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Histological and Histochemical Studies on Uterus of Adult Bakerwali Goat in Different Phases of Estrous Cycle

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ABSTRACT

The uterus (horns and body) was lined with the simple columnar to pseudostratified columnar epithelium. The mean height of the epithelium was less in follicular phase. The mean epithelial height of corpus uteri was higher than that of cornua uteri irrespective of the phase of estrous cycle but mean epithelial height was more in luteal phase than in follicular phase both in body and horn. The lining epithelium showed strong reaction for Alcian Blue. The intense PAS positivity was seen at supranuclear zone of glandular epithelium and at basement membrane. Mild lipid and cholesterol reaction was seen at apical and basal borders of lining epithelium. Endometrial glands were lined by simple columnar epithelium. In some cases these glands penetrate and reached upto stratum vasculare. Proliferation of endometrial glands were seen in follicular phase whereas in luteal phase the endometrial glands became compact and were seen in secretory phase both in uterine horns and uterine body. The glandular epithelium height also increased and showed apical blebs in the luteal phase of the cycle in Bakerwali goat. The lumina of glands in luteal phase were engorged with secretions. The luminal diameter decreased in luteal phase in both uterine horn and body. Mucosal folds were in close apposition to each other in cornua uteri as compared to corpus uteri. Endometrial glands were more in corpus uteri than in cornua uteri. The thickness of tunica muscularis was significantly higher in luteal phase and increased towards the uterine body.

HIGHLIGHTS

- The height of the epithelium was less in follicular phase and increased towards the corpus uteri.
- Proliferation of endometrial glands was seen in follicular phase.

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

The uterus of goat is bicornuate type and occupies prime position as it is responsible for sperm transport, luteolysis and control of cyclicity, provides an environment for nourishment of the zygote. It modifies itself to accept the conceptus and to provide protection throughout pregnancy and also helps in expulsion of foetus and foetal placenta at time of parturition. In the horn and body of the uterus, the wall is divided into the *Tunica mucosa* (endometrium), *Tunica submucosa* (myometrium), *Tunica muscularis* (perimetrium) and *Tunica serosa* (Hafez and Hafex, 2000). Despite its importance that it affects the fertility, the volume of work done on the uterus with respect to different phases of estrus cycle, especially in Bakerwali goat is very marginal, that's why the study was planned.

MATERIALS AND METHODS

Fifty genitalia of apparently healthy, non-pregnant cyclic adult Bakerwali goats were collected from local slaughter

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houses immediately after sacrifice. The samples were classified into follicular and luteal phase depending upon the status of ovary (06 samples in each group). The specimen were fixed in 10% neutral buffered formalin for 24-48 hours and then processed for routine paraffin technique. The 6 µm sections were stained with Harris Hematoxylin and Eosin for routine histomorphology (Luna, 1968), Van Gieson and Verhoeff's and Masson's Trichrome method to differentiate Collagen and elastic fibres respectively (Mallory, 1942), Gomori's method to demonstrate reticular fibres. For histochemical studies the sections were stained by Periodic acid Schiff method (PAS) to demonstrate carbohydrate, Alcian Blue - PAS method (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).

RESULTS AND DISCUSSION

The uterine wall comprised of endometrium, myometrium and perimetrium as described by Bhattacharya and Saigal (1984), Singh and Prakash (1990), Uppal and Roy (2002) in buffalo and Poyam *et al.* (2011) in goat. The tunica mucosa was thickest layer of uterine horn and uterine body. It comprised of lamina epithelialis and propria submucosa. The uterine cavity irrespective of horns and body was lined with the simple columnar to pseudostratified columnar epithelium (Fig. 1 & 2). Similar observations were recorded by Sundarvadanan and Venkatswami (1973) and Sweta *et al.* (1993) in buffalo and Poyam *et al.* (2011) in goat reported pseudostratified columnar epithelium. The epithelium contained the columnar cells, goblet cells and basal cells. The basement membrane was reticular in nature.

