

HISTOLOGICAL STUDY OF UTERUS DURING VARIOUS PHASES OF ESTROUS CYCLE IN OSMANABADI GOAT

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Abstract: The height of epithelium appeared to be increased from proestrous to diestrous. The height and thickness of mucosal folds appeared minimum in diestrous phase as compared to other phases of estrous cycle. The endometrial glands were interspersed in the highly vascular stroma. The number of uterine glands per cubic mm showed significant decreasing trend from follicular phase to luteal phase. The glands appeared straight with narrow lumen at the end of proliferative phase while the cellular hypertrophy caused wideness in the lumen of gland during luteal phase. Highest numbers of endometrial glands were present in proliferative phase as compared to luteal phase. The cells in the glands showed increasing pattern from follicular to luteal phase. Thickest endometrium was seen in follicular phase than the luteal phase of reproductive cycle.

Keywords: Histology, Uterus, phases of estrous, Osmanabadi Goat.

INTRODUCTION

There is very close relationship between fertility and functions of genital organs and hormonal statuses and histological and histochemical changes of reproductive organs. Considering the importance of uterus in reproduction, the present study is undertaken to study the histology of uterus in Osmanabadi goat during various stages of estrous cycle.

MATERIAL AND METHODS

The study was conducted on 24 healthy Osmanabadi goats at different stages of estrous cycle. The genitalias were brought to the laboratory of the department of Anatomy in ice pack thermos for the further study. The complete genital tract was cleaned, cleared off its fascia and washed in normal saline. It was preserved in ice in order to examine it critically. The stage of estrous of the animal was determined by the expertise opinion of Gynecologists on the basis of the surface morphological features of the ovaries. The presence of graffian follicle, corpus luteum and blood clot of ruptured follicle on the surface of the ovary was used as the criteria for classification of phases of estrous as follicular, luteal and the stages of cycle as proestrous, estrous, metestrous and diestrous [plate-1, 2,3,4] as per the method suggested by Roberts [1971]. The detail method of classification was as under.

[A] Follicular Phase

Group-I: Proestrous: The ovaries with matured follicles clearly visible and slightly bulging on the surface.

Group-II: Estrus: The ovaries with graffian follicles intensely projecting on the surface.

[B] Luteal phase

Group III: Metestrous: The ovaries having blood clots at the site of ruptured follicles on the surface.

Group IV: Diestrous: The ovaries with corpus luteum bulging on the surface.

After classification and identification of the phase of estrous, the genitalia were spread flatly in the enamel tray. The tissue pieces of 3-5mm thickness were cut from different parts of uterus and preserved in 10% neutral buffered formaline.

The tissue preserved in 10% neutral buffered formaline were then processed for routine paraffin embedding method and were cut at 3-5 μ thickness. [Singh and Sulochana, 1978]. For sectioning, manually operating rotary microtome was used. Following methods were used for staining these sections. Harri`s haematoxylin and eosin for general histological observations [Mukherjee, 1990]. Crossman`s modification of Mallory`s triple stain for muscle, collagen, and elastic fibers [Singh and Sulochana, 1978].

Masson`s Trichrome stain for collagen and muscle fiber [Mukherjee, 1990] Verhoeff`s stain for collagen and elastic fibers [Mukherjee, 1990] Silver impregnation stain method [Singh and Sulochana, 1978]. The micrometry on histological structure of uterus from the stained sections was conducted by using ocular micrometer.

The data obtained were subjected to statistical analysis as per standard procedure adopted by [Panse and Sukhatme, 1967].

RESULT AND DISCUSSION

In the present study, histological observations were made on the uterus of Osmanabadi goat during various phases of estrous cycle i.e. proestrous, estrous, metestrous and diestrous.

The histoarchetecture of uterus in Osmanabadi goat comprised of three layers from within outward as endometrium or stratum mucosa, myometrium and perimetrium or serosa [Plate-1]. These findings are in agreement with the reports of Junsqueira and Carneiro [1971] and Bloom and Fawcett [1975] in Human, Sane *et al.* [1982] in goat, DiFiore [1989] in human, Banks [1993] in bovine, Dhale [2001] in Osmanabadi goat and Banubakode [2002] in goat.

