

Review Article

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## Morphogenesis of Mammary Gland in Indian Buffalo (*Bubalus bubalis*): A Review on Prenatal Study

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### ABSTRACT

The mammary gland develops through several distinct stages. The first transpires in the embryo as the ectoderm forms a mammary line that resolves into placodes which leads to various stages like mammary bud, primary sprout, secondary and tertiary sprouts. Simultaneously the connective tissue whorls, fat pad, teat and gland sinus and other associated structures like sweat and sebaceous glands are also developed. Regulated by epithelial/mesenchymal interactions, the placodes descend into the underlying mesenchyme and produce the ductal structure of the gland present at birth. These processes require numerous signaling pathways that fuel the dramatic changes in the gland occurring during prenatal life. Our knowledge of morphogenesis of mammary gland and its biology may reveal the ways to manipulate the development of mammary gland to enhance milk production in mature buffalo and to treat various diseases.

#### Keywords

Buffalo, Mammary gland, Mammary bud, Primary Sprout, Fat pad, Gland sinus

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### Introduction

Buffalo breed needs a systematic genetic improvement programme and efficient measures for their conservation (Singh, 2006). This breed plays a significant role in rural livelihood of India in terms of draught, milk and manure. The name “Black Gold” has emerged as synonym for the one very popular breed of buffaloes i.e. Murrah, which serves as capital reserve or cash crops to rural folk by producing economic stability, livelihood security and social status. The mammary

gland distinguishes mammals from all other animals with its unique anatomical structure that secretes milk for the nourishment of the newborn (Jenkinson, 2003). They are complex secretory organs composed of a number of different cell types: epithelial cells that grow from the teat into a fat pad, formed by adipocytes and infiltrated by vascular endothelial cells, fibroblasts and immune cells (Akers, 2002). This article focuses primarily on the epithelial changes during prenatal life of Indian buffalo (*Bubalus bubalis*). Here, we review how basic morphogenesis shape the

structure of the gland at each stage of development.

## **Morphogenesis of mammary gland**

### **Mammary line**

Anderson (1978) reported that mammary band developed at 9 mm CRL in sheep, 5 mm CRL in goat and 14 mm CRL in cattle. Similarly, Carroll (1980) and Ellis (1998) observed that the mammary gland formed from an invagination of ectoderm at 1.4 cm CVRL in cow embryo. Challana *et al.*, (2014) reported that the earliest recognizable stage of mammary gland development in the present study was mammary line found in 1.2 cm CVRL (34 days) buffalo foetus on the ventral side in the inguinal region posterior to umbilicus. Whereas, Akers (2002) described that the mammary band was formed at 30<sup>th</sup> day in bovine foetuses. However, Jenkinson (2003) reported that the mammary band run along both sides of the midline in the inguinal region at day 40 in sheep foetuses.

Anderson (1978) found mammary line at 10 mm CRL in sheep, 10 mm CRL in goat and 17 mm CRL in cattle and mammary crest was observed at 15 mm CRL in sheep, 28 mm CRL in goat and 19 mm CRL in cattle. Panchal *et al.*, (1998a) reported that a pair of mammary line appeared in the inguinal region on either side of mid ventral line, medial to thigh at 38 days (1.0 cm CRL) in buffalo foetuses. Whereas, Singh (2000) observed four mammary anlagen on the ventral abdominal wall caudal to umbilicus between the hind limbs in buffalo foetus at 90-109 days of gestation.

Challana *et al.*, (2014) reported that the proliferation of mesenchymal cells resulted in condensation of mammary line into mammary hillock at 1.7 cm CVRL (37 days) of fetal age. The mammary hillock was cone shaped.

Akers (2002) reported that mammary streak became the mammary line by fifth week of development in bovine foetuses. Jenkinson (2003) noticed the mammary line in the inguinal area posterior to umbilicus at 40 days in sheep foetuses. Whereas, McGeady *et al.*, (2006) stated that the mammary line extended from the forelimb buds to the hind limb buds at 30 days of gestation in bovine foetus. However, Vaish (2012) reported that the initiation of the development of the mammary gland was observed as mammary line in the inguinal region on either side of the median line, medial to thigh in 4.50 cm CRL and discoid shaped mammary crest in 5.00 cm CRL goat foetuses.

