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Immunohistochemical identification of interstitial cells of cajal (ICCs) in prenatal bovine intestine

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The gastrointestinal (GI) motility, the main characteristic feature of GIT smooth muscle, plays a vital role in digestion, absorption and storage of feed in animals. This GIT motility is the intrinsic electrical properties of the smooth muscle mass. This electrical activity originates from the specialized smooth muscles referred as the interstitial cells of cajal (ICC). ICC is mesodermal in origin (Young *et al*. 1996). The ICC exhibits rhythmical and spontaneous oscillation in their transmembrane electrical potentials. This property of the spontaneous electrical rhythmicity, in combination with their electrical connection to the smooth muscle mass, imparts to ICC their role as electrical pacemaker of the gut. In the prenatal non-functional GIT, ICC may play certain functional properties or not still unknown. No literature was found in case of bovine prenatal ontogenesis of ICC. Keeping in view the common GIT pathology, the present work was undertaken.

Aborted fetuses (24) were collected from Tangra slaughterhouse, maintained by Kolkata Municipal Corporation. The Crown rump length (CRL) of the foetuses was measured in cm with the help of a graduated tape from the crown of the head to the base of the tail along the vertebral column and subsequently the age was determined using the formula of Richardson (1980). Depending on the CRL, the foetuses were grouped into four groups, viz. 86–95 days, 116–132 days, 152–163 days and 178–185 days and the number of animals was 6 in each group. The abdomen was incised and the total intestine was separated out. The desired samples were collected from different parts of intestine and were kept in deep freeze. For immune-histochemical study, the cryostat sections of 5µm thickness were obtained from frozen samples. The sections were also obtained from paraformaldehyde fixed low melted paraffin blocks with 5µm thickness.

Frozen sections were fixed in ice-cold para formaldehyde at (4% wt/vol in 0.1 M phosphate buffer) 4°C for 25 min. After that the steps were similar for both cryostat and paraffin sections. These tissues were incubated for 30 min with 100 µl of 0.3% H_2O_2 in methanol at room temperature to quench endogenous peroxidase activities. Tissue sections were then incubated for 1 h in 1% goat serum albumin in 0.1 M PBS (pH 7.2) at room temperature to block non-specific antibody binding activity. After subsequent washing with phosphate buffer saline (PBS), the sections were incubated overnight at 4°C in humid chambers with rabbit polyclonal antibody raised against human kit protein (1: 100 dilutions in 0.1 M PBS). Immunoreactivity was detected 1hr incubation with secondary antibody (Horseradish peroxidase conjugated goat anti- rabbit immunoglobulin, 1: 2000). Slides were then rinsed three times in PBS and the sections were treated with DAB solution for 30 min. The sections were mounted in glycerin jelly. Statistical analysis was done by using one-way Anova. Photography and morphometric studies were done with microscope and Leica Qwin software.

In the present study, the ICC was detected in different regions and morphologically characterized into three categories depending on their site, viz. ICC related to submucosal or Meissner's plexus (ICC-SMP), ICC related to intramuscular region (ICC-IM) and ICC related to Auerbach's plexus (ICC-AP). The ICC-SMP was found in the form of a network of cells, ICC-IM was present parallel to the muscle fibre and ICC-AP was multipolar and presented in the form of a network. Hudson *et al*. (1999) reported three types of c-kit immunoreactive cells in equine GIT.

Small intestine: All the 3 segments of small intestine expressed c-kit immunoreactivity against ICC from 116–132 days (avg. fourth month) of gestational age onwards (Fig.1). Out of the expressed ICC, morphologically ICC-IM and ICC-AP were detected but ICC-SMP was absent. Maria *et al*. (2007) reported ICC ontogenesis in the human ileum at 7–9 weeks of gestation. Fintl *et al.* (2004) also conducted similar experiment in equine and found that at 6 months of gestation a network of ICC was present in the Myenteric plexus of both small and large intestine.

It was observed that the ICC-AP was located either where the myenteric element was absent or on the circular muscle

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Table 1. Mean (± SE) values of ICC count (per mm^2) in different segments of intestine in different age groups of foetus

Component of intestine	116–132 day	152–162 day	178–185 day	P value
Duodenum	66.67^{zc} ± 1.54	75.17^{yc} ± 2.55	85.83^{xc} ±1.08	P<0.01
Jejunum	58.33^{zd} ±1.05	68.33^{yd} ± 0.95	74.50^{xd} ± 0.76	P<0.01
Ileum	79.33^{zb} ±1.08	84.50^{yb} ± 0.43	91.83^{xb} ± 0.70	P<0.01
Ileocecal junction	41.17^{ze} ± 0.95	47.67^{ye} ±1.05	54.00^{xe} ± 0.58	P<0.01
Cecocolic junction	88.33^{za} ±1.15	96.83^{ya} ± 0.60	104.50^{xa} ± 0.76	P<0.01
Cecum	37.50^{zf} ±1.52	43.83^{yb} ± 0.60	51.00^{xf} ± 0.89	P<0.01
Colon	33.67^{zg} ± 0.76	38.33^{yg} ± 0.49	46.50^{xg} ± 0.76	P<0.01
P value	P<0.01	P<0.01	P<0.01	N=6

Values bearing different superscripts viz., x, y, z in a row and a, b, c, d, e, f, g in a column differ significantly. P, Significant value; N, number of replicates.

site of the plexus or on the longitudinal muscle site of the plexus. ICC-IM was found along the longitudinal and circular muscle fiber. The number of ICC recorded in the different age groups of foeti was significantly high in duodenum than any other part of small intestine in individual foetus and also among the foetuses (Table 1).

