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Histology and Histochemistry of Oviduct of Adult Bakerwali Goat in Different Phases of Estrus Cycle

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ABSTRACT

Twelve genitalia of apparently healthy, non pregnant adult Bakerwali goat were collected from local slaughter houses immediately after sacrifice. Sections were stained with different staining methods. The oviduct of Bakerwali goat was lined with pseudostratified columnar ciliated epithelium in infundibulum and ampulla whereas pseudostratified columnar nonciliated epithelium in isthmus. The thickness of tunica mucosa, tunica muscularis of oviduct increases towards the isthmus and was significantly higher in luteal phase of estrus cycle whereas height of epithelium was significantly higher in luteal phase but decreased towards the layer of muscles in the terminal part of isthmus. The height of lining epithelium was significantly higher in luteal phase than follicular phase in all three segments of oviduct. In the luteal phase, apical blebs with PAS positive material were seen above lining epithelium. The cytoplasm of glandular and lining epithelium showed strong reaction with Alcian blue and mild reactivity for bound lipids with Sudan Black B. Tunica muscularis showed moderate reaction for Alcian blue showing presence of acidic mucopolysaccharides and mild reaction for bound lipids with Sudan Black B.

Keywords: Bakerwali goat, estrus cycle, histochemistry, histology, oviduct

India's vast genetic resources in goats are reflected by the availability of 20 breeds with a population of 140.5 million (18th Livestock census, 2007) which is the second largest population of goat in the world. State of Jammu & Kashmir has a population of 20.6 million goats with unique breeds. One such breed is Bakerwali which is known for its habit to migrate over long distances, strongly built body potentially to resist diseases. Bakerwali is reared by Bakerwali tribe. It is mainly found in hilly tracts of Poonch, Jammu, Rajouri, Udhampur and Kathua (FAO, 2010). The paucity of information on histology and histochemistry of oviduct of adult Bakerwali goat prompted to conduct the detailed study on its various segments during follicular and luteal phases of estrus cycle.

MATERIALS AND METHODS

Twelve genitalia of apparently healthy, non pregnant adult Bakerwali goat were collected from local slaughter houses immediately after sacrifice. These were classified into follicular and luteal phase. The specimen were fixed in 10% neutral buffered formalin for 24-48 hours and then processed for routine paraffin technique. The 5-6 µm sections were stained with Harris Hematoxylin and Eosin for routine histomorphology (Luna, 1968), Van Gieson and Verhoeff's method to differentiate Collagen and elastic fibres respectively (Mallory, 1942), 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 (Luna, 1968). 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 (Snedecor

and Cochran, 1994) to find out "t" test using 16.0 version of SPSS software.

RESULTS AND DISCUSSION

The wall of the oviduct of Bakerwali goat presented four layers viz., tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa from lumen outwards. The tunica mucosa was further subdivided into lamina epithelialis and lamina propria. Lamina propria and tunica submucosa were blended together because of absence of distinct lamina muscularis mucosae and formed propria submucosa (Fig. 1). This corresponded closely with the earlier description in goat (Joshi, 1974; Joshi *et al.,* 1977; Singh and Prakash, 1990), sheep (Singh and Madan, 1989) and other domestic animals (Trautman and Fiebiger, 1957; Dellmann and Eurell, 1998).

Tunica mucosa was characterised by presence of longitudinal folds throughout the length of the oviduct. It was highly folded and the folds were categorised into primary, secondary and tertiary folds (Fig. 2). Similar findings were reported by Trautman and Fiebiger (1957) in other domestic animals and Natarajan *et al.* (2003) in buffalos.

In follicular and luteal phase, the mucosal folds of infundibulum and ampulla were lined by pseudostratified ciliated columnar epithelium (Fig. 7) whereas isthmus was lined by non-ciliated pseudostratified columnar epithelium. Similar findings were reported by Joshi (1974); Joshi *et al.* (1977) in goat. In the present study the epithelium consisted of ciliated and secretory cells. Nayak and Ellington (1977) also reported bovine infundibular epithelium contained both secretory and ciliated cells. The height of lining epithelium was significantly higher in luteal phase than follicular phase in infundibulum, ampulla and isthmus (Table 2). This is in agreement with the findings of Shalini and Sharma (2003) in Gaddi goats. Contrary to this, Pathak *et al.* (2012) observed taller epithelium in follicular phase in sheep.