In the present study the mean epithelial height was higher in luteal phase (29.35 ± 0.85 µm in uterine body and 22.29 ± 0.85 µm in uterine horn) than in follicular phase (27.69 ± 1.04 µm in uterine body and 20.30 ± 0.89 µm in uterine horn) irrespective of region of uterus. This could be attributed to the effect of progesterone during luteal phase after estrogen priming in follicular phase. The mucosal folds lie in close opposition to each other in uterine horns as compared to corpus uteri (Fig. 1). The lining epithelium invaginated into propria submucosa forming endometrial glands (Fig. 8). These endometrial glands were more in the corpus uteri than in cornuae. Similar findings were reported by Trautmann and Fiebiger (1957), Dellmann and Eurell (1998) in other domestic animals and Suri and Sharma (2005) in Gaddi goat.

The Lamina propria submucosa comprised of loose connective tissue as also reported by Singh and Prakash (1990) in goat, Uppal and Roy (2002) in buffalo and Suri and Sharma (2005) in Gaddi goat. The propria was occupied by the glands which were few in superficial zone (zona spongiosum) and compactly arranged in deep zone (zona compactum) (Fig. 9). The endometrial stroma composed of mainly collagen fibres and reticular fibres which also surrounded the endometrial glands and their proportion progressively increased towards the tunica muscularis. The lining epithelium showed strong reaction for Alcian Blue indicating presence of AMPS and PAS positive reaction was seen only in the basement membrane of lining epithelium (Fig. 4). Contrary to this, Joshi *et al.* (1983) and Roy and Saigal in goats (1986) observed strong PAS reaction at apical and basal borders of lining epithelium. Mild lipid and cholesterol reaction was seen at apical and basal borders of lining epithelium in the present study irrespective of uterine cornuae and corpus uteri as also reported by Shalini (1997) in Gaddi goat.

Endometrial glands were simple, coiled tubular glands formed due to invagination of the endometrial lining epithelium (Fig. 3) as reported by Trautmann and Fiebiger (1957) and Dellmann and Eurell (1998) in other domestic animals. They were lined by simple columnar epithelium. This is in agreement with the observations of Ramchandraiah *et al.* (1980a) in sheep, Singh and Prakash (1990) in goat, Suri and Sharma (2005) in Gaddi goat and Poyam *et al.* (2011) in goat. Glands reached up to the inner circular muscle layer of tunica muscularis. In some cases these glands penetrate and reach stratum vasculare as also reported by Suri and Sharma (2005) in Gaddi goat. Proliferations of glands were seen more in follicular phase whereas in luteal phase the endometrial glands became compact (Fig. 6) and were seen in secretory phase both in uterine horn and uterine body. Similar findings were reported by Sundarvadanan and Venkatswamy (1973) in buffalo. The glandular epithelium height also increased in luteal phase due to secretory activity. The glandular epithelium, nuclear height and glandular diameter in uterine horn and body were significantly higher in luteal phase than in follicular phase of estrous cycle.

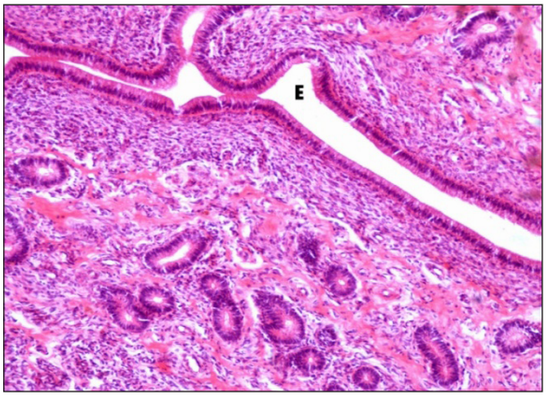

Fig. 1: Uterine Horn of Adult Bakerwali goat showing simple columnar epithelium (E). H&E × 100

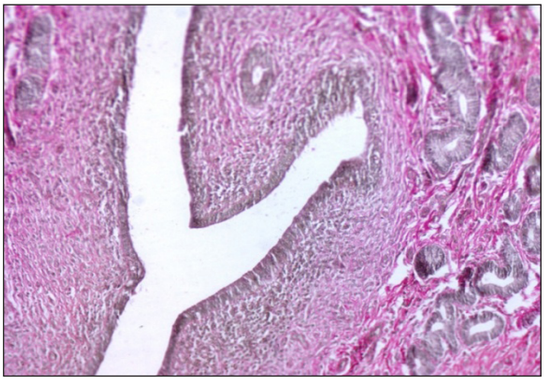

Fig. 2: Uterine Horn of Adult Bakerwali goat showing collagen fibres around the endometrial glands. Von Geison & Verhoff's × 100

