were shorter and narrower, with a large number of cervical folds (Tables 1 and 2). Considering the morphology of the cervical folds, the ring form was more frequent at ages < 6 months, 6 - 12 months and 1 - 2 years and the flap form at ages 2 - 3, 3 - 4 and > 4 years (Fig. 1).

Depth of penetration

Cervical penetration correlated positively with the len-

Table 1. Effect of age on gross anatomy of the cervix in Sanjabi ewes

	< 6 month	6 - 12 month	1- 2 year	2 - 3 year	3 - 4 year	> 4 year
Length (cm)	$3.93^{a} \pm 0.25$	$4.17^{a} \pm 0.44$	$4.36^{a} \pm 0.18$	$6.36^{b} \pm 0.34$	$6.62^{b} \pm 0.23$	$7.58^{c} \pm 0.17$
Number of folds (n)	$5.62^{a} \pm 0.58$	$5.33^{a} \pm 0.32$	$4.92^{a} \pm 0.37$	$4.42^{b} \pm 0.47$	$4.34^{b} \pm 0.22$	$3.36^{c} \pm 0.43$
Width (cm)	$1.10^{a} \pm 0.07$	$1.14^{a} \pm 0.04$	$1.27^{b} \pm 0.08$	$1.31^{b} \pm 0.06$	$1.33^{b} \pm 0.04$	$1.57^{c} \pm 0.10$
Penetration (cm)	$1.31^{a} \pm 0.33$	$1.57^{a} \pm 0.37$	$2.42^{b} \pm 0.58$	$2.79^{b} \pm 0.26$	$4.22^{c} \pm 0.45$	$4.58^{c} \pm 0.69$
Distance of folds (cm)						
EOU*-1st	$0.74^{a} \pm 0.04$	$0.77^{a} \pm 0.02$	$0.93^{b} \pm 0.04$	$0.95^{b} \pm 0.07$	$1.08^{c} \pm 0.06$	$1.11^{c} \pm 0.08$
1st - 2nd	$0.54^{a} \pm 0.07$	$0.60^{a} \pm 0.03$	$0.72^{b} \pm 0.06$	$0.76^{b} \pm 0.05$	$0.88^{c} \pm 0.08$	$0.93^{c} \pm 0.06$
2nd –3rd	$0.46^{a} \pm 0.09$	$0.51^{a} \pm 0.05$	$0.63^{b} \pm 0.06$	$0.68^{b} \pm 0.04$	$0.82^{c} \pm 0.04$	$0.85^{c} \pm 0.05$

Values with different superscripts (a, b, c, and d) within rows are significantly different ($P < 0.05$) (mean ± S.D.).
*External os uteri

gth and width of the cervix, ($r = 0.93$; $P < 0.01$ and $r = 0.88$, respectively) and negatively with the number of cervical folds ($r = -0.61$; $P < 0.01$). Penetration into the body of the uterus (expressed as the maximum depth of penetration) was achieved in 0/156 (0 %) at ages < 6 month and 6 - 12 months, 11/156 (7.05 %) at ages 1 - 2 and 2 - 3 year, 19/78 (24.35 %) at ages 3 - 4 year and 41/78 (52.56 %) at ages > 4 year. The maximum depth of penetration was related to age and cervical grade ($P < 0.05$). With the use of catheter, this increase started to be significant ($P < 0.05$) after 3 - 4 years of age. The average depth of penetration of grade 1 cervices was ($P < 0.05$) greater than grade 3 with the depth of penetration of grade 2 cervices being intermediate (Table 2).

Distances between cervical folds and the type of external os

The spacing between the folds was variable. At the ages < 6 months and 6 - 12 months the distance between the external os uteri (EOU) and the first cervical fold, between the first and the second cervical fold and between the second and the third cervical fold was ($P < 0.05$) shorter than another ages (Table 1). The distance between cervical folds in grade 1 cervices was ($P < 0.05$) greater than other grades (Table 2). The cervices with flap os were the most common, accounting for 146/468 (31.2%) of the total os examined, followed by papilla 121/468 (25.9%) and duckbill 112/468 (23.9%). The rose (55/468; 11.7%) and slit (34/468; 7.3%) type os were the least common. At ages < 6 and 6 - 12 months, the flap os (44.9% and 41%, respectively) was observed more frequently; with the papilla and duckbill os being more frequent in older ewes.