### **Mammary bud**

Anderson (1978) described mammary hillock at 20 mm CRL in sheep, 29 mm CRL in goat and 21 mm CRL in cattle. Whereas, mammary bud was observed at 25 mm CRL in sheep, 30 mm CRL in goat and 25 mm CRL in cattle. Carroll (1980) reported mammary bud at 3.0 cm CRL in cow foetuses. Turner and Huynh (1991) reported that mammary bud was most often elongated and ovoid in shape and was carried outward with the developing teat rather than beginning to invade the underlying mesenchymal tissue in cattle embryo. Challana *et al.*, (2014) reported that the mammary bud is the prominent stage of mammary embryonic development in buffaloes. It was observed in 2.6 cm CVRL (41 days) buffalo foetus. The later was ovoid in shape with its long axis perpendicular to the surface of the foetus.

Ellis (1998) observed that the mammary hillock and bud were the prominent stages of mammary embryonic development in ruminants. Panchal *et al.*, (1998a) in buffalo foetuses and Akers (2002) in bovine foetuses noticed the mammary bud at 55 days (4.20 cm CVRL) and 43 days, respectively and

Jenkinson (2003) found mammary bud at 40 days in sheep foetuses. McGeady *et al.*, (2006) described that mammary crest developed into mammary bud at 30 days of gestation in bovine foetus. However, Hyttel *et al.*, (2010) stated that the mammary bud lengthened and branched from day 58 onwards in bovines.

Chaurasia *et al.*, (2012) observed formation of mammary bud between 4.4 to 6.0 cm CRL (44-49 days) and the cells of the mammary bud progressed deeper into the dermis at 7.9 cm CRL in goat foetus. Similarly, Vaish (2012) observed that 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 6.70 cm CRL goat foetus.

### **Primary sprout**

Anderson (1978) described primary sprout at 56 days of gestation in sheep, 50 days in goat and 80 days in cattle. Similarly, Carroll (1980) found the primary sprout at 12.0 cm CRL in cow foetuses.

Panchal *et al.*, (1998a) reported that primary sprout got elongated and entered deep into the teat base at 102 days (14.30 cm CRL). At 108 days (16.0 cm CRL), the luminization of the primary sprout was found in its proximal rounded end. The luminization progressed towards the distal end of the duct near the teat apex in 120 days (18.50 cm CRL) buffalo foetuses. Similarly, Singh (2000) found that the canalization of primary sprout started in the centre at the proximal end and preceded to distal end at 120 to 146 days in buffalo foetuses.

Akers (2002) described the primary sprout at day 80 in bovine. Jenkinson (2003) noticed primary sprout at 15.0 cm CRL (60 days) in

sheep foetus. The later was canalized and extended up to full length of the duct. Chaurasia *et al.*, (2012) observed that the primary sprout sunk deeper into the teat base at 12.5 cm CRL (64 days) in goat foetuses. Later on luminization of the primary sprout took place at 12.8 cm CRL and showed that luminized primary sprout continued its development deep into the future glandular part of the udder. Similarly, Vaish (2012) reported the primary sprout at 11.50 cm CRL in goat foetuses. This structure got extended as a cellular cord and entered deep into the base of the teat at 12.50 cm CRL. The luminization of the primary sprout from the base towards the apex of the teat was observed in 17.60 cm CRL and 12.8 cm CRL in goat foetuses by (Vaish, 2012) and (Chaurasia *et al.*, 2012), respectively. Whereas Challana *et al.*, 2014 revealed that the mesenchymal cells surrounding the mammary bud proliferated to form the outward projection of mammary tissue which developed to epidermal cone (primitive teat) at 6.7 cm CVRL (58 days) in the inguinal region of buffalo foetus. The epidermal cells of the bud elongated by rapid cell proliferation into the underlying mesenchymal tissue along the length of the teat to form primary sprout at 7.4 cm CVRL (62 days) of fetal age. These cells got extended as cellular chords and enter deep into the base of teat at 10.7 cm CVRL (77 days). The primary sprout gave rise to the teat cistern, gland cistern and major duct system of mammary gland.

### **Secondary sprout**

Anderson (1978) found secondary sprouts at 59 days in sheep, 60 days in goat and 90 days in cattle foetuses. Forsyth *et al.*, (1999) and Jenkinson (2003) noticed numerous secondary sprouts at 80 days of sheep foetuses. Singh (2000) reported secondary sprout at 254 days in buffalo foetuses. However, Akers (2002) described the

secondary sprout at 90 days in bovine foetuses.

