

Histomorphology of Endometrium during Follicular and Luteal Phases of Estrous Cycle in Umblachery Cow

S. Sakemohammed¹, S. Paramasivan^{*2}, S. Sivagnanam³, S. Sathesh Kumar⁴, N.Narmatha⁵ and A.S.Selvaramesh⁶

Department of Veterinary Anatomy, Veterinary College & Research Institute, Orathanadu – 614 625, Thanjavur
Tamil Nadu Veterinary and Animal Sciences University

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ABSTRACT

The histomorphological study was performed on the endometrial tissue obtained by biopsy from adult Umblachery breed of cows reared at District Livestock Farm, Korukkai, Thiruvarur district of Tamil Nadu. The endometrium of uterus consisted of four distinct layers from within outwards namely, lamina epithelialis, stratum compactum, stratum spongiosum and stratum basalis. The endometrium was lined with simple columnar epithelium during follicular phase with the occasional patches of pseudostratified epithelium in between the folds of endometrium, while a typical pseudostratified columnar type was observed during luteal phase of estrous cycle. The endometrial glands were relatively more in number in stratum basalis than in stratum spongiosum during follicular phase of estrous cycle, and further increased in numbers during luteal phase. The endometrial glands were highly coiled and branched during the luteal phase as compared to follicular phase. The histomorphological and micrometrical parameters confirmed that the endometrium was more active and secretory during luteal phase than the follicular phase of estrous cycle in Umblachery cow.

Keywords: Histomorphology, Endometrium, Oestrous Cycle, Umblachery Cow

INTRODUCTION

The Umblachery cattle, a medium to small sized breed indigenous to the coastal districts of Tamil Nadu, India, namely Thiruvarur, Nagapattinam and Thanjavur, has long been celebrated for its remarkable strength and robustness (Rajendran *et al.*, 2008). With an estimated population of 2,83,000 within its breed tract, the Umblachery cattle embodies the essence of resilience and adaptability. The reproductive system of the Umblachery cattle is a corner stone of its genetic potential and sustainability. This system, comprising intricate structures and processes, holds the key to the breed's continued prosperity and genetic diversity.

In mammals, the endometrium of the uterus

plays a critical role in fertility and adapts through various phases of the estrus cycle. The uterus not only influences ovarian structures but also actively participates in different physiological events, including the production of uterine milk, creating a conducive environment for sperm and accommodating vital nutritional conditions for blastocyst development (Shahrooz *et al.*, 2013). The functional layer of the endometrium undergoes specific molecular, enzymatic, morphological and structural changes under the influence of ovarian hormonal fluctuations during the estrus cycle.

Surprisingly, there is a dearth of information on anatomy of female reproductive organs in white cattle and no reports are available on Umblachery cow as this animal is under conservation and also the slaughter of cow is banned in Tamil Nadu. Hence, this study is designed with the aim to investigate the histomorphology of the female reproductive tract during the follicular and luteal phases of the estrous cycle.

1. PG Scholar, Dept. of Veterinary Anatomy, VCRI, Orathanadu - 614 625. 2. Professor and Head, Dept. of Veterinary Anatomy, VCRI, Udumalpet, 3. Associate Professor and Head, Dept. of Veterinary Anatomy, VCRI, Theni, 4. Dean, Faculty of Basic Sciences, TANUVAS, 5. The Dean, VCRI, 6. Assistant Professor and Head, Dept. of Animal Genetics and Breeding, VCRI.

*Corresponding Author E-mail: paramsanatomy@gmail.com

MATERIALS AND METHODS

The adult Umblachery breed of cows reared at District Livestock Farm, Korukkai, Thiruvavur district of Tamil Nadu were utilised for the study. The histomorphological studies on the endometrium of Umblachery cattle were conducted on two groups, Group I : follicular phase (n=10) and Group II : luteal phase (n=10) of estrous cycle. Based on the presence of dominant ovarian follicles and corpus luteum in the ovary examined through per rectal palpation and further confirmed by ultrasonographic examination, the phases of the estrous cycle were determined (Satheshkumar and Kathiresan, 2018).

The uterine tissue samples were collected by using Albuchin's uterine biopsy catheter. The endometrial biopsy samples collected were washed in normal saline and fixed in various fixatives *viz.*, 10 per cent neutral buffered formalin (NBF), Bouin's fluid and Zenker's solution for further procedures to study the histomorphological and histochemical features of female reproductive tract in Umblachery cows.

