HISTO-ARCHITECTURAL CHANGES IN PLACENTAL EPITHELIUM DURING GESTATION IN BUFFALOES (*BUBALUS BUBALIS*)

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ABSTRACT

The present study was conducted on placentomes of 20 buffaloes (n=20) ranging from 38 to 243 days of gestation. Their gestational age was determined by measuring the curved crown rump length (CRL) of foetii using an inelastic thread as per formula given by Soliman (1975) in bovines. Depending on the CRL and estimated age of the foetuses, the samples were divided into 3 groups. The placental tissues fixed in 10% NBF were processed and stained accordingly. The present observation revealed that the cryptal epithelium was cuboidal during early and mid pregnancy but transformed to squamous during late pregnancy especially at the tip of the maternal septa. This reduction in size and nature of epithelium during late pregnancy was in order to shorten the distance between foetal and maternal tissue for substance exchange by diffusion. The trophoblastic epithelium transformed from cuboidal during early and mid pregnancy to stratified epithelium in the arcade region due to accumulation of binucleated giant cells that were involved in phagocytosis of extravasated erythrocytes in the arcade region. The phagocytosis released iron from the RBC that were probably being utilized by the developing fetus as very strong reaction for acid phosphatase was observed in the arcade region and iron in the form of fine granules were observed by special staining.

Keywords: *Bubalus bubalis*, buffaloes, buffalo placenta, cryptal epithelium, trophoblastic epithelium

INTRODUCTION

Buffalo has been an integral part of livestock husbandry in Asia for over five thousand years producing draft power, milk, meat and hides (Nanda and Nakao, 2003). The buffalo, considered as India's milking machine, plays a significant role in Indian economy as it alone accounts for about 50% of total milk production thus forming the backbone of India's dairy industry (Das *et al*., 2008). Unfortunately, buffalo did not receive the attention of researchers in accordance with its merits, which resulted in decline in its production (Nanda and Nakao, 2003). The major impediment to its efficient production is its poor reproduction. The development and delivery of a healthy offspring are important milestones of reproductive

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performance. The normal development of a healthy offspring requires the most protective environment which is provided by the placenta. The placenta is an organ for physiological exchange between foetus and dam which acts as a selective barrier and as an endocrine organ. Its structure enables foetal nutrition, excretion and respiration to take place without permitting transfer of molecules of high molecular weight, particulate matter and blood cells (McGeady *et al*., 2006). Placental transport capacity must keep pace with increasing demand of foetus for normal foetal growth and this transport across the feto-maternal apposition occurs via the epithelial covering of the villi and crypts, hence the present work was undertaken to elucidate the changes in epithelial coverings, if any, with gestation in buffaloes.

MATERIALS AND METHODS

The present study was conducted on placentomes of 20 buffaloes (n=20) ranging from 38 to 243 days of gestation, obtained from slaughter house, post mortem materials and clinics. The gestational age was determined by measuring the curved crown rump length (CRL) of foetii using an inelastic thread as per formula given by Soliman (1975) in bovines.

$$Y = 28.66+4.496\ X \quad (CRL<20\ cm)$$
$$Y = 73.544+2.256\ X \quad (CRL\geq 20\ cm)$$

Where Y is the age in days and X is the CRL in cm.

Depending on the CRL and estimated age of the foetuses, the samples were divided into 3 groups:

Group 1 = CRL upto 20 cm
Group 2 = CRL>20 cm to 40 cm
Group 3 = CRL>40 cm

The placental tissues fixed in 10% neutral buffered formalin were processed by acetone-benzene schedule to obtain 5 to 7 µ paraffin sections. The sections were stained with hematoxylin and eosin for routine morphology, periodic acid Schiff (PAS) for neutral-mucopolysaccharides, Alcian blue (pH 2.5) for acid-mucopolysaccharides and Prussian blue for ferric iron (Luna, 1968).

