Haryana Vet. (December, 2024) **63(2),** 157-161 ISSN 0033-4359 (P) **Research Article**

HISTOLOGICAL CHANGES IN GRAVID UTERUS DURING DIFFERENT STAGES OF PREGNANCY IN BARBARI GOAT

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ABSTRACT

The present study was conducted on the 18 gravid uteri of Barbari goats. Each foetus was measured for its crown rump length (CRL) in centimeters with the help of nylon thread. The weight, crown-rump length and approximate age of the foeti were measured. These uteri were divided into three groups consisting six uteri in each *viz*. early gestation period (0 day-50 days), mid-gestation period (51 days-100 days) and late gestation period (101 days-till full term). The small pieces of the gravid uterus were collected and fixed in 10% neutral buffered formalin (NBF) processed by routine paraffin embedding technique. The sections were stained with hematoxylin and eosin for general histoarchitecture. The uterine wall was comprised of three layers (inner to outer) i.e. endometrium, myometrium, and perimetrium. The endometrium showed mucosal folds and was lined by simple columnar epithelium, but at some places, pseudostratified columnar epithelium was also observed. The endometrial glands were interspersed in the highly vascular stroma. The myometrium was the thickest tunic of the uterus and mainly composed of inner circular and outer longitudinal bundles of smooth muscle fibers separated by connective tissue. Perimetrium was the outermost layer consisting of loosely arranged connective tissue, numerous lymph, blood vessels, and nerves.

Keywords: Goat, Histogenesis, Pregnancy, Uterus

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The domestic goat is a sociable, inquisitive, and intelligent species, which has been used for its meat, milk, skin and fur (Verma *et al.*, 2022). Uterus is an important place for reproduction, successful implantation of the embryo into the endometrium and maintenance of intrauterine environmental homeostasis during pregnancy are key steps in successful mammal reproduction (Bazer *et al.*, 2018). Maintaining immune homeostasis between the embryo and mother during embryo attachment and pregnancy (Johns *et al.*, 2021), enhancing antioxidant activity at the mother-fetus interface (Hussain *et al.*, 2021), and promoting placental angiogenesis and development (Maltepe *et al.*, 2015) is essential. Thus, the uterus plays a crucial role during the entire pregnancy.

The aim of the present study was to documenting information on the progressive histological changes in the gravid horn of the uterus during the entire pregnancy in Barbari goat. The results of this study, besides filling the lacuna in the scientific literature, on the changes in the uterus during the pregnancy period will also form the foundation stone for the gynecologist, goat breeders, physiologists, pathologists, clinicians, biochemists, and other who are engaged with the reproductive problems of goat.

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MATERIALS AND METHODS

The present study was conducted on 18 gravid uteri of Barbari goats collected from local slaughter houses, Mathura (U.P.), and aborted cases presented at TVCC, DUVASU, Mathura. Each fetus was measured for its crown-rump length in centimeters with the help of a nylon thread as per the technique described by Harvey (1959) and weighed in grams on the digital analytical balance. The approximate age of embryos/foeti was estimated by using the formula given by Singh *et al.* (1979) in goats, W1/3= 0.009 (t-30) where W= body of fetus in gram and t = age of fetus in days. For fetuses below 30 days, the approximate age of foeti was estimated by its crown-rump length, and morphological features (Verma *et al.*, 2021 and Verma *et al.*, 2023).

These gravid uteri were divided into three groups viz; early gestation period (0 day-50 days), mid gestation period (51 days-100 days), and late gestation period (101 days-till full Term), each group consisted of 6 gravid uteruses. The small pieces of the tissues were collected from different parts of the gravid horn (apex and base on both sides) and body of the tissue and fixed in 10% neutral buffered formalin (NBF). The tissues were processed by routine paraffin embedding technique and stained with Hematoxylin and Eosin stain for general histoarchitecture

(Luna 1968). The micrometrical observations were conducted in all the uteri used for histological studies. The data on micrometrical observations were subjected to statistical analysis (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The uterine wall of the gravid horn consisted of three different layers *viz*. endometrium, myometrium, and perimetrium. The endometrium was the innermost mucosal layer. The myometrium was the middle musculo-vascular layer and a thin outer serous covering, the perimetrium similar to observations of Bagade *et al.* (2018) in Osmanabadi goat. The endometrium consisted of luminal and glandular epithelial cells surrounded by supporting stromal cells in domestic animals (Spencer *et al.*, 2004).