After complete fixation was achieved, the collected tissues were processed by routine alcohol-xylene schedule and paraffin blocks were prepared as per the standard histological procedures (Singh and Sulochana, 1996). The tissue sections were cut at 3-5 μm thickness using Leica semi-automatic rotary microtome. The paraffin sections were subjected to various standard staining methods *viz.*, Haematoxylin and Eosin, Modified Mallory's stain, Verhoeff's method for elastic fibres, Gomori's method for reticular fibres, Masson's trichrome and combined Alcian blue-PAS technique (Bancroft and Stevens, 1996) and the observations on normal histomorphology and histochemistry were recorded.

RESULTS AND DISCUSSION

The endometrium of uterus histologically consisted of four distinct layers from within outwards namely, lamina epithelialis, stratum compactum, stratum spongiosum and stratum basalis (Fig. 1). The later 3 layers combined formed the lamina propria connecting the subjacent myometrium. The thickness of endometrium in Umblachery cow was recorded as $1654.2 \pm 105.503 \mu\text{m}$ during follicular phase which increased to $1753.2 \pm 162.84 \mu\text{m}$ during luteal phase of estrous cycle (Table 1).

1. Lamina epithelialis

The endometrium was lined with simple columnar epithelium during follicular phase with the occasional patches of pseudostratified epithelium was also noticed in between the folds of endometrium. The observations are in corroboration with Jagan *et al.* (2022) in indigenous cattle who observed that the epithelium may be simple columnar or pseudostratified columnar. In contrast to the current observation, Karimi *et al.* (2017) in buffalo noticed the pseudostratified columnar epithelium over the entire surface of endometrium.

The simple columnar epithelium in the follicular phase showed tall columnar cells with vesicular elongated basal nucleus and basal cells close to the basement membrane which were oval to round in shape with thin cytoplasm and condensed spherical nucleus (Fig.2). The basal location of these cells in between tall columnar cells gave the appearance of pseudo-stratification. However, tall columnar type of epithelium observed in the endometrium during follicular phase changed to typical pseudostratified columnar type (Fig.3) during luteal phase as reported by Wang *et al.* (2007) in bovines. The cells appeared as arranged into three to four layers in the epithelium. The cells were tightly packed with oval shaped nuclei located at basal half of the cells leaving more cytoplasm at luminal side of the cells. The cytoplasm of these cells was acidophilic and packed with coarse secretory granules.

The surface epithelium over the uterine caruncles also showed simple columnar epithelium in follicular phase and pseudostratified columnar epithelium in luteal phase and appeared same as that of inter-caruncular area of uterus. In contrast, the lining epithelium was simple columnar without any ciliated cells over the caruncles in African buffaloes as observed by Schmidt *et al.* (2006). The differences in the height and structure of epithelial cells are attributed to the variation in secretory activity during different phases of estrous cycle, as well as differences in species and breed of animals.

The apical border of these epithelial cells often displayed modifications like striated borders, cilia, or secretory blebs, indicating their secretory or absorptive nature (Fig. 3). The average thickness of the surface epithelium of endometrium was $11.1 \pm 0.99 \mu\text{m}$ during follicular phase and the value increased to $18.2 \pm 1.17 \mu\text{m}$ during the luteal phase

of estrous cycle. Similarly, Katare *et al.* (2015) reported the increase in epithelial height during luteal phase of estrous cycle in goats.

2. Stratum compactum

The stratum compactum was noticed just beneath the surface epithelium made up of dense cellular arrangement intermingled with the collagen, reticular and elastic fibres (Fig. 4). The fibroblasts, neutrophils, plasma cells and mast cells were displayed in stratum compactum during both the phases of estrous (Uppal and Roy, 2002). Bonnett *et al.* (1991) also have noticed the stratum compactum in the endometrial biopsy tissues collected from Holstein-Friesian dairy cows and reported the occurrence of mononuclear cells in great numbers in this layer. The number and functions of these cells are correlated well with the inflammatory changes during follicular phase and postpartum involution.

The thickness of this subepithelial layer increased with more amount of connective tissue fibres oriented parallel to the basement membrane of epithelium along with high infiltration of connective tissue cells during follicular phase. Whereas, the thickness of fibrous layer decreased substantially due to the formation of endometrial glands in expanded stratum spongiosum during luteal phase (Fig.5).

