The umbilical cord (UC), a connection between foetus and mother, is responsible for nutritional exchange and protect the vessels running through it. The UC matrix (Wharton’s jelly) represents an excellent alternative non-invasive source of mesenchymal stem cells (MSC) in equines for treatment of a number of diseases. The UC derived cells could be serially passaged up to 30 passages without alterations in morphology, indicating a high proliferative potential of these cells. The findings of present study may help in identifying the proper site in Wharton’s jelly for efficient collection and isolation of MSC for various downstream applications.

**MATERIALS AND METHODS**

The present study was conducted on UC of six foals of either sex at full term foaling of Thoroughbred mares. Length of the UC was measured and the tissues were collected from umbilical vein (UV) and umbilical arteries (UA) surrounded by Wharton’s jelly at the foetal and placental sides. The tissues were also collected at the sites of anastomosis of umbilical arteries (UA) and umbilical veins. The tissues were fixed in 10 per cent neutral buffered formalin and processed for light microscopy. Paraffin sections (5-6 µ) were stained with routine Harris’ hematoxylin and cosin stain, Gomori’s method for reticulum, Weigert’s method for elastic fibres, McManus’ method for glycogen (PAS), Alcian blue method for muco-substances (pH 2.5), PAS-Alcian blue method for mucosubstances (pH 2.5), colloidal iron method (Luna, 1968) and Crossman’s trichrome stain for collagen fibres (Crossman, 1937).

**RESULTS AND DISCUSSION**

**Gross anatomy:** The umbilical cord extended from placenta to umbilicus of the foal. However, the length was measured 55 cm to 65 cm only upto the point of ligation. The UC, divided into amniotic portion towards foetal side and allantoic portion towards placental side, was mainly comprised of two central UA and two peripheral UV along with allantoic duct (urachus) and Wharton’s jelly towards juxtaplacental region. However, a single umbilical vein formed by fusion of two veins was observed towards juxtafoetal portion. The thickness of walls of umbilical artery was greater than that of umbilical vein. Histo-architecture of umbilical artery, vein and Wharton’s jelly varied at different sites of extent and presented varying qualitative affinities for mucopolysaccharides.

**Key words:** Foal, Histomorphology, Umbilical artery, Umbilical vein, Urachus
The UC had a tortuous course due to flexuous orientation of umbilical blood vessels. Chirality or natural spiraling of the vessels with four to five turns over the entire length along with slight twisting of the UC has been considered normal due to foetal movements (Schafer, 2004). Longer UC reported in male foals and foals from older mares (Whitwell and Wood, 1992) had been associated with a risk of excessive torsion of the cord or entrapment around the foetus. The UC having two UA and two UV measured 40-45 cm in ruminants (Carambula et al., 1997), 50 cm in bovine and buffaloes (Ferreira et al., 2009).

In cut section, the lumen of UA was smaller than that of UV (Fig. 1 b). Anastomosis of two UV into a single UV was a common feature however, anastomosis between UA was also observed in one case (Fig. 1c). In contrast, the vessels did not exhibit anastomosis in buffaloes. However, the cut section of the UC presented a line parallel disposition in juxtafoetal porion, an inverted crux constellation in middle porion and an isosceles trapezoid pattern toward juxtaplacental portion (Ferreira et al., 2009). Remnant of yolk sac could not be traced during the present study.

Microscopic observations: The lumen of UV was larger and of irregular shape in contrast to that of buffalo where elliptical lumen was observed (Singh et al., 2012; Ferreira et al., 2009). The thickness of walls of UA was greater than that of UV. The UA was comprised of tunica intima, media and externa/adventitia (Fig. 2). The endothelium of tunica intima was regular and well developed having a single layer of cells resting on a basement membrane. These cells had eosinophilic cytoplasm and their basophilic nuclei protruded into the lumen. The subendothelial connective tissue was small and PAS positive. A subendothelial cushion had been reported to be consisted of an inner proteoglycan layer having an abundance of proteoglycan ground substance and a thicker outer musculo-elastic layer having predominance of smooth muscle cells and elastic fibres (Stary et al., 1992). Internal elastic layer was irregular but distinct due to presence of interrupted elastic fibres (Fig. 3).

Tunica media was thick having different arrangements of smooth muscle fibres along with fibroblasts, few collagen and fine short elastic fibres (Fig. 2). The muscle fibres were strongly PAS positive; however, their arrangement was not uniform throughout the media. The thicker tunica adventitia was constituted mainly by circular and longitudinal layers of smooth muscles, collagen fibres, fibroblasts and very few elastic fibres. The concentration of collagen fibres increased towards longitudinally oriented muscle fibres and towards periphery. The strongly PAS positive fibroblasts were loosely arranged towards periphery of the tunica externa adjacent to WJ and were separated by bundles of collagen fibres. Fine blood vessels representing vasa-vasorum were also observed. There was comparatively more distribution of acidic mucopolysaccharides towards tunica intima and part of tunica media except the regions of smooth muscles where a more distribution of neutral mucopolysaccharides was observed. The concentration of neutral mucopolysaccharides increased progressively towards tunica externa; however, the reaction for acidic mucopolysaccharides was very weak. Alcian blue and colloidal iron reactivity was more towards luminal surface and tunica media especially the region of fibroblasts.

