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Original Research

A Study of Histochemical Distribution in Full Term Placental Cotyledon of Gir Cows and Jaffrabadi Buffaloes

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Abstract

The present study was conducted to compare the histochemical distribution in the fetal placenta of Gir cows and Jaffrabadi buffaloes. It has shown that there is a great variation in weight, cotyledon numbers, and diameter of the cotyledon of the placenta in Gir cow and Jaffrabadi buffalo. Acid mucopolysaccharides, neutral polysaccharides, and sudanophilic staining reveal that the distribution of carbohydrate and lipid in cotyledons are similar in Gir and Jaffrabadi animals but the difference was observed between small and large size cotyledon in both species. Alizarin Red staining shows that calcium is not present in a detectable amount in small and large cotyledons of both species.

Keywords: Cotyledon, Gir Cow, Histochemistry, Jaffrabadi Buffalo, Placenta

Introduction

Understanding of bovine placental development is essential for veterinarians, pathologists, diagnosticians, and researchers. Lesions of diagnostic significance can be recognized for many economically important infectious abortifacient diseases, and there is growing evidence that pregnancy failure of cloned calves is due in part to unexplained placental failure (Manzoor *et al*., 2018). Placentology and placental pathology are becoming of increasing importance. Acid mucopolysaccharides (AMPS) present in the connective tissue stroma and walls of the blood vessels are an important element on the metabolism of the villi, taking part in the regulation of the water metabolism and maintenance of the electrolyte and acid-base balance. Transport of metabolic products, as well as defense mechanisms involved in bacterial and viral infections of the tissues, are also dependent on the mucopolysaccharides (Majewski *et al*., 1963). Binucleated cells (BNCs) of placenta contained periodic acid-Schiff (PAS)-positive granules and different stages of BNC migration and fusion with uterine epithelial cells were stated a strong homology between water buffalo and cattle BNCs concerning cell morphology, protein expression, glycosylation pattern, and characteristics of cell migration and fusion (Carvalho *et al.,* 2006). Therefore, this study was carried out to show the distribution pattern of carbohydrate, lipid, and calcium in the fetal cotyledon of Gir cows and Jaffrabadi buffaloes, which help to distinguish the health and pathological placental condition leads to post-partum problems to the dam.

Materials and Methods

The present work was carried out at the Department of Veterinary Physiology & Biochemistry, College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh. The whole full-term placenta was collected after parturition randomly from Gir cows and Jaffrabadi buffaloes (10 each), maintained at Cattle Breeding Farm, JAU, Junagadh. The mean parity for both the species was between 4 to 6. The placental cotyledonary tissues were collected in 10 % neutral buffered formalin and kept for at least 24-48 hours for fixation. Further, these tissues were processed by the routine method of dehydration in graded alcohol, clearing in xylene, and embedding in paraffin. Tissue sections of 5-6 µm thicknesses were prepared and routinely stained by different special staining method, PAS- Alcian Blue at pH 2.5 (Luna, 1968) for acid and neutral mucopolysaccharides, Oil Red O (Luna, 1968) and Sudan Black B (Chayen *et al.,* 1969) for lipid and Alizarin Red S (Prophet *et al.,* 1994) for calcium distribution. Histological changes were observed in small and large size fetal cotyledon, by using a light microscope. The cotyledons having diameter less than 5 cm and those having more than 7 cm were considered as small and large cotyledons, respectively (Padodara *et al*., 2020).

Results and Discussion

The distribution of neutral and acid mucopolysaccharides in the small and large cotyledon of Gir cow and Jaffrabadi buffalo were demonstrated by Periodic Acid Schiff (PAS) and Alcian Blue (AB), respectively (Fig. 1A & 1B). Strongly PAS-positive cytoplasmic granules were found in the trophoblastic epithelium of the villous arcade area in the large cotyledon of Gir cows and Jaffrabadi buffaloes compare to the small-sized cotyledons. A strong reaction of neutral mucopolysaccharides showed for tunica intima of blood vessels was observed. A weak alcinophilic reaction was found at the basement membrane of the villous and in the center of the villi in the large cotyledon of both species compared to the small one. High activity of neutral mucopolysaccharides indicates it containing glycogen thus confirming that a high level of metabolism would be expected in placental trophoblasts where considerable activity concerning transport, degradation, and synthesis of materials is taking place. A similar reaction of PAS was observed during the mid and late phase of pregnancy and some of the migrating giant cells showed alcinophilic granules at mid-phase in buffaloes (Ranjan *et al.,* 2013) and acid mucopolysaccharides (glycosaminoglycans, GAGs) content increased in term placenta than young placenta in human (Lee *et al.,* 1973). BNCs are distributed randomly throughout the trophoblast. They are characterized by a dark-stained cytoplasm as a result of the presence of ribosomes and mature large Golgi bodies (Wango *et al.,* 1990). Large membrane-bound granules which occupy almost half of the volume of BNCs are produced by those Golgi bodies and can be detected in tissue sections using PAS stain (Rodriguez *et al.,* 2004).