The endometrium comprised of mucosa and mucosal folds. The surface epithelial lining consisted of simple columnar epithelium, but at some places pseudostratified columnar epithelium rested on the basement membrane in between the intercaruncular areas was observed [Plate-2]. Similar observations were reported by Kalkar [1971] in buffalo, Agarwal and Bhattacharya [1980] and Banubakode [2002] in goats. The 'Clear cells', having clear space around the dark basophilic nuclei and were marked between the epithelial cells and basement membrane. This finding confirmed the views of Joshi [1974] in goats. The endometrium was composed of two zones differing in structure and function [Plate-3]. The superficial layer or the functional zone and the thin deep layer or the basal zone. The superficial part of functional zone consisted of richly vascular loose connective tissue while the deep part of functional zone was having less cellular loose connective tissue. Similar observations were recorded by Joshi [1974] in goats and Dellmann and Brown [1986] in domestic animals. The endometrial stroma was highly cellular, thick, edematous, vascular and interspersed with glands. The uterine glands contained simple columnar epithelium and distributed throughout endometrium, except the caruncular area, Similar observations were made by Kalkar [1971] and Chawan [1987] in buffalo and Dellmann and Brown [1986] in domestic animals. The cellular proliferation of the gland was continued during entire proliferative phase. At the end of proliferative phase, glands appeared straight with narrow lumen [Plate-3]. The straight tubular glands were increased in size and length. While the straight narrow glands became tortuous and the lumen was dilated towards the end of secretory or luteal phase [Plate-4]. The glands were expanded and compactly arranged. Similar observations were noted by Dellman and Brown [1987] and Banks [1993] in bovine and Bloom and Fawcett [1975] in human and Heranjal *et al.* [1976] in buffalo. The glandular hypertrophy was associated with the cellular hypertrophy due to accumulation of secretory products in the luteal phase. These observations are in agreement with findings of Singh and Sharma [1985] in buffalo and Banks [1993] in bovine. In metestrous phase, there was glandular hyperplasia along with intense hypertrophy. Similar observations were recorded by Heranjal *et al.* [1976] in buffalo, Singh and Sharma [1985] in buffalo, Banks [1993] in bovine and Banubakode [2002] in goats, They observed significant increase in the glandular proliferation during luteal phase. Highest number of endometrial glands were present in proliferative phase and lowest during late luteal phase. This finding is in toto similar with the observations of Jankiraman *et al.* [1976] in water buffalo. Wider glands were noted in luteal

than follicular or estrous phase. This finding is in parallel with the observations made by Jankiraman *et al.* [1976] in water buffalo.

The epithelium was rested on superficial layer of basement membrane or lamina propria-submucosa. It was made up of loosely arranged connective tissue. Preferably, collagen and elastic fibers were found abundant. These observations are in agreement with the reports made by Stinson *et al.* [1962] in bovine. Kalkar [1971] in buffalo, Bloom and Fowcett [1975] in human, and Chawan [1987] in buffalo

The height of epitheliums was increasing from follicular phase to luteal phase. There was no significant statistical difference in the height of epithelium of uterus in different phases of estrous cycle. This finding is in concurrence of Joshi [1974] in goats. The average height of epithelium of uterus irrespective of phase of estrous cycle varied from $9.78 \pm 0.82 \mu$ to $11.90 \pm 1.18 \mu$ with a mean of $10.78 \pm 1.58 \mu$. However, Wordinger *et al* [1971] recorded greater epithelial height [$20.84 \pm 0.66 \mu$] in bovine endometrium, Bhattacharya and Saigal [1984] reported 19.01 to 38.94 μ in goat uterus and Ramchandriah [1986]^b recorded 18 to 20 μ in ewes. This might be due to the species and breed variation. The height of epithelium of uterus in proestrous, estrous, metestrous and diestrous was $9.78 \pm 0.82 \mu$, $10.32 \pm 1.11 \mu$, $11.13 \pm 1.30 \mu$, $11.90 \pm 1.18 \mu$ respectively. [Table-1] Similar were the findings of Ramchandriah *et al.* [1980]^a in ewes and Singh and Sharma [1985] in buffalo. They attributed that the increasing trend of epithelial height from follicular to luteal phase was due to the increased estrogen induced protein. The present observations on the change of height of epithelial cells during estrous cycle in Osmanabadi goat correlates with the finding of Bloom and Fawcett [1975] in human and Banubakode [2002] in goat. They mentioned that such changes in cell height during different phases of estrous cycle are biphasic. There was cellular proliferation continued during entire proliferative phase and mitosis was observed in the cells of the epithelial lining. The mitosis was rare during secretory phase. The present observations on the change in the epithelial cells of uterus during estrous cycle in goat is in concurrence with the findings of Junsqueira and Carneiro [1971] in human.

