Original article

ANATOMICAL AND HISTOLOGICAL STUDIES ON THE CERVICAL CANAL OF RAHMANI SHEEP

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Summary

Macroscopic and microscopic findings of Rahmani sheep cervical canal were evaluated for designing a trans-cervical catheter. The study was carried out on 160 cervices divided into four groups: (Group A; young follicular, Group B; old follicular, Group C; young luteal and Group D; old luteal). All morphometric parameters of cervical canals of each group including length, average length and the external os diameter and shape were measured and tabulated. Epoxy casts were made to demonstrate the anatomic variations of each group. Data collected were statistically analysed using ANOVA. One hundred and twenty tissue sections from each group were sectioned and stained with Harris haematoxylin and eosin, Masson’s trichrome stain for demonstration of collagen fibres, Alcian blue pH 2.5 for demonstration of acidic mucins and PAS technique for demonstration of neutral mucins. In conclusion, the current study is the first report on the morphometric structure of the cervical canal of native Egyptian Rahmani ewes.

Key words: catheter guide, cervical canal, epoxy cast, morphometric parameters, Rahmani ewe

INTRODUCTION

The major Egyptian breeds of sheep are Ossimi, Rahmani and Barki. Rahmani ewes are small to medium sized sheep with coarse wool and fatty tail. All Egyptian breeds are raised mainly for wool, milk and lamb production (Marai et al., 2009).

Artificial insemination using frozen-thawed spermatozoa enhances the production of genetically superior lambs by maximising the utilisation of superior rams. However, the conception rates in sheep following insemination using frozen-thawed spermatozoa are still unsatisfactory (Kershaw et al., 2005). Transcervical artificial insemination in sheep is still limited with unsatisfactory pregnancy rates due to presence of the cervical rings that act as insulating barrier during artificial insemination (More, 1984) and the narrow cervical lumen especially at the level of the first three cervical folds (Halbert et al., 1990). Unfortunately, those factors hinder the insertion of the insemi-
nating pipette into the lumen of the cervi-
cal canal. On the other hand, laparoscopic
insemination in ewes increases the preg-
nancy rates using frozen-thawed sper-
matozoa but its application requires highly
qualified operator and expensive tools
(Ax et al., 2000). Ovine cervical canal is a
long tubular organ with highly tortuous
lumen and has 4 to 7 cervical rings that
are a barrier to any contaminants (Fukui et
al., 1978). Age and breed variations are
the two commonest factors affecting the
ewe cervix morphometry, the shape of
cervical folds varies from a ring in young
ewes while becoming a flap shape in the
older ewes (Kaabi et al., 2006). The aver-
age length of the ewe cervical canal found
by More (1984) was 6.7 cm with presence
of six annular folds forming crypts that
can hold spermatozoa, while Epbleston et
al. (1994) found that the average length of
the ewe’s cervical canal was 4.87 cm in
Merino breed with 3.97 average numbers
of cervical folds. The cervical folds ap-
pear as funnel-shaped arrangement that
project caudally into the lumen of the cer-
vical canal; the second cervical fold is
eccentric while the other folds appear
concentric (Naqvi et al., 2005). This anat-
omical configuration hinders the passage
of the inseminating pipette during insemi-
nation. Histologically, the cervical canal
of sheep is lined by non-secretory column-
ar epithelium with mucous secreting gob-
let cells; the cervical stroma is composed
of collagen bundles running in different
directions with many fibroblasts, blood
capillaries and few smooth muscle cells.
The thick muscular coat of smooth mus-
cles was demonstrated and covered exter-
nally by serosa (Dobson, 1988).

The aim of the current study was to re-
cord the morphometric parameters as well
as the histological criteria including the histochemical mucins types and the sub-
mucosal collagen fibres area of the cervi-
cal canal of Rahmani breed ewes during
both follicular and luteal phases of the
estrous cycle in either young or old ani-
mals to obtain sufficient data for manufac-
turing a suitable inseminating pipette and
facilitate the proper manipulation of the
cervix.

