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Erythrophagocytosis in placentomes of buffalo

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The iron required by the developing foetus comes from mother via placenta during pregnancy. There are reports on the transfer of iron from mother to foetus during pregnancy in carnivores (Malassiné 1977), cow (Murai and Yamauchi 1986) and sheep (Myagkaya and Vreeling 1976). In sow, the iron does not appear to cross the placenta to enter the foetal circulations (Douglas *et al.* 1972). But such informations are lacking in buffalo, hence the present study was conducted to reveal the mechanism of its transfer from mother to the foetus.

The present study was conducted on placenta of 31 pregnant buffaloes ranging from 38 to 243 days of gestation. The placentomes were collected from centre and tip of the pregnant uterine horn (Schmidt *et al.* 2006) of non-descript buffaloes sacrificed at slaughter house, Delhi. The stage of pregnancy was determined by measuring the curved crown rump length (CCRL) of foetuses using an inelastic thread as a curved line along the vertebral column between the most anterior part of frontal bone to the ischiatic tuberosity (Edward 1965).

The approximate age of the foetuses was determined on the basis of their CVRL as per formula given by Soliman (1975) in bovines.

The placental tissue samples were fixed in 10% neutral buffered formalin (10% NBF). The samples were processed for paraffin blocks preparation by acetone benzene schedule and the sections of 5–7 µm were obtained on glass slides. These paraffin sections were stained with Haematoxylin and eosin (H&E) for routine morphology and Prussian blue for ferric iron (Luna 1968). The tissue samples were also processed for semi-thin sections (0.5–2.0 µm) and stained with 1% methylene blue.

In the present study, the maternal septal epithelium in the arcade region (the region where the tip of the maternal septa interdigitates with the base of the chorionic villi) was found to be cuboidal throughout early and mid pregnancy that transformed to squamous during late pregnancy at 65 cm CCRL (220 days) (Figs 1, 2). Raja Ram (1982) also reported a change in epithelium from cuboidal to squamous during late pregnancy in buffalo. These epithelial cells were darkly stained and seemed to be in the process of degeneration (Fig.2). The chorionic trophoblastic epithelium in the arcade zone was much taller and appeared stratified (Fig.1). Similar observations were recorded by Sharma *et al*. (1983) in buffalo soon after parturition. Uninucleate and binucleate giant cells were found to be intermingled with chorionic epithelial cells (Figs 5, 6).

Extravasation of blood in the arcade region was first observed at 49 cm CCRL (184 days) (Fig.3). Björkman (1954) described the appearance of hematoma in the bovine placenta in the third month of gestation. Murai and Yamauchi (1986) also reported extravasation of blood at 150 days of gestation in bovine placentomes near the arcade area.

Clustering of giant cells around extravasated erythrocytes was observed in the arcade area near the villous base during late pregnancy at 65 cm CCRL (220 days) (Figs 2,4,5 and 6). Wimsatt (1951) had also reported that the giant cells in certain parts of the placenta of sheep were erythrophagocytic and assisted in making iron available to the foetus. Murai and Yamauchi (1986) reported that in cow, clusters of mononuclear trophoblast cells extended into the hematoma and occasionally ingested erythrocytes.

With advancing gestation at 49 cm CCRL (184 days) intense acid phosphatase activity was observed in the trophoblastic epithelium especially at the villous base in the arcade area and it was weak elsewhere (Fig.7). Murai and Yamauchi (1986) also detected strong acid phosphatase activity in the trophoblast cells particularly in those lining the base of the stem villi facing the hematoma. Earlier histochemical studies have postulated the role of acid phosphatase in the breakdown and ingestion of blood cells (Burton *et al.* 1976). This might be the reason that strong acid phosphatase activity was observed in the present study. Since acid phosphatase is a lysosomal enzyme, its increased activity indicated erythrophagocytosis by the lysosomes.

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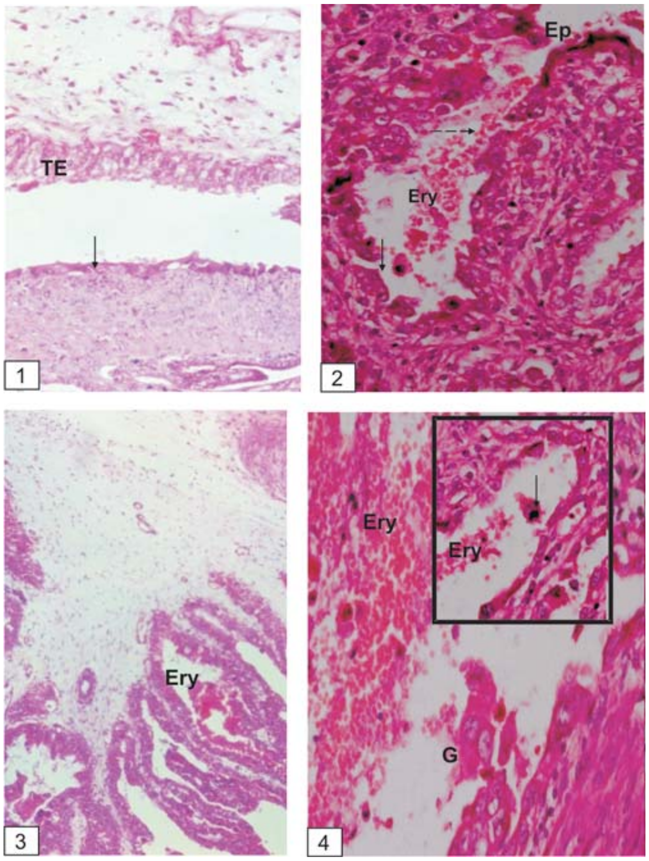
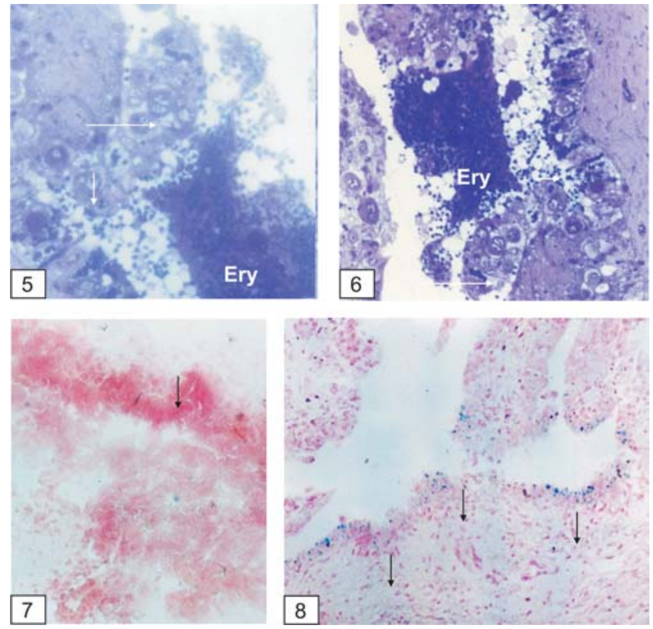


