

## **Morphology of the interstitial cells of Cajal of the human ileum from foetal to neonatal life**

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### **Abstract**

The so-called interstitial cells of Cajal myenteric plexus (ICC-MP), interstitial cells of Cajal intramuscular (ICC-IM) and interstitial cells of Cajal deep muscular plexus (ICC-DMP) are the three types of ICC endowed within the intestinal muscle coat where they play different roles in gut motility. Studies on ICC ontogenesis showed ICC-MP in the human ileum by 7–9 weeks while information on ICC-IM and ICC-DMP in fetuses and newborns are not exhaustive. Functional recordings in the fasting state of prematurely born babies aged 28–37 weeks showed immature ileal motility. To gain more information on the time of appearance of the three ICC types in the human ileum and on the steps of the acquisition of mature features, we studied by c-kit immunohistochemistry fetuses aged 17–27 weeks and newborns aged 36–41 weeks. In parallel, the maturative steps of enteric plexuses and muscle layers were immunohistochemically examined by using anti-neuron specific enolase (NSE), anti-S-100 and anti- $\alpha$  smooth muscle actin ( $\alpha$ SMA) antibodies. The appearance and differentiation of all the ICC types were seen to occur in concomitance with those of the related nerve plexuses and muscle layers. ICC-MP appeared first, ICC-IM and ICC-DMP later and their differentiation was incomplete at birth. In conclusion, the ICC-MP, the intestinal pacemaker cells, in spite of absence of food intake, are already present during the foetal life and the ICC-IM appear by pre-term life, thus ensuring neurotransmission. The ICC-DMP and their related nerve plexus and smooth muscle cells, *i.e.* the intestinal stretch receptor, begin to differentiate at birth. These findings might help in predicting neonatal ileal motor behaviour and in interpreting the role of ICC abnormalities in the pathophysiology of intestinal motile disorders of neonates and young children.

**Keywords:** ICC differentiation • immunohistochemistry • man

### **Introduction**

In the intestinal muscle coat of laboratory mammals and human beings, there are three different popula-

tions of interstitial cells of Cajal (ICC) which interconnect to each other forming networks. These cells are also in contact with nerve endings and smooth muscle cells. Correct ileal motility depends on the role played by each ICC type [1]. According to physiological data, the ICC named ICC-MP (or, according to some Authors, ICC-AP or ICC-My) which are located in between the circular and longitudinal muscle layers

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and around myenteric plexus ganglia and nerve strands, are responsible for the peristalsis [2, 4]. A second ICC population named ICC-DMP forms a dense network in close contact with the nerve plexus named deep muscular plexus (DMP) and the innermost rows of the smooth muscle cells of the circular muscle layer (the so-called ICL). This ICC population, together with the DMP and the ICL is present only in the ileum and probably represents the intestinal stretch receptor [5–7]. Finally, a third ICC population forms a network within the thickness of the longitudinal and circular muscle layers. These intramuscular ICC, named ICC-IM, are similar to the ICC-IM located at other gut levels and are usually considered to be involved in neurotransmission [8].

During foetal life, in the absence of food intake, the presence of all the ICC types might not be necessary. Moreover, in newborns, before feeding or when their alimentation is only liquid, one or more ICC population might not have differentiated yet. Until now it has been ascertained that the ICC-MP appear very early in the human ileum, by 7–9 weeks [9–10]. Conversely, reports on the presence of ICC-IM in foetuses and newborns are not exhaustive, but it can be presumed they differentiate later than the ICC-MP [11]. The presence of ICC-DMP in full-term neonates has been reported [12], but images are not shown. No information is available on the age of appearance of both DMP and ICL and whether these structures are already differentiated at birth. Physiological studies on human foetuses are obviously lacking and the only available information regards functional reports on babies prematurely born at 28–37 weeks. According to these studies, ileal motility is not the same as that of newborns or adults, although the response to feeding is appropriate [13], the motor behaviour in the fasting state is immature [14, 15]. Whether the differences between foetuses, neonates and adults reflect either the incomplete organization of ICC and enteric plexus networks and/or the immaturity of ICC, neurons and smooth muscle cells is unknown.

To gain information on the ICC morphology and organization during foetal life and in the pre-term and at-term neonates is of particular interest in order to predict the ileal motile behaviour of the babies, especially of those prematurely born and allowed to survive in the incubator and artificially fed. This information might also help in interpreting data reported for Hirschsprung's disease [16, 20] and for several infantile intestinal malformation or malfunctions

[21, 26]. The aim of the study was to investigate in the human beings the timing of appearance and maturation of each ICC population, in parallel with that of smooth muscle cells and nerve structures. c-kit, neuron specific enolase (NSE), S-100 and  $\alpha$  smooth muscle actin ( $\alpha$ SMA) immunohistochemistry was studied in human foetuses aged by 17–27 weeks and pre-term and at-term newborns aged 36–41 weeks.

## Materials and methods

Full thickness small bowel specimens were obtained at autopsy from 20 human foetuses (gestational age between 17 and 27 weeks) and seven newborns (aged between 36 and 41 weeks). Foetal specimens were obtained from either spontaneous or therapeutic termination of pregnancy, after parental consent and ethical approval from the local committee had been obtained. The gestational ages were estimated based upon anatomical criteria according to the Carnegie staging system and the crown rump length, head circumference and foot length. The live born babies died within the first week of life. For adult tissue, specimens of small intestine were collected from two patients operated on for pancreatic carcinoma.