In the Bakerwali goat the epithelial apical border was irregular with numerous cytoplasmic projections. The secretory material accumulated in the supranuclear zone and also formed apical blebs along the microvillus border of the epithelium indicating apocrine mode of secretions (Fig. 3). Shalini and Sharma (2003) in Gaddi goat also reported secretory cells were having steamers or blebs in luteal phase. In follicular and luteal phase, the apical borders of lining epithelium showed strong PAS positive reaction and strong lipid reaction.

The lamina propria of the oviduct of Bakerwali goat continued with the submucosa constituting propria submucosa as there was no intervening muscularis mucosae. This layer was primarily formed of loose connective tissue, rich in blood vessels, collagen fibres (Fig. 4) and elastic fibres. Elastic fibres were sparse and seen mainly in the wall of blood vessels. Similar observations were reported by Dellmann and Eurell (1998) and Rajput and Sharma (1997) in sheep.

Oviductal glands were mainly found in tubal part of infundibulum, ampulla and isthmus. The glands were simple tubuloalveolar type formed by the invagination of lining epithelium into propria –submucosa. In follicular phase, the glands were oval or round in cross section. The lining epithelium of glands was pseudostratified columnar. The glandular diameter, epithelium height and nuclear height were significantly ($P \leq 0.05$) higher 60.45 ± 5.25 µm, 18.68 ± 0.81 µm and 7.61 ± 0.53 µm in luteal phase than 42.13 ± 4.72 µm, 16.18 ± 0.78 µm and 4.05 ± 0.36 µm in follicular phase (Table 2). The number of oviduct glands increased from infundibulum to isthmus part. Similarly the glandular diameter, epithelium and size of nucleus also showed increased portion from anterior to posterior (ampulla to isthmus) (Table. 2). Oviductal glands had higher glandular diameter, epithelium height and nuclear height in luteal phase than in follicular phase.

In follicular and luteal phases the apical borders of lining epithelium showed strong PAS positive reaction. The apical blebs were also PAS positive indicating presence of mucopolysaccriades. The secretory border and basement membrane was also moderately PAS positive (Figs. 5, 10). Similar findings were reported by Singh and Prakash (1990) in goat and Shalini and Sharma (2003) in Gaddi goat. The pseudo glands at the base of mucosal folds were also strongly PAS positive. The cytoplasm of glandular and lining epithelium showed strong reaction with Alcian blue (Fig. 11) and mild reactivity for bound lipids with Sudan Black B. The secretory material was also positive for carbohydrates and lipids (Fig. 10). The reaction was similar in infundibulum, ampulla and isthmus. Moderate lipid reaction was observed at apical borders of mucosal

folds of infundibulum (Fig. 6) where as strong lipid reaction was observed at apical borders of mucosal folds of isthmus (Fig. 12). On the contrary, Joshi *et al.* (1977) and Shalini and Sharma (2003) reported mild presence of acidic mucopolysaccharides irrespective of region but exhibited strong reaction in follicular phase in Gaddi goats.

Tunica muscularis of infundibulum contained only two or three layers of circularly arranged muscle fibres which were loosely arranged. In between muscle bundles collagen and reticular fibres were also interspersed (Fig. 4). This is in agreement with the findings of Singh and Prakash (1990) in goat and Trautman and Fiebiger (1957) in other domestic animals. In ampulla the number of circularly arranged muscle fibres increased in numbers (5 to 8) and small blood vessels also invaded between them. Elastic fibres were present only in the tunica intima of blood vessels (Fig. 8). In isthmus the muscle fibres layers were increased to 10-12, running in spiral and interwoven manner. The outer longitudinally arranged muscle layer was also observed in isthmus. Similar findings were reported by Singh and Prakash (1990) in goat.