MATERIALS AND METHODS

Cervices collection

One hundred and sixty non-pregnant ewe
cervices (Rahmani breed) were collected
from the local abattoir between September
2016 to October 2017. Cervices were di-
vided according to the age (young; less
than 18 month-old; old; 18–48 months of
age) and the stage of estrous cycle (fol-
licular and luteal; depending on the ovar-
ian structures) into four groups: Group A
(young follicular, n=40), Group B (old
follicular, n=40), Group C (young luteal,
n=40) and Group D (old luteal, n=40).

Monitoring the shapes of the external
cervical os

All uteri were transported to the labora-
tory 2–3 hours post-slaughter, cervices
were excised and separated cranially at
the level of the uterine body and caudally
at the level of the posterior vagina, and
the external os was exposed by making a
longitudinal incision through the anterior
vagina and the cervix (Fig. 1). The shape
of the external os was observed and classi-
fied into four types (Halbert et al., 1990).
The identified four types were: flab, duckbill, rosette and papilla.

Cervical canal measurements

All morphometric parameters including
1) average length of cervical canal taken
at the level of the external opening to its
union with the body of the uterus; 2) average length of cervical canal at different levels; 3) average diameter of cervical canal and 4) the external os diameter in each group were measured using Vernier caliper. The average number of cervical folds was estimated and recorded.

**Epoxy injection technique**

Uterine specimens were flushed by injection of warm sterile normal saline from the body of the uterus toward the vagina to remove any mucus or debris followed by injection of E151N20 epoxy resin (Schummer, 1951). Specimens were left for 3–4 days in the refrigerator for hardening, then dissected carefully to declare the epoxy casts of the cervical canal.

**Tissue architecture**

Collected cervical tissue samples were fixed in 10% neutral buffered formalin for 72 h. Samples were trimmed and processed by ascending series of alcohols, cleared in xylene, infiltrated with Paralast synthetic wax and blocked out into Para plast tissue embedding media. Sections of 3–5 µm were cut by rotatory microtome. The sections were stained with Harris haematoxylin and eosin as a general staining method, Masson’s trichrome stain for demonstration of collagen fibres, Alcian blue pH 2.5 for demonstration of acidic mucins and PAS technique for demonstration of neutral mucins (Bancroft & Stevens, 2010). Ten random non-overlapping fields from each tissue section of different stains in different groups with a total 120 tissue sections from each group per stain were analysed using Leica application suite for slide analysis equipped with full HD microscopic camera (Leica Biosystems, Germany).

**Statistical analysis**

Data concerning average length of cervical canal, average length of cervical canal at different levels, external os diameter and average diameter of cervical canal at different levels were analysed by one-way analysis of variance (ANOVA) using SPSS 10.0 Means ± SEM were calculated. Average number of cervical folds and different shapes of external os were analysed using the chi-square test.

**RESULTS**

**Shapes of the external os and number of cervical folds**

Different shapes of the external os were monitored according to age and stage of the estrous cycle (Fig. 2). The flap shape
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was predominant in Group A (34/40; 85.0%), duckbill shape was frequent in group B (31/40; 77.5%), a shape of papilla was common in group C (28/40; 70.0%) and the rosette shape was prevailing in Group D (29/40; 72.5%). The average number of cervical folds was 6 in 38/40 or 95.0% of Group A, 36/40 (90.0%) of Group B and 35/40 (87.5%) of Group D, while 7 fold were found out in 37/40 (92.5%) of Group C.

Average external os diameter and cervical canal length

The external os diameter was significantly (P<0.05) higher in group B (1.2±3.3 cm) compared to 0.67±2.1, 0.40±1.2 and 0.73±0.9 cm respectively for Group A, C and D (Table 1).

The average diameter of the cervical canal was significantly higher in group B (1.80±0.42 cm) than in other groups (0.71±2.7, 0.78±3.1 and 1.10±3.7 cm for Groups A, C and D, respectively) at the level of 2nd cervical fold. There was no significant difference (P>0.05) in the average diameter of cervical canal at the level of 4th fold between Groups B and D (1.30±3.3 and 1.20±2.8 cm, respectively) but both were significantly higher than diameters recorded in Groups A and C (0.61±1.9 and 0.57±1.7 cm, respectively).