Bowel specimens were opened at the anti-mesenteric border and a circumferential segment corresponding to the mid-gut was fixed in 10% neutral formalin and paraffin-embedded in each case. For wax embedding, tissues were dehydrated through an ascending series of alcohol (1 × 5 min each), cleared in HistoClear (2 × 5 min) and then left over-night in molten wax at 56°C prior to embedding. Sections 4  $\mu$ m thick were stained with hematoxylin & eosin in order to evaluate the adequacy of the samples and to exclude the possible presence of autolytic changes.

For immunohistochemistry, consecutive 4  $\mu$ m sections of full-thickness loops of the small intestine were collected and immunolabelled. Immunohistochemical analysis was performed using the detection Kit-Polymer (Novocastra, Newcastle upon-Tyne, UK) according to the manufacturer's instruction. The sections were deparaffined in xylol and a descending series of alcohol (less than 1 min per each) and then re-hydrated in distilled water. The endogenous peroxidase was inhibited by Novocastra Peroxidase Block RE7101 Kit, followed by the protein block by Novocastra Protein Block RE7102. This was followed by incubation with the primary antibodies for 45 min at room temperature, rinsing in a phosphate buffered solution (PBS), incubation with the post-primary block RE7111 for 20 min at room temperature and final incubation with NovoLink Polymer RE7112 for 20 min at room temperature. Immunoreactivity was detected at room temperature by the addition of 3,3'-diaminobenzidine (DAB, Novocastra) as a substrate. All the immunolabelled sections were counterstained by Harris

hematoxylin. No staining was observed when the respective primary antibody was omitted.

The primary antibodies used, and their respective dilutions, are listed in Table 1.

## Results

### Interstitial cells of Cajal (ICC) and c-kit-immunoreactivity (c-kit-IR)

#### Foetuses aged 17 weeks

At this age, the myenteric plexus elements are arranged in long and large groups of cells and only occasionally are fragmented in ganglia. In these foetuses, the ICC are exclusively located in between the circular and longitudinal muscle layers and, therefore, can be identified as ICC-MP (Fig. 1A). Close to the myenteric plexus elements, the ICC form an almost uninterrupted monolayer of cells apposed to either the circular muscle side or the longitudinal muscle side of the plexus (Fig. 1A and B). It should be noted that the ICC-MP located either where myenteric elements are absent (Fig. 1A), or on the circular muscle side of the plexus (Fig. 1C) are circularly orientated and those located on the longitudinal muscle side of the plexus are longitudinally orientated (Fig. 1C). Although rarely, it is also possible to see ICC obliquely orientated, apparently located within the plexus and crossing it from the circular to the longitudinal side (Fig. 1B). Most of the ICC-MP have an ovoid shape and one, or a maximum of two, short processes (Fig. 1C and D). When the processes are two, they start at the opposite poles of the cell and the ICC appears as spindle-shaped (Fig. 1C). Rarely, the ICC located on the two sides of a ganglion have short processes by which they connect to each other (Fig. 1F). c-kit-IR appears as a thin, faintly labelled ring corresponding to the cell contour. Processes are also labelled. All of the c-kit-IR cells identified as ICC are smaller than the mast cells, which are round-shaped, have a spherical nucleus and a clear cytoplasm (Fig. 1E).

#### Foetuses aged 19–27 weeks

By 19 weeks, the spindle-shaped ICC are more frequent; most of them are larger and longer than the mast cells (Fig. 1G) and than the ICC at 17 weeks. The ICC located close to the ganglia might have a triangular body due to the presence of a third process