The thickness of tunica muscularis in infundibulum was 46.20 ± 2.38 µm, 48.95 ± 1.69 µm; ampulla was 124.30 ± 3.75µm, 260.70 ± 24.62 µm and isthmus was 278.30 ± 18.63 µm, 371.80 ±18.97 µm in follicular and luteal phase respectively (Table 1). In adult Bakerwali goat the thickness of tunica muscularis increased gradually from infundibulum to isthmus. Similar trends were observed in tunica muscularis by Rajput and Sharma (1997) in Gaddi sheep, Nataranjan *et al.* (2003) in buffalo and Pathak *et al.* (2012) in sheep. Pathak *et al.* (2012) also reported increased thickness of tunica muscularis in follicular phase as compared to luteal phase.

Tunica muscularis showed moderate reaction for Alcian blue showing presence of acidic mucopolysaccharides and mild reaction for bound lipids with Sudan Black B. Similar findings were observed by Shalini and Sharma (2003) in Gaddi goats. On the contrary, Rajput (1995) in Gaddi sheep and Nataranjan *et al.* (2003) in buffalo reported mild reaction with Alcian Blue. Endothelium of blood vessels showed positive reaction for carbohydrates.

Tunica serosa comprised of mainly collagen (Fig. 9), reticular fibres and smooth muscle fibres. Fine blood vessels were also observed. Thickness of tunica serosa in infundibulum was 199.10 ±10.06 µm in luteal phase and 62.15 ± 2.90 µm in follicular phase (Table 1). The present findings are comparable with the findings of Dellman and Eurell (1998) who also observed many blood vessels and nerves in the tunica serosa of oviduct of other domestic animals. Similar findings were also observed by Rajput and Sharma (1997) in Gaddi sheep, Nataranjan *et al.* (2003) and Ayen *et al.* (2012) in buffalo.

Tunica serosa showed moderate reaction for Alcian blue indicating presence of acid mucopolysaccharides. Basement membrane underlying endothelium of blood vessels showed positive reaction with PAS. Similar findings were observed by Shalini and Sharma (2003) in Gaddi goats. On the contrary, Rajput (1995) in Gaddi sheep and Nataranjan *et al.* (2003) in buffalo reported mild reaction with PAS and Alcian Blue.

Table 1: Micrometry of oviduct of Adult Bakerwali goat in different phases of estrus cycle.

Parameters	Oviductal segments	Follicular phase	Luteal phase
Thickness of Tunica mucosa (µm)	Infundibulum	103.2 ± 3.16^{a} (77 – 120)	239.5 ± 26.09^{b} (110 – 440)
	Ampulla	269.50 ± 10.51^{a} (220 – 330)	478.50 ± 34.05^{b} (275 – 770)
	Isthmus	667.70 ± 28.73^{a} (550 – 792)	896.50 ± 63.00^{b} (605 – 1210)
Thickness of Tunica muscularis (µm)	Infundibulum	46.20 ± 2.83 (22 – 77)	48.95 ± 1.69 (33 – 55)
	Ampulla	124.30 ± 3.75^{a} (110 – 165)	260.70 ± 24.62^{b} (110 – 440)
	Isthmus	278.30 ± 18.63^{a} (220 – 385)	371.80 ± 18.97^{b} (275 – 462)
Thickness of Tunica serosa (µm)	Infundibulum	62.15 ± 2.90 (44 – 88)	199.10 ± 10.06 (132 – 297)
	Ampulla	137.50 ± 13.65 (88 – 220)	234.30 ± 24.82 (110 – 330)
	Isthmus	211.5 ± 12.11 (165 – 280)	314.05 ± 19.26 (198 – 440)

*Mean with different superscripts differ significantly (P ≤ 0.05)

Table 2: Micrometry of different components of oviduct in Adult Bakerwali goat in different phases of estrus cycle.

