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# RESEARCH ARTICLE

## HISTOMORPHOGENESIS OF MAMMARY GLAND IN PRENATAL NONDESCRIPT GOAT

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Development, Goat, Mammary gland, Mammary line, Mesenchymal tissue, Prenatal.

#### **ABSTRACT**

The study was conducted in eighteen prenatal female goats comprising group I, II and III having Crown rump length, up to 10 cm, 10-20cm and 20+ cm respectively. The initiation of the development of the mammary gland was observed as mammary line in the foetus of group I (4.50 cm CRL). The mammary line was made up of several (4-5) layers of epidermal cells, developed on the stratum germinativum. The further proliferation of cells lead to the formation of mammary hillock observed in the foetus of group I (5.20 cm CRL). The mammary bud remained below the epidermis and was ovoid, encapsulated by concentric arrangement of mesenchymal tissue. The initiation of the teat development appeared in the foetus of group I (6.70 cm CRL). The epidermal cells of the bud proliferated in the deeper mesenchyme to form the primary sprout. The luminization of the primary sprout proceeded from the base towards the apex of the teat in the foetus of group II (17.60 cm CRL). In group III (28.00 cm CRL), the cavity of the teat cistern was increased in size and the cells started to rearrange into definitive bilayered epithelium. The entire tissue next to the stratum germinativum was mesenchymal in nature. With the advancement of age the spherical masses of mesenchymal cells differentiated into adipose tissue called as fat pad, noticed in group II (20.00 cm CRL). In group III (36.00 cm CRL) the development of the adipose tissue became extensive throughout the udder tissue above and around the gland cistern and duct system. Traces of elastic fibers were noticed in the interwhorl connective tissue area of the fat pad in the foetus of group II (20.00 cm CRL) and distribution of elastic fibers were sparse and scattered in the connective tissue septae of the developing gland. However, distinct lamina of elastic fibers were observed in the blood vessel.

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## INTRODUCTION

The mammary gland of goat is a cutaneous, exocrine, compound tubuloalveolar gland and is derived from a modification of sweat glands. Each gland appears in early embryonic life as clumps of cells proliferating from a longitudinal ectodermal ridge along the milk line to form the buds (Panchal and Vyas, 2005). The developmental study on mammary gland in prenatal stage is essential to understand the basic information vis a vis application in other fields. Research work have been conducted on histological and developmental aspects of mammary gland of buffalo (Panchal *et al.*, 1998 a & b, 1999) and ultratructural studies on bovine mammary gland (Nickerson and Pankey, 1983) and of buffalo (Chaurasia, 2010). However, there is paucity of literature on histomorphogenesis of mammary gland in prenatal and postnatal goat.

Keeping in view the above facts in mind, the present study has been undertaken.

### **MATERIALS AND METHODS**

The present investigation was conducted in 18 prenatal female goats. The gravid uteri for prenatal mammary gland samples were procured from small animal abattoir, Jabalpur. The samples were collected immediately after slaughter of the animals. The female foetuses after collection from the gravid uteri were placed in 3 groups as per their crown-rump length (CRL). Group I comprised of foetuses up to 10 cm CR length, group II between 10<sup>+</sup> to 20cm CR length and group III included the foetuses above 20<sup>+</sup> cm CR length. The approximate age of foetuses were calculated by using following formulae based on Soliman (1975).

Y=28.66 +4.496 x (CRL< 20 cm) Y=73.544 + 2.256x (CRL>20cm)

Where Y is the age of foetus in days.

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The observation was done on the histological sections of the developing mammary gland of prenatal goats (*Capra hircus*) ranging between 4.50 cm to 36.00 cm CR length. In group I, the initial development of mammary gland was observed in female foetuses of 4.50cm, 5.00cm, 5.20, 6.70 cm, 7.20 cm and 9.20 cm CR length. In group II, foetuses having 11.50 cm, 12.50 cm, 16.50 cm, 17.60 cm, 18.50 cm and 20.00 cm CR length and in group III, goat foetuses of 28.00 cm, 28.50 cm, 30.00 cm, 33.00 cm 34.00 cm and 36.00 cm CR length were studied.