**Table 1** Antibodies

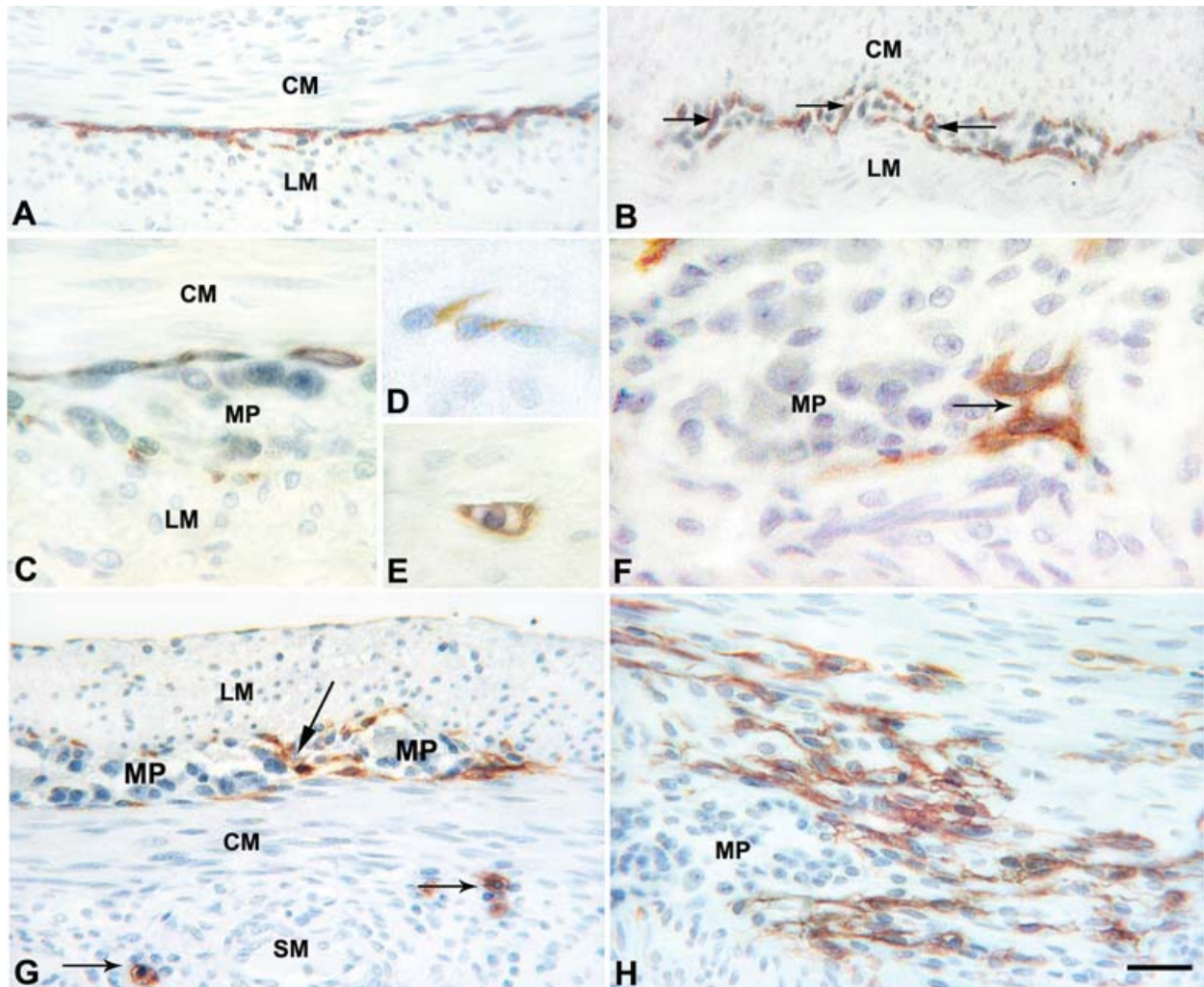
Antigen	Clone	Supplier	Dilution
$\alpha$ -SMA	1-A4	Dako	1:100
NSE	Polyclonal	Dako	1:100
C-Kit	CD-117	Dako	1:300
S-100	Polyclonal	Dako	1:7000

and clearly form a network (Fig. 1H). By 20–22 weeks, the ICC size is further increased (Fig. 2A); many of these cells have an oval or triangular body and at least three processes (Fig. 2B) by which they form a network (Fig. 2C and d), and a complete envelope to the ganglia. By 22–27 weeks, the ICC processes become longer and give rise to secondary branches (Fig. 2E). Frequently, one process can be seen in the circular muscle layer (Fig. 2F). The c-Kit labelling is intense at this stage.

#### Neonates aged 36–41 weeks

In the pre-term babies aged 36 weeks, some of the ICC-MP are mainly spindle-shaped, their size is increased and their processes are richly branched (Fig. 3A). In the newborns aged 38–41 weeks, the ICC-MP have large bodies, mainly oval or triangular in shape, and both primary and secondary processes have numerous, thin ramifications (Fig. 3B). By 36 weeks, the ICC-IM begin to differentiate and to colonize the circular muscle layer. At first, the ICC-IM are very close to the myenteric plexus region (Fig. 3C); later on, by 38 weeks, several ICC-IM are present at different levels of the circular muscle layer, from the outermost to the innermost portion of this layer (Fig. 3D). At 39 weeks, most of the ICC-IM has a fusiform shape (Fig. 3E) and at 41 weeks each process is often bifurcated (Fig. 3F). In the babies aged 38–41 weeks, some ICC-IM are present also within the longitudinal muscle layer, and others are present close to the nerve strands that, passing through the connective septa, connect the myenteric with the submucous plexus (Fig. 3G). These latter cells are small, have an oval body and one-two short processes. Exceptionally, spindle-shaped ICC can be seen close to the submucosal border of the circular muscle layer (Fig. 3G and H). However, they cannot be unequivocally considered as ICC-DMP.