Parameters	Oviductal segments	Follicular phase	Luteal phase
Epithelial height (µm)	Infundibulum	24.75 ± 0.74^{a} (19.04 – 11.90)	28.92 ± 1.05^{b} (21.42 – 35.70)
	Ampulla	18.46 ± 0.91^{a} (11.90 – 23.80)	26.29 ± 1.33^{b} (16.66 – 35.70)
	Isthmus	9.76 ± 0.56^{a} (7.14 – 11.90)	19.28 ± 1.20^{b} (11.90 – 23.80)
Nucleus height (µm)	Infundibulum	8.33 ± 0.39^{a} (7.14 – 9.52)	8.57 ± 0.27^{b} (7.14 – 9.52)
	Ampulla	7.02 ± 0.37^{a} (4.76 – 9.52)	7.38 ± 0.54^{b} (4.76 – 11.90)
	Isthmus	5.24 ± 0.32^{a} (4.76 – 7.14)	7.49 ± 0.36^{b} (4.76 – 9.52)
Glandular Diameter (µm)	Infundibulum	-	-
	Ampulla	42.13 ± 4.72^{a} (23.80 – 71.40)	60.45 ± 5.25^{b} (35.70 – 83.30)
	Isthmus	66.99 ± 3.37^{a} (47.60 – 95.20)	75.21 ± 2.45^{b} (59.50 – 95.20)
Glandular Epithelial height (µm)	Infundibulum	-	-
	Ampulla	16.18 ± 0.78^{a} (11.90 – 19.04)	18.68 ± 0.81^{b} (11.90 – 23.80)
	Isthmus	20.83 ± 0.59^{a} (16.66 – 23.80)	27.61 ± 1.67^{b} (19.04 – 35.70)
Glandular Nuclear height (µm)	Infundibulum	-	-
	Ampulla	4.05 ± 0.36^{a} (2.38 – 4.76)	7.61 ± 0.53^{b} (4.76 – 11.90)
	Isthmus	8.09 ± 0.53^{a} (4.76 – 9.52)	9.52 ± 0.39^{b} (7.14 – 11.90)

*Mean with different superscripts differ significantly (P ≤ 0.05)

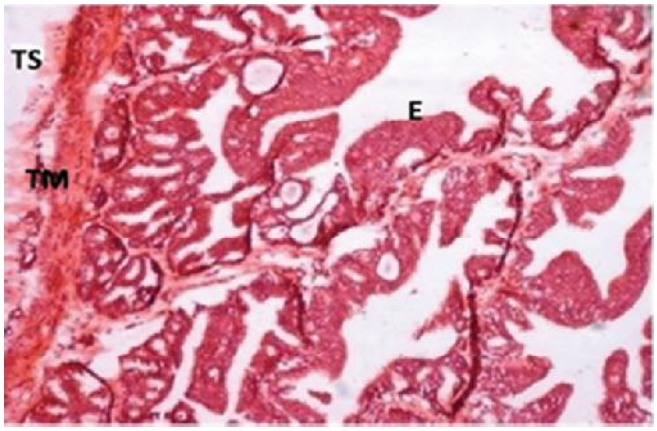

Fig. 1: Oviduct of Adult Bakerwali goat showing different layers, lamina epithelialis (E), Tunica muscularis (TM) and Tunica serosa (TS). H&E × 100

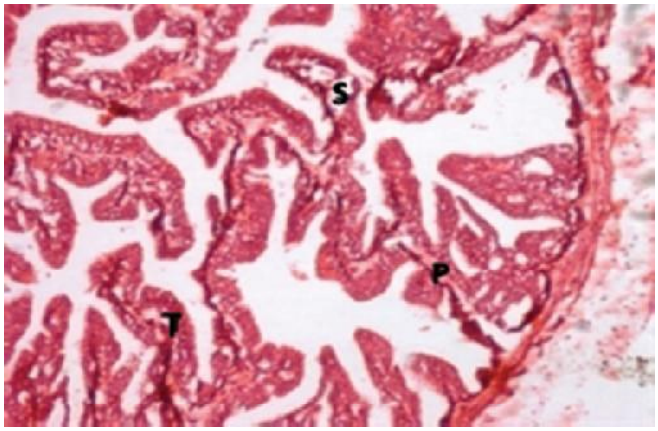

Fig. 2: Infundibulum of Adult Bakerwali goat in follicular phase showing primary (P), secondary (S) and tertiary mucosal folds (T). H&E × 100