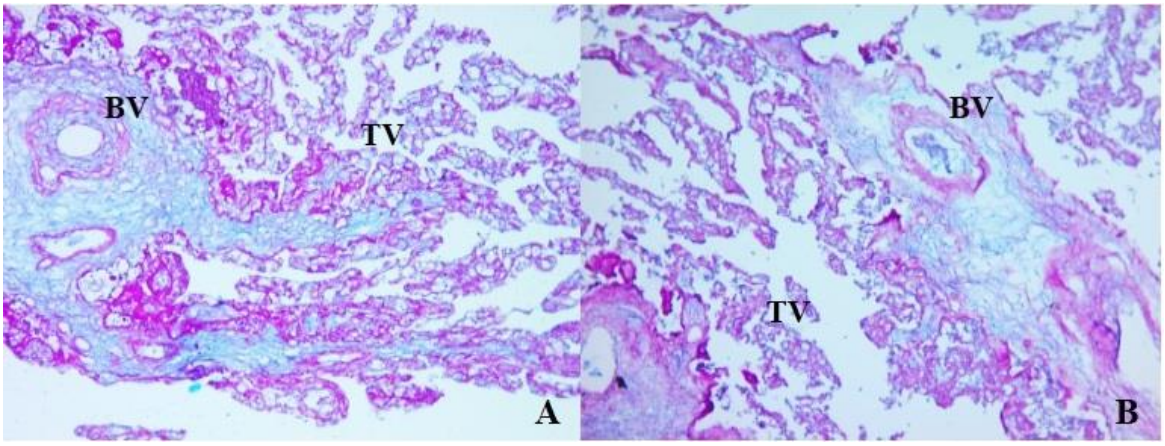

Figure 1: Periodic Acid Schiff's & Alcian Blue staining for NMPS and AMPS demonstration in the A) large cotyledon & B) small cotyledon: Tissues showing the strong reaction of the periodic acid Schiff (red for NMPS and blue for AMPS) in center of the tertiary villous (TV) and the villi (BV-blood vessel) (PAS-AB X 100).

The histochemical investigation of calcium was demonstrated by Alizarin Red stain but calcium could not be found deposited in any parts of the small and large fetal cotyledon of Gir cow as well as Jaffrabadi buffalo (Fig.2A & 2B). Although, very few and little globular pattern calcium deposits were observed only in large-sized fetal cotyledon in both the species. The cardinal feature of calcium metabolism in the fetus is the active placental transport of large quantities of calcium, whereas PTH and calcitonin do not cross the placenta. Fetal calcium levels suggest that ionized calcium is transferred from the mother to the fetus at a rate of 50 mg/day at 20 weeks of gestation to a maximum of 330 mg/day at 35 weeks of gestation. The resultant fetal hypercalcemia suppresses the fetal parathyroid and stimulates fetal calcitonin release. The 25-hydroxy vitamin D appears to cross the placenta freely but the placental permeability of 1,25 $(OH)_2D$ is questionable. With birth, the placental source of calcium terminates abruptly and the serum calcium level declines, perhaps aggravated by hypoparathyroidism and/or hyper-calcitonemia residual from fetal life (Kumar and Kaur, 2017). This could be the reason for the absence of calcium in the full-term expelled placental cotyledons. However, in disagreement with our findings, few researchers have reported the presence of calcium deposits in the placenta at various gestational stages. The probable explanation for this is explained by Wallingfordb *et al.* (2018), who stated that the deposition of calcium in the placenta can be considered as a marker of viral infection, but the mechanisms, histo-anatomical specificity, and pathophysiological significance of placental calcification are poorly understood. Similarly, Agababov *et al.* (2007) hypothesized that the increased placental calcification at early embryonic life might be caused due to nanobacterial infection, and it is named pathological placental calcification (PPC).

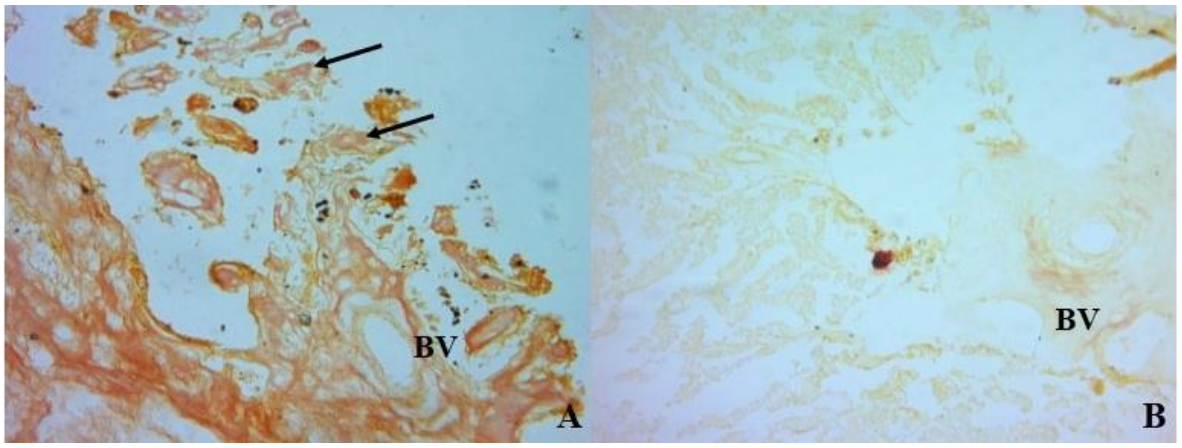

Figure 2: Alizarin Red staining (X 100) for calcium deposition in A) large cotyledon & B) small cotyledon: Globular pattern of very little calcium (arrow mark) deposition found at the boundary of villi in large cotyledon, BV-Blood vessels (Alizarin Red X 100).