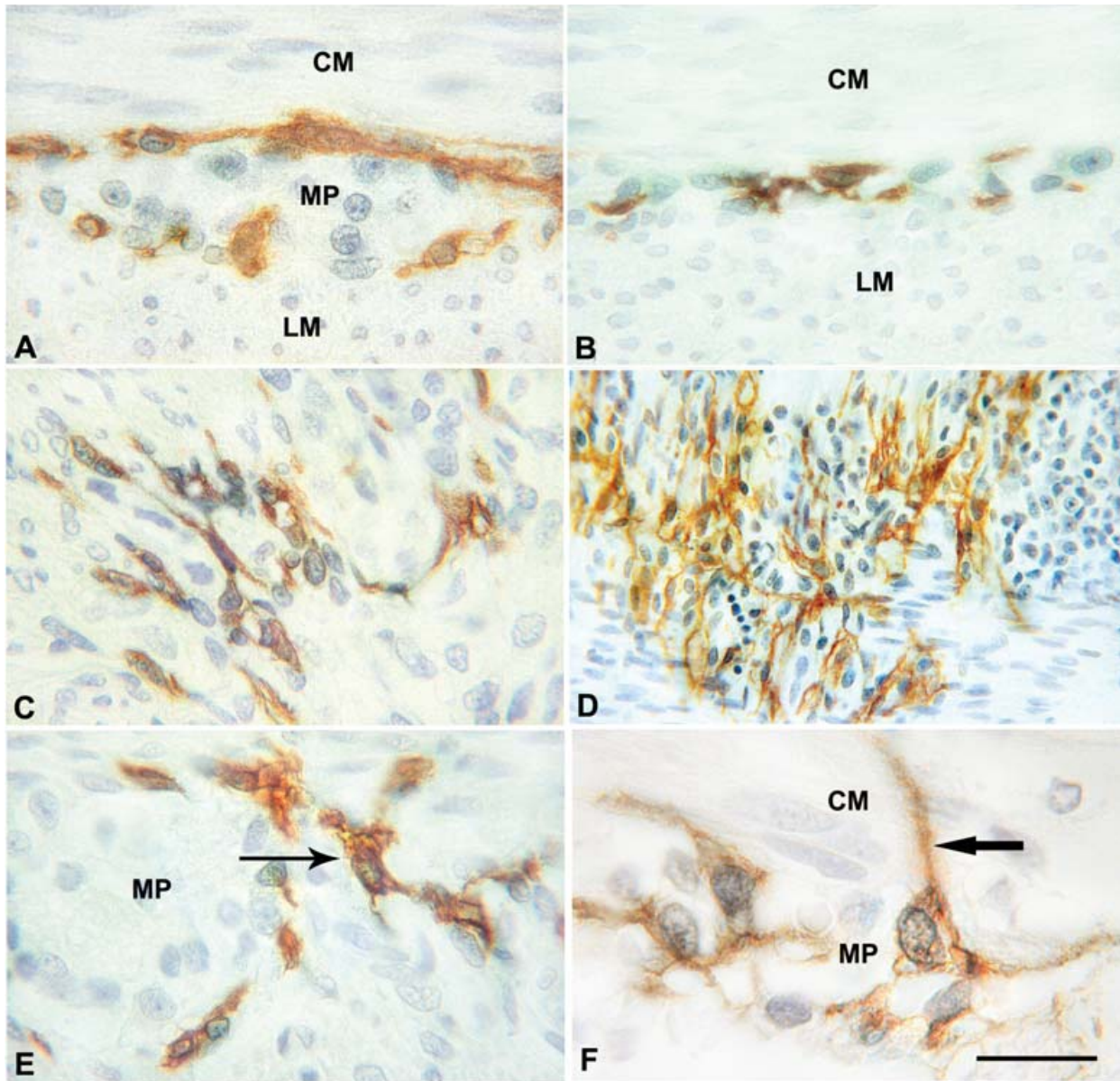


**Fig. 1** c-kit immunohistochemistry. **A–F**: Foetuses aged 17 weeks. **G** and **H**: foetuses aged 19 weeks. **A**: aligned ICC located in between the circular and longitudinal muscle layers. These ICC can be considered ICC-MP. **B**: ICC located on both sides of a group of myenteric plexus elements. *Arrows* indicate ICC apparently located within the plexus. **C**: detail of the ICC-MP closely apposed around a ganglion. These cells have an oval shape and two short processes starting at the opposite poles of the cell. **D**: two ICC-MP with one c-kit-IR short process. **E**: one c-kit-IR mast-cell. **F**: two interconnected (*arrow*) ICC-MP. **G**: one ICC (*long arrow*) apparently dividing the group of myenteric plexus elements into two ganglia. *Short arrows* indicate submucosal mast cells. **H**: The network formed by the ICC-MP. **CM**: circular muscle layer; **LM**: longitudinal muscle layer; **MP**: myenteric plexus; **SM**: submucosa. Bar: **A, B, G, H** = 50  $\mu\text{m}$ ; **C–F** = 20  $\mu\text{m}$ .

