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Anatomical studies on cervix of adult Bakerwali goat during different phases of estrus cycle

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Abstract

The present investigation was aimed to study the histology and histochemistry of cervix during different phases of estrus cycle. Twelve genitalia of apparently healthy, non pregnant cyclic adult (2-3 years of age) Bakerwali goat was collected from local slaughter houses of Jammu region during year 2014. Histological study revealed that the cervix was lined with pseudostratified columnar epithelium and the epithelial height was higher in the follicular phase ($26.49 \pm 1.72 \mu m$) as compared to luteal phase ($22.69 \pm 1.60 \mu m$). The cervical epithelium invaginate into the lamina propria submucosa to form simple coiled tubular cervical glands. They were lined with simple tall to low columnar epithelium in follicular and luteal phase respectively. The inner, outer diameter of cervical gland and glandular epithelium height was significantly ($P \le 0.05$) higher in the follicular phase of estrus cycle. Intense PAS reaction was seen in the supranuclear zone of lining, glandular epithelium and cervical secretions. The muscular tunic of the cervix contained inner circular and outer longitudinal muscle layer which was infiltrated with large number of blood vessels and nerves. Tunica serosa comprised of irregular dense fibrous connective tissue which contained loosely arranged collagen fibres. It was mildly positive for PAS, cholesterol and bound lipids.

Keywords: Bakerwali goat, cervix, estrus, histology, histochemistry

1. Introduction

A cervix uteri is the lower, narrow portion of the uterus where it joins with the top end of the vagina. It is cylindrical or conical in shape and protrudes through the upper anterior vaginal wall ^[4, 7, 13]. It play an important roles as a sphincter of the uterus. The uterine cervical epithelium protects the upper reproductive tract from harmful bacteria and foreign debris providing a physical barrier, secretions containing bactericidal and virucidal agents and a pathogen-dependent direct immunomodulation ^[10]. During estrus, it takes part in direct spermepithelium interaction ^[16], as well as in the signal reception from seminal fluid ^[12]. Keeping in mind the paucity of literature on anatomical studies on the internal female genitalia of adult Bakerwali goat in different phases of estrus cycle prompted the present study.

2. Materials and Methods

Twelve genitalia of apparently healthy, non-pregnant adult (2-3 years of age) Bakerwali goat were collected from local slaughter houses of Jammu region during year 2014 immediately after sacrifice and depending upon the structure present on surface of ovary these were classified into follicular and luteal phase. The specimen were fixed in 10% neutral buffered formalin for 24-48 hours and processed for routine paraffin technique. The 5-6 µm sections were stained with Harris Hematoxylin and Eosin for routine histomorphology ^[5], Van Gieson and Verhoeff's method to differentiate Collagen and elastic fibres respectively ^[6], Gomori's method to demonstrate reticular fibres and Masson's Trichrome technique to demonstrate Collagen fibres. For histochemical studies the sections were stained by Periodic acid Schiff method (PAS) to demonstrate carbohydrate, Diastase Digestion to demonstrate glycogen, Alcian Blue method plus PAS (pH-2.5) to demonstrate acidic and neutral mucopolysaccharide, Sudan Black B method to demonstrate bound lipids and Schultz method to demonstrate cholesterol ^[5]. The micrometrical parameters were recorded using calibrated ocular micrometer duly calibrated with stage micrometer to elucidate various components of oviduct. All the recorded data were put to Standard Statistical procedures [15]. The data was analyzed using 16.0 version of SPSS software. For comparison between follicular and luteal phase 'paired t test' was applied.

3. Results and Discussion

The cervix uteri of Bakerwali goat had the usual three coats or endometrium, myometrium and perimetrium as also described by Dellmann and Eurell^[1] and Priedkalns^[9] in domestic animals. The cross-sections of the cervix revealed that the mucosa was thrown into several finger like projections called plicae longitudinales as described by Shalini et al. [14] in Gaddi goat and Poyam *et al.*^[8] in goat. The longitudinal folds (cervical crypts) of opposite sides lie in close apposition with each other but did not interdigitate as observed by Shalini et al. [14] in Gaddi goat. At some points the primary folds were branched to give rise to secondary folds. The core of folds comprised of thick collagen fibres reinforced with fine reticular fibres but elastic fibres were not observed. Fine reticular fibers were also reported in the basement membrane. Many engorged blood vessels were also seen in core of cervical crypts in follicular phase as also observed in Gaddi goats ^[14].