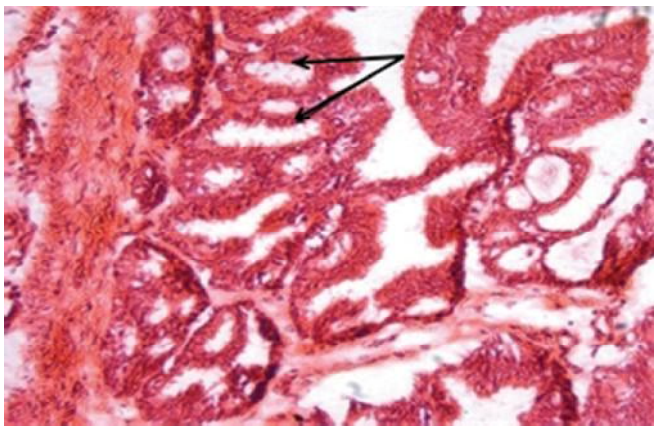

Fig. 3: Infundibulum of Adult Bakerwali goat in luteal phase showing apical blebs. H&E × 100

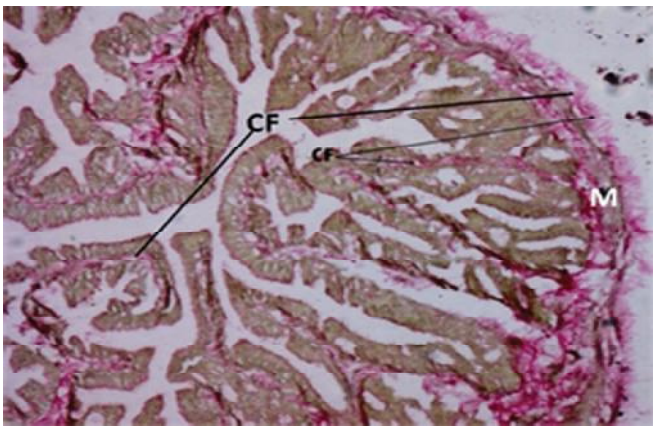

Fig. 4: Infundibulum of Adult Bakerwali goat in follicular phase showing collagen fibres (CF) in core of mucosal folds, Tunica serosa and muscle fibres (M) in Tunica muscularis. Von Geison and Verhoff's × 100

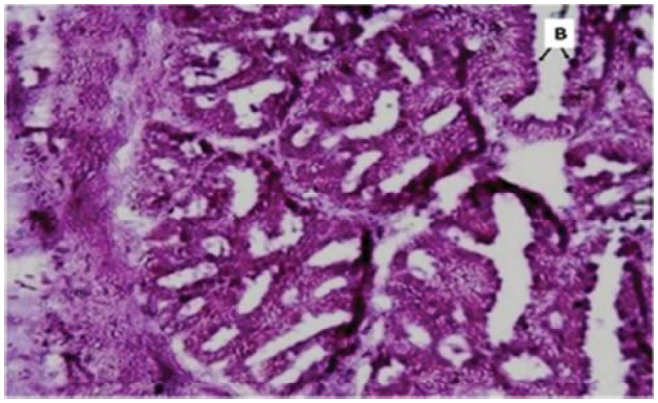

Fig. 5: Infundibulum of Adult Bakerwali goat showing PAS +ve reaction in apical boders (B). PAS × 200

