

Histopathological observations in cervix of dystocia affected vis-à-vis normally calved buffaloes

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ABSTRACT

The present study was to assess the histopathological alterations of cervix in relation to dilatation in normally calved, dystocia and uterine torsion affected PGF₂α induced buffaloes. Biopsy specimens from the caudal part of the cervix were obtained from 27 pluriparous buffaloes at exactly the day of calving, immediately after calving, 24 h after calving, immediately after uterine detorsion and 24 h after uterine detorsion and medical treatment. The animals were divided into 6 groups based on the type of calving; group 1: normally calved buffaloes (n = 3); group 2: dystocia with fully dilated cervix (n = 5); group 3: dystocia but cervix failed to dilate (n = 4); group 4: torsion of uterus with fully dilated cervix (n = 5); group 5: torsion of uterus with incomplete dilatation of cervix (n = 6); and group 6: contracted cervix (n = 4). Compared to group 1, the cervical histopathology of dystocia and uterine torsion affected buffaloes revealed severe damage to epithelium with edema and hemorrhage in connective tissue. The smooth muscle cells showed coagulative necrosis, vacuolar degeneration and appeared hypertrophied. The elastic and collagen fibres were much denser. Very few polymorphonuclear cells could be traced. The inflammatory changes were chronic in groups 3, 5 and 6 leading to inability of cervix to dilate completely. Administration of PGF₂α did not induce further cervical dilatation. In conclusion, severe cervical damage in the cases of dystocia and uterine torsion indicated progression towards severe inflammation and possibly failure of cervix to dilate on its own followed by per-vaginal delivery of fetus.

Key words: Buffalo, Cervix, Dystocia, Histopathology

Dilatation of cervix at or near term is the result of mechanisms that are very similar to an inflammatory reaction (Stjernholm-Vladic *et al.* 2004). The resultant disruption of the extra cellular matrix softens the cervix and then actively causes funneling, shortening and subsequently dilatation of the cervix. The opening of the cervix during parturition was described in cows from initiation of the parturition until vaginal expulsion of the fetus (Breeveld-Dwarkasing *et al.* 2002). However, failure of cervix to dilate completely is a major obstacle in per-vaginum fetal delivery in buffaloes resulting in dystocia. In the parturient cow the cervical diameter is influenced by smooth muscular tissue from within the cervix resulting in increased vascularity (Breeveld-Dwarkasing *et al.* 2003b). The epithelial lining of cervix

composed of stratified squamous to cuboidal cells with normal blood vessels on the day of parturition (Eibert *et al.* 2007). Honparkhe *et al.* (2009) demonstrated distorted epithelium, severe venous congestion and hemorrhages in uterine torsion affected buffaloes. The smooth muscles cells showed coagulative necrosis with much fragmentation of elastic fibres. These differences in the organization of smooth muscle cells could imply a different functionality during cervical dilatation. The present study was, therefore, conducted to evaluate the histopathological alterations in relation to dilatation of cervix in normally calved, dystocia and uterine torsion affected dairy buffaloes.

MATERIALS AND METHODS

Selection of animals: Normally calved buffaloes (3) and dystocia affected dairy buffaloes (24) were studied. Complete history with regard to their age, stage of gestation, duration of labor, colicky symptoms and previous handling or medication of the buffaloes, if any, were recorded. All the buffaloes were in their second to fourth parity. All the animals were examined clinically to determine the nature and cause of dystocia, based upon which the buffaloes were divided

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into six groups. Group 1 included normally calved buffaloes ($n = 3$). Group 2 ($n = 5$) contained buffaloes having dystocia with fully dilated cervix where per-vaginum delivery was achieved through mutation and traction after correction. Group 3 comprised buffaloes having dystocia where cervix failed to dilate ($n = 4$). Group 4 included buffaloes having uterine torsion with fully dilated cervix to achieve per-vaginum delivery of fetus ($n = 5$). Group 5 included buffaloes having uterine torsion with incomplete dilatation of cervix ($n = 6$) while group 6 comprised buffaloes with contracted cervix ($n = 4$). Detorsion of uterus in groups 4 and 5 was achieved by Sharma's modified Schaffer's method (Singh and Nanda 1996). The buffaloes belonging to groups 3, 5 and 6 were subjected to caesarean section (Roberts 1971).

Collection of biopsy of cervix: The biopsy of cervix was collected with a properly sterilized cervical biopsy punch immediately before and 24 h after delivery through per-vaginum in group 1. In groups 2 and 3, biopsy samples were taken immediately before and after delivery and 24 h of treatment, respectively. For groups 4 and 5, biopsy samples were taken immediately after successful uterine detorsion and delivery and 24 h of treatment, respectively, whereas in group 6, samples were taken immediately at arrival and 24 h of treatment. The tissues were fixed in 10% buffered formalin till further processing. The fixed tissues were processed for histomorphological studies through routine alcohol benzene schedule (Lillie 1965), infiltrated and embedded in paraffin wax. The sections (5–7 μm) were cut and stained by Haematoxylin and Eosine (Luna 1968) and examined for changes.