The lining epithelium of cervical crypts was pseudostratified columnar epithelium (Fig. 1, 2). Contrary to this, Rajput ^[11] in sheep and Shalini *et al.* ^[14] in Gaddi goat reported tall columnar epithelium with goblet cells. However, Poyam *et al.* ^[8] in goat had observed pseudostratified columnar epithelium. In present study, the epithelial height was significantly higher in the follicular phase than in luteal phase of estrus cycle (Table 1). The similar trends were observed by Joshi *et al.* ^[3]

in goats whereas contrary trends were observed by Joshi [2] in goat, Rajput^[11] in sheep and Shalini et al.^[14] in Gaddi goat. Most of the cervical crypts had broad luminal end with collagen fibres extending upto their tip (Fig. 6). Contrary to this, Shalini et al. [14] in Gaddi goat observed narrow luminal ends. The lamina propria submucosa comprised of dense collagen and reticular fibres. The smooth muscle fibres were rarely seen in Bakerwali goat. The cervical glands were formed due to the invaginations of the lining epithelium into the propria submucosa. These glands were simple coiled tubular mucus type lined with simple tall columnar epithelium. The nuclei were oval and basally located. Cervical glands were seen to extend upto tip of cervical crypts in follicular phase (Fig. 3). Cervical glands were also surrounded by dense collagen fibres and fine reticular fibres (Fig. 6). The glands were seen in phase of secretion in follicular phase which was evident by presence of mainly apical blebs, luminal secretions. The cytoplasm of glandular epithelium was foamy and nuclei were pressed against the basement membrane (Fig. 4). In luteal phase, the glands were lined by low columnar epithelium and lumen of the gland was not filled with mucoid secretions (Fig. 7). In present study, the diameter of cervical glands and glandular epithelium height was significantly higher in the follicular phase than in luteal phase (Table 1). Similar observation was made by Poyam et al. [8].

Table 1: Micrometry of different components	cervix in Adult Bakerwali goat in different	ent phases of estrus cycle.
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Parameters	Follicular phase	Luteal phase
Height of lining onithelium (um)	26.49 ± 1.72^{a}	$22.69 \pm 1.60^{\text{b}}$
Height of lining epithelium (µm)	(14.28 - 47.60)	(7.14 - 47.60)
Outer diameter of Cervical gland (µm)	284.9 ± 24.66^a	215.97 ± 16.84^{b}
Outer diameter of Cervical grand (µm)	(132 - 605)	(77 – 440)
In the diameter of Company all allowed (1997)	211.57 ± 25.15^{a}	147.77 ± 16.27^{b}
Inner diameter of Cervical gland (µm)	(55 - 550)	(22 – 352)
Clandylar anithalium haight (um)	73.86 ± 0.61^{a}	67.99 ± 1.22^{b}
Glandular epithelium height (µm)	(71.4 – 83.3)	(57.12 - 83.3)
Length of muchans of Comrised aland (um)	5.79 ± 0.22	5.39 ± 0.19
Length of nucleus of Cervical gland (µm)	(4.76 - 7.14)	(4.76 – 7.14)

*Mean with different superscripts differ significantly ($P \leq 0.05$)

Intense PAS reaction was seen in the supranuclear zone of lining, glandular epithelium and cervical secretions indicating presence of carbohydrates (Fig. 5). Cytoplasm showed intense reaction for Alcian Blue indicating presence of acidic mucopolysaccharides (Fig. 8). Poyam *et al.* ^[8] in goats also reported similar findings with PAS and PAS- Alcian Blue pH 2.5. Presence of glycogen was also observed. Moderate lipid reaction was seen in apical and basal borders of epithelium of cervical crypt and gland whereas intense lipid reaction was seen in cervical secretions. Similar reactions were seen for glycogen and lipids in Gaddi goat by Shalini *et al.* ^[14].

The muscular tunic of the cervix contained inner circular and outer longitudinal muscle layer which was infiltrated with large number of blood vessels and nerves as also described by Dellmann and Eurell^[1] in domestic animals. Coarse collagen fibres and fine reticular fibres were reported in outer longitudinal layer of tunica muscularis encircling individual muscle bundle (Fig. 9, 10). Trautman and Fiebiger^[17] described circular layer of muscles to be rich in elastic fibres in other domestic animals. In present study elastic fibres were not observed. The myometrium showed mild with PAS and for bound lipids with Sudan Black B.

In present study, tunica serosa comprised of irregular dense fibrous connective tissue which contained loosely arranged collagen fibres (Fig. 9, 10). However, Shalini *et al.* ^[14] in Gaddi goat and Rajput ^[11] in sheep observed that tunica serosa comprised of loose connective tissue lined by mesothelial lining having blood vessels and lymphatics. Perimetrium was mildly positive for PAS, cholesterol and bound lipids.