Endometrium

The endometrium was the inner mucosal lining of the uterus which constituted the maternal component of the placenta. It comprised of surface epithelial lining and endometrial stroma containing uterine glands as observed earlier by Igwebuike (2009) in goat and Uppal and Roy (2002) in buffalo uterus.

Surface epithelium

At 23 days of pregnancy the surface epithelium was denuded from most of the area of endometrium. Whenever present, the epithelium was pseudostratified columnar and appeared in patches and could also be seen around the opening of the uterine glands. Supra-nuclear region of the epithelial cells was highly eosinophilic with basal nuclei as described by Bagade *et al.* (2018) in Osmanabadi goat. Uppal and Roy (2002) in buffalo and Bagade *et al.* (2018) in Osmanabadi goat reported that surface epithelium varied from simple columnar to pseudo-stratified columnar during early pregnancy.

The micrometrical observations on endometrium, myometrium and perimetrium has been summarized in table 1 and 2.

In the II group, the surface epithelium could not be observed at 51, 53, 65, 85 and 90 days of pregnancy but at 93 days of pregnancy surface epithelium appeared in a discontinuous manner (Fig. 1). In III group, the surface epithelium reappeared as pregnancy progressed, however denudation was seen in isolated patches. The epithelium was simple columnar to pseudostratified in nature (Fig. 2). In the apical segment there was non-significant change where as in middle segment there was significantly ($p<0.05$) increased in height of the epithelium.