Wharton’s jelly surrounding the vessels was mainly comprised of bundles of collagen fibres, fibroblasts, large number of blood capillaries and connective tissue cells. The network of collagen bundles was dense and compact towards the umbilical vessels and became loose towards the outer epithelium. The fibroblast cells were more in number towards the umbilical vessels than towards amnion and were mostly stellate-shaped with large cytoplasmic processes positive for mucopolysaccharides. The connective tissue cells of different types having different nuclear morphology were observed as reported in buffalo (Singh et al., 2012). Some larger cells were triangular or star-shaped having less basophilic nuclei and strongly eosinophilic cytoplasmic processes. A few round cells with differently stained nuclei were also observed. The connective tissue of WJ and the cytoplasmic processes of all the cell types were weakly PAS positive, mildly positive for neutral mucopolysaccharides and negative for acidic mucopolysaccharides.

The section of UV at the site of anastomosis revealed almost similar histological features except few differences (Fig. 4). The lumen of one of anastomosing UV was drastically obliterated and all the tunics were not clearly distinguishable. Tunica externa was thicker than tunica media but was comparatively thin as compared to juxtaplacental position. Bundles of smooth muscles and fibroblasts present at the periphery of tunica externa of both the veins merged towards intervacular position where concentration of collagen fibres was also maximum. Vasa vasorum was observed towards externa.
The UV towards juxtafoetal position had similar structure except that number of blood capillaries increased towards periphery of the externa and distribution of collagen and elastic fibres was comparatively lesser.

The UA of juxtaplacental area also presented smaller lumen of varying shapes. The endothelium was similar to that of UV (Figs. 5, 6). The subendothelial connective tissue was larger in size and contained smooth muscles and showed a weak reaction for acidic mucopolysaccarides and moderate reaction for Alcian blue. Internal elastic membrane was better developed than that of UV and the concentration of elastic fibres was comparatively increased (Fig. 3). The tunica media was almost equal or slightly larger in dimension than that of the tunica externa. The smooth muscle bundles were oriented in different directions and were strongly PAS positive. A distinct external elastic membrane was absent; however, fragmented elastic fibres were observed. The smooth muscle fibres of tunica externa were oriented
longitudinally towards deeper portion and loosely arranged with large connective tissue towards the periphery and showed a more pronounced positive reaction for neutral mucopolysaccharides. Glycosaminoglycans comprising of chondroitin sulfate, heparin sulphate, dermantan sulphate, keratin sulphated and hyaluronic acid with the predominance of the latter had been demonstrated (Bankowski et al., 1996). The collagen fibres were comparatively more in tunica intima than tunica media with maximum concentration towards tunica externa (Fig. 6). The concentration of elastic fibres was drastically increased towards tunica media and tunica externa than that of their respective counterparts of the UV. The tunica externa was almost equal to tunica media.

The WJ surrounding UA was almost of same thickness and shared histological features with that of UV region except that smaller blood vessels and blood capillaries were more numerous towards periphery (Figs. 7, 8). The smooth muscles and fibroblast were comparatively more in number than that of UV. In addition, small nerve bundles cut in different profiles and some structures resembling to ganglion were also observed towards periphery of the WJ (Fig. 7). These structures presented a strong reaction for PAS and neutral mucopolysaccharides. The nerves had been reported to be absent in the UC (Marzioni et al., 2004). The lumen of UA was further reduced at the site of anastomosis and it was present in the form of varying shapes of star. The WJ showed less blood vascularity but a predominance of smooth muscles at intervascular region which was strongly PAS positive for neutral mucopolysaccharides. The UA and its surrounding WJ towards juxtafoetal portion had histological features similar to that of juxtaplacental region.

The allantoic duct presented lumen of irregular shape and was lined by simple cuboidal to low columnar epithelium (Fig. 9). The less basophilic nuclei of varying shapes were oriented in mid portion of the epithelium. The eccentric nucleoli were visible only in few nuclei. The cytoplasm was eosinophilic, dense and finely granular. A few cells containing darker elongated nuclei were also interspersed in between the cells. All the cells were strongly positive for PAS, neutral mucopolysaccharides and colloidal iron especially towards the supranuclear portion and weakly positive for acidic mucopolysaccharides. The duct was surrounded by loose irregular connective tissue having predominance of collagen fibres along with few reticular and fine elastic fibres (Fig. 9). The connective tissue cell population was drastically reduced. The blood vascularity in the form of fine blood capillaries was also reduced as compared to adjacent portion of WJ.

REFERENCES


