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## B Satya Prasad

Veterinary Assistant Surgeon,  
Directorate of Animal  
Husbandry, Labbipet,  
Vijayawada, Andhra Pradesh,  
India

## Manda Srinivas

Department of Veterinary  
Gynaecology & Obstetrics  
N.T.R. College of Veterinary  
Science, Gannavaram (A.P.)  
Sri Venkateswara Veterinary  
University, Tirupati,  
Andhra Pradesh, India

## G Venkata Naidu

Professor & Head, Super  
Speciality Veterinary Hospital,  
SVVU, Visakhapatnam,  
Andhra Pradesh, India

## V Vaikunta Rao

Professor & Head, Veterinary  
Clinical Complex, CVSc,  
Tirupati, Andhra Pradesh, India

## Corresponding Author:

### Manda Srinivas

Department of Veterinary  
Gynaecology & Obstetrics  
N.T.R. College of Veterinary  
Science, Gannavaram (A.P.)  
Sri Venkateswara Veterinary  
University, Tirupati,  
Andhra Pradesh, India

## Silicone moulds for morphometric studies on cervical canal of Nellore sheep

**B Satya Prasad, Manda Srinivas, G Venkata Naidu and V Vaikunta Rao**

### Abstract

The cervical canal of the ewe does not allow easy transcervical passage of insemination instruments during artificial insemination and embryo transfer programmes. The purpose of the present study was to examine, the gross structure of the cervix in ewe lambs and adult ewes of Nellore sheep breed popular in Andhra Pradesh. Reproductive tract was incised within 15 minutes of slaughter of ewe lambs and adult ewes. The cervical canal was filled with silicone sealant and moulds were obtained from 6 ewe lambs and 6 adult ewes in follicular phase of estrous cycle. The results were showed a significant difference ( $P<0.01$ ) in the length (cm), weight (gm), number of folds (cm), height of fold, diameter of fold (cm), internal diameter of fold (cm) and distance from os to folds (cm) between the ewe lambs and adult ewes.

**Keywords:** Silicone moulds, morphometry, cervix, sheep

### 1. Introduction

Recent reproductive technologies have opened up many possibilities and one among these technologies includes artificial insemination (AI) with frozen semen. Existing native sheep could be upgraded by use of fresh or short-term preserved semen from elite rams through fixed time artificial insemination (FTAI). AI of sheep has been in extensive use for over the last six decades in major sheep rearing countries, but the complex anatomy of the cervix compromised the fertility. Fertility following AI with frozen semen was limited due to the inability of sperms to transit the complex cervix. However, the impetus as of now was to develop the non-invasive transcervical insemination technique (Halbert *et al.*, 1990) [5].

Sheep cervix is characterised by sphincter like structure with a thick wall. Cervical canal has three to four annular transverse prominences called as annular rings. These rings are folds and fit into each other to close to cervix securely and provides barrier to transcervical artificial insemination (Hafez, 1993) [4]. To avoid the problems associated with transcervical artificial insemination (TCAI), many scientists have focused on the development of new TCAI catheters and conducting studies on the anatomy of cervix in the recent past (Kaabi *et al.*, 2006) [6]. Breed, age, parity and physiological state influenced the length of the ovine cervix was described in the earlier studies. The mean length of the cervical canal also recorded differences in the morphology of the external cervical os between animals (Kershaw *et al.*, 2005) [7]. The objective of the present study was to determine the morphometry of the cervical canal of Nellore sheep, which is popular native indigenous sheep breed of Andhra Pradesh and mainly reared for its meat.

### 2. Material and Methods

Reproductive organ of Nellore ewes lambs (n=6) and adult ewe (n=6) in follicular phase of estrous cycle (based on ovarian status) were collected from slaughter house and excised with help of scissors and scalpel within 15 min after collection. The organs were washed with normal saline, packed in ice cubes and then transported in an igloo box to the laboratory for further studies.

Silicone sealant (Pidilite Industries Ltd., Mumbai, India) was used for casting the cervical moulds as per the procedure described by Halbert *et al.* (1990) [5]. An incision was made in the body of uterus through which the sealant was slowly injected by applying gentle pressure to avoid distortion or damage in the cervical canal. After filling with sealant the vagina was tied and the specimens were placed on a flat surface at 5 °C for 24 hrs for polymerization of the sealant. An incision was made in the vagina to expose the cervical os. The tissue overlying the annular folds was carefully dissected using a scalpel and forceps.

The annular folds were grasped with forceps and slowly cut in succession from lumen to the outer surface in order to avoid any pressure that could damage the silicone mould. The hardened mould was removed carefully from the cervical canal. A total of 12 complete moulds were recovered so that 6 each were available for studies from ewe lambs and ewes, respectively.

The various measurements of length, number of folds, height of the first two-folds, diameter of first two-folds, internal circumference (converted to the diameter) measured between folds at the midpoint, distance from os to first two-folds and were made using vernier calipers as per the procedure described by Eppleston *et al.* (1994) [2] and all the values are recorded in centimetres. The statistical analysis of data was done by adopting computer software programmed for Windows XP (version 15.0, SPSS Inc. Munich) and Excel (version 2007 Microsoft) and as per the procedure described by Snedecor and Cochran (1994) [9].