### Adult

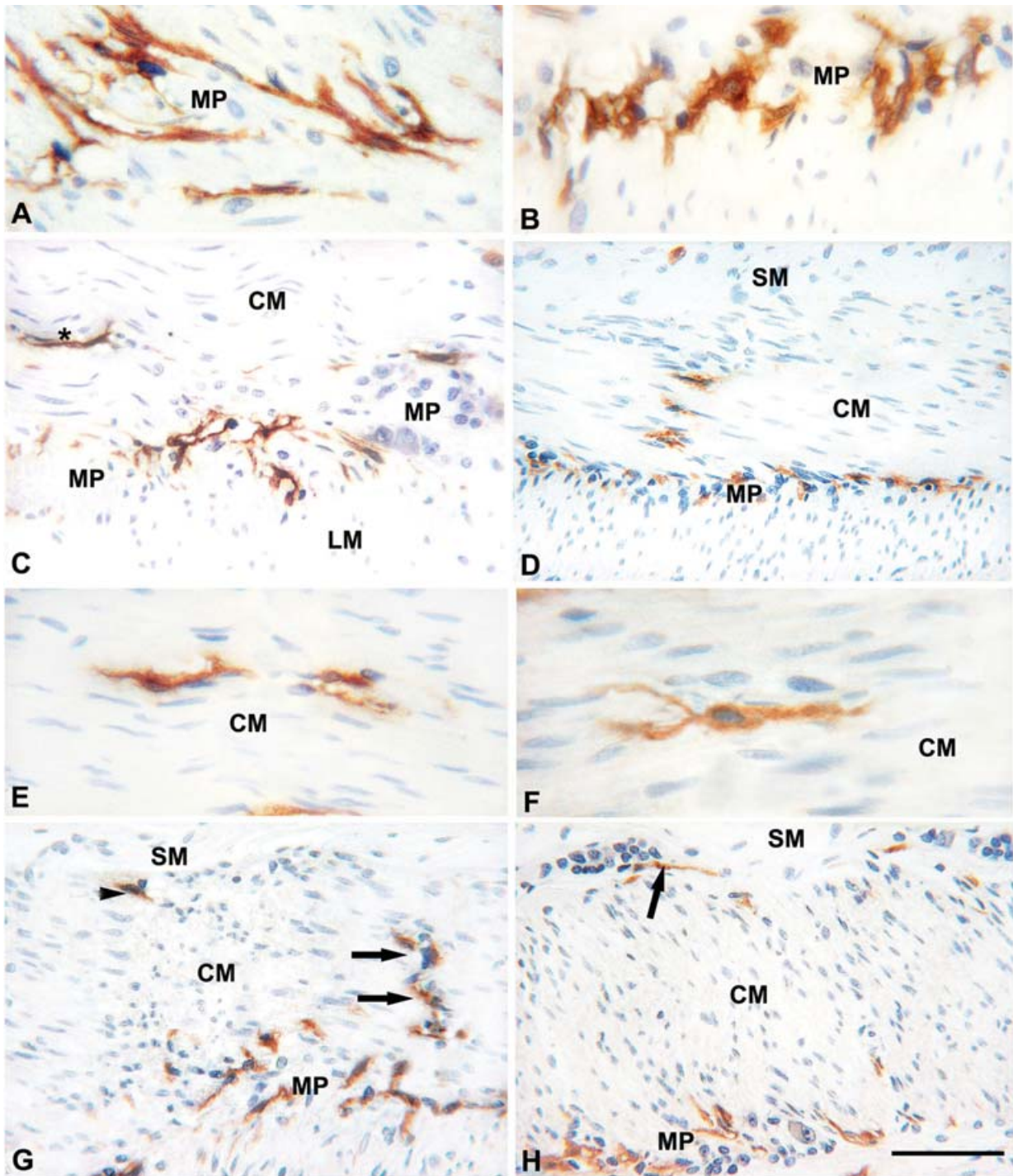
In the adult, all the three ICC populations are present and, with the exception of the ICC-DMP, are intensely labelled (Fig. 4A). The ICC-MP have an oval or triangular body and all of them are rich in long and

ramified primary and secondary processes, by means of which they contribute to form a network (Fig. 4B). Both the ICC-IM (Fig. 4C) and the ICC-DMP (Fig. 4D) have an oval body with at least two long processes.