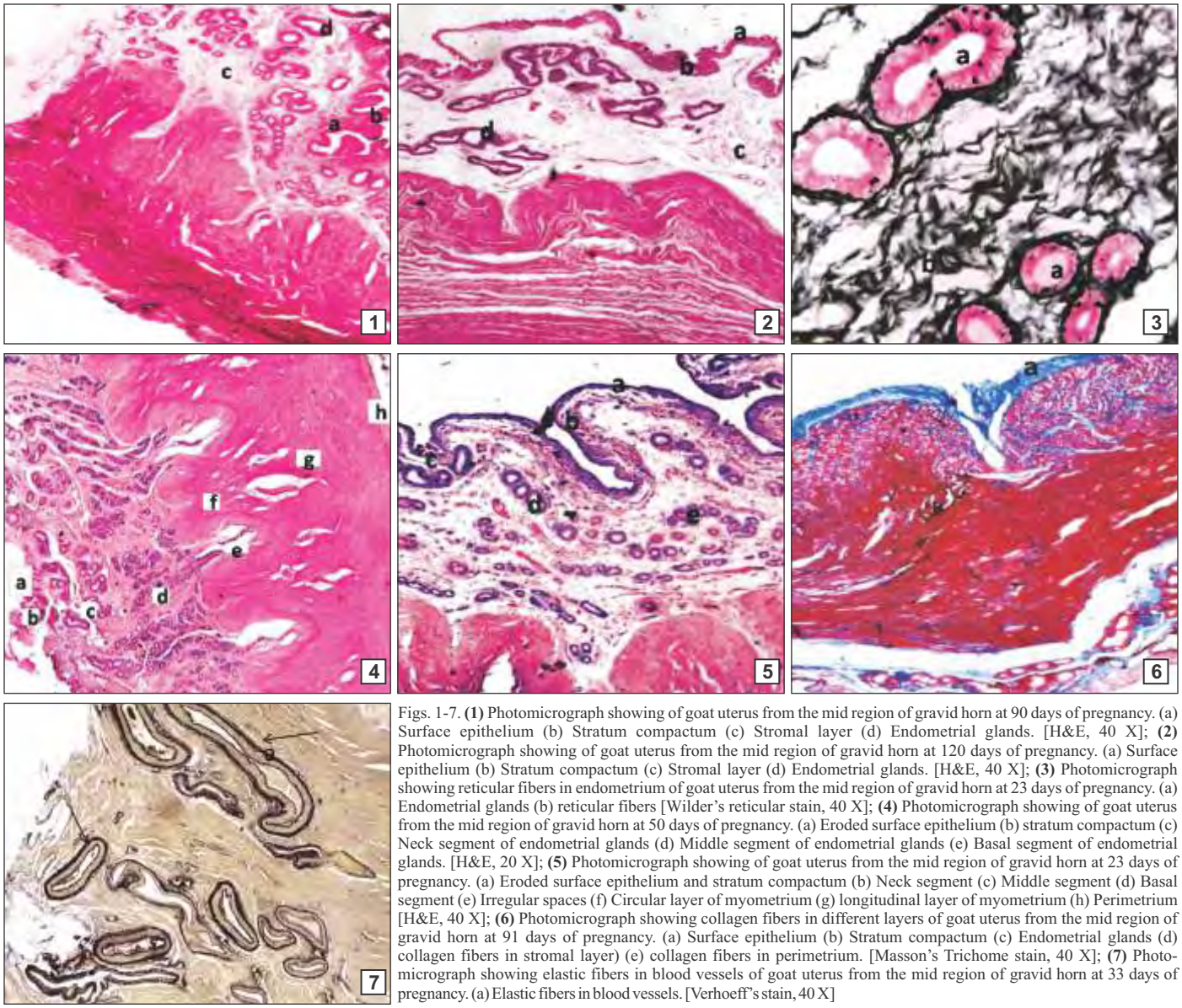
Lamina Propria

The lamina propria comprised of sub epithelial or stratum compactum zone and endometrial stromal zone as reported by Skjerven (1956) in cattle and Uppal and Roy (2002) in buffalo. The subepithelial or stratum compactum zone was unevenly thickened and appeared relatively denser and more cellular than the deeper portion. Stratum compactum zone was rich in blood capillaries and was denser during different stage of pregnancy. The lamina propria in the uterus was consisted of network of reticular and collagen fibers. At this stage of pregnancy, many isolated thick reticular fibers were observed in between stromal loose connective tissue (Fig. 3) similar to observation made by Chandra (1973) in pregnant buffalo and Igwebuike and Ezeasor (2014) in West African Dwarf goat. Elastic fibers could not be detected in blood vessels during 23 days of pregnancy. Many isolated thick reticular fibers were observed in this layer but their number was more in superficial zone as compared to deeper zone. At this stage, body and middle portion of gravid horn having more blood vessels as compared to apical portion. The size and number of blood vessels were increased in number with advancement of pregnancy.

The lamina propria became increasingly edematous with the advancement of pregnancy. The conspicuous feature at this stage was the presence of isolated lymph nodules in the stroma. The stroma was identified in two different zones *viz*. superficial and deeper zone. The superficial zone was rich in blood capillaries and was denser and showed no edematous changes. The deeper zone such as stromal zone consisted of several endometrial glands with some loose connective tissue, revealed large irregular spaces suggestive of increased oedema as observed by Igwebuike and Ezeasor (2014) in West African Dwarf goat. The intercaruncular areas of the endometrium contained large numbers of branched, coiled uterine glands (Fig. 4). The size, diameter and branching of endometrial glands were increased as pregnancy progressed as observed earlier by Igwebuike (2009) and Igwebuike and Ezeasor (2014) in West African Dwarf goat. The thickness of endometrium has been summarized in tables (3). In the apical segment there was non-significant change where as in middle segment there was significantly ($p<0.05$) increased in height of the endometrium.

Myometrium

At 23 days of pregnancy the myometrium was composed of inner circular and outer longitudinal muscle layers. Between the circular and longitudinal muscle layers many blood vessels were found known as stratum