At the level of the sixth fold, the average diameter of both Groups B and D

Fig. 2. Epoxy casts of the cervical canal of different groups (left) and external os appearance (right):
1. Flap (Group A; young follicular); 2. Papilla (Group B; young luteal);
3. Duckbill (Group C; old follicular); 4. Rosette (Group D; old luteal).
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The average length of cervical canal was significantly (P<0.05) higher in Group B (8.4±3.2 cm) than in all other groups (4.8±1.32, 4.6±2.73 and 5.2±1.77 cm for Group A, C and D, respectively) as shown in Table 2.

The average length of the cervical canal between external os and 1st fold, 1st and 2nd folds, 2nd and 3rd folds, 3rd and 4th folds, 4th and 5th folds and 5th to 6th folds (1.20±4.8 and 0.90±8.7 cm) was significantly higher than that recorded in Groups A and C (0.47±1.5 and 0.42±1.2 cm) (Table 1).

<p>| Table 1. Cervical canal diameter in Rahmani ewes (cm) at different levels. Data are presented as mean ± SEM, n=40 |</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>Group A (young follicular)</th>
<th>Group B (old follicular)</th>
<th>Group C (young luteal)</th>
<th>Group D (old luteal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>External os diameter</td>
<td>0.67±0.15</td>
<td>1.2±0.26</td>
<td>0.40±0.14</td>
<td>0.73±0.35</td>
</tr>
<tr>
<td>Cervical canal diameter at the 2nd cervical fold level</td>
<td>0.71±0.13b</td>
<td>0.78±0.14b</td>
<td>1.80±0.42a</td>
<td>1.10±0.45b</td>
</tr>
<tr>
<td>Cervical canal diameter at the 4th cervical fold level</td>
<td>0.61±0.20b</td>
<td>0.57±0.11b</td>
<td>1.30±0.36a</td>
<td>1.20±0.47a</td>
</tr>
<tr>
<td>Cervical canal diameter at the 6th cervical fold level</td>
<td>0.47±0.11b</td>
<td>0.42±0.18b</td>
<td>1.20±0.53a</td>
<td>0.90±0.22a</td>
</tr>
</tbody>
</table>

Different superscripts within the same row are significantly different at P<0.05.

<p>| Table 2. Average length of cervical canal at different levels. Data are presented as mean ± SEM, n=40 |</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>Group A (young follicular)</th>
<th>Group B (old follicular)</th>
<th>Group C (young luteal)</th>
<th>Group D (old luteal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical canal length between external os and 1st fold</td>
<td>0.4±0.15b</td>
<td>0.9±0.18a</td>
<td>0.3±0.17b</td>
<td>0.5±0.22b</td>
</tr>
<tr>
<td>Cervical canal length between 1st fold and 2nd fold</td>
<td>0.7±0.13b</td>
<td>1.2±0.21a</td>
<td>0.5±0.13b</td>
<td>0.7±0.41b</td>
</tr>
<tr>
<td>Cervical canal length between 2nd fold and 3rd fold</td>
<td>0.6±0.14b</td>
<td>1.7±0.17a</td>
<td>0.4±0.11b</td>
<td>0.5±0.23b</td>
</tr>
<tr>
<td>Cervical canal length between 3rd fold and 4th fold</td>
<td>0.7±0.17b</td>
<td>1.4±0.15a</td>
<td>0.3±0.18b</td>
<td>0.5±0.16b</td>
</tr>
<tr>
<td>Cervical canal length between 4th fold and 5th fold</td>
<td>0.6±0.17b</td>
<td>1.5±0.18a</td>
<td>0.4±0.15b</td>
<td>0.6±0.19b</td>
</tr>
<tr>
<td>Cervical canal length between 5th fold and 6th fold</td>
<td>0.8±0.17b</td>
<td>1.3±0.12a</td>
<td>0.4±0.17b</td>
<td>0.4±0.21b</td>
</tr>
<tr>
<td>Cervical canal length</td>
<td>5.2±1.8</td>
<td>4.5±1.3</td>
<td>8.4±2.1</td>
<td>4.8±1.4</td>
</tr>
</tbody>
</table>

Different superscripts within the same row are significantly different at P<0.05.
were 0.9±1.8, 1.2±3.1, 1.7±3.7, 1.4±1.5, 1.5±0.8 and 1.3±2.2 cm respectively for Group B. Interestingly, all average lengths of Group B were significantly higher than those of Groups A, C, D at the same levels of the cervical canal (P<0.05; Table 2).