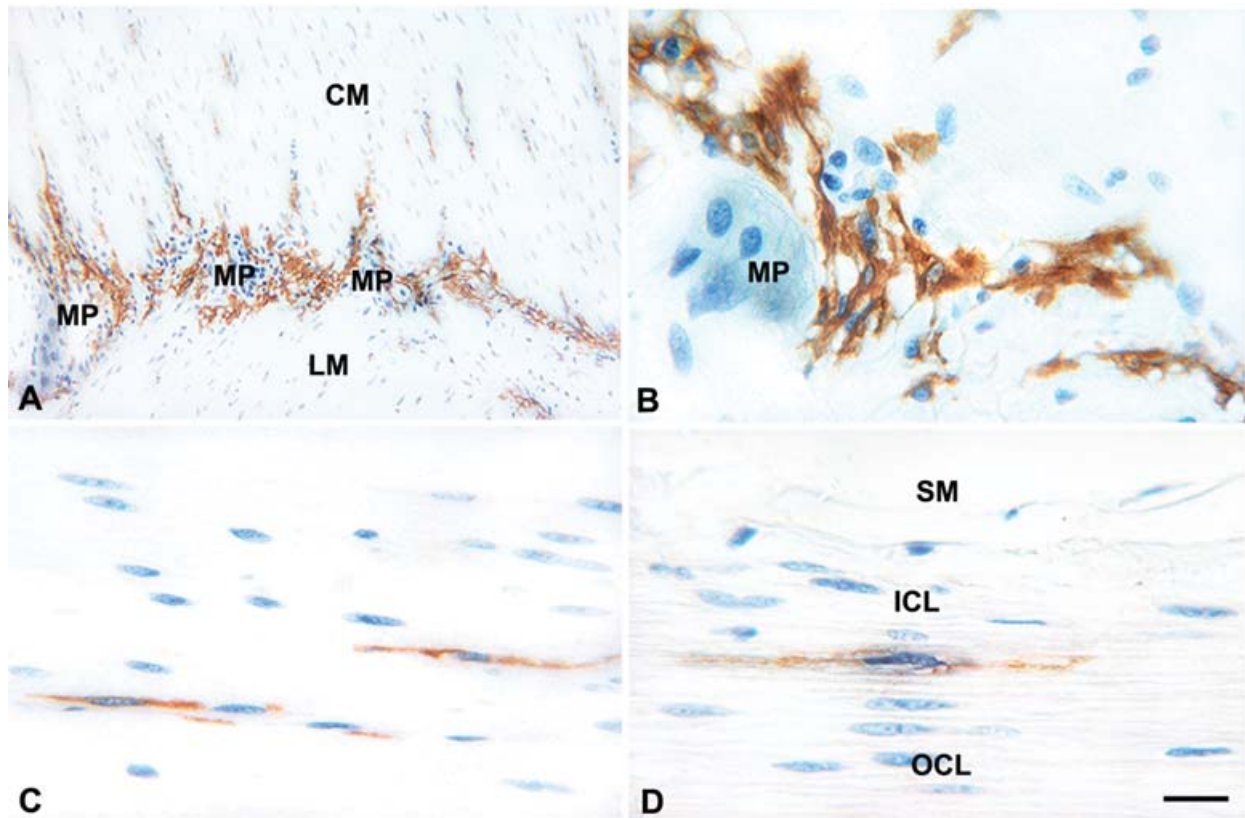


**Fig. 2** c-kit immunohistochemistry. **A–C**: Foetuses aged 20 weeks. **D**: Foetus aged 22 weeks. **E** and **F**: Foetuses aged 27 weeks. **A**: spindle-shaped ICC-MP enveloping a ganglion. The ICC located on the circular muscle side of the plexus are circularly orientated and those located on the longitudinal muscle side of the plexus are longitudinally orientated. **B**: an ICC-MP with at least three processes. **C**: The ICC-MP network. **D**: The ICC-MP network. **E**: ICC-MP, one of which has four processes. **F**: one ICC with three processes. One of these (*arrow*) enters the circular muscle layer. **CM**: circular muscle layer; **LM**: longitudinal muscle layer; **MP**: myenteric plexus. Bar: **A–C**, **E**, **F** = 20  $\mu\text{m}$ ; **D** = 50  $\mu\text{m}$ .





**Fig. 3** c-kit immunohistochemistry. **A–H:** Neonates aged 36–41 weeks. **A:** 38 weeks. Highly ramified ICC-MP. **B:** 41 weeks. The primary processes of the ICC-MP are rich in secondary processes. **C:** 36 weeks. One ICC-IM (*asterisk*) located within the circular muscle layer near the myenteric plexus. **D:** 38 weeks. Three ICC-IM located at different levels of the circular muscle layer. **E:** 39 weeks. Two spindle-shaped ICC-IM. **F:** 41 weeks. An ICC-IM with two processes, one of which bifurcates. **G:** 39 weeks. c-kit-IR cells (*arrows*) in a connective septum of the circular muscle layer. The *arrowhead* indicates a c-kit-IR cell at the submucosal side of the circular muscle layer. **H:** 39 weeks. The *arrow* indicates a presumptive ICC-DMP. **CM:** circular muscle layer; **LM:** longitudinal muscle layer; **MP:** myenteric plexus; **SM:** submucosa. Bar: **A, B, E, F** = 50  $\mu$ m; **C, D, G, H** = 20  $\mu$ m.



**Fig. 4** c-kit immunohistochemistry. Adults. **A:** the ICC are mainly concentrated at the myenteric plexus level, but the intramuscular ones are also numerous. **B:** the network formed by ICC-MP, which are richly branched and connected to each other. **C:** two spindle-shaped ICC-IM. **D:** one spindle-shaped ICC-DMP. This cell is faintly labelled. **CM:** circular muscle layer; **LM:** longitudinal muscle layer; **MP:** myenteric plexus; **SM:** submucosa, **ICL:** inner circular muscle layer; **OCL:** outer portion of the circular muscle layer. Bar: **A** = 100  $\mu\text{m}$ ; **B-D** = 20  $\mu\text{m}$ .

### Smooth muscle cells, muscle layers and $\alpha$ -smooth muscle actin immunoreactivity ( $\alpha$ SMA-IR)

#### In the foetuses aged 17–27 weeks

$\alpha$ SMA-IR is faint (Fig. 5A), except for the inner row of the circular muscle cells that is more intensely labelled than the other rows at 27 weeks (Fig. 5B). However, the subdivision of the circular muscle layer in two separate portions is not identifiable. *In the pre-term babies aged 36 weeks* the two portions of the circular muscle layer (named the inner one, ICL, and the outer one, OCL) are clearly recognizable. At this age, the ICL is made up by only one-two rows of intensely labelled smooth muscle cells (Fig. 5C). *In*