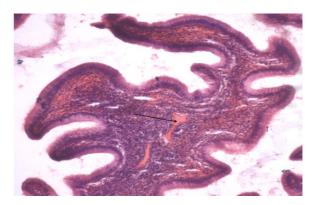


Fig 1: Cervix of Adult Bakerwali goat in follicular phase showing pseudostratified columnar epithelium and engorged Blood vessels (arrow) in cervical crypt. H&E X 100

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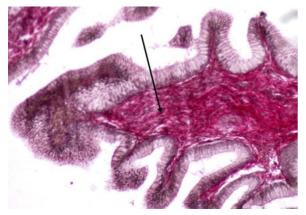


Fig 2: Cervix of Adult Bakerwali goat in luteal phase showing dense collagen fibres extended upto tip of cervical crypt. Von Geison & Verhoff's X 100

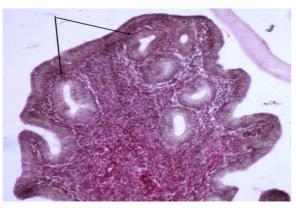


Fig 3: Cervical glands extended upto tip of cervical crypt (arrow) in follicular phase. Von Geison & Verhoff's X 100

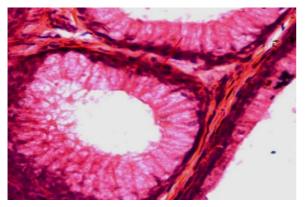


Fig 4: Cervical gland of Adult Bakerwali goat in follicular phase showing nucleus pressed towards the base and cytoplasm engorged with mucus secretions. H&E X 400

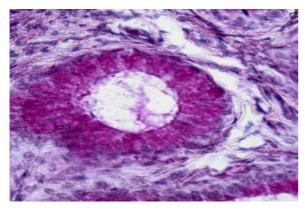


Fig 5: Cervical gland of Adult Bakerwali goat in follicular phase showing strong PAS reaction in apical blebs and foamy cytoplasm. PAS X 400

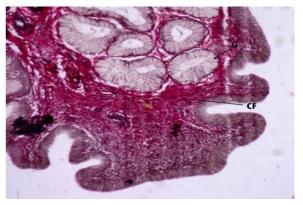


Fig 6: Cervical crypt of Adult Bakerwali goat in follicular phase showing dense collagen fibres (CF) surrounding individual glands (G) as well as extending upto tip forming broad end. Von Geison & Verhoff's X 100

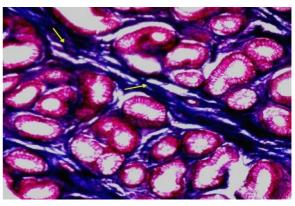


Fig 7: Cervix of Adult Bakerwali goat in luteal phase showing empty cervical glands surrounded by dense collagen fibres. Masson's Trichrome X 100

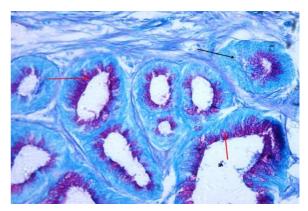


Fig 8: Apical borders of glandular epithetium and lining epithelium show intense PAS reaction and cytoplasm showing intense Alcian Blue reaction in Luteal phase. PAS & Alcian Blue (pH-2.5) X 100

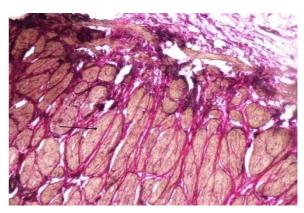


Fig 9: Cervix of Adult Bakerwali goat in luteal phase showing fine collagen fibres and muscles.Von Geison & Verhoff's X 100

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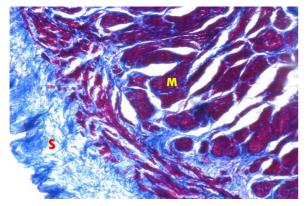


Fig 10: Cervix of Adult Bakerwali goat in follicular phase showing longitudinal layer of myometrium (M) and perimetrium (S). Masson trichrome X 100

4. Conclusion

The cervix uteri of Bakerwali goat had the usual three coats or endometrium, myometrium and perimetrium. The cervical crypts of opposite sides lie in close apposition with each other in luteal phase but did not interdigitate. The core of folds comprised of thick collagen fibres reinforced with fine reticular fibres extended upto tip of cervical crypt. Most of the cervical crypts had broad luminal end. Many apical blebs, luminal secretions were observed in follicular phase. Cervical glands were seen to extend upto tip of cervical crypts in follicular phase. The diameter of cervical glands and glandular epithelium height was significantly higher in the follicular phase than in luteal phase. Intense PAS reaction was seen in the supranuclear zone of lining, glandular epithelium and cervical secretions. Tunica muscularis and serosa was mildly positive for PAS and bound lipids.

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