The endometrium of uterus composed of mucous membranous folds projecting into the lumen. This finding is in concurrence with Sane [1982] in goats. The height of mucosal folds in proestrous estrous, metestrous and diestrous phase were recorded as $168.18 \pm 15.35 \mu$, $170.61 \pm 14.85 \mu$, $172.16 \pm 13.21 \mu$ and $154.27 \pm 16.56 \mu$ respectively [Table-1]. Minimum height of the folds was observed in diestrous phase [$154.27 \pm 16.56\mu$]. The average height of mucosal folds irrespective of phase of estrous cycle is $166.30 \pm 21.27 \mu$ with a range of

154.27 \pm 16.50 μ to 172.16 \pm 13.21 μ . Bhattacharya and Saigal [1984]^b reported maximum number of uterine folds in endometrium during diestrous in goats. The increase in number of folds may be the reason for decrease in the height of mucosal folds of uterus within the particular space. No relative reference was found in the available literature regarding the height of mucosal fold in uterus during various stages of estrous cycle, hence could not be compared.

Thickness of mucosal folds of uterus showed no significant difference during various stages of estrous cycle. The average thickness of mucosal folds in proestrous, estrous, metestrous and diestrous was 246.38 \pm 16.96 μ , 219.71 \pm 28.41 μ , 214.35 \pm 29.65 μ , 197.80 \pm 7.07 μ respectively. [Table-1]. Minimum thickness of mucosal folds was observed in diestrous phase. The average thickness of mucosal folds irrespective of estrous phase was 261.23 \pm 31.81 μ which ranged between 197.80 \pm 7.07 μ to 246.38 \pm 16.96 μ . Bhattacharya and Saigal [1984]^b reported maximum number of uterine folds in endometrium during diestrous. The increase in number of folds may be the reason for decrease in the thickness of mucosal folds of uterus within the particular space. No relative reference was found in the available literature regarding the thickness of mucosal fold in uterus of goat, during various stages of estrous cycle, hence could not be compared.

The uterine stroma was found to be highly cellular and composed of many mucous secreting glands. [Plate-3] The number of uterine glands per cubic mm showed no significant statistical difference with respect to different phases of estrous cycle. Their number was decreasing from follicular phase to luteal phase. The number of uterine glands per cu mm was 49.44 \pm 1.91, 44.52 \pm 1.40, 39.61 \pm 1.35 and 35.64 \pm 1.29 in proestrous, estrous metestrous and diestrous respectively. [Table-2] The overall average number of glands per cubic mm irrespective of phase of estrous cycle was 42.30 \pm 1.92 with a range of 35.64 \pm 1.29 to 49.44 \pm 1.19.

The observations regarding decrease in the number of glands from follicular to luteal phase in the present study are in full agreement with the findings made by Banubakode [2002] in goats, However, this observation is in contrast with the observations made by Ramchandriah *et al.* [1980]^a in ewes. They stated that increase in number of acini could be due to increase in coiling of gland. Singh and Sharma [1985] in buffalo reported decreasing trend in the number of superficial glands per cubic mm from follicular to luteal phase, which is similar to the present observations in Osmanabadi goat. The uterine glands become more branched and coiled due to hyperplasia of cells in diestrous phase. Similar were the findings of Banks

[1993] in bovine. The glands were branched, coiled, straight tubular and holocrine in nature. These findings are in agreement with those reported by Bloom and Faucett [1975] in human, Chawan [1987] in buffalo and Banubakode [2002] in goat.

The cells in the glands showed increasing pattern from follicular to luteal phase. Though the statistical difference during various stages of estrous cycle was non-significant. The number of cells in endometrial glands were 93.50 ± 8.11 , 97.17 ± 4.01 , 102.17 ± 5.93 and 103.33 ± 8.48 respectively in proestrous, estrous, metestrous and diestrous showing increasing trend [Table-2]. The overall average of cells irrespective of estrous phase was 99.04 ± 9.72 and ranged between 93.50 ± 8.11 to 103.33 ± 8.48 . In luteal phase, there was maximum number of cells in the endometrial gland and glandular hyperplasia along with intense hypertrophy. Those observation are in agreement with the reports of Bank [1993] in bovine, Banubakode [2002] in goats, The cell height reached maximum in luteal phase in the present study, may be due to influence of progesterone. Similar observations were made by Jankiraman *et al.* [1976] in water buffalo.