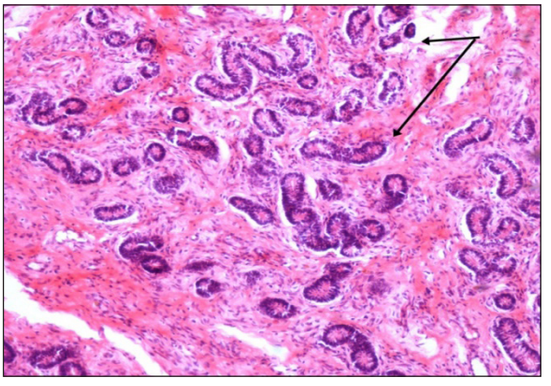

Fig. 3: Uterine Horn of Adult Bakerwali goat showing proliferative phase in deep zone of endometrial glands (arrow). H&E × 100

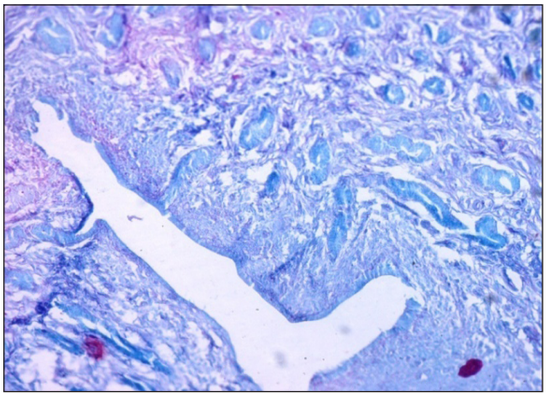

Fig. 4: Uterine Horn of Adult Bakerwali goat showing strong Alcian Blue reaction in lining epithelium. PAS plus Alcian Blue (pH-2.5) × 100

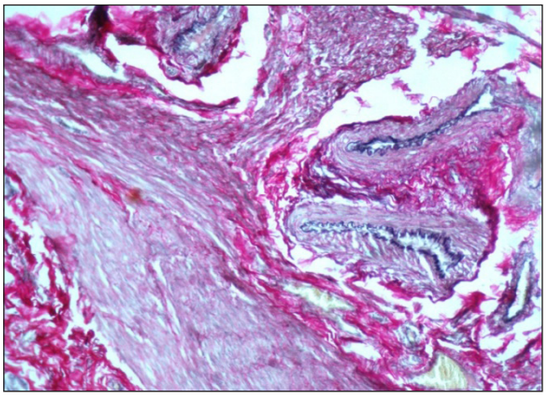

Fig. 5: Uterine Horn of Adult Bakerwali goat showing inner circular layer of myometrium and stratum vasculare. Von Geison and Verhoff's × 100

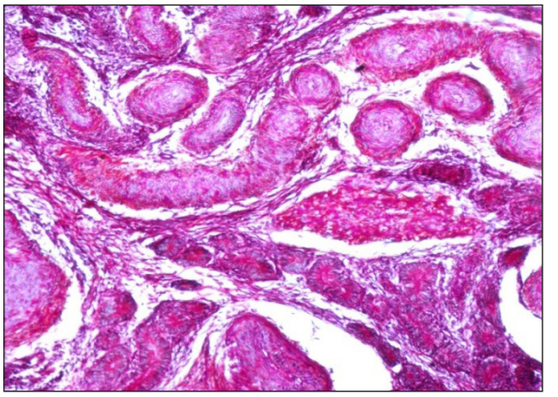

Fig. 6: Uterine Horn of Adult Bakerwali goat showing endometrial glands and blood vessels in the vascular layer. Masson's trichrome × 200

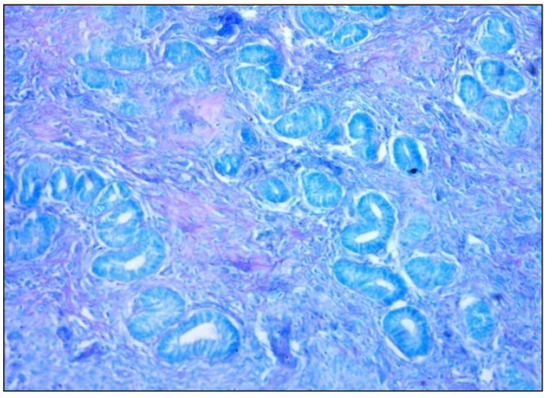

Fig. 7: Uterine Horn of Adult Bakerwali goat showing Alcian Blue +ve reaction in cytoplasm of glands. PAS plus Alcian Blue (pH-2.5) × 100