Morphologically, ICC was a mixture of bipolar and stellate shaped. There was a limited and inconsistent degree of branching of ICC from the myenteric plexus region into the longitudinal muscle layer of the foetus. However, this branching became more prominent and consistent in the advanced age group of the foetus (Fig. 1). The orientation of ICC-IM was parallel towards the long axis of the circular muscle fiber. Marquez *et al*. (2006) identified ICC in the jejunum of adult *Bos taurus* by using polyclonal anti C-kit antibodies and recorded similar distribution pattern. The present morphometric studies determined that ICC had a fusiform shaped presenting cytoplasmic prolongation and ICCs were associated with the myenteric plexus. Nevertheless, the presence of widespread ICC in the longitudinal muscular layer of the jejunum differed from previously described studies of other mammals.

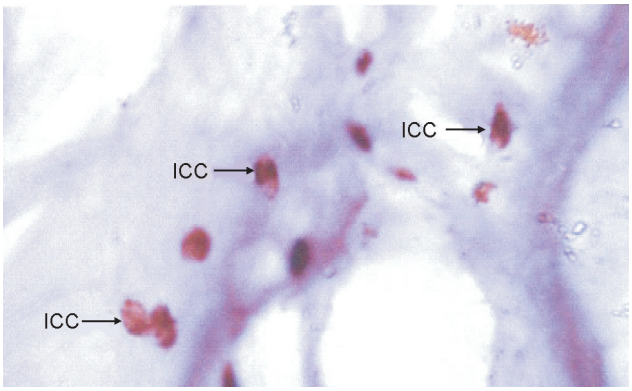

Fig. 1. Photomicrograph showing kit receptor immunohistochemistry of ICCs in the ileum of bovine foetus (6 month). X100.

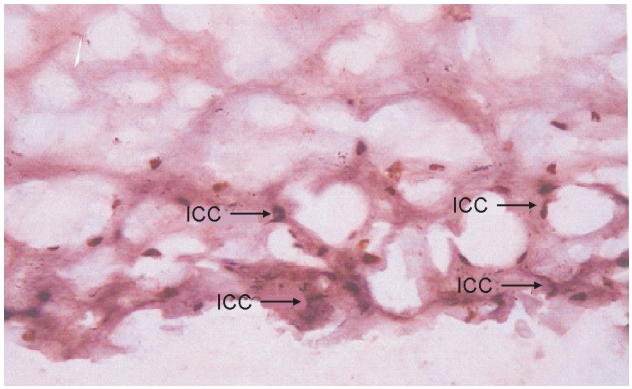

Fig. 2. Photomicrograph showing kit receptor immunohisto chemistry of ICCs in the ceco-colic junction of bovine foetus (5 month). X40.

Ileo-cecal and ceco-colic junctions: At the ileo-cecal junction, the ICC was multipolar and their processes were directed towards the muscle fibre. ICC-SMP was not detected. At the ceco-colic junction, majority of ICC in the circular muscle layer was bipolar in shape, but in the older age they appeared stellate shaped. The number of ICC was counted (Table 1) and it was observed that the highest population was recorded in ceco-colic junction in all age groups and the number increased significantly.

Large intestine: In Cecum, both bipolar and stellate shaped ICCs were found in the Auerbach's plexus (Fig. 2). The distribution and density of ICC showed no apparent significant change among the three age groups. In colon, the presence, distribution and morphology of ICC was almost similar to that of the Cecum. Fintl *et. al*. (2004) reported similar network of ICC in the large intestine of adult equines.

SUMMARY

Interstitial cell of cajal (ICC) was detected using C-kit immunoreactivity in different regions of prenatal foetal intestine. The ICC was first detected as early as four months of foetal age. The ICC was bipolar to stellate shaped and mostly concentrated around myenteric plexus, innervating their fibers in the longitudinal and circular muscle layers. The number of ICC varied significantly in different regions of intestine. ICC at sub mucosal plexus was not detected in the small intestine but present in the large intestine. Highest population of ICC was detected at ceco-colic junction. We are the first worker to report the ontogenesis of ICC in prenatal bovine intestine. These results suggested the correlation of ICC related pathophysiology in adult.

REFERENCES

Fintl C, Pearson G T, Ricketts S W, Mayhew I G and Hudson N P H. 2004. The development and distribution of the interstitial cells of cajal in the intestine of the equine fetus and neonate. *Journal of Anatamy* **205** (1): 35–44.

Hudson N P H, Pearson G T, Kitamura N and Mayhew I G. 1999. An immunohistochemical study of interstitial cells of cajal (ICC) in the Equine gastrointestinal tract. *Research in Veterinary Science* **66** (3): 265–71.

Maria S, Faussone P, Maria G, Vannucchi R A, Aleksandra S and Paola M. 2007. Morphology of the interstitial cells of cajal of the human ileum from fetal to neonatal life. *Journal of Cell Morphology* **11** (3): 482–94.

Marquez S G, Galotta J M, Portiansky E L and Barbeito C G. 2006. Characterization of interstitial cells of cajal in bowel of cattle *(Bos taurus)*. *Veterinary Research Communication* **30** (3): 221–29.

Richardson C. 1980. Personal Communication. *Veterinary Reproduction and Obstetrics*. pp 9–59. Arthur, Noakes and Pearson, 6th edn. ELBS, London.

Young H M, Ciampoli D, Southwell B R and Newgreen D F. 1996. Origin of interstitial cells of cajal in mouse intestine. *Developmental Biology* **180** (1): 97–107.