The fresh unfixed tissue samples were quickly frozen in liquid nitrogen. These samples were subjected to cryostat sectioning at -20°c using cryostat microtome. The cryostat sections of 10 to 12 µm thickness were obtained on glass slides and incubated with the required incubating medium for demonstration of acid phosphatase activity (Barka and Anderson, 1963).

The Haematoxylin and eosin stained sections of the buffalo placentomes were used to record the micrometrical observations with the help of Filar and ocular micrometer duly calibrated with stage micrometer. The average values of all parameters were subjected to statistical analysis (Snedecor and Cochran, 1994). The desired photographs were taken using digital photographic microscope.

RESULTS AND DISCUSSION

Cryptal epithelium

At very initial stage of development, up to 5.5 cm CRL (53 days) the lining epithelium of maternal caruncle within the developing primary crypt was cuboidal to low columnar whereas it was denuded elsewhere on the caruncular surface

(Figure 1). The epithelium of the maternal crypts became continuous cuboidal from 9 cm CRL (69 days) onwards (Figure 2 and Figure 4). In cow, a single layer of cuboidal cryptal epithelium was reported by Lee *et al.* (1986) thus confirmed the present findings.

The nuclei of cryptal epithelial cells were spherical to oval and lightly stained during early pregnancy. The cytoplasm was lightly stained (Figure 2 and Figure 4). Schmidt (2005) in buffalo and Eurell and Frappier (2006) in bovines also reported similar observations.

The cryptal epithelium was observed to be cuboidal from cryptal base to its tip throughout the period in Group 2. However, at 31 cm CRL (143 days) slight denudation of epithelium was observed which was due to the migration and fusion of giant cells of trophoblastic origin as maximum migration of giant cells was observed at this stage (Ranjan and Singh, 2013).

The epithelium continued to be cuboidal till 170 days of gestation (43 cm CRL). After that, the epithelium transformed to simple squamous at cryptal base and at the tip of the maternal septa, elsewhere it was low cuboidal (Figure 5 and Figure 6). The nuclei were flattened and the cytoplasm was more eosinophilic. This reduction in size and nature of epithelium during late pregnancy was in order to shorten the distance between foetal and maternal tissue for substance exchange by diffusion thus meeting the increasing demand for substances by rapidly growing foetus towards the end of gestation (Woicke *et al*., 1986). The histological observation regarding change in epithelial nature and size was further strengthened by micrometrical observations on cryptal epithelial height which revealed a negative correlation coefficient with age (Table 1 and Table 2).

The average nuclear diameter in cryptal epithelium of the placentome was 8.83±0.17 µm, 8.09±0.23 µm and 7.75±0.36 µm in Group 1, 2 and 3 respectively. The decrease in the diameter was due to change in the epithelium from cuboidal to squamous. Least variation in nuclear diameter was observed in Group 1 (3.74%) while the coefficient of variation was more in Group 3 (10.45%). The correlation coefficient between age and nuclear diameter was negative (-0.670) that was significant at 0.01 level indicating a marked change in nuclear diameter (Table 1 and Table 2).

Trophoblastic epithelium

During early pregnancy, the villi consisted of cuboidal trophoblastic epithelium with darkly stained nuclei and eosinophilic cytoplasm. Cuboidal to columnar trophoblastic epithelium along with giant cells throughout the pregnancy had been reported earlier in ruminants (Igwebuike, 2004), bovines (Greenstein *et al*., 1958) including buffalo (Sharma *et al*., 1983) which confirms the present observation.

At 13 cm CRL (87 days) simple cuboidal epithelium with dark stained nuclei and eosinophilic cytoplasm was observed which was in conformity with the findings of Sharma *et al.* (1983) in buffalo (Figure 3). From 17 cm CRL (105 days) the secondary and tertiary villi showed continuous cuboidal epithelium (Figure 4) but the villous base (arcade region) started showing stratification which became more pronounced with advancing gestation (Figure 5). Similar observations were made by Sharma *et al.* (1983). The stratification was due to accumulation of binucleated giant cells in the arcade region (Figure 7) which was more pronounced in late gestation in order to phagocytise the extravasated erythrocytes (Figure 9) in the arcade area near the villous base in order to release iron in the form of fine granules

from the erythrocytes and make it available to the developing foetus (Ranjan *et al*., 2012). In the present observation, extravasation of erythrocytes (Figure 8) along with a very intense reaction for acid phosphatase (Figure 10) was observed in the arcade region. Simultaneously, iron in the form of fine granules was observed which was probably being utilized by the developing fetus (Figure 11).