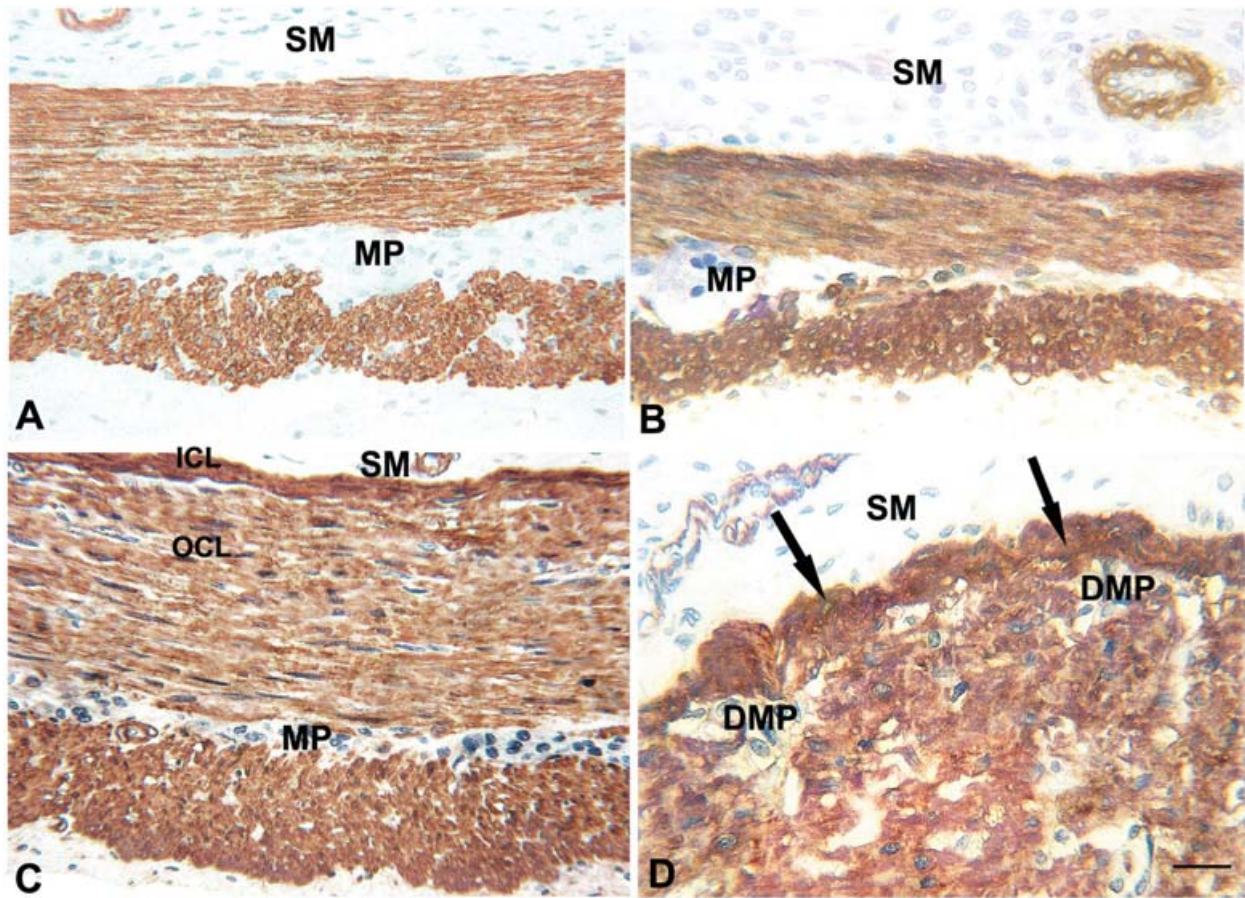
*the at-term babies aged 38–41 weeks*, the ICL is thicker and intensely labelled (Fig. 5D).

### Nerve structures and NSE- and S-100-IR

#### In the foetuses aged 17–27 weeks

Both myenteric and submucous plexuses are present; intramuscular nerves, however, are rare and the DMP is absent (Fig. 6A and B). *In the newborns aged 36–41 weeks*, the intramuscular nerves are numerous (Fig. 6E–G) and a primitive DMP can be seen by 36 weeks (Fig. 6C and D). At 17 weeks, only some of the neurons are NSE-IR, most of which are faintly labelled, and some others, especially those at the submucous plexus, are NSE-negative (Fig. 6E).





**Fig. 5**  $\alpha$  smooth muscle actin immunohistochemistry ( $\alpha$ SMA-IR). **A:** 22 week-old foetus.  $\alpha$ SMA-IR is faint and the circular muscle layer is not subdivided into two portions. **B:** 27 week-old foetus. The circular muscle layer is not subdivided into two portions;  $\alpha$ SMA-IR is faint except for the inner row of the circular muscle cells that is intensely labelled. **C:** 36 week-old newborn.  $\alpha$ SMA-IR is intense and the circular muscle layer is subdivided into two portions. The innermost portion is intensely labelled but is still thin. **D:** 39 week-old newborn. The inner portion of the circular muscle layer (arrows) is thick and intensely  $\alpha$ SMA-IR. **MP:** myenteric plexus; **SM:** submucosa; **DMP:** the area where the deep muscular plexus is located; **ICL:** inner circular muscle layer; **OCL:** outer circular muscle layer. Bar: **A–C** = 50; **D** = 20  $\mu$ m.