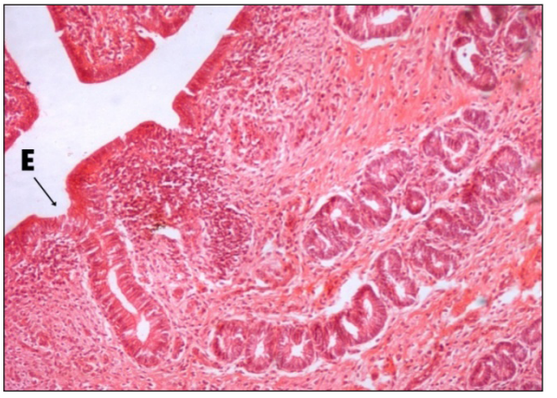

Fig. 8: Uterine body of Adult Bakerwali goat showing proliferation of glands (E) in follicular phase. H&E × 100

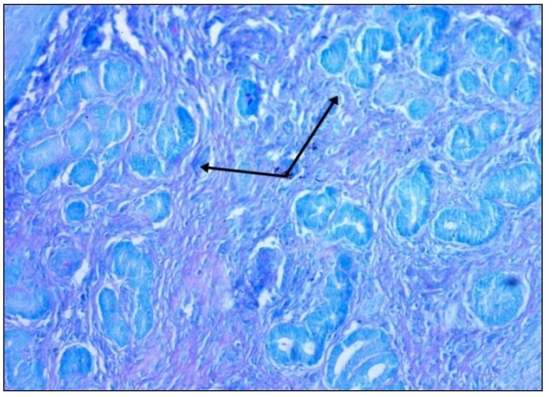

Fig. 9: Uterine body of Adult Bakerwali goat showing compact zone of endometrial glands (arrow) in follicular phase. PAS & Alcian Blue (pH-2.5) × 100

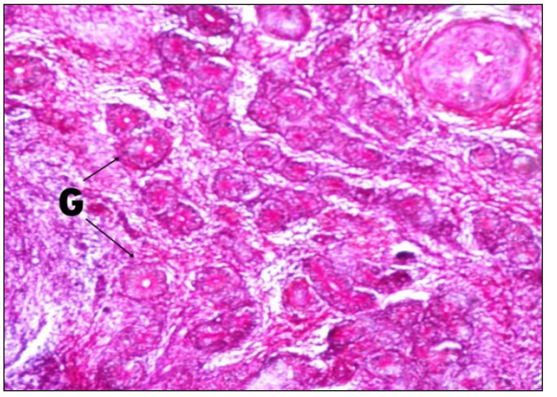

Fig. 10: Proliferative phase of endometrial glands (G) showing strong PAS reaction at apical borders. PAS × 100

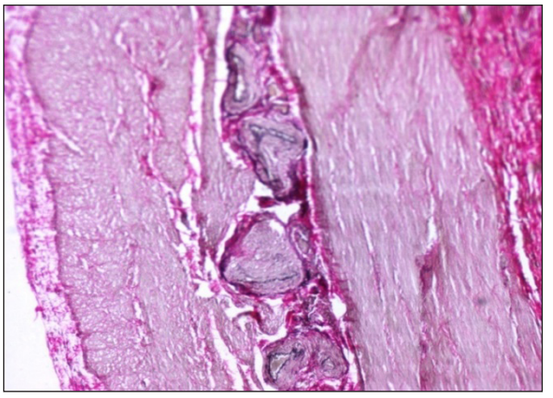

Fig. 11: Uterine body of Adult Bakerwali goat showing elastic fibres. Von Geison and Verhoff's × 100