The lipids were demonstrated by Oil Red O stain and Sudan Black B in the small and large cotyledon of Gir cow and Jaffrabadi buffalo (Fig.3A & 3B). Both the cotyledonary tissues showed a strong Sudanophilic reaction and the Oil Red O positive lipids were observed in both the species. This positive reaction indicates the probable site for

steroidogenesis and the storage of lipid hormone in cotyledonary tissues. Following our findings, a similar reaction was observed in buffaloes by Ranjan *et al.* (2013). They observed strong reactions for lipids at trophoblastic and cryptal epithelium, with advancing gestation the maternal septa and villous core showed moderate activity. Also, traces of lipids were found during early pregnancy in buffalo that increased with gestation. The lipid content was more on the fetal side as compared to the maternal side (Prasanth Babu, 2008). Bjorkman (1954) observed that the cryptal epithelium and the trophoblastic epithelium were rich and poor respectively in fatty materials.

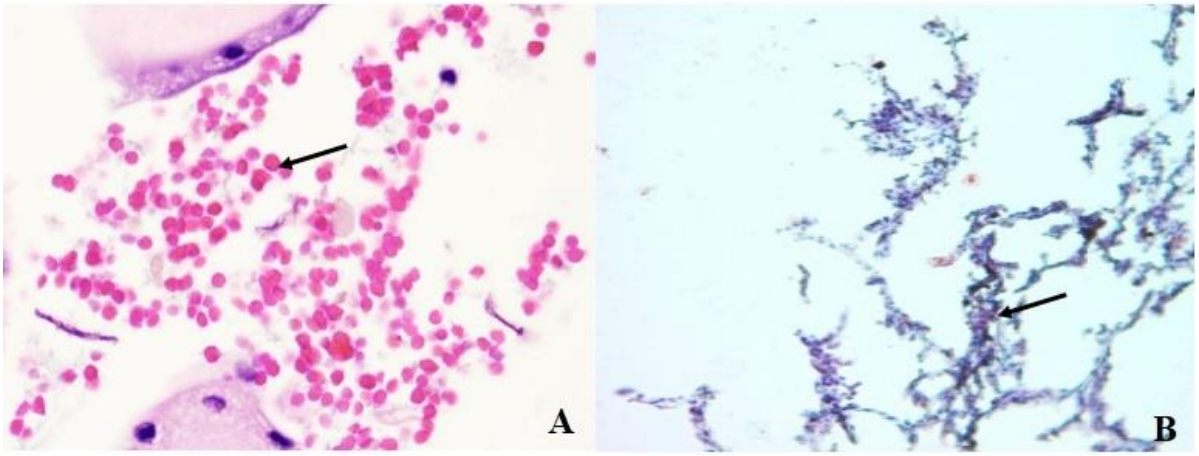

Figure 3: A) Oil Red O staining (X 400) for lipid deposition in cotyledon: Tissues showing the strong reaction of the lipid part in the middle of the villous (arrow mark) and the villi (Oil Red O X 400); B) Sudan Black B staining (X 100) for lipid deposition in cotyledon: Distribution of lipid content in trophoblastic epithelium (arrow mark) at tertiary villi (Sudan Black B X 100).

Conclusion

The histochemical investigation of the distribution of neutral and acid mucopolysaccharides in the small and large cotyledon of Gir cow and Jaffrabadi buffalo revealed strong PAS-positive cytoplasmic granules were found in the trophoblastic epithelium of the villous arcade area in the large cotyledon of Gir cows and Jaffrabadi buffaloes compare to the small-sized cotyledons. A strong reaction of neutral mucopolysaccharides showed for tunica intima of blood vessels was observed. A weak alcinophilic reaction was found at the basement membrane of the villous and in the center of the villi in the large cotyledon of both species compared to the small one. High activity of neutral mucopolysaccharides indicates it containing glycogen thus confirming that a high level of metabolism would be expected in placental trophoblasts where considerable activity concerning transport, degradation, and synthesis of materials is taking place. The calcium distribution was demonstrated by Alizarin Red stain but calcium could not be found deposited in any parts of the small and large fetal cotyledon of Gir cow as well as Jaffrabadi buffalo. Although, very few and little globular pattern calcium deposits were observed only in large-sized fetal cotyledon in both the species. The lipids were demonstrated by Oil Red O stain and Sudan Black B in the small and large cotyledon of Gir cow and Jaffrabadi buffalo. In both species studied, the small and large cotyledonary tissues showed strong Sudanophilic reaction and Oil Red O positive lipids.

Conflict of Interests

There is no conflict of interest.

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