The nucleus of trophoblastic epithelial cell was darkly stained with eosinophilic cytoplasm (Figure 3 and Figure 4). Similar findings were reported by Dellmann (1993) in mare and Sharma *et al.* (1983) in buffalo. Throughout the pregnancy several uninucleate and binucleate giant cells with darkly stained nuclei and vacuolated cytoplasm and few multinucleate cells with vacuolated cytoplasm were observed within the trophoblastic epithelium (Figure 3 and Figure 4).

The average epithelial height of the trophoblastic epithelium was 17.68±1.67 µm, 16.24±0.65 µm and 21.32±1.57µm in Group I, II and III respectively. This increase in epithelial height might be due to stratification of the trophoblastic epithelium at the villous base observed during mid and late pregnancy. The coefficient of variation was consistent in Group II (8.99%). The correlation coefficient between age and trophoblastic epithelium was positive (0.393) but was non-significant at any level (Table 1 and Table 2).

Table 1. Micrometrical observations on trophoblastic epithelial height, cryptal epithelial height and nuclear diameter.

Groups	Trophoblastic epithelium		Cryptal epithelium		Nuclear diameter	
	Mean±S.E	C.V	Mean±S.E	C.V	Mean±S.E	C.V
Group 1	17.68±1.67	18.89	8.06±0.64	15.88	8.83±0.17	3.74
Group 2	16.24±0.65	8.99	7.48±0.29	10.03	8.09±0.23	6.43
Group 3	21.32±1.57	16.51	7.51±0.21	6.26	7.75±0.36	10.45

Table 2. Correlation coefficient between age (CRL) and other parameters.

Sl. No.	Parameters	Correlation coefficient
1.	Trophoblastic epithelium	0.393[c]
2.	Cryptal epithelium	-0.125[c]
3.	Nuclear diameter	-0.670[a]

a- Correlation is significant at 0.01 level (2-tailed)

b- Correlation is significant at 0.05 level (2-tailed)

c- Correlation is non-significant at any level.

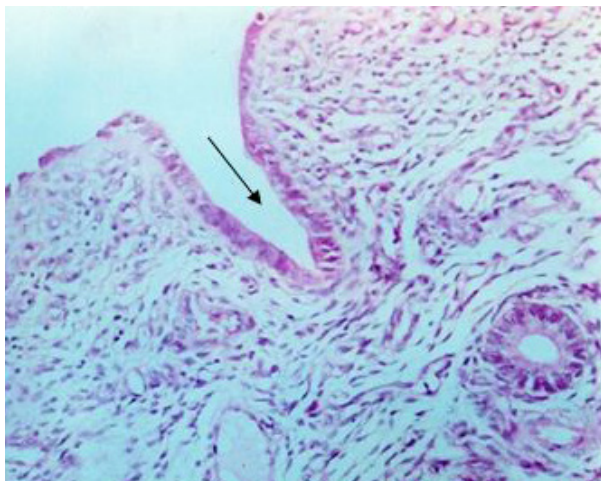

Figure 1. Photomicrograph of caruncle of 5.5 cm CRL (53 days) showing primary crypt (arrow) lined by simple cuboidal to low columnar epithelium . H and E X200.