Discussion

The present study is the first report on anatomical structure of cervix of native Iranian sheep. The results of the present study indicated that, values of 7.58 cm for the cervix length in adult ewes and 3.93 cm in ewe lambs had the lowest average number of cervical folds: 3.36 and 5.62, respectively. While in Malpura and Kheri breeds, Naqvi et al. (2005) found values of 5.36 cm for the cervix length in adult ewes and 3.87 cm in ewe lambs which had the lowest average number of cervical folds: 3.48 and 3.24, respectively. The mean length of the cervical canal has been described as, 6.3, 5.35 and 6.54 cm and an average number of cervical folds of 4, 4.89 and 5.8 (Fukui and Roberts, 1978; Halbert et al., 1990; Kershaw et al., 2005), respectively. This suggests that the variation in the results for the length of the cervical canal and number of cervical folds in different breeds could be due to differences in the age and breed size used between studies. The results of the present study indicated that change in cervical folds shape, depends on the age of the ewe. The folds change from a ring shape in ewe lambs to a flap shape in older ewes.

Table 2. Effect of cervical grade on the gross anatomy of the cervix in Sanjabi ewes

	Cervical grade (n)		
	1 (148)	2 (213)	3 (107)
Length (cm)	$6.70^{a} \pm 0.96$	$5.30^{b} \pm 1.2$	$4.90^{c} \pm 1.20$
Number of folds	$3.80^{c} \pm 0.86$	$4.50^{b} \pm 1.06$	$4.80^{a} \pm 0.98$
Width (cm)	$1.50^{a} \pm 0.15$	$1.30^{b} \pm 0.16$	$1.20^{c} \pm 0.13$
Penetration (cm)	$3.90^{a} \pm 0.97$	$2.60^{b} \pm 1.08$	$2.20^{c} \pm 0.95$
Distance of folds (cm)			
EOU*-1st	$1.08^{a} \pm 0.12$	$0.92^{b} \pm 0.13$	$0.88^{c} \pm 0.13$
1st - 2nd	$0.88^{a} \pm 0.11$	$0.72^{b} \pm 0.14$	$0.67^{c} \pm 0.15$
2nd –3rd	$0.79^{a} \pm 0.13$	$0.62^{b} \pm 0.16$	$0.60^{c} \pm 0.15$

Different letters (a, b and c) in the same rows indicate significant differences ($P < 0.05$) (mean ± S.D.).
*External os uteri

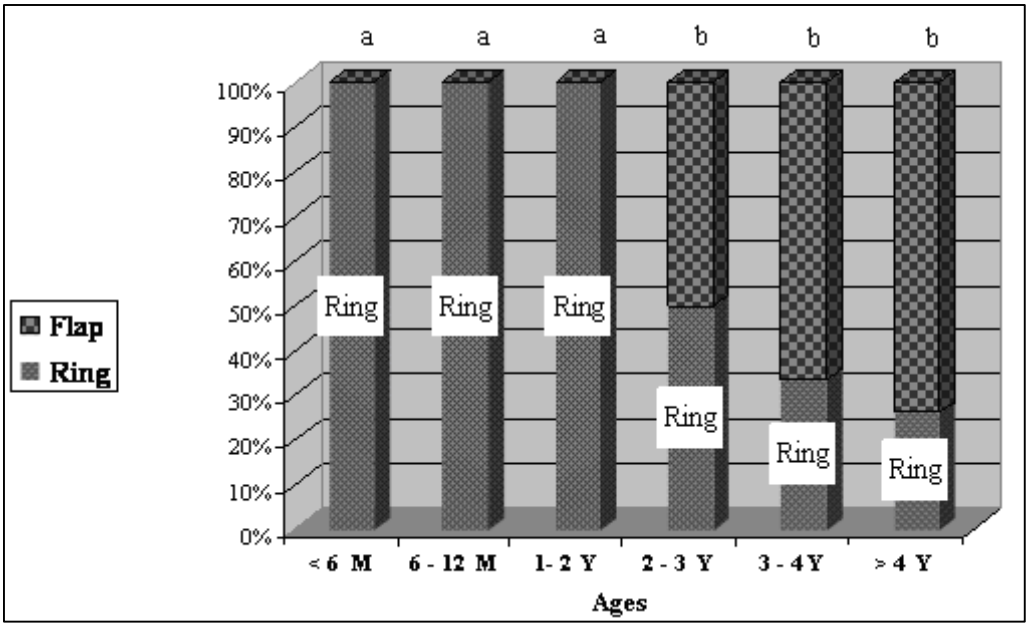

Figure 1. Effect of age on morphology of the cervical folds in Sanjabi ewes. Different letters (a and b) indicate significant differences between ages ($P < 0.05$).