RESULTS AND DISCUSSION

Histopathological observations on cervix of normally calved buffaloes (group 1): On the day of calving, the cervical mucosa was lined with simple, tall, columnar mucinogenic secretory epithelium with slight desquamation (Fig. 1A). The epithelial cells stained red indicating the presence of mucopolysaccharides. The propria-submucosa comprised of loose, irregular, edematous connective tissue with hemorrhage and polymorphonuclear cells in the subepithelial as well as in deeper areas. Macrophages, plasma cells, and pigmented cells with lot of inflammatory reaction were observed particularly in perivascular areas. The collagen and smooth muscle fibres were hypertrophied and dense under epithelium. Collagen fibre disorientation was common. The elastic fibres were few, running parallel to collagen fibres with evident elastolysis. The observations are in conformity to the findings of Stockham and Scott (2002) who also reported increased vascularity, edema and dissolution of collagen bundles from mid-gestation up to parturition. Based on the present observations, it is evident that the cervical dilatation is associated with dissolution of collagen fibre bundles which further was associated invariably with

increased mucopolysaccharides in the matrix. Increased concentration of mucopolysaccharides at parturition results in the induction of cervical softening that promotes longitudinal division of the collagen fibres in cervix (Fosang *et al.* 1984). The dissolution of collagen fibres, enhancing the process of cervical dilatation is further justified with the marked increase in the number of polymorphonuclear cells, which are known as a potent source of collagenase (Tenore 2003). Within 24 h after parturition, the cervical epithelium nearly regained its height and secretory activity (Fig. 1B). The edema, venous congestion and hemorrhages were reduced. The propria-submucosa was denser and more organized with compacted collagen fibres. At places, however, degenerative collagen tissues and hemorrhage were evident. Elastic content in connective tissue was negligible showing complete elastolysis. The cellular components decreased significantly and fibroblasts predominated. Similar changes in muscle fibres have been reported by Kumar (1990) in buffalo cervixes.

Histopathological observations on cervix of dystocia affected buffaloes with complete cervical dilatation (group 2): In dystocia affected buffaloes, there was partial loss of cervical epithelium and in few cases there was complete denudation and it was highly necrosed (Fig. 1C). There was severe hemorrhage and venous congestion in lamina propria with mild edema. At certain locations, the inflammatory reaction was seen. The smooth muscle cells showed coagulative necrosis, vacuolar degeneration and appeared hypertrophied. The pathological changes observed may probably be due to pressure atrophy caused by dystocia, however, the changes were not severe enough to prevent complete cervical dilatation. Immediately after delivery, there was slight lowering of cervical epithelium and tissue hemorrhages increased further as evident from frequently encountered blood pools, possibly due to manual fetal traction (Fig. 1D). Like group 1, polymorphonuclear cells were considerably and fibroblasts appeared in plenty. Collagenolysis and elastolysis were evident as indicated by irregular collagen fibres and few elastic fibres. The smooth muscle fibres appeared vacuolated and separated. The results are in consonance with the findings of Dhindsa *et al.* (2009) in dystocia affected buffaloes.

Histopathological observations on cervix of dystocia affected buffaloes with incomplete cervical dilatation (group 3): The histopathological findings in this group were more or less similar to that in group 2 except that the degeneration of vascular wall due to circulatory arrest was more severe leading to hypoxic anoxia of the cervical tissue (Fig. 1E). After 24 h of treatment, muscle layers showed no change in compactness, but degeneration was further advanced all over (Fig. 1F). Collagen degradation was pronounced. Edema, congestion and hemorrhage were much extensive in submucosa. The polymorphonuclear cells were absent. Necrotic foci were observed in deeper layers.

Prostaglandin treatment was not effective to induce further dilatation of the cervix sufficient to affect per-vaginum delivery of the fetus. Breevald-Dwarkasing *et al.* (2003a) reported increased collagen degradation and smooth muscle denaturation in the cervix of animals that suffered a delay in calving leading to incomplete dilatation of cervix. The

buffaloes in this group were referred for caesarean section.