McGeady *et al.*, (2006) stated that eight to twelve secondary sprouts radiate from the gland sinus into the surrounding tissue after the fourth month in bovine foetuses. Whereas Chaurasia *et al.*, (2012) reported secondary sprouts at 13.8 cm CRL (68 days) in goat foetuses. Challana *et al.*, 2014 found that the secondary sprouts were budding off at different angles to the proximal end of the primary sprout in various directions between 14-17 cm CRL (69- 78 days) in goat foetuses. These ducts were anlagen of the duct system of the udder. The secondary sprouts appeared as irregular shaped hollow sacks at 15 cm CVRL (96 days) of fetal age. These ducts would form the duct system of the mammary gland. The lumen of secondary sprouts began to grow by cellular degeneration of epithelial cells of primary sprout at various angles into the surrounding mesenchyme at 18.2 cm CVRL (110 days) as the rapidly growing epithelial cells were unable to obtain nutrients. The secondary sprouts gave rise to tertiary branches at 21.2 cm CVRL (122 days) and further branching of the tertiary ducts was observed at 24.1 cm CVRL (130 days) that proceeded towards the fat pad.

### **Teat formation**

Anderson (1978) described development of teat at 6.0 cm CRL (46 days) in sheep and 8.0 cm CRL (65 days) in cattle foetuses. Sheffield (1988) reported development of teat at 80 day of gestation in bovine foetuses. Panchal *et al.*, (1998a) found that the development of teat was initiated by an elevation of epidermal cone surrounding the mammary bud at 5.70 cm CRL in buffalo foetuses whereas, Akers (2002) stated that the teat development by day 65 in bovine foetuses. Chaurasia *et al.*, (2012) and Vaish (2012) observed a papilla like teat

discernible on either side of midline in inguinal region between two thighs at 9.5 cm CRL (58 days) and 6.70 cm CRL, respectively in goat foetuses. Challana *et al.*, (2014) found that as the age advanced, gradually the epidermal cone got elevated along the mammary bud and the development of teat was initiated at 6.7 cm CVRL (58 days). With increasing age, the length of teat also increased.

### **Fat pad**

Sheffield (1988) and Panchal *et al.*, (1999a) reported formation of fat pad at 80 and 189 days in cattle and buffalo foetuses, respectively. Whereas Forsyth *et al.*, (1999) and Jenkinson (2003) found fat pad at day 140 and day 80 respectively, in sheep foetuses.

Singh (2000) found fat pad at 120 days of fetal life in buffalo. Whereas, McGeady *et al.*, (2006) described formation of mammary fat pad on the base of the developing mammary gland at 180 day of gestation in cattle. Chaurasia *et al.*, (2012) and Vaish (2012) reported that the fat pad developed by branching of the secondary sprouts and differentiation of spherical masses of mesenchymal tissue at 19.5 cm and 20.0 cm CRL, respectively in goat foetuses. Challana *et al.*, (2014) found that the mesenchymal cells at the base of developing mammary gland differentiated into fat pad at 45.5 cm CVRL (176 days) of buffalo foetus.

### **Gland cistern**

Anderson (1978) described gland cistern at 23.0 cm CRL (130 days) in cattle foetus. Whereas, Carroll (1980) found gland cistern at 17.00 to 21.00 cm CRL in cow foetus. Jenkinson (2003) found the gland cistern near the proximal end of primary sprout at day 80 in sheep foetuses. Chaurasia *et al.*, (2012)

noticed highly proliferative growth of gland cistern and all the ducts were luminized at 38.6 cm CRL (139 days). Whereas, Vaish (2012) observed gland cistern between the fat pad at 17.60 cm CRL goat foetuses. Challana *et al.*, (2014) reported that the initiation of gland cistern was observed in 15 cm CVRL (96 days) buffalo foetus. The continual growth of the lumen of the primary sprout pushed back the cells lining the primary spout towards glandular tissue of developing mammary gland resulting in formation of fully developed gland cistern at 45.5 cm CVRL (176 days) in buffalo foetuses.

### **Teat cistern**

Anderson (1978) observed the teat cistern at 30.0 cm CRL (110 days) in cattle. Whereas, Carroll (1980) found the teat cistern at 17.00 to 21.00 cm CRL in cow foetuses. The streak canal was the distal most part of the primary sprout that canalized at last but lumen was not fully formed up to 152-182 days in buffalo foetuses (Singh, 2000). Challana *et al.*, (2014) found that the canalization of primary sprout proceeded downward and initiated the formation of teat cistern in 15 cm CVRL (96 days) of buffalo foetus. Well distinct teat cistern was noticed at 24.1 cm CVRL (130 days). The lumen of teat cistern continued downward to form teat canal at 21.2 cm CVRL (122 days) of buffalo foetus.

Akers (2002) described the streak canal and teat cistern at 100 days in bovine foetuses. Jenkinson (2003) reported the teat cistern at 80 days in sheep foetuses. At day 140 of fetal age the entire gland greatly increased in size due to lengthening of the primary sprout which developed to teat and gland cisterns.