Previous studies speculated that morphological changes based on age could be explained by the effect of parturition, which cause the cervix to expand and, probably, the rupture of most of the cervical folds (Dun, 1955; Kaabi et al., 2006). These results revealed that increase in the depth of cervical penetration in older ewes, is corrected with shape of cervix. Kaabi et al. (2006) indicated that besides the morphometric changes in the cervix, the change in the shape of the cervical folds could be the most determining factor in the increasing the cervical penetration based on age.

Eppleston et al. (1994) demonstrated that one of the important factors affecting the fertility of frozen-thawed ram semen inseminated into the cervix of sheep was the depth of insemination. Breed, age, parity and physiological state influence the depth of cervical penetration (Windsor, 1995; Kershaw et al., 2005; Kaabi et al., 2006). The study of Kershaw et al. (2005) revealed that the maximum depth of penetration of the cervix was reduced in luteal ewes compared to non-luteal ewes. However, there was no effect of the stage of the cycle on the grade of the cervix. The depth of cervical penetration in this study was influenced by the age and the grade of the cervical lumen. Important finding in current study is the greater depth of cervical penetration at 3-4 y of age years and older. Thus, a practical point of view would favor the application of AI and ET, at ages > 4 year, or may be at 3-4 years. The depth of cervical penetration correlated positively with the length and width of the cervix, and negatively with the number of cervical folds. The number of cervical folds decreased with age, influencing cervical penetration. Previous studies also reported that cervical penetration in different breeds was negatively correlated with the number of folds (Eppleston et al., 1994; Kaabi et al., 2006). Nevertheless, in the ewe breeds analyzed by Kershaw et al. (2005) the maximum depth of penetration was not related to the number of cervical folds although the presence of complete or incomplete folds as well as interdigitations were amongst the influencing factors. This difference in cervical penetration can be explained by the fact that the sheep cervix is highly complex (Kershaw et al., 2005; Kaabi et al., 2006). The values for depth of penetration obtained by Kaabi et al. (2006) with a straight catheter, ranging from 2.34 to 4.45 cm, are similar to the values in the present study. However, Kershaw et al. (2005) found that with a straight catheter, depth of penetration ranged from 1.27 to 3.57 cm. A cervix with a simple arrangement of the internal folds allowed the passage of pipette easier than one with a complex arrangement of internal folds.

The analysis of the distances between cervical folds showed that at the ages < 6 months and 6–12 months the distance between the cervical folds was significantly shorter than at other ages. Naqvi et al. (2005) reported that the distance between the os and the first cervical fold was greater in adult ewes than in ewe lambs, although smaller than values reported in the present study. This may be attributed to the comparatively smaller size of the native semi-arid region breeds than large size breed used for present study. We also found an increase in the distance between the cervical folds, especially at

3-4 years of age, which would facilitate the use of the catheter. Shorter distances narrow the lumen and make it difficult to locate the orifices of the cervical folds, especially when using the straight catheter (Kaabi et al., 2006). Halbert et al. (1990) indicated that cervical penetration improves when the distance between the first two cervical folds is greater. Wulster-Radcliffe et al. (1999) showed that the shape of the external cervical os and the size of the cervical canal are the major physical barriers to transcervical embryo transfer in sheep. These results showed that the distribution of os types differed with age, with rose types more common in adult ewes, and flap os types more common in ewe lambs. The difference in os types with age may be contributable to a morphological alteration at parturition, increasing in size and complexity (Dun, 1955; Kershaw et al., 2005). As the categorization of os type is based on the arrangement of fibrous flaps that surround the opening of the cervix, it is possible that partial or complete prolapse of the first cervical fold may influence the classification of the os (Leethongdee et al., 2007). The incidence of cervical prolapse is greater in parous ewes (Kershaw et al., 2005) and therefore the more complex os types such as rose may be more common in older multiparous ewes.