Histopathological observations on cervix of uterine torsion affected buffaloes with complete cervical dilatation (group 4): In majority of the cases, there was distortion of cervical epithelium with little secretory activity (Fig. 1G). Blood vessels showed congestion and degeneration of its wall

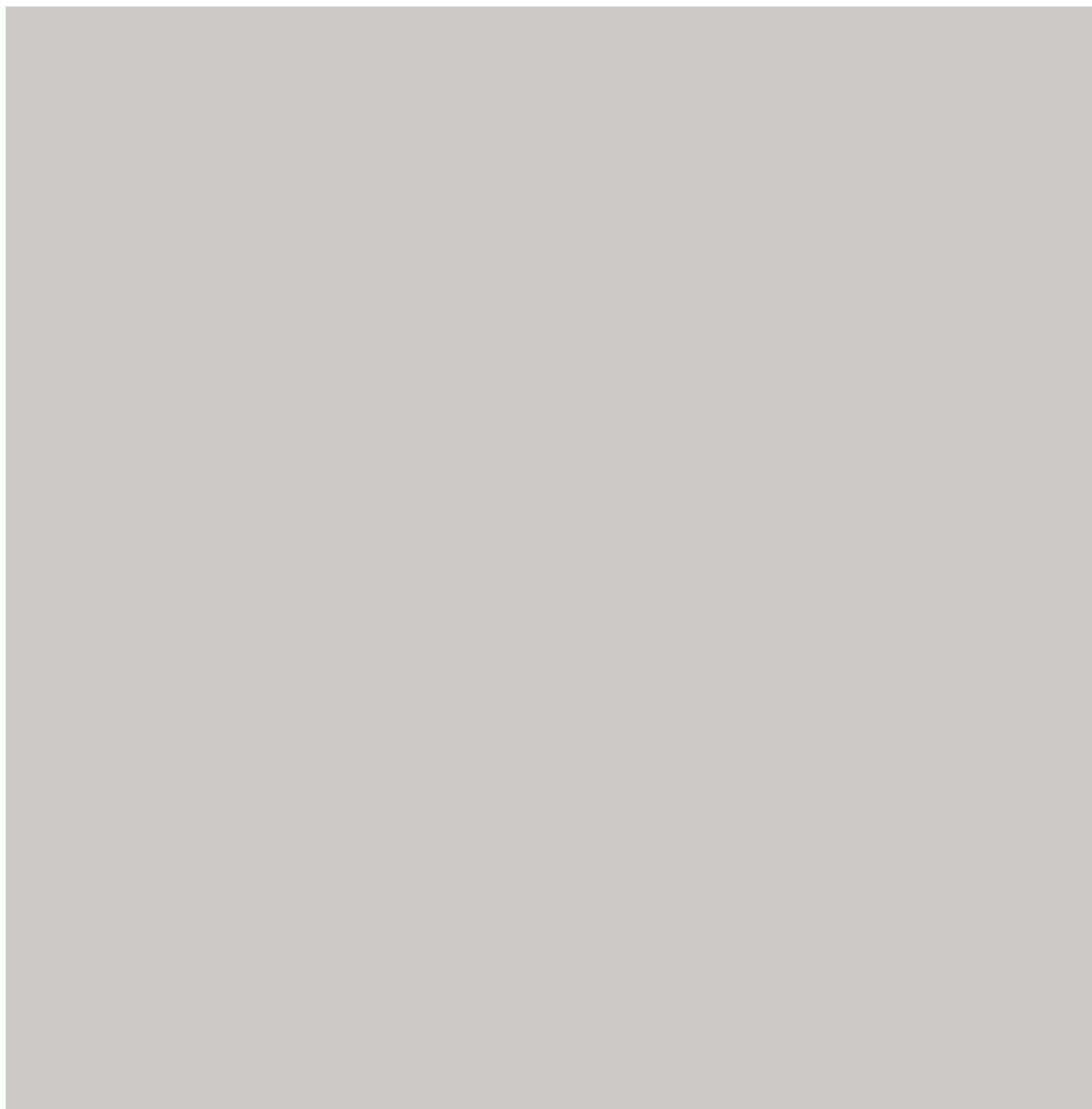


Fig. 1 (A–L). Histopathological alterations in the cervical tissue of normally calved (group 1, plates A and B) and dystocia affected buffaloes (groups 2, plates C and D; 3, plates E and F; 4, plates G and H; 5, plates I and J and 6, plates K and L). Plates A and B, C and D, G and H, indicate marked number of polymorphonuclear cells (→), edema (!), deranged collagen and elastic muscle fibres (*), occasional necrosis (#), while plates E and F, I and J, K and L, indicate few polymorphonuclear cells (<), marked fibrosis and congestion (>), and intact collagen and elastic fibres (^). H & E×300.