The values of thickness of endometrium were statistically significant from each other in various phases of estrous cycle. The thickness of endometrium during various stages of estrous cycle was $497.36 \pm 19.91\mu$, $738.81 \pm 54.13 \mu$, $784.78 \pm 41.29 \mu$ and $1002.94 \pm 34.98 \mu$ in proestrous, estrous, metestrous and diestrous respectively [Table-3]. An increasing trend in the thickness of endometrium was observed in the present study. The overall average thickness of endometrium irrespective of phase was $755.97 \pm 55.92 \mu$ with a range of $497.36 \pm 19.91 \mu$ to $1002.94 \pm 34.98 \mu$.

During proliferative phase or follicular phase, there was increase in thickness of endometrium. Mitosis was observed in epithelial cells. In secretory or luteal phase, there was further thickening of endometrium. This was largely attributed to the edema of stroma and accumulation of secretion in the uterine glands. Similar observations were recorded by Bloom and Fawcett [1975] in human. During secretory phase, the endometrium was found to become much thicker due to increased secretory activity of the gland and edematous fluid in the stroma. These observations are in toto agreement with the observations reported by DiFiore [1989] in human.

The myometrium of uterus was mainly composed of inner circular & outer longitudinal bundles of smooth muscle fibers separated by connective tissue. The myometrium was the thickest tunic of uterus. In the present study, three layers were presented i.e. stratum submucosum [circular], stratum vasculare and stratum subserosum [longitudinal]. Similar

observations were recorded by EL- Sheikh and Abdelhadi [1970] in Egyptian buffalo, Bloom and Fawcett [1975] in human, Sane *et al.* [1982] in goat, Chawan [1987] in buffalo, Dellman and Brown [1987] and Banks [1993] in bovine.

The thickness of myometrium in proestrous, estrous, metestrous and diestrous was $614.28 \pm 14.11 \mu$, $597.30 \pm 23.56 \mu$, $641.56 \pm 14.41 \mu$, and $629.12 \pm 20.89 \mu$ respectively [Table-3]. The overall average thickness of myometrium irrespective of phase was $620.55 \pm 55.27 \mu$ and ranged between $597.30 \pm 23.56 \mu$ to $641.56 \pm 14.41 \mu$. There was gradual increase in the thickness of myometrium from follicular to luteal phase. Similar observations were recorded by Shukla [1973] in buffalo. The thickness of circular muscles was observed to be increased more during proliferative to luteal phase. This increase in thickness of circular muscle may be due to hypertrophy of muscle cells during all stages and considerable hyperplasia during late luteal phase as observed by Shukla *et al* [1973] in buffalo.

Perimetrium was the outmost layer consisted of loosely arranged connective tissue, numerous lymph, blood vessels and nerves. Smooth muscle cells were present in the perimetrium. Similar findings were observed by Sane *et al.* [1982] in goat, Dellmann and Brown [1986] in bovine and Chawan [1987] in buffalo.

Non-significant statistical difference was observed in perimetrium during various phases of estrous cycle. Thickness of perimetrium in proestrous, estrous, metestrous and diestrous was $39.79 \pm 4.97 \mu$, $44.76 \pm 3.85 \mu$, $47.25 \pm 4.59 \mu$ and $39.19 \pm 4.69 \mu$, [Table-3]. The overall mean irrespective of phase was $42.74 \pm 6.53 \mu$ and ranged between $39.79 \pm 4.97 \mu$ to $47.25 \pm 4.59 \mu$. Similar micrometrical readings were observed irrespective of estrous phase of uterus by Dhale [2001] in Osmanabadi goat.