McGeady *et al.*, (2006) and Hyttel *et al.*, (2010) described teat cistern at 120 days of gestation in mammals. Chaurasia *et al.*, (2012) noticed the teat canal at 21.7 cm CRL

(91 days) in goat foetuses. The lumen of teat cistern became wide and increased height of the primary and secondary mucosal folds were also noticed at this age. However, Vaish (2012) reported teat cistern in 34.00 cm CRL in goat fetuses and Challana *et al.*, (2014) found that the rosette of furstenberg was observed at 39.5 cm CVRL (163 days) at junction of teat canal and streak canal and keratin plug was found at 45.5 cm CVRL (176 days) of buffalo fetuses

### **Micro vascularization**

Carroll (1980) observed that blood vessels were formed in the mesenchyme surrounding the primary sprout at 17.00 to 19.00 cm of cow foetuses. Akers (2002) stated that the blood vessels began to form at day 80 of gestation in bovines.

Jenkinson (2003) reported that Nerves and small blood vessels formed in close association with each bundle of developing adipose tissue at day 80 and elastic fibres at day 90 in sheep foetuses. Chaurasia *et al.*, (2012) and Vaish (2012) reported that the numerous blood capillaries invaded the bud at 4.4 cm to 6.0 cm CRL (44 -49 days) and 7.20 cm CRL in goat foetuses, respectively.

The small blood vessels were observed in the mesenchymal tissue of mammary bud at 2.6 cm CVRL (41 days) of buffalo foetus.

At 18.2 cm CVRL (110 days), nerves and small blood vessels were found in close association with connective tissue whorl. With advancement of fetal age, the numerous blood vessels were seen around secondary sprout and developing gland and teat cistern. At 69 cm CVRL (229 days), blood and lymphatic vessels along with nerve bundles were present throughout the mammary gland (Challana *et al.*, 2014).

## **Skin and its appendages**

Bhayani *et al.*, (1992) observed a distinct epidermis and dermis between 74-115 days of gestation in buffalo. Panchal *et al.*, (1999b) observed increased in cell layer of epidermis for 3-4 at 73 days of gestation to 12-15 at 191 days of buffalo foetus. Singh (2006) revealed that the skin was comprised of distinct epidermis and dermis at 5.5 cm CVRL (53 days) in bovine foetus.

Malik *et al.*, (1996) reported that the epithelium was divided into 4 layers on stratum germinativum, stratum granulosum, stratum intermedium and stratum basale in goat foetus. Challana *et al.*, (2014) found that the skin was comprised of distinct epidermis and dermis at 6.7 cm CVRL (58 days) buffalo foetuses. Epidermis was 4-5 cell layers thick at 6.7 cm CVRL (58 days) which was increased to 7-8 cell layers at 7.4 cm CVRL (62 days) and 12-14 at 10.7 cm CVRL (77days). With advancing fetal age, thickness of epidermis decreased and became 4-5 cell layers at 18.2 cm CVRL (110 days). Dermis was clearly divided in two layers at 18.2 cm CVRL (110 days). Outer layer i.e. towards epidermis was loosely arranged whereas the inner layer was dense. The thickness of dermis increased with advancement of fetal age.

Carroll (1980) observed hair follicles began at 120 days of cow foetus. Bhayani *et al.*, (1992) and Panchal *et al.*, (1999b) reported first evidence of hair follicle at 115-120 days and 102 days respectively in buffalo foetuses. McGeady *et al.*, (2006) described that the hair follicles began to develop around the 120<sup>th</sup> day of gestation in bovines. Challana *et al.*, (2014) Jenkinson (2003) observed the presence of hair follicles along the entire length of the teat at 80 days of gestation in sheep foetuses. Chaurasia *et al.*, (2009) reported the hair follicle on the teat at 65 days

in goat foetuses. Challana *et al.*, (2014) observed that the primordia of hair follicle at 6.7 cm CVRL (58 days) buffalo foetus.

Bhayani *et al.*, (1992) observed the sweat gland at 140-150 days buffalo foetuses. Panchal *et al.*, (1999b) reported that the primordium of sweat glands at 139 day in buffalo foetus. Chaurasia *et al.*, (2009) observed the sweat glands for the first time at 83 days in goat foetuses.

Panchal *et al.*, (1999b) reported the sebaceous gland at 158 days in buffalo foetuses. Whereas Jenkinson (2003) reported sebaceous glands associated with each primary follicle at 120 days in sheep foetuses. Chaurasia *et al.*, (2009) reported bunch of sebaceous gland around the hair follicles in 117-139 days goat foetuses. Challana *et al.*, (2014) found that the primordia of sweat and sebaceous glands were found at 21.2 cm CVRL (122 days) of buffalo foetus.

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