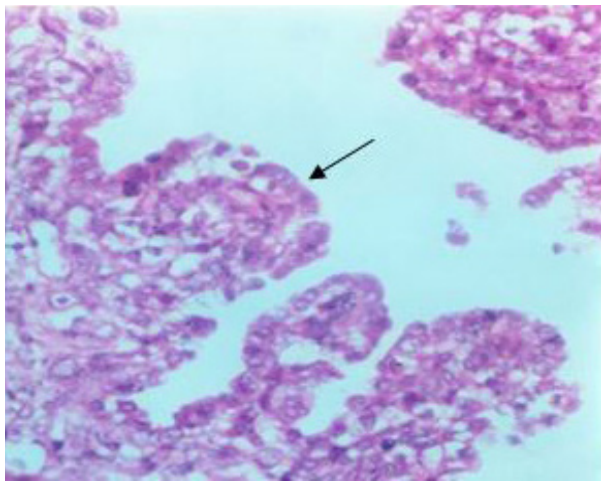

Figure 2. Photomicrograph of buffalo placenta of 9 cm CRL (69 days) showing cuboidal cryptal epithelium (arrow). H and E X400.

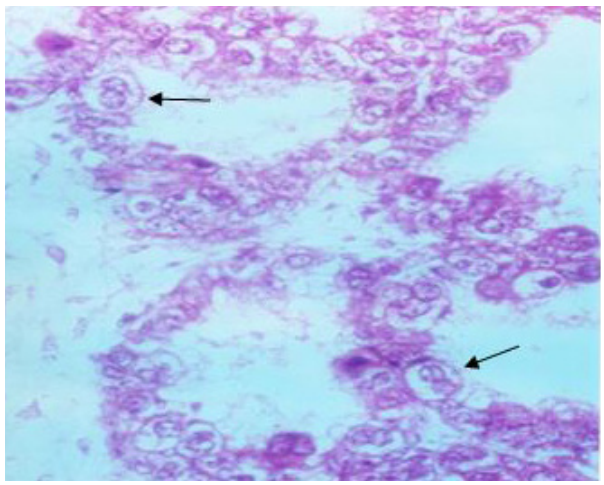

Figure 3. Photomicrograph of buffalo placentome of 13 cm CRL (87 days) showing uninucleate and binucleate giant cells (arrow) within the trophoblastic epithelium. H and E X400.

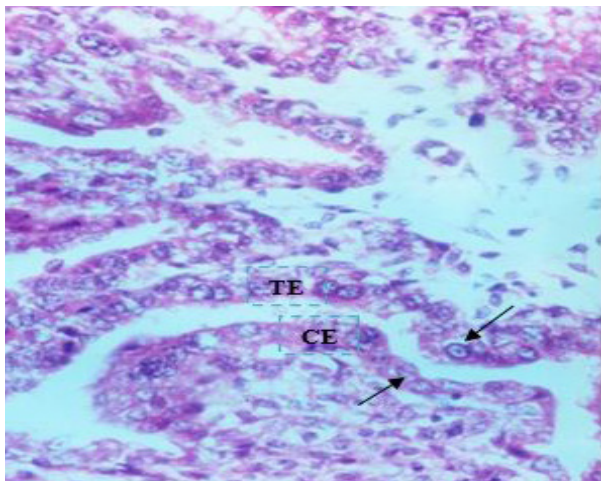

Figure 4. Buffalo placentome of 17 cm CRL (105 days) showing cuboidal trophoblastic (TE) and cryptal epithelium (CE) and giant cells (arrow). H and E X400.

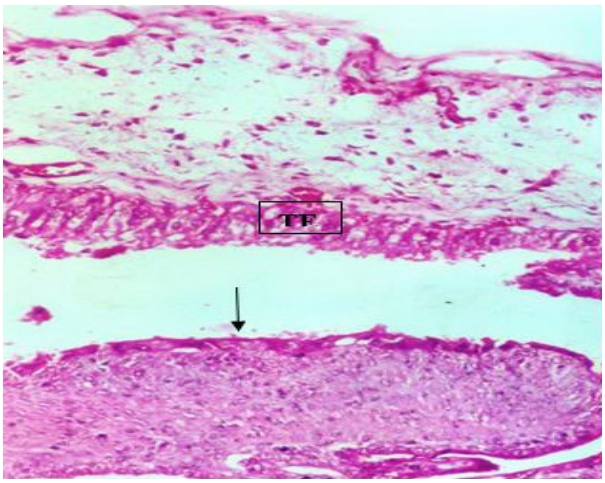

Figure 5. Photomicrograph of buffalo placentome of 40 cm CRL (164 days) showing club shaped ending of maternal septa (arrow) with squamous epithelium and stratified trophoblastic epithelium (TE) at the villous base. H and E X200.