Epoxy casts of the cervical canal

Epoxy cast for each group was dissected carefully to be a clear model during the future manufacture of the inseminating catheter (Fig. 2).

Histological findings

Histologically, cervical mucosal epithelium of surveyed ewes was composed of simple columnar secretory cells and occasionally was ciliated non-secretory or stratified columnar epithelium. Regarding mucins dynamics in terms of area percentage of expression and reactivity in the secretory epithelial cells, the highest percentage of reactivity of Alcian blue stain PH 2.5 was demonstrated in the middle cervical region of both young follicular (Group A) and old luteal ewes (Group D) attaining up to 28.5% and 26.5% respectively from the secretory epithelium (Fig. 3). However, the least reactive regions were the cranial cervical part of both young and old follicular ewes (Group A and B; Fig. 3).

In terms of reactivity of epithelial mucins to PAS technique, the highest reactivity was limited to the caudal region of the cervical tube of both old follicular (Group B) and old luteal (Group D) ewes occupying up to 22% of the secretory epithelial area. However, PAS reactivity was nearly absent in other regions of different groups (Fig. 4). Masson’s trichrome staining of different cervical regions of all groups demonstrated enormous amount of collagen fibres in submucosal layer of all

![Fig. 3. Expression levels of acidic mucins by cervical epithelium in caudal part of the cervix of the old luteal ewes (A) as well as in follicular stages (B) and in cranial segments of the cervix of old follicular ewes (C) as well as in ewes in follicular stages (D). Alcian blue pH=2.5, 400×](image)
regions of different groups. Moreover, the middle cervical region demonstrated the highest area percentage of collagen fibres as well as optical density values in different surveyed ewes of each group (Fig. 5).

The examined tissue sections of the middle cervical region of young luteal (Group C) and old luteal ewes (Group D) recorded the highest mean value of collagen fibres percentage among all examined groups. However, the highest optical density values of demonstrated collagen fibres were recorded in Group B, D and C respectively (Table 3).

**DISCUSSION**

The current study is considered the first report on the anatomical and the mor-
phometric structure of the cervical canal of native Egyptian ewes.

The results showed that the shape of the external cervical os differed according to the age and the stage of estrus cycle. On the other hand, Cruz et al., (2014) found that the duckbill shape was the most predominant in Santa Ines ewes, and rosette and flap (Kershaw et al. (2006) or spiral shape (Naqvi et al., 1998) were the predominant shapes in other breeds – this difference is attributed to the breed variation. The average number of cervical folds recorded in the present study was 6 in Groups A, B and D while it was 7 in Group C. Cruz et al. (2014) reported that the number of cervical folds ranged from 3–7 per cervix (mean 4.4±1.06) depending on the age, where ewes at age <6 months or 6–12 months had narrower cervices with large number of folds. In line with our results, Gultiken et al. (2009) found no significant difference (P>0.05) between number of the cervical folds in lambs (5.16±0.93) and ewes (4.73±0.7).

Our results showed that the average length of cervical canal was significantly higher in old follicular ewes (Group B) than in other groups. Similarly, Habibibzad et al. (2015) found that the length of cervical canal varied from 3.93±0.25 cm in young lambs <6 months of age to 7.58±0.17 cm in ewes older than 4 years in Sanjabi breed. Naqvi et al. (2005) added that the length of the cervix was 5.36 cm in mature ewes while it was 3.87 cm in young ewes. These variations in results of the length of the cervical canal can be attributed to breed size variation, age and the stage of estrous cycle. Also, our study demonstrated that the external os diameter was significantly higher in Group B. Cruz et al. (2014) reported different diameters of the external os depending on the different shapes of external os and age of ewes. Interestingly, our results demonstrated a significant variation in both diameter and length of cervical canal at different levels of cervical folds.