### **RESULTS AND DISCUSSION**

#### **Mammary Line**

The initiation of the development of the mammary gland was observed as mammary line in the inguinal region on either side of median line, medial to thigh in the foetus of 4.50 cm CR length (Fig.1). The mammary line was made up of several (4-5) layers of epidermal cells, proliferating from the stratum germinativum (Fig.2). The study is in accordance with the findings of Carrol (1980) in cow embryo, who observed that the mammary line occurred as a thickening of the epidermis, 5-6 cell layers deep which fused into the surrounding two layered epidermis quickly.

#### Mammary Bud

The small spherical elevation appeared along the mammary line, due to profuse proliferation of the germinal epithelium. This stage of development of the mammary bud was mammary crest which was discoid shaped noticed in the foetus of 5.00 cm CR length (Fig. 3 and 4). The further proliferation of cells lead to form the mammary hillock akin to half lunar crescent shaped observed in the foetus of 5.20 cm CR length (Fig. 5). The stratum germinativum in the area of mammary hillock rounded off and submerged in the underlying tissue due to continuous proliferation of the new cells to form the complete mammary bud in the foetus of 6.70 cm CR length (Fig.6). This bud remained deep to the epidermis and was of ovoid shape. It was encapsulated by concentric arrangement of mesenchymal cells. The histological elements of the mammary bud were malpighian layer (stratum germinativum) resting on the basement membrane and a group of central cells . In the present investigation, it was observed that the prenatal development of the mammary gland was initiated in the form of mammary line, followed by mammary crest, mammary hillock and mammary bud. The present study was in similar trend with the report of Turner (1952) and Kon and Cowie (1961) in cow, Arey (1954) and Langman (1985) in human, Scott (1971) in mouse and Panchal (1998a) in buffalo.

### **Teat Development**

The initiation of teat development appeared in the female foetus at 6.70 cm CR length (Fig.7), where as Turner (1952) and Kon and Cowie (1961) observed the first trace of teat development at 8.40 cm CR length in cowwhereas Panchal (1998a) noticed at 5.70 cm CR length in female buffalo foetus. The mammary bud linked with epidermal cells as a cellular plug. Proliferation of mesenchymal cells surrounding the

mammary bud projected outward over the tissue forming a conical papilla or primordial teat, which was observed in the foetus of 7.20 cm CR length. Numerous blood capillaries in the mesenchymal tissue were observed in the core of the developing cone.

## **Primary Sprout**

The epidermal cells of the bud proliferated in the deeper mesenchyme of the developing gland to form the primary sprout. In the present study the initiation of primary sprout was observed in the foetus of 11.50 cm CR length. Turner (1952) and Kon and Cowie (1961) observed the primary sprout in cattle at 12.00 cm - 15.00 cm CR length. This structure got extended as a cellular cord and entered deep into the base of the teat at 12.50 cm CR length. The luminization of the primary sprout was seen in the foetus of 16.50 cm CR length. The luminization of the primary sprout proceeded from the base towards the apex of the teat was observed in the foetus of 17.60 cm CR length (Fig.8). According to Turner (1952) and Kon and Cowie (1961) in cattle the primary sprout canalization at this stage might be due to widening of the sprout resulting in the formation of a lumen. Carrol (1980) reported primary sprout in cow foetus at 12.00 cm CRL stage. Panchal (1998a) also reported primary sprout in buffalo foetus at 12.00 cm CR length. The cross-section of the mid region of the developing teat revealed that at 17.60 cm CR length the centrally placed lumen was surrounded by several layers of epithelial cells (Fig. 9 and 10). At the age of 18.50 cm CR length the lumen of the developing teat cistern enlarged. The lining cell layers were reduced to 2-3 cell layer in the foetus of 20.00 cm CR length and the lumen increased in size. The cells were large columnar with round or oval nuclei (Fig. 11). In late gestation at 28.00 cm CR length the cavity of the teat cistern increased with histodifferentiation of the cells into the definitive cell types. The cells of the teat cistern started to rearrange into bilayered epithelium (Fig. 12 and 13). Later on the development proceeded by increased diameter of the lumen of the teat cistern in foetuses of 30.00 cm and 33.00 cm CR length, the cells were differentiated in the typical bilayered epithelium with 3-4 mucosal folds. In foetuses of 34.00 cm and 36.00 cm CR length, well developed teat cistern was present with five mucosal folds. The epithelium was bilayered with luminal cuboidal and basal columnar cells (Fig. 14).