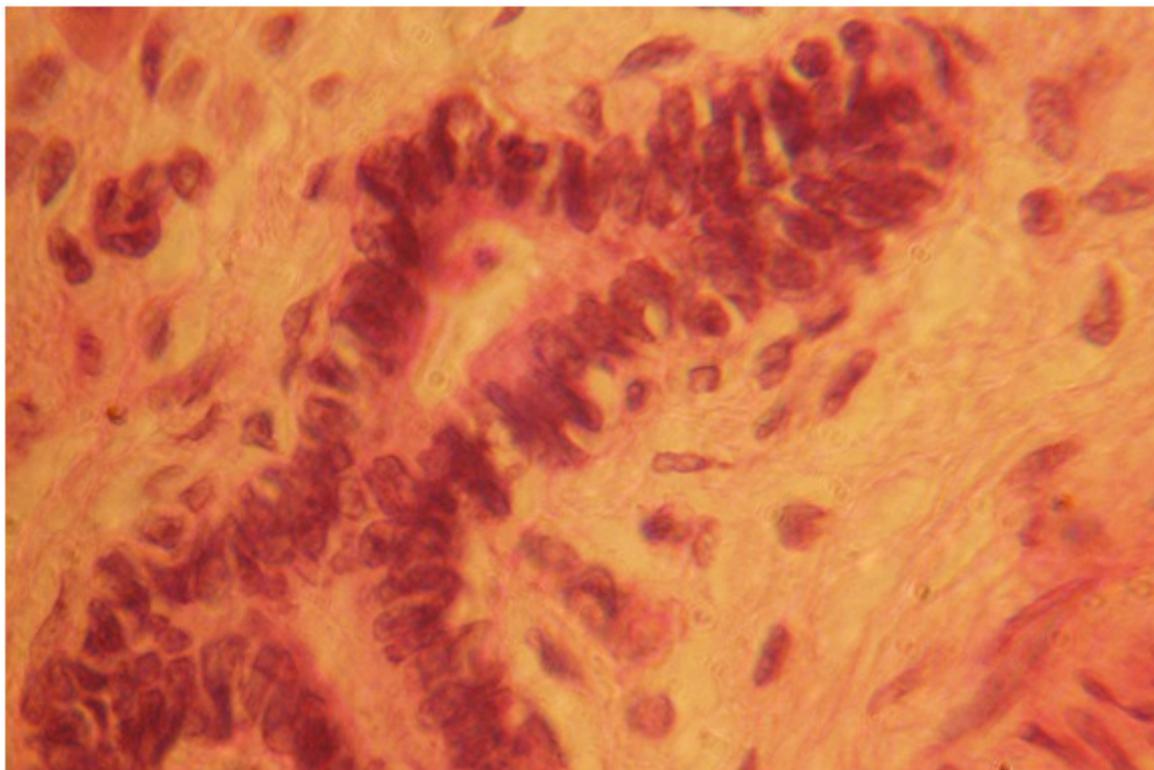


Plate No 1 Histological structures of Uterus [H and E 100x]

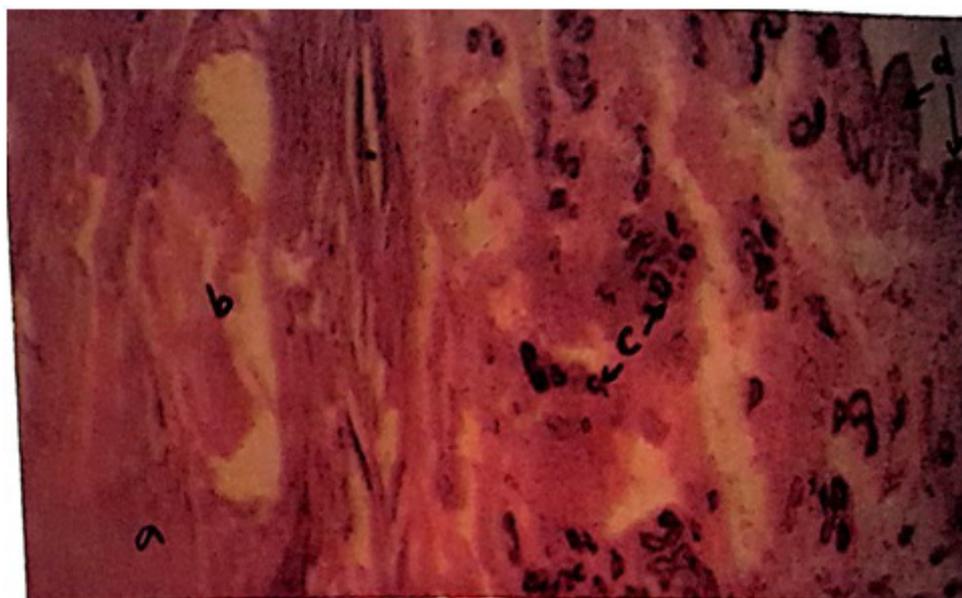


Plate No. 2 Microphotograph of uterus during metestrous phase showing
[Masson's Trichrome 10 X]