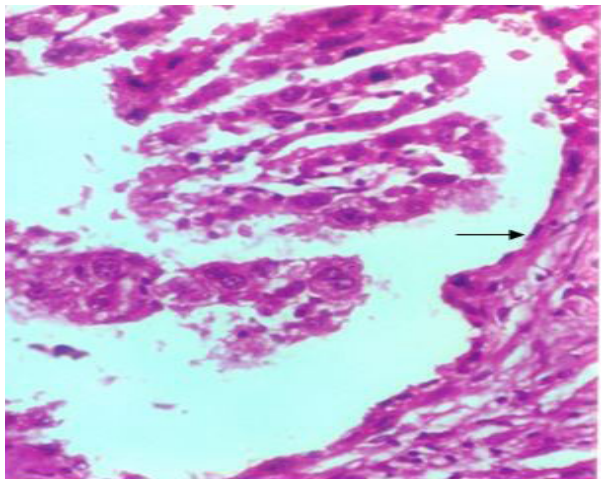

Figure 6. Photomicrograph of buffalo placentome of 43 cm CRL (170 days) showing presence of squamous epithelium at the cryptal base (arrow). H and E X400.

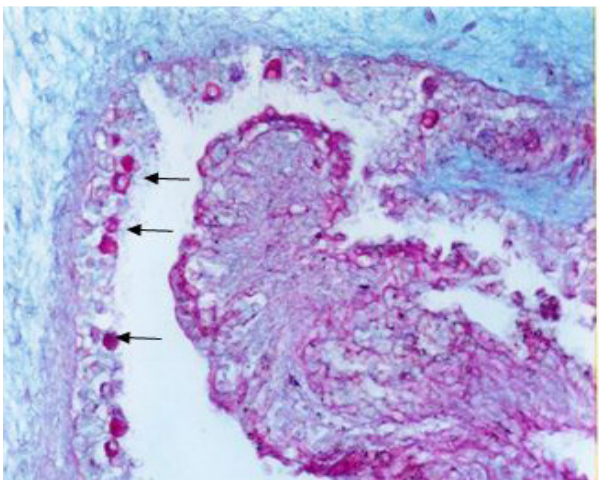

Figure 7. Photomicrograph of buffalo placentome of 40 cm CRL (164 days) showing aggregation of PAS-positive giant cells in trophoblastic epithelium (arrow). PAS-AB X200.

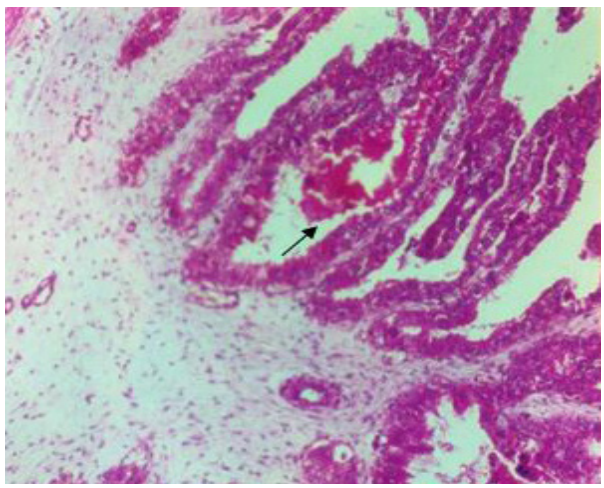

Figure 8. Photomicrograph of buffalo placentome of 49 cm CRL (184 days) showing extravasation of blood (arrow) in the arcade region. H and E X100.