Figs 1–4. **1.** Photomicrograph showing low cuboidal cryptal epithelium (arrow) and stratified trophoblastic epithelium (TE) at the villous base in buffalo placentome of 40 cm CCRL (164 days). H & E ×200. **2.** Buffalo placentome of 65 cm CCRL (220 days) showing squamous cryptal epithelium (Ep) with darkly stained nuclei, point of extravasation of blood from maternal septa (broken arrow) and presence of uninucleate giant cell (arrow) within the extravasated blood (Ery). H & E ×400. **3.** Buffalo placentome of 49 cm CCRL (184 days) showing extravasation of erythrocytes (Ery) in the arcade region. H & E ×100. **4.** Extravasation of erythrocytes (Ery) in the arcade region and its phagocytosis by binucleate giant cell (G) in 65 cm CCRL (220 days) buffalo placentome. H & E X400. [Inset: Buffalo placentome of 65 cm CVRL (220 days) showing extravasated erythrocytes (Ery) and presence of binucleate giant cell (arrow) that seems to erythrophagocytose the blood cells. H & E ×400.]



Figs 5–8. **5.** Semithin section of buffalo placentome of 65 cm CCRL (220 days) showing clustering of giant cells (arrow) around extravasated erythrocytes (Ery) in the arcade region. Methylene blue ×400. **6.** Semithin section of buffalo placentome of 65 cm CCRL (220 days) showing extravasated erythrocytes (Ery) surrounded by giant cells (black arrow) in the arcade region. Numerous erythrocytes are also present within the trophoblastic epithelium (red arrow) in the arcade area. Methylene blue ×200. **7.** 49 cm CCRL (184 days) buffalo placentome showing strong ACPase activity in the trophoblastic epithelium (arrow) of arcade region. Azodye method ×200. **8.** Buffalo placentome of 65 cm CCRL (220 days) showing presence of iron in the form of fine granules (arrow) in trophoblastic epithelium of arcade region. Prussian blue ×200.

Essner (1960), Morselt *et al.* (1973) and Myagkaya *et al.* (1984) had also demonstrated the acid phosphatase activity in erythrophagocytizing trophoblast in ovine placenta.

Finally, iron in the form of fine granules were observed in the trophoblastic epithelium of the villous base in the arcade area during late pregnancy at 65 cm CCRL (220 days) (Fig.8). The presence of iron in the trophoblastic cells had also been reported in cow (Weeth and Herman 1952 and Björkman and Sollen 1960) and in buffalo (Raja Ram 1982 and Sharma *et al.* 1983).

Extravasation of blood, its subsequent erythrophagocytosis by the giant cells and its breakdown with the involvement of acid phosphatase from lysosomes was reported to be responsible for making iron available to the foetus (Seal *et al.* 1972, Burton *et al.* 1976 and Murai and Yamauchi 1986). Extravasation of the maternal blood and its resorption and digestion by the trophoblastic epithelium at the base of villi is stated to be a physiological way of providing supply of iron to the foetus in ruminants (Hradecky *et al.* 1987).

In the present study, extensive extravasation in the arcade area, subsequent clustering of giant cells around erythrocytes and intense acid phosphatase activity in the trophoblastic epithelium of arcade area and finally presence of iron in the trophoblastic epithelium of arcade area were observed. All these events indicated active erythrophagocytosis and subsequent breakdown to provide iron to the foetus. Thus the arcade area seems to constitute an important route for the transfer of iron from mother to foetus in buffalo.

SUMMARY

The present study was conducted on placenta of 31 pregnant buffaloes ranging from 38 to 243 days of gestation

to reveal the mechanism of transfer of iron from mother to the foetus in buffalo. The maternal septal epithelium in the arcade region changed from cuboidal to squamous with advancing pregnancy. These epithelial cells were darkly stained. The chorionic trophoblastic epithelium in the arcade zone appeared stratified. Extravasation of blood in the arcade region was first observed from 49 cm CCRL (184 days) onwards. Clustering of giant cells around extravasated erythrocytes was observed in the arcade area near the villous base. With advancing gestation intense acid phosphatase activity was observed in the trophoblastic epithelium especially at the villous base. Iron in the form of fine granules were observed in the trophoblastic epithelium of the villous base in the arcade area during late pregnancy

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