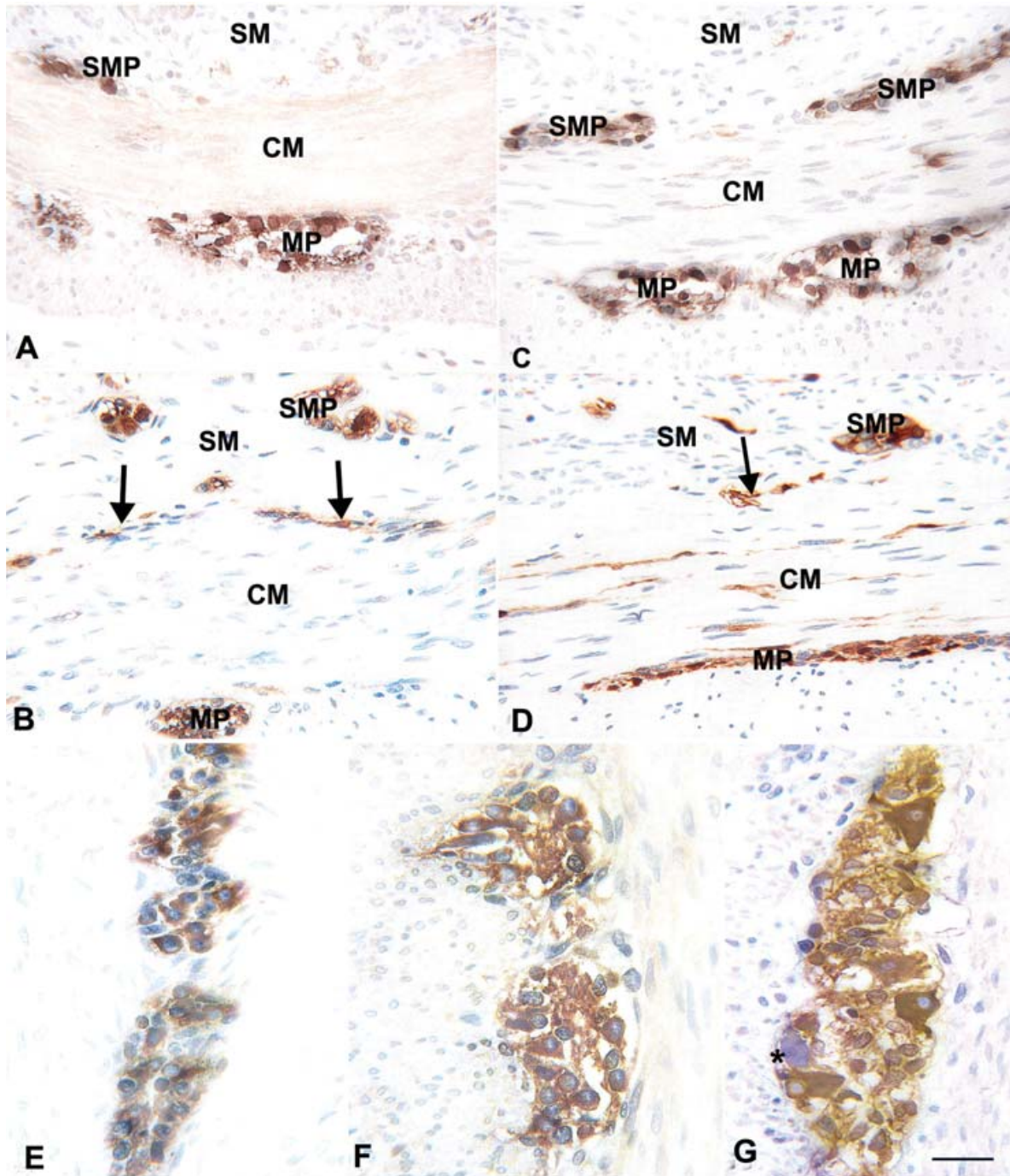
All neurons are small and have a round or piriform body. By 20 weeks, some of the neurons are more differentiated; their body is increased in size and often triangular in shape (Fig. 6F). By term, not all neurons are differentiated yet, as only some of them are intensely NSE-IR and the others are faintly labelled or still NSE-negative (Fig. 6G). However, all neurons, are increased in size.

In the Table 2, a timeline to show at which week of development these different cell types appear within the human gut.

## Discussion

The present findings, obtained in the human ileum of fetuses aged 17–27 weeks and newborns aged 36–41 weeks, confirm data from the literature on the early detection of the ICC-MP during foetal life. At 17 weeks, they already form a continuous network, but still have an immature morphology. These ICC progressively differentiate up to birth, and even later, by giving raise to numerous processes and increasing in size. Conversely, the ICC-IM appear at 36 weeks





**Fig. 6** neuron specific enolase (NSE-IR) and S-100 (S-100-IR) immunohistochemistry. **A** and **B**: NSE-IR; **C** and **D**: S-100-IR. **A** and **C**: 22 week-old foetus. Both the myenteric and the submucous plexuses are present. **B** and **D**: 36 week-old foetus. At the level of the future DMP there are several nerve fibers (*arrows*). **E-G**: NSE-IR. **E**: 17 week-old foetus. All neurons are small and mainly round in shape. Some of them are NSE-negative and some are faintly labelled. **F**: 20 week-old foetus. Some of the neurons are round in shape, but those more intensely labelled have a triangular or piriform body. Some neurons are still NSE-negative. **G**: 39 week-old newborn. Some neurons are faintly labelled and others are still NSE-negative (asterisk). The neurons intensely NSE-IR have a large body and numerous processes. **CM**: circular muscle layer; **MP**: myenteric plexus; **SM**: submucosa, **SMP**: submucous plexus. Bar: **A-D** = 50  $\mu$ m; **E-G** = 20  $\mu$ m.

**Table 2** Time course of cell type appearance

Cell types	Gestational age		
	17-27 weeks	36 weeks	38-41 weeks
ICC-MP	present	present	present
ICC-IM Circ. muscle		present	present