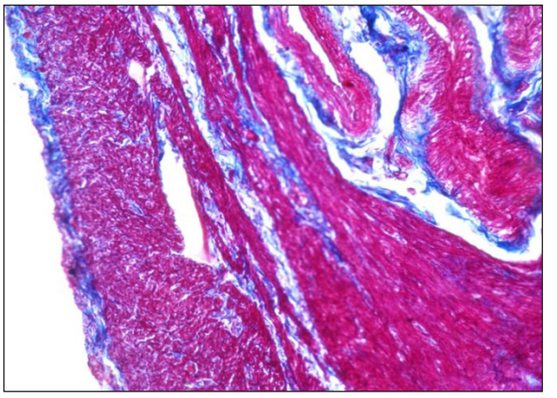

Fig. 12: Uterine body of Adult Bakerwali goat showing presence of collagen fibres. Masson's Trichrome × 100

The glandular diameter was recorded as 107.43 ± 2.87 µm in follicular phase and 125.40 ± 5.74 µm in luteal phase and was also comparatively higher in corpus uteri than in cornua uteri. The luminal diameter was significantly higher in follicular phase than in luteal phase of estrus cycle indicating higher epithelium due to secretary activity of luteal phase. Ramchandraiah *et al.* (1980a) in sheep and Suri and Sharma (2005) in Gaddi goat observed increased glandular epithelium height, luminal and glandular diameter in uterine horn and body during luteal phase. In present study luminal diameter was higher in follicular phase it may be because the lumina of glands were engorged with secretions and glandular epithelium also showed apical blebs in the luteal phase of the cycle in Bakerwali goat. Alcian Blue positive reaction was seen in cytoplasm of glandular epithelium indicating presence of acidic mucopolysaccharides irrespective of cycle and region of the uterus (Fig. 7). On the contrary, Dhale (2001) and Poyam *et al.* (2011) showed moderate to intense activity of PAS-Alcian blue (2.5 pH) in mucosal and glandular epithelium of horn and body of uterus. In luteal phase PAS positive reaction was seen in supranuclear zone of the gland and in basement membrane of glandular epithelium whereas reactivity was more intense in the endometrial glands of uterine body (Fig. 10) as also observed by Shalini (1997) in Gaddi goat. Contrary to this, Sundarvadanan and Venkatswamy (1973) observed a broader zone of PAS positive material during the follicular phase than in the luteal phase and superficial glands were strongly reactive as compared to basal glands.

The tunica muscularis comprised of two layers namely inner circular and outer longitudinal layer of smooth muscles. The inner circular layer in its outer third contained a very well developed vascular layer (stratum vasculare) (Fig. 5). This is in agreement to findings of Trautmann and Fiebiger (1957) and Dellmann and Eurell (1998) in other domestic animals and Uppal and Roy (2002) in buffalo. It was thicker in uterine body as compared to uterine horns. The muscle bundles were arranged in form of several interwoven sheets. Collagen fibres were observed in outer layer and more in uterine horn. The reticular fibres were fine and few. Elastic fibres were reported only around the blood vessels as also observed by Shalini (1997) in Gaddi goat (Figs. 11, 12). The tunica muscularis was moderately positive for glycogen and mild for bound lipids and cholesterol irrespective of phase of cycle and region of uterus. This confirmed the observations of Bhattacharya and Saigal (1984) in goat's myometrium.

The *Tunica serosa* comprised of loose connective tissue layer covered by mesothelial cell layer. The fibro-architecture comprised mainly of collagen fibres. Few elastic fibres and reticular fibres could be seen in the tunica serosa. The thickness of *Tunica serosa* was higher in luteal phase in uterine horn and body. On contrary, Agarwal and Laloraya (1978) in mammals did not find elastic fibres and showed increased thickness of perimetrium in follicular phase. The tunica serosa showed mild reactivity for PAS indicating mild presence of carbohydrates and bound lipids. Similar findings were observed by Rajput (1995) in Gaddi sheep. Intima of blood vessels showed reactivity with Alcian blue. Very mild reaction was seen for cholesterol in both luteal and follicular phase in horn and body of uterus.

CONCLUSION

Proliferation of endometrial glands was observed in follicular phase whereas in luteal phase they were compact. Due to sceretory activity epithelial height increased and lumen of glands were engorged during luteal phase. The histomorphological parameters viz. glandular epithelial height, corresponding nuclear height and glandular diameter were recorded significantly higher during follicular phase. Strong positive lipid reactivity was seen in glandular epithelium of corpus uteri in follicular phase whereas strong PAS activity was observed in basement membrane of endometrial glands during luteal phase.

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