The variation in length and diameter of cervical canal at different levels was one of the main obstacles during artificial insemination as it differs according to age and breed (Naqvi et al., 2005). The length of cervical canal especially at the level of

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group A (young follicular)</th>
<th>Group B (old follicular)</th>
<th>Group C (young luteal)</th>
<th>Group D (old luteal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean alcian blue reactivity area, %</td>
<td>cranial 0.30±0.12b</td>
<td>1.5±0.28b</td>
<td>8.6±4.1b</td>
<td>11.5±2.3a</td>
</tr>
<tr>
<td></td>
<td>middle 28.4±1.3c</td>
<td>15.0±2.3b</td>
<td>9.2±4.3c</td>
<td>26.5±2.4a</td>
</tr>
<tr>
<td></td>
<td>caudal 18.7±1.5a</td>
<td>17.0±2.5a</td>
<td>11.8±4.6b</td>
<td>19.7±2.6a</td>
</tr>
<tr>
<td>Mean % of submucosal collagen</td>
<td>cranial 56.4±2.2a</td>
<td>55.0±2.3a</td>
<td>59.1±2.7a</td>
<td>53.2±2.6a</td>
</tr>
<tr>
<td></td>
<td>middle 62.2±3.1c</td>
<td>62.4±3.2a</td>
<td>69.4±3.5a</td>
<td>66.2±2.9a</td>
</tr>
<tr>
<td></td>
<td>caudal 59.2±4.3c</td>
<td>58.7±3.9a</td>
<td>56.5±2.8a</td>
<td>56.2±4.5a</td>
</tr>
<tr>
<td>Mean OD of collagen fibres</td>
<td>cranial 0.51±0.09a</td>
<td>0.48±0.05a</td>
<td>0.55±0.04a</td>
<td>0.45±0.03a</td>
</tr>
<tr>
<td></td>
<td>middle 0.61±0.05a</td>
<td>0.73±0.06a</td>
<td>0.69±0.07a</td>
<td>0.70±0.08a</td>
</tr>
<tr>
<td></td>
<td>caudal 0.57±0.12a</td>
<td>0.68±0.14a</td>
<td>0.65±0.18a</td>
<td>0.56±0.14a</td>
</tr>
</tbody>
</table>

Values with different superscripts in the same row were significantly higher at P<0.05.
the external os to the first cervical fold and that from first to second cervical fold was greater than that reported either in Karayaka ewes (Gultiken et al., 2009) and tropical breeds (Naqvi et al., 2005). For the first time, cervical canal measurements were recorded in our study using epoxy injection not by making silicone molds as done by Naqvi et al. (2005).

Several authors have discussed the mucins dynamics in cervical region of Merino strain and other breeds (Becher et al., 2004). It was reported qualitatively that sialomucins were found in the basal part of cervical folds unlike sulfomucins which were limited to the tips of mucosal folds (Pluta et al., 2011). The current study quantified the area percentage of total sialomucins as well as sulfomucins in examined tissue sections for each region. It is evident that the middle and caudal cervical regions of cervical canal play a critical role in mucins production levels unlike the cranial cervical region. The greater mucins expression levels in these regions is expected to aid in higher sperm entrapment and guidance in middle and caudal cervical regions. The higher mucins levels also provide a higher protection as a physical barrier against harmful pathogens in these regions. In agreement with Pluta et al. (2011) neutral mucins were generally less expressed in cervical canal of ewes. However, current investigation recorded that they were mostly expressed and limited to the caudal part of cervical canal in old ewes unlike negative expression in young ewes.

There was no record in available literature for the relationship between collagen fibres’ area percentage, density and reproductive state of cervical region of ewes. The current study reported that the submucosal collagen fibre area percentage remodelling was directly associated with the optical density values in examined regions of cervical canal in each group. The middle part of cervical canal demonstrated a higher amount of mature collagen bundles than other cervical regions in different stages of surveyed Rahmani ewes. However, it was evident that the reproductive stage with the expected hormonal state of each group had no significant effect on submucosal collagen bundles remodelling. These findings were in agreement with previous reports on cyclic changes of human cervix (Petersen et al., 1991) and cow cervix (Breeveld-Dwarkasing et al., 2011).

In conclusion, the current study provided a complete illustration about the anatomical configuration and histological architecture of the cervical canal of the Egyptian Rahmani ewes to facilitate the future manufacturing a trans-cervical catheter for this breed.

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