3. Stratum spongiosum

The stratum spongiosum of endometrium was richly vascular and consisted of a loose connective tissue with many fibrocytes, macrophages and mast cells, eosinophils, lymphocytes and plasma cells as reported by Uppal and Roy (2002) in buffaloes. In follicular phase, this layer of lamina propria was oedematous, more vascular and particularly pervaded with more leucocytes, plasma cells and mast cells (Fig. 6). Jagan *et al.* (2022) in indigenous cattle who explained two different layers in the endometrium as outer functional zone and inner basal zone based on the number of glands present.

4. Stratum basalis

The stratum basalis was densely cellular and consisted of many small to large blood vessels and closely packed uterine glands. These observations are in agreement with the findings reported by Eurell and Frappier (2006) in ruminants and Bandyopadhyay *et al.* (2007) in bovine. The stratum basalis was observed as intensely stained areas with

numerous coiled tubular endometrial glands surrounded by connective tissue stroma. In the deeper zone of the endometrium, the uterine glands were more coiled as compared to the glands present in the superficial zone (Fig. 6). The endometrial glands were relatively more in number in stratum basalis than in stratum spongiosum during follicular phase, but were distributed evenly in increased numbers during luteal phase in Umblachery cows.

5. Endometrial glands

The endometrial glands in Umblachery cow were noted as simple tubular, coiled tubular and occasionally branched in type which were distributed in the stratum spongiosum and stratum basale of endometrium (Fig. 1,6). Uppal and Roy (2002) have also observed these glands as coiled tubular in cyclic buffaloes but were simple tubular type of glands in neonatal calves and prepubertal buffaloes. The branched tubular glands were showing coiled terminal ends towards the deeper zones. The uterine glands were well developed with multiple branching and initiation of secretory activity during luteal phase as also reported by Bandyopadhyay *et al.* (2007) in bovines.

The occurrence of tubular sections of the superficial glands were greater with a wider lumen in stratum spongiosum. They were lined with epithelium that consisted of either pseudostratified tall columnar or simple tall columnar. The basal uterine glands, on the other hand, more in number, smaller in cross section and had a narrower lumen during follicular phase of estrous cycle indicating the proliferative stage as reported by Espejel and Medrano (2017) in dairy cows.

The endometrial glands were highly coiled and branched during the luteal phase as compared to follicular phase (Fig. 7). The tubular glands located in stratum spongiosum grow and radiate into many branched with terminal coils in the stratum basale was also reported by Yamaguchi *et al.* (2021) in human endometrium. The lining epithelial cells of uterine glands in the stratum spongiosum were tall columnar to pseudostratified type and in the stratum basalis they were simple columnar type during the follicular phase. These glandular cells were tall columnar or stratified columnar type during luteal phase and packed with more amount of secretory materials in the apical half and nuclei were restricted at the basal half (Fig. 8). This is in agreement with the reports of Espejel and Medrano (2017). The

diameter of endometrial glands was $40.5 \pm 1.10 \mu\text{m}$ in stratum spongiosum and $26.2 \pm 1.28 \mu\text{m}$ in stratum basale during the follicular phase but the same parameters increased to $52.9 \pm 4.17 \mu\text{m}$ in stratum spongiosum and $47.8 \pm 3.36 \mu\text{m}$ in stratum basale during the luteal phase of the estrous cycle.

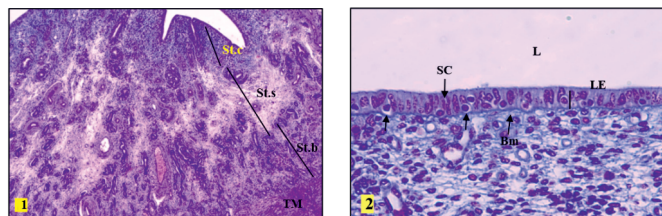


Fig. 1. Endometrium of cow during follicular phase of estrous cycle, LE - Lamina epithelialis, St.c - Stratum compactum, St.s - Stratum spongiosum, St.b - Stratum basale, TM - Tunica muscularis. **H&E x 40.** **Fig. 2.** Simple columnar epithelium with columnar cells and basal cells (arrow) in endometrium of Umblachery cow during follicular phase. LE - Lamina epithelialis, SC - Simple columnar epithelium, Bm - Basement membrane, L - Lumen. **Masson's trichrome x 400.**