### 3. Results and Discussion

Silicone cervical moulds could be readily prepared from cervixes of 6 estrus ewe lambs and 6 estrus adult ewes (Figure 1). Assessment of cervical moulds included length, weight, number of folds, height of the folds (first and second fold), diameter of the fold (first and second fold), internal diameter of the fold (first and second fold), distance from the os of the fold (first and second fold) and are presented in the Table.1

**Table 1:** The Mean  $\pm$  SE of parameters related with cervical moulds in ewe lamb (n=6) and adult ewe (n=6)

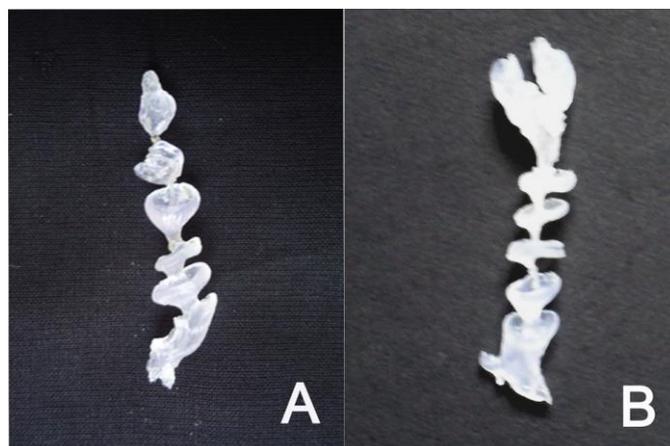
Parameters	Ewe Lambs (n=6)	Adult Ewes (n=6)
<b>Assessment of cervical moulds</b>		
Length (cm)	3.34 $\pm$ 0.26 <sup>a</sup>	4.48 $\pm$ 0.33 <sup>b</sup>
Weight (cm)	0.32 $\pm$ 0.28 <sup>a</sup>	0.58 $\pm$ 0.11 <sup>b</sup>
Number of folds (cm)	3.53 $\pm$ 0.27 <sup>a</sup>	4.54 $\pm$ 0.27 <sup>b</sup>
<b>Height of folds</b>		
First-fold (cm)	0.16 $\pm$ 0.02 <sup>a</sup>	0.30 $\pm$ 0.01 <sup>b</sup>
Second-fold (cm)	0.15 $\pm$ 0.01 <sup>a</sup>	0.27 $\pm$ 0.02 <sup>b</sup>
<b>Diameter of fold</b>		
First-fold (cm)	0.20 $\pm$ 0.02 <sup>a</sup>	0.60 $\pm$ 0.02 <sup>b</sup>
Second-fold (cm)	0.29 $\pm$ 0.03 <sup>a</sup>	0.63 $\pm$ 0.05 <sup>b</sup>
<b>Internal diameter of fold</b>		
First-fold (cm)	0.09 $\pm$ 0.02 <sup>a</sup>	0.20 $\pm$ 0.01 <sup>b</sup>
Second-fold (cm)	0.11 $\pm$ 0.01	0.18 $\pm$ 0.01
<b>Distance from the os to fold</b>		
First-fold (cm)	0.18 $\pm$ 0.03 <sup>a</sup>	0.38 $\pm$ 0.01 <sup>b</sup>
Second-fold (cm)	0.13 $\pm$ 0.02 <sup>a</sup>	0.39 $\pm$ 0.02 <sup>b</sup>

<sup>a</sup> Mean bearing different superscripts within the row differ significantly ( $P < 0.01$ )

Analysis of variance revealed a significant difference ( $P < 0.01$ ) in the length (cm), weight (gm), number of folds, height of fold, diameter of fold (cm), internal diameter of fold (cm) and distance from os to folds (cm) between the ewe lambs and adult ewes.

The number of cervical rings in moulds was in line with the findings of Halbert *et al.* (1990) [5] and Eppleston *et al.* (1994) [2] who reported similar length of cervix as observed in the present study while Cruz Junior *et al.* (2014) [1] reported similar cervical ring diameter in Santa Ines ewes. Divergently, in Merino ewes, Malpura and Kheri ewes, Karayaka ewes (Eppleston *et al.*, 1994; Naqvi *et al.*, 2005 and Gultiken *et al.*, 2009) [2, 8, 3] reported higher morphometric values for different parameters of cervical moulds. It was opined that most of the

anatomical studies on ovine cervix had been performed on a small group of ewes of specific breeds. The ewes and ewe lambs in the present study could not be randomly selected and were small in number; therefore, decisive conclusions about the differences in the cervical moulds in comparison with the previous studies could not be made (Halbert *et al.*, 1990) [5]. Elsewhere, some of the morphometric studies on cervical canal were conducted during breeding and non breeding seasons and opined that increased estradiol concentrations during the follicular phase caused cervical relaxation.



**Fig 1:** Silicone moulds made from the cervix of Nellore sheep. A: Ewe lamb B: Ewe

### 4. Conclusion

It is concluded from the present study that the findings would be beneficial to the researcher to improve the lambing rate of Nellore ewes following transcervical artificial insemination (TCAI). The present research recorded assessment of cervical canal anatomy of Nellore breed of sheep in Andhra Pradesh by using silicone moulds.

### 5. Acknowledgments

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