# Differentiation of Mesenchyme

The entire tissue next to the stratum germinativum was mesenchymal in nature, which was diffuse and undifferentiated in the initial stage of development. The embryonic tissue was made up of loosely connected spindle shaped cells. In the foetus of 11.50 cm and 12.50 cm CR length, the gland parenchyma revealed formation of the whorls composed of centrifugaly disposed cells. The whorls which were observed in clusters in the foetus of 17.60 cm CR length with centrally located few adipose cells (Fig. 15 and 16). Further development demonstrated that the central cells of the connective tissue were lipocytic in nature in the foetus of 18.50 cm CR length (Fig. 17 and 18). These whorls were associated with blood capillaries and were separated by fibroblasts (Fig. 19 and 20).

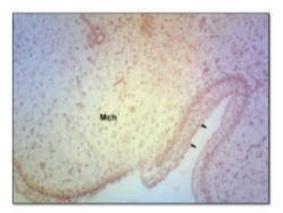


Plate 1: Photomicrograph-cross section of skin of goat in the inguinal region, Group I (4.50 cm CRL) showing mammary line ( t ), mesenchymal tissue (Mch). H&E X 100.

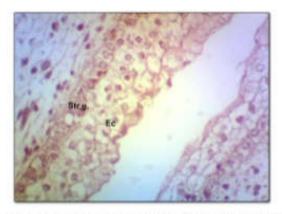


Plate 2: Photomicrograph-cross section of mammary line, Group I (4.50 cm CRL) showing 4-5 layers of epidermal cells (Ec) on stratum germinativum (Str.g.). H&E X 400.

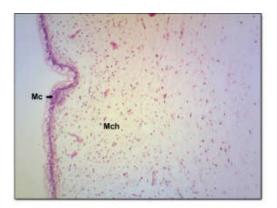


Plate 3: Photomicrograph-cross section of skin in the inguinal region, Group I (5.00 cm CRL) showing mammary crest (Mc), mesenchymal tissue (Mch). H&E X 100

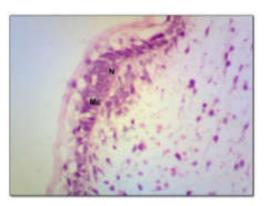


Plate 4: Photomicrograph-cross section of mammary crest (Mc), Group I (5.00cm CRL) showing condensed nuclei (N). H&E X 400.

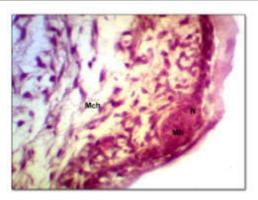


Plate 5: Photomicrograph-cross section of mammary hillock (Mh), Group I (5.20 cm CRL) showing condensed nuclei (N), mesenchymal tissue (Mch). H&E X 400.

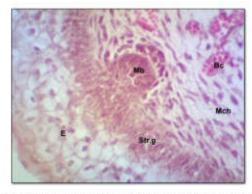


Plate 7: Photomicrograph-cross section of mammary bud (Mb), Group I (6.70cm CRL) developing from stratum germinativum (Str.g) into the underlying mesenchymal tissue (Mch), numerous blood capillaries (Bc) and epidermis (E). H&E X 400.

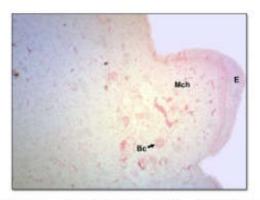


Plate 8: Photomicrograph-cross section of skin, Group I (7.20 cm CRL) showing initial development of teat in the form of cone, epidermis (E) mesenchymal tissue (Mch) and numerous blood capillaries (Bc). H&E X 40.