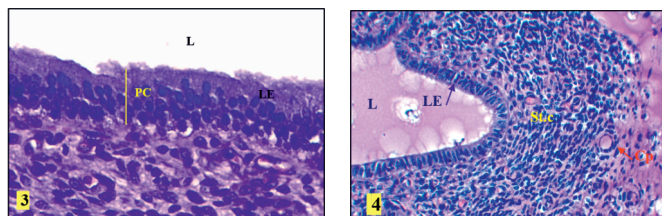


Fig. 3. Pseudo stratified columnar epithelium over the endometrium of Umblachery cow during luteal phase. LE - Lamina epithelialis, PC - Pseudo stratified columnar epithelium, L - Lumen. **H&E x 400.** **Fig. 4.** Surface epithelium of endometrium showing invagination to form uterine glands. Cp - Capillaries, L - Lumen, LE - Lamina epithelialis, St.c - Stratum compactum. **H&E x 100.**

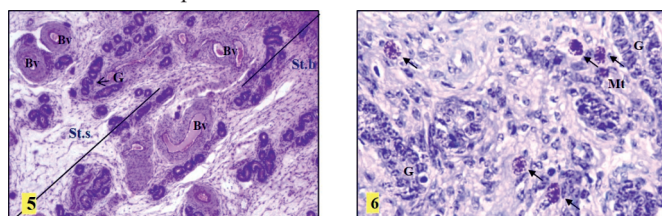


Fig. 5. Endometrial glands and blood vessels in endometrium during luteal phase in Umblachery cow, St.s - Stratum spongiosum, St.b - Stratum basale, G - Endometrial glands, Bv - Blood vessels. **H&E x 40.** **Fig. 6.** Presence of mast cells in endometrium during follicular phase of Umblachery cow, Mt - Mast cells, G - Endometrial glands. **Toluidine blue x 400.**

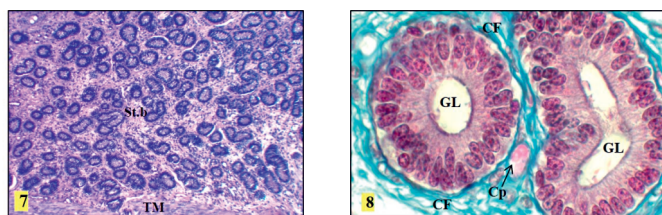


Fig. 7. Coiled endometrial glands in the stratum basale during luteal phase in Umblachery cow, St.b - Stratum basale, TM - Tunica muscularis. **H&E x 40.** **Fig. 8.** Pseudo stratified columnar epithelium of endometrial glands during luteal phase in Umblachery cow, GL - Glandular lumen, Cp - Capillaries, CV - Collagen fibres. **Modified Mallory's x 1000.**

Table: 1. Micrometrical parameters of endometrium during follicular and luteal phases (μm)

S. No.	Micrometrical parameters	Follicular phase	Luteal phase	P Value
1	Thickness of endometrium	1654.2 ± 105.503	1753.2 ± 162.84	0.285
2	Height of uterine epithelium	11.1 ± 0.99	18.2 ± 1.17	0.197
3	No. of glands in Stratum spongiosum	22.7 ± 2.75^a	36.0 ± 3.59^b	0.004**
4	No. of glands in Stratum basale	86.7 ± 10.00	74.6 ± 10.25	0.204
5	Diameter of glands in Stratum spongiosum	40.5 ± 1.10^a	52.9 ± 4.17^b	0.009**
6	Diameter of glands in Stratum basale	26.2 ± 1.28^a	47.8 ± 3.36^b	0.00001**
7	Luminal diameter of glands in Stratum spongiosum	11.9 ± 0.56^a	20.7 ± 2.70^b	0.002**
8	Luminal diameter of glands in Stratum basale	14.0 ± 1.36	15.7 ± 3.47	0.327
9	Epithelial density of glands in Stratum spongiosum	21.7 ± 1.52^a	34.5 ± 3.40^b	0.001**
10	Epithelial density of glands in Stratum basale	22.4 ± 1.35^a	37.7 ± 2.47^b	0.000019**

The abundant glands with more coiled nature, wide lumen with more secretory materials appeared as released into the lumen of the glands are indicative of the secretory luteal phase of estrous cycle and well correlated with the influence of progesterone. Similar findings were also supported by Ohtani *et al.* (1993) in bovine endometrial cells. The micromorphological observations confirmed that the endometrium was more active and secretory during luteal phase than the follicular phase in Umblachery cow. The histomorphological findings of this study will serve as a baseline information for understanding the reproductive physiology and

pathology of Umblachery cattle and helpful for implementing reproductive strategies for conservation of this indigenous breed.

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