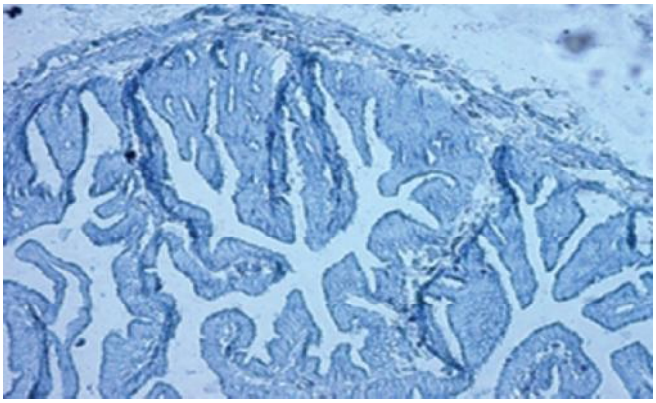

Fig. 6: Infundibulum of Adult Bakerwali goat showing mild lipid reaction at apical borders of mucosal folds. Sudan Black B × 100

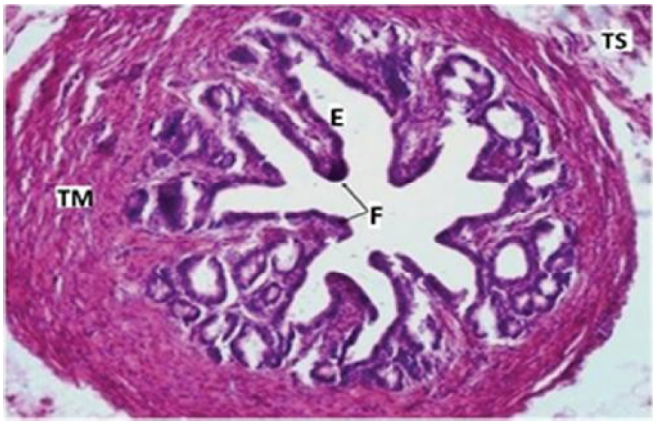

Fig. 7: Ampulla of Adult Bakerwali goat in luteal phase showing mucosal folds (F) with pseudostratified columnar epithelium (E), thick Tunica muscularis (TM) and Tunica serosa (TS). H&E × 100

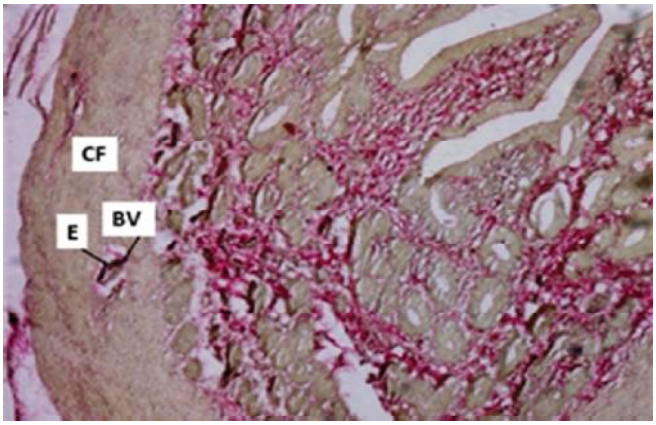

Fig. 8: Ampulla of Adult Bakerwali goat in follicular phase showing fine collagen fibres (CF) in Tunica muscularis and elastic fibres (E) in Tunica intima of blood vessel (BV). Von Geison and Verhoff's × 100

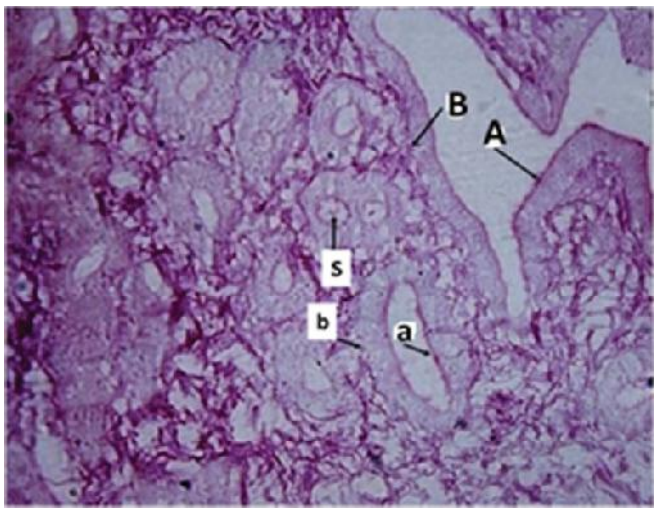

Fig. 9: Ampulla of Adult Bakerwali goat in luteal phase showing Collagen fibres (CF) in central core of mucosal folds, Tunica muscularis and Tunica serosa. Masson's Trichrome × 100