Figs. 1-7. **(1)** Photomicrograph showing of goat uterus from the mid region of gravid horn at 90 days of pregnancy. (a) Surface epithelium (b) Stratum compactum (c) Stromal layer (d) Endometrial glands. [H&E, 40 X]; **(2)** Photomicrograph showing of goat uterus from the mid region of gravid horn at 120 days of pregnancy. (a) Surface epithelium (b) Stratum compactum (c) Stromal layer (d) Endometrial glands. [H&E, 40 X]; **(3)** Photomicrograph showing reticular fibers in endometrium of goat uterus from the mid region of gravid horn at 23 days of pregnancy. (a) Endometrial glands (b) reticular fibers [Wilder's reticular stain, 40 X]; **(4)** Photomicrograph showing of goat uterus from the mid region of gravid horn at 50 days of pregnancy. (a) Eroded surface epithelium (b) stratum compactum (c) Neck segment of endometrial glands (d) Middle segment of endometrial glands (e) Basal segment of endometrial glands. [H&E, 20 X]; **(5)** Photomicrograph showing of goat uterus from the mid region of gravid horn at 23 days of pregnancy. (a) Eroded surface epithelium and stratum compactum (b) Neck segment (c) Middle segment (d) Basal segment (e) Irregular spaces (f) Circular layer of myometrium (g) longitudinal layer of myometrium (h) Perimetrium [H&E, 40 X]; **(6)** Photomicrograph showing collagen fibers in different layers of goat uterus from the mid region of gravid horn at 91 days of pregnancy. (a) Surface epithelium (b) Stratum compactum (c) Endometrial glands (d) collagen fibers in stromal layer) (e) collagen fibers in perimetrium. [Masson's Trichome stain, 40 X]; **(7)** Photomicrograph showing elastic fibers in blood vessels of goat uterus from the mid region of gravid horn at 33 days of pregnancy. (a) Elastic fibers in blood vessels. [Verhoeff's stain, 40 X]

Table 1. Variation of thickness of different layer of endometrium (µm) in gravid horn

Sr. No.	Apical Segment		Middle Segment	
	Height of Epithelium	Total Thickness	Height of Epithelium	Total Thickness
Group - I (Mean ± SE)	13.13±0.22	398.32±24.61	13.58^{a} ± 0.20	623.83^{a} ± 26.42
Group - II (Mean ±SE)	13.48± 0.19	450.59± 24.97	13.79^{ab} ± 0.34	481.98^{ab} ± 43.07
Group - III (Mean ± SE)	14.58± 0.84	496.28± 34.62	15.52^{c} ± 0.62	500.45^{c} ± 34.14

Table 2. Variation of thickness of different layer of myometrium (µm) in gravid horn

Sr. No.	Apical segment (Thickness)			Middle Segment		
	Circular muscle layer	Longitudinal muscle layer	Total thickness	Circular muscle muscle layer	Longitudinal layer	Total thickness
Group- I (Mean ± SE)	195.51^{a} ± 16.62	130.43^{a} ± 18.10	325.94^{a} ± 34.33	328.75^{a} ± 13.58	223.13^{a} ± 7.75	569.89^{a} ± 20.31
Group- II (Mean ± SE)	274.50^{b} ± 8.42	180.27^{ab} ± 4.26	454.77^{b} ± 12.42	338.75^{a} ± 13.58	241.82^{ab} ± 16.07	625.40^{ab} ±2 4.79
Group -III (Mean ± SE)	399.86^{c} ± 26.82	321.07^{c} ± 29.61	720.93^{c} ± 55.42	399.32^{bc} ± 19.63	294.80^{c} ± 6.43	694.13^{c} ± 20.79

Table 3. Variation of thickness of different layer of body of uterus (µm)

Group		Group-I (Mean±SE) (Range)	Group-II (Mean±SE) (Range)	Group-III (Mean±SE) (Range)
Endometrium	Height of surface epithelium	13.22^{a}±0.11	13.23^{ab}±0.16	14.39^{c}±0.30
	Total thickness	462.12±19.37	498.64±29.03	711.61±49.29
Myometrium	Circular muscle layer	350.89^{a}±14.87	397.56^{b}±9.94	469.23^{c}±7.67
	Longitudinal muscle layer	265.33^{a}±20.57	310.49^{b}±13.53	378.20^{c}±6.09
	Total thickness	616.23^{a}±32.98	708.05^{b}±21.85	847.43^{c}±13.53
Perimetrium	Total thickness	24.21± 0.21	32.21 ± 0.23	39.28 ± 0.41

Table 4. Variation of thickness of different layer of perimetrium (µm) and total thickness of uterine wall (µm)