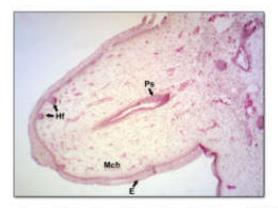


Plate 11: Photomicrograph-sagittal section of developing teat, Group II (17.60 cm CRL) showing luminized primary sprout (Ps), developing hair follicles (Hf), epidermis (E), mesenchymal tissue (Mch). H&E X 40.

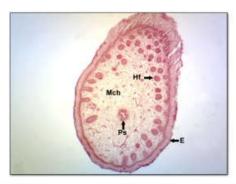


Plate 13: Photomicrograph-cross section of developing teat in the mid region, Group II (17.60 cm CRL) showing luminized primary sprout (Ps), surrounded by mesenchymal tissue (Mch), developing hair follicles (Hf) and epidermis (E). H&E X 40.



Plate 14: Photomicrograph-cross section of developing teat in mid region, Group-II (17.60 cm CRL) showing luminized primary sprout (Ps), surrounded by mesenchymal tissue (Mch), blood vessels (Bv), developing hair follicles (Hf) and epidermis (E). H&E X 100.

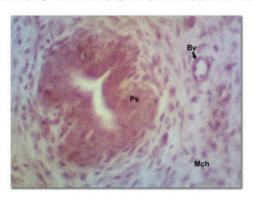


Plate 15: Photomicrograph-cross section of developing teat in mid region, Group II (17.60 cm CRL) showing luminized primary sprout (Ps), surrounded by mesenchymal tissue (Mch), blood vessels (Bv). H&E X 400.



Plate 17: Photomicrograph-cross section of teat in mid region, Group II (20.00 cm CRL) showing teat cistern (Tc), lined by multilayered epithelium (Ep), surrounded by developing connective tissue (Ct), blood vessels (Bv), developing hair follicles (Hf) and epidermis (E). H&E X 100.

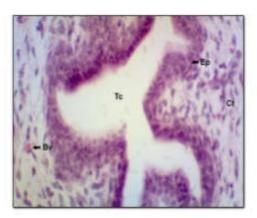


Plate 18: Photomicrograph-cross section of teat in mid region, Group II (20.00 cm CRL) showing developing teat cistern (Tc), lined by multilayered epithelium (Ep) surrounded by developing connective tissue (Ct) and blood vessels (Bv). H&E X 400.



Plate 19: Photomicrograph-cross section of teat in mid region, Group III (28.00 cm CRL) showing developed teat cistern (Tc) lined by rearranging bilayered epithelium (Ep), surrounded by developing connective tissue (Ct) and blood vessels (Bv). H&E X 100.

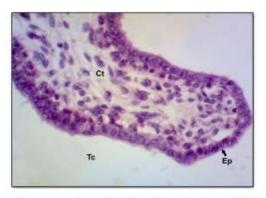


Plate 20: Photomicrograph-cross section of teat in mid region Group III (28.00 cm CRL) showing fold of teat cistern (Tc), lined by rearranging bilayered epithelium (Ep) with developing connective tissue (Ct). H&E X 400.

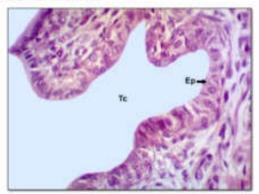


Plate 22: Photomicrograph-cross section of teat in mid region, Group III (34.00 cm CRL) showing, teat cistern (Tc), lined by bilayered epithelium (Ep). H&E X 400.

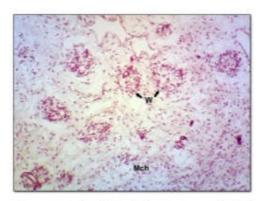


Plate 23: Photomicrograph-cross section of developing mammary gland, Group-II (17.60 cm CRL) showing connective tissue cell whorls (W) in mesenchymal tissue (Mch). H&E X 100.

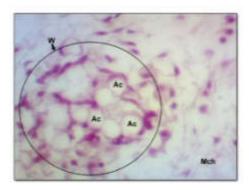


Plate 24: Photomicrograph-cross section of developing mammary gland, Group II (17.60 cm CRL) showing Adipose cells (Ac) aggregations in the whorl (W) and mesenchymal tissue (Mch). H&E X 400.