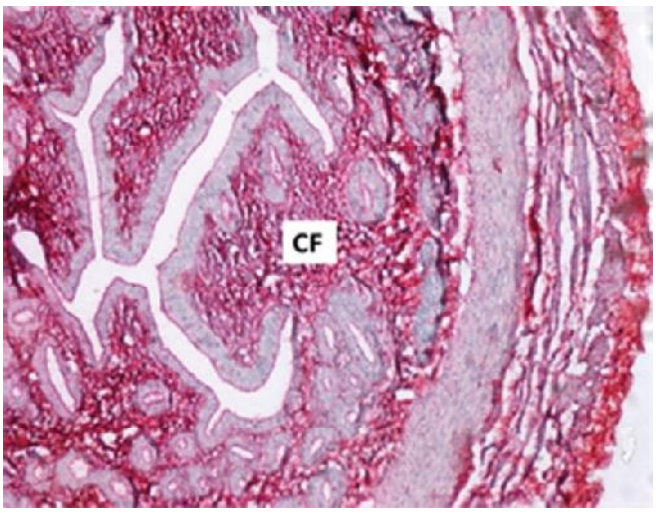

Fig. 10: Ampulla of Adult Bakerwali goat in follicular phase showing intense PAS +ve reaction for apical border of mucosal folds(A), apical border of oviductal glands (a), secretions (S) and moderate reaction for basal border of mucosal folds (B), basal border of oviductal glands (b). PAS × 200

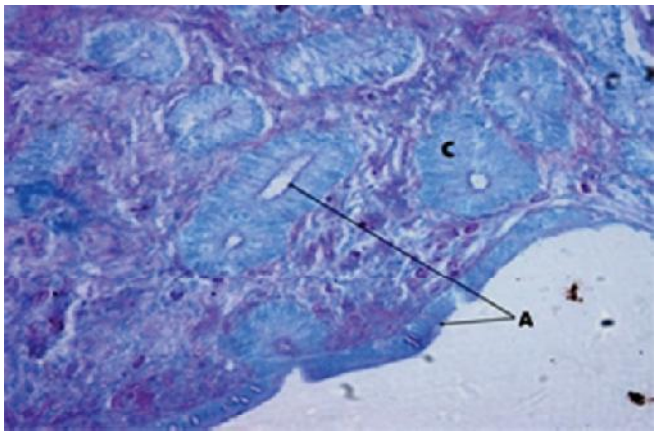

Fig. 11: Isthmus of Adult Bakerwali goat showing PAS +ve glandular and lining apical borders (A), Alcian Blue +ve glandular and lining cytoplasm. × 200

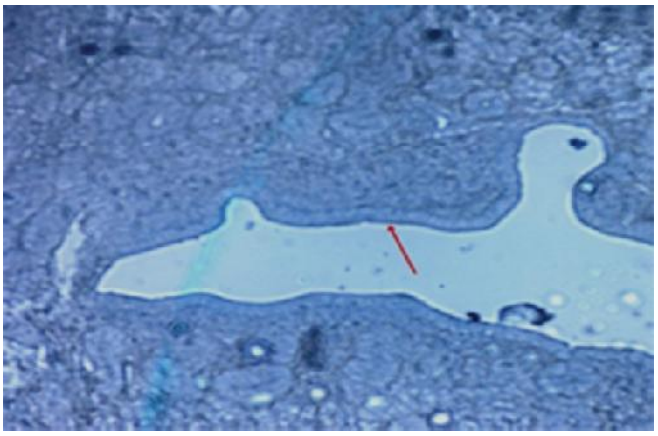

Fig. 12: Isthmus of Adult Bakerwali goat showing strong lipid reaction at apical border of lining epithelium. Sudan Black B × 100

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