with severe hemorrhages. Red blood cells were dispersed throughout in the tissue spaces. The cellular content of connective tissue decreased considerably to fewer cells mainly fibroblasts. Deeper layer of propria-submucosa showed relatively denser collagen tissue than the superficial layers. Inter-fibrous ground substance in deeper layers demonstrated mucopolysaccharides. Majority of the muscle fibres were relatively thinner as compared to group 1 and were separated by collagenous strands. Vacuolations were lacking in majority of the areas. The connective tissue presented little amount of cellular components. The collagen and elastic fibres were irregularly placed as in group 2. Similar changes were reported by Kumar (1990) on the cervical tissue in buffaloes affected with uterine torsion. Immediately after delivery, blood vessels showed engorgement and hemorrhages and exhibited degenerative changes compared to the samples taken immediately after uterine detorsion (Fig. 1H). The cellular component, noticeably the polymorphonuclear cells reduced. Extravascular blood was predominant in connective tissue and heavily infiltrated with active fibroblasts. The collagen as well as elastin content were very little, loose and degenerative type. This implies widespread, diffused collagenolysis and elastolysis. Individual muscle fibres were loosened and inter-fascicular areas were heavily engorged with blood. The widespread hemorrhage and infiltration of polymorphonuclear cells immediately after delivery following uterine detorsion could be attributed to the release of pressure (tourniquet) over some of the blood vessels, which caused flush of blood into the weakened vascular walls leading to their rupture. Koets *et al.* (1998) reported increased release of polymorphonuclear cells in cervical epithelium in cows with twisted uterus. It also implies that circulatory arrest of these vessels was not severe and longstanding to cause permanent damage in the cervix. The polymorphonuclear cell infiltration seemed to be the result of inflammatory processes, perhaps in response to tissue insult caused during delivery by manipulative correction and traction in the present study.

Histopathological observations on cervix of uterine torsion affected buffaloes with incomplete cervical dilatation (group 5): Biopsy sample immediately after detorsion of uterus revealed a significant change with necrosis and degeneration of cervical epithelium (Fig. 1I). It may be due to uterine torsion induced prolonged circulatory arrest (ischemia) leading to hypoxic degeneration and irreparable necrosis of the cervical tissue. The stroma of propria-submucosa showed relatively denser collagenic tissue. The elastic fibres were more intense in the vicinity of blood vessels similar to that in group 3. Congestion, hemorrhage, degeneration of vascular wall, few polymorphonuclear cells and active fibroblasts were observed. A comparison of above observations with those of groups 1, 2, 3 and 4 revealed extensive destruction of cervical epithelium with necrosis, congestion, hemorrhage, pronounced degeneration of

vascular wall accompanied with rare occurrence of polymorphonuclear cells. Dilatation of cervix depends on the resistance caused by visco-elastic properties of cervix (Breeveld-Dwarkasing *et al.* 2003b), which may be disturbed due to permanent cervical ischemia, since all the cases reported in this group exhibited labor pains 36 to more than 72 h earlier. Edema, congestion and hemorrhage were evident but 24 h after PGF₂α treatment cellular components were almost negligible, which indicated that intramuscular administration of PGF₂α had no useful effect in causing dilatation of cervix; rather it seemed to further advance the collagen density (Fig. 1J). The polymorphonuclear cells were not present as in group 3. Also it appeared to cause severe tissue loss due to hemorrhage and increased cellular breakdown and phagocytic activity. Breeveld-Dwarkasing *et al.* (2002) reported that cervical dilatation is governed by the forces induced by uterine contractions. However, fetal death in all the buffaloes of this group could have impaired the uterine contractions. Bryman *et al.* (1996) have reported indifferent responsiveness of cervical tissue to prostaglandin administration. Therefore, buffaloes in this group were also subjected to caesarean section.

Histopathological observations on cervix of dystocia affected buffaloes subjected to cesareotomy (group 6): In this group, histopathological examination of cervix revealed that the collagen fibres were dense indicating very little collagenolysis (Fig. 1 K, L). Cellular components were rare except fibroblasts and pigmented cells. The smooth muscle fibres were degenerated and in the deeper layers necrotic foci could also be seen. No polymorphonuclear cells could be traceable. The elastic muscle fibres also remained compact. Severe inter-muscular edema, inter-muscular fibrin deposition, congestion, necrosis, fibrosis and hemorrhages were present. Even 24 h after PGF₂α treatment, the findings were similar to that in delayed cases of groups 3 and 5.

From the above study, it can be summarized that increase in severity of cervical histopathological alterations following dystocia and uterine torsion were indicative of progression towards severe inflammation and necrotic changes in cervix leading to failure of cervix to dilate. Even administration of PGF₂α was not sufficient to induce complete cervical dilatation. It is, therefore, recommended to handle dystocias as early as possible to avoid tissue damage and further complications.

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