Sr. No.	Perimetrium		Total Thickness of uterus		
	Apical segment	Middle Segment	Apical Segment	Middle Segment	Body
Group- I (Mean ± SE)	40.44±0.58	40.94±0.46	1193.79^{a}±45.12	1778.47±67.95	1958.27^{ab}±57.59
Group- II (Mean ± SE)	42.29±0.73	42.10±0.99	1404.92^{ab}±39.48	1808.14±26.32	1987.59^{a} ± 23.86
Group -III (Mean ± SE)	43.40±2.04	42.96±1.50	1984.55^{c}±132.21	1937.20±56.52	2201.66^{c}±15.32

vasculare as observed by Dellmann (1996) in ruminants. The stratum vasculare showed considerable increase in its thickness and its component blood vessels underwent extensive hypertrophic changes (Fig. 5) similar observation was made by Chandra (1973) in pregnant buffalo. The size and number of blood vessels increased as pregnancy advanced. The outer longitudinally arranged muscle fibers constituted a thinner layer as compared to circular muscles layer (Fig. 5). The thickness of myometrium was first increased by hypertrophy during early to mid-pregnancy followed by hyperplasia during late pregnancy. Shynlova *et al.* (2010) observed that increased in size of myometrium may be due to hyperplasia of uterine smooth muscle cells (SMCs) within the myometrium in rat during late pregnancy. The diameter of the individual muscle cells increased several times as pregnancy advanced. Similar observations were made by Bagade *et al.* (2018) in Osmanabadi goat.

In group II, hypertrophy of the myometrium was observed. At 51 and 53 days of pregnancy more hypertrophies observed in circular muscle layer than longitudinal muscle layer. In between the muscle fibers many bundle of collagen fibers were observed. These collagen fibers more numerous in longitudinal muscle layer as compared to circular layer (Fig. 6). In group III, numbers of collage fibers were more as compared to second group. The blood vessels constituting the stratum vasculare had very large number of elastic fibers (Fig. 7). Elastic fibers were more in tunica externa as compared to tunica interna. Collagen fibers increased whereas elastic fibers decreased with advancement of pregnancy. It may be that when the pregnant uterine horn expended due to developing foetus, the elastic fibers tend to be stretched beyond their elastic limits and as a result they degenerate, these seems to be followed by profuse regeneration of collagen fibers that are much more resistant to stretch which are required for great stability on the expending uterus as reported by Gulia (2003) in goat and Bagade *et al.* (2018) in Osmanabadi goat.

The thickness of circular muscles fibers and longitudinal muscles fibers were increased significantly ($P<0.05$) with the advancement of pregnancy (Table 2). The total thickness of apical, middle and body portion of the myometrium was increased significantly with advancement of pregnancy (Table 2 and 3).

Perimetrium

The perimetrium consisted of outer most epithelial covering which rested on a connective tissue layer. The epithelium was simple squamous to simple cuboidal as observed by Chandra (1973) in gravid uterus of buffalo. The connective tissue layer consisted of loosely arranged collagenous fibers along with reticular and few elastic fibers (Fig. 6). The collagen fibers were found in the form of bundles and few collagen fibers were continued within the myometrium (Fig. 6). Numerous capillaries were observed under the serous coat. Numerous lymph and blood vessels were also observed in the perimetrium. The above histoarchitectural changes in perimetrium support the great stability for expanding of uterus as observed earlier by Gulia *et al.* (2003) in pregnant goat and Bagade *et al.* (2018) in Osmanabadi goat. There was non-significant changes in thickness of perimetrium and total thickness of uterine wall increased significantly ($P<0.05$) with advancement of pregnancy (Table 4).

CONCLUSIONS

The surface epithelium was denuded from most of the area of endometrium during early pregnancy. The epithelium was simple columnar to pseudostratified columnar during mid-pregnancy. At 93 days of pregnancy surface epithelium was reappearing in a discontinuous manner and in late pregnancy isolated patches of denudation was noticed. The thickness of myometrium was first increased by hypertrophy during early to mid pregnancy followed by hyperplasia during late pregnancy. This arrangement changed at the onset of the pregnancy, when the pregnant uterine horn expended due to developing foetus, the elastic fibers tend to be stretched beyond their elastic limits and as a result they degenerate, these seems to be followed by profuse regeneration of collagen fibers that are much more resistant to stretch which are required for great stability on the expending uterus.

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