a. Myometrium b. Stratum Vasculare c. Endometrial gland d. Mucosal fold

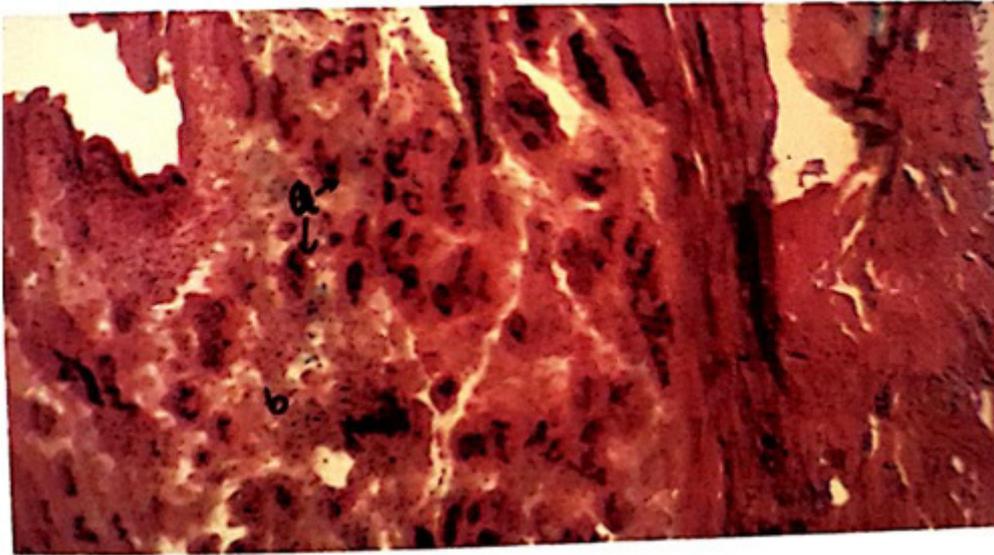


Plate No. 3 Microphotograph of uterus during diestrous phase showing [Masson's Trichrome 10 X]

a. Endometrial glands b. Endometrial stroma

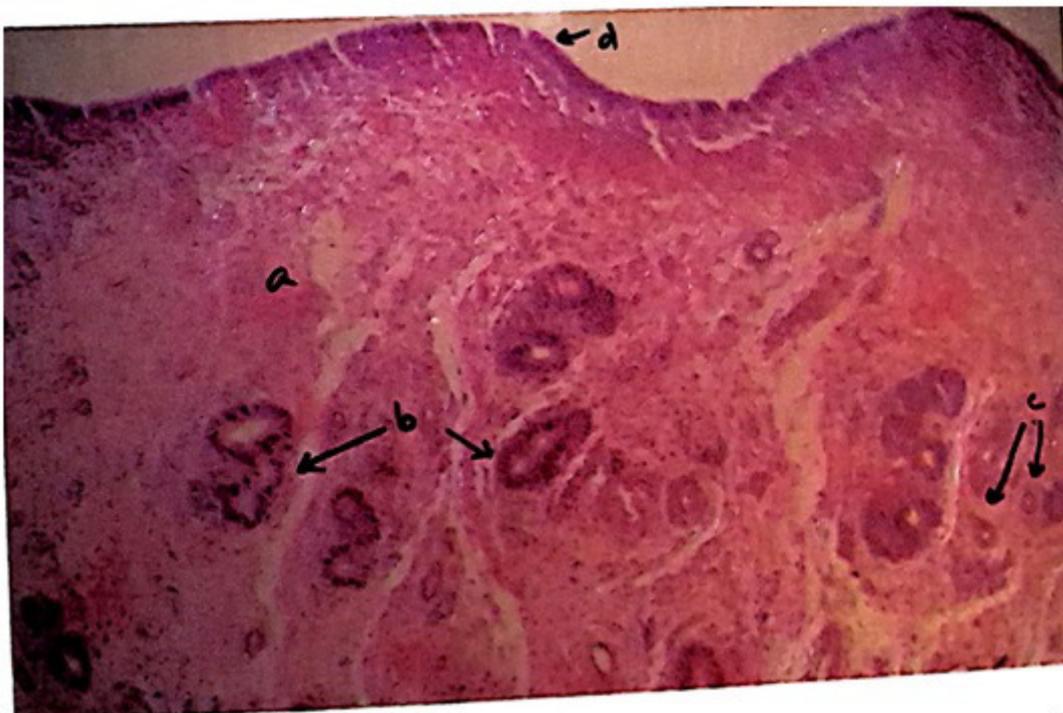


Plate No. 4 Microphotograph of uterus during proestrous phase showing [HE 10 X]

a. Endometrium b. Uterine glands c. blood vessels d. surface epithelium

Table 1: Showing range, Mean \pm SE values of height of epithelium [μ], height of mucosal fold [μ] and thickness of mucosal fold [μ] of uterus in different phases of estrous cycle in Osmanabadi goat.