In conclusion, (i) a practical point of view would favor the application of reproductive biotechnologies at ages > 4 years, and may be at 3-4 years of age, because the cervix is more penetrable to catheter at these ages; (ii) ewe age and cervical grade are the factors that most determinant of cervical penetration; (iii) the results provide a framework for future manipulative studies regarding the application of reproductive biotechnology in Sanjabi ewes.

Acknowledgements

The authors thank Mr. M. Mirshamsi for assistance and the Director of the Bisetoon and Mahidasht abattoirs for providing reproductive tracts used for this study, and also the staff who assisted in ewe age determination.

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Communicating editor: *Mohammad Javad Zamiri*

تاثیر سن و نوع گردن رحم بر ساختار آناتومی، ریخت شناسی و عمق نفوذ گردن رحم در گوسفندان نژاد سنجابی

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چکیده این مطالعه به منظور تعیین تفاوت های آناتومیک و ریخت شناسی بین انواع مختلف گردن رحم گوسفندان نژاد سنجابی و نیز بررسی ارتباط بین مشخصات گردن رحم و میزان نفوذ در گردن رحم انجام شد. تعداد ۴۶۸ دستگاه تولید مثلی در ۶ گروه سنی متفاوت پس از کشتار جمع آوری و برای بررسی های ساختاری به آزمایشگاه انتقال داده شدند. شکل منفذ خارجی گردن رحم در هر نمونه مورد بررسی و بر اساس یکی از ۵ نوع، آویخته، نوک پستانی، نوک مرغابی، گل رزی و شکافی طبقه بندی شد. چین های گردن رحم شمارش و نوع گردن رحم به طور کامل بررسی و به گردن رحم های نوع ۱، ۲و ۳ درجه بندی شدند. اختلاف معنی داری از نظر طول و تعداد چین های گردن رحم در سنین کمتر از ۶ ماه و۱۲- ۶ ماه نسبت به سنین ۳- ۲، ۴- ۳ و بیشتر از ۴ سال وجود داشت ($P < ۰/۰۵$). گردن رحم سنین کمتر از ۶ ماه و۱۲- ۶ ماه و گردن رحم های نوع ۳ کوتاه تر، تنگ تر و هم چنین تعداد چین ها در آنها بیشتر بود. میزان نفوذ تحت تاثیر نوع گردن رحم بود و در گردن رحم نوع ۱ (گردن رحم های ساده) نسبت به نوع ۳ (گردن رحم های با پیچیدگی زیاد) بیشتر بود. میزان نفوذ به درون گردن رحم همبستگی مثبتی با طول و عرض گردن رحم (به ترتیب ۰/۹۳ = r و ۰/۸۸ = r؛ $P < ۰/۰۱$) و همبستگی منفی با تعداد چین های گردن رحم (۰/۶۱ - =r؛ $P < ۰/۰۱$) داشت. در سنین کمتر از ۶ ماه و ۱۲- ۶ ماه فاصله چین ها بطور معنی داری نسبت به بقیه سنین کمتر بود($P < ۰/۰۵$). منفذ خارجی گردن رحم در سنین کمتر از ۶ ماه و۱۲- ۶ ماه به شکل آویخته، در سنین۲- ۱ و۳- ۲ سال به شکل نوک پستانی، در سنین ۴ - ۳ سال به شکل نوک مرغابی و در سنین بالاتر از ۴ سال به شکل گل رزی به میزان بیشتری مشاهده شد. در این مطالعه متناسب با افزایش سن میش ها، به دلیل افزایش اندازه و کاهش تعداد چین های گردن رحم و پیچیدگی حداقل، میزان نفوذ سوند تلقیح از کانال گردن رحم نیز افزایش یافت. از نقطه نظر کاربردی نوع گردن رحم به عنوان یک فاکتور مهم در تعیین میزان نفوذ در گردن رحم گوسفندان سنجابی است و برای کاربرد در برنامه های بیوتکنولوژی تولید مثل سنین بیشتر از ۴ و شاید ۴-۳ سال به خاطر افزایش میزان نفوذ به درون آن پیشنهاد می شود.