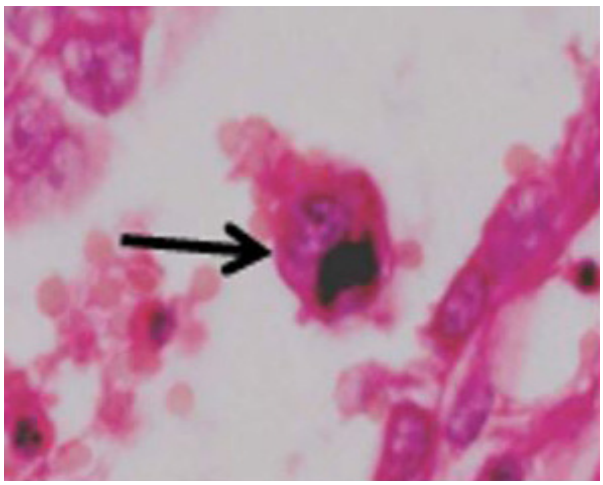

Figure 9. Binucleate giant cell showing phagocytosis of extravasated erythrocytes (arrow). H and E X400.

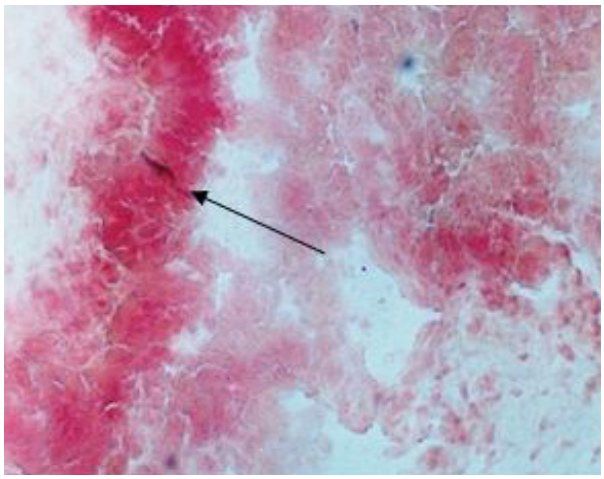

Figure 10. Photomicrograph of buffalo placentome of 49 cm CRL (184 days) showing strong ACPase activity in the trophoblastic epithelium (arrow) of arcade region. Azodye method X200.

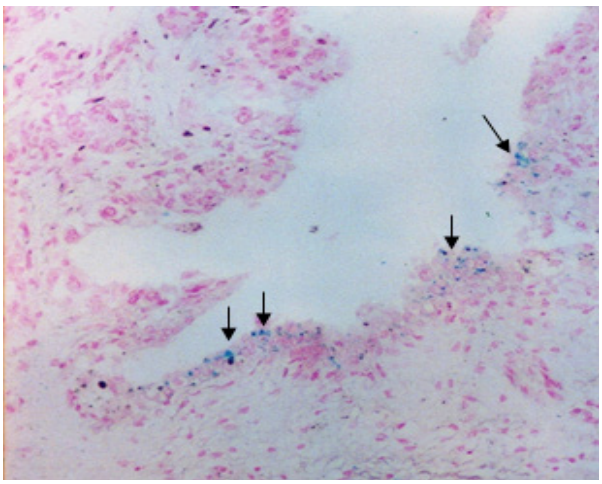

Figure 11. Photomicrograph of buffalo placentome of 65 cm CRL (220 days) showing presence of iron in the form of fine blue granules (arrow) in trophoblastic epithelium of arcade region. Prussian blue X200.

CONCLUSION

The change in cryptal epithelium from cuboidal to squamous shortened the distance in the feto-maternal apposition with gestational age in buffalo and enhanced the rate of exchange of materials by diffusion thus meeting the increasing demand for substances by rapidly growing foetus towards the end of gestation. The stratification of trophoblastic epithelium in the arcade region during mid and late pregnancy was due to accumulation of migrating giant cells which were responsible for phogocytising the extravasated erythrocytes to supply iron to the developing foetus. Hence, based on the observations it can be concluded that epithelial coverings play a major role in feto-maternal apposition and exchange of metabolites for normal fetal growth and placental functioning throughout the gestational age.

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