Sr.No.	Groups	Parameters					
		Height of epithelium[μ]		Height of mucosal fold[μ]		Thickness of mucosal fold[μ]	
		Range	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE
1	Group I	8.15-12.82	9.78 \pm 0.82	114.38-210.28	168.18 \pm 15.35	192.66-294.82	246.38 \pm 16.96
2	Group II	7.1-14.19	10.32 \pm 1.11	127.14-209.26	170.51 \pm 14.85	105.54-294.52	219.71 \pm 28.41
3	Group III	8.34-15.21	11.13 \pm 1.30	129.92-211.26	172.16 \pm 13.21	109.88-288.38	214.35 \pm 29.65
4	Group IV	8.06-15.78	11.90 \pm 1.18	108.18-213.68	154.27 \pm 16.56	172.22-220.98	197.80 \pm 7.07
5	Overall mean	10.78 \pm 1.18		166.30 \pm 21.27		261.23 \pm 31.81	
6	'F' value	0.69		0.30		0.80	
7	CD value	3.31		44.45		66.48	
8	Statistics	NS		NS		NS	

NS: Non-significant

Group-I: Proestrous

Group-II: Estrous

Group-III: Metestrous

Group-IV: Diestrous

Table 2: Showing range, Mean \pm SE values of number of uterine glands and number of cells in the endometrial gland of uterus in different phases of estrous cycle in Osmanabadi goat.

Sr.No	Groups	Parameters			
		No. of uterine glands		Cells in the endometrial glands	
		Range	Mean \pm SE	Range	Mean \pm SE
1	Group I	40.18-54.35	49.44 \pm 1.91	69-122	93.50 \pm 8.11
2	Group II	35.24-53.32	44.52 \pm 1.40	89-116	97.17 \pm 4.01
3	Group III	29.06-48.35	39.61 \pm 1.35	89-128	102.17 \pm 5.93
4	Group IV	27.47-47.46	35.64 \pm 1.29	85-132	103.33 \pm 8.48
5	Overall mean	42.30 \pm 1.92		99.04 \pm 9.72	
6	'F' value	1.82		0.44	
7	CD value	22.96		20.31	
8	Statistic	NS		NS	

NS: Non-significant

Group-I : Proestrous

Group-II: Estrous

Group-III: Metestrous

Group-IV: Diestrous

Table 3: Showing range, Mean [\pm SE] values of thickness of endometrium [μ], myometrium [μ] and perimetrium [μ] of Uterus in different phases of estrous cycle in Osmanabadi goat.

Sr.No	Groups	Parameters					
		Thickness of endometrium [μ]		Thickness of myometrium [μ]		Thickness of perimetrium [μ]	
		Range	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE
1	Group I	432.68-552.20	497.36 \pm 19.91	566.96-656.48	614.28 \pm 14.11	29.84-59.68	39.79 \pm 4.97
2	Group II	596.80-971.40	738.81 \pm 54.13	522.20-671.40	597.30 \pm 23.56	28.52-60.02	44.76 \pm 3.85
3	Group III	590.76-880.28	784.78 \pm 41.29	596.80-686.32	641.56 \pm 14.41	31.32-58.94	47.25 \pm 4.59
4	Group IV	869.80-1119	1002.94 \pm 34.98	552.04-701.24	629.12 \pm 20.89	27.40-59.08	39.19 \pm 4.69
5	Overall mean	755.97 \pm 55.92		620.55 \pm 55.27		42.74 \pm 6.53	
6	'F' value	27.50		1.04		0.65	
7	CD value	116.87		55.27		15.65	
8	Statistics	HS		NS		NS	

NS: Non-significant
 Group-I: Proestrous
 Group-II: Estrous

HS: Highly-Significant
 Group-III: Metestrous
 Group-IV: Diestrous

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