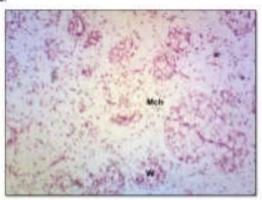


Plate 25: Photomicrograph-cross section of developing mammary gland, Group II (18.50 cm CRL) showing more connective tissue cell whorls (W), mesenchymal tissue (Mch). H&E X 100.

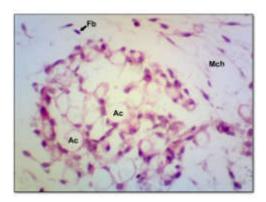


Plate 26: Photomicrograph-cross section of mammary gland, Group II (18.50 cm CRL) showing large adipose cells (Ac) in the cell whorls (W) with peripheral mesenchymal tissue (Mch) and fibroblasts (Fb) H&E X 400.

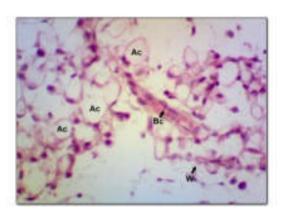


Plate 27: Photomicrograph- cross section of mammary gland, Group II (18.50 cm CRL) showing blood capillary (Bc) entering in to the cell whorls (W), Adipose cells (Ac). H&E X 400.

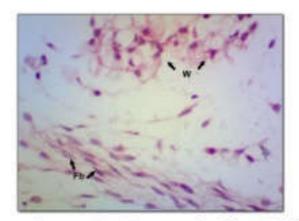


Plate 28: Photomicrograph-cross section of mammary gland, Group II (18.50 cm CRL) showing parallel arrangement of fibroblasts (Fb) around cell whorl (W). H&E X 400.

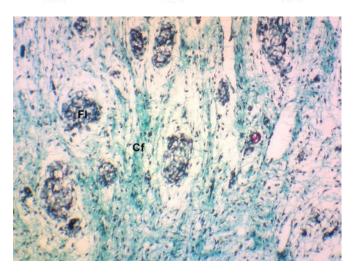


Plate 82: Photomicrograph-cross section of mammary gland, Group II (17.60 cm CRL) showing collagen fibers (Cf) in between the developing fat lobules (Fl). Masson's Trichrome stain x 100

With the advancement of age the spherical masses of mesenchymal tissue which formed in the developing gland differentiated into adipose tissue as fat pad, seen in the foetus of 20.00 cm CR length. The present finding is in line with the report of Panchal (1998b) in buffalo foetus. In 36.00 cm CR

length foetus, the development of the adipose tissue became very extensive throughout the udder tissue above and around the gland cistern and duct system. The gland cistern was first observed in the foetus of 17.60 cm CR length which became larger in 18.50 cm CR length. In the foetus of 20.00 cm CR

length the well defined gland cistern was seen between the fat pad, which was lined by undifferentiated layer of epithelium (Plate 30 and 31).

The udder tissue deep to the stratum germinativum of the foetus was mesenchymal in group I. The embryonic tissue was comprised of loosely arranged spindle shaped cells and very thin reticular fibers. The network of these fibers was distributed throughout the mesenchymal tissue area of the gland. The mesenchymal cells had a large vesicular nuclei. Differentiation of mesenchyme and occurrence of collagen fibers was observed in the foetus of 17.60 cm CR length (Fig. 21). Whereas, in buffalo foetus, the traces of collagen fibers were observed at 12.50 cm CR length (Panchal, 1998b). The collagen fibers were not in the form of bundles but were sparsely distributed. The initiation of formation of collagen strands around the connective tissue whorls was observed in the foetus of 20.00 cm CR length. The scanty elastic fibers were noticed in the inter-whorl connective tissue area of the fat pad in 20.00 cm CR length. Distribution of elastic fibers was sparse and scattered in the connective tissue septae of the developing gland. However, distinct lamina of elastic fibers was observed in the tunica intima of the blood vessel. Panchal (1998) observed the traces of elastic fibers in buffalo foetus at the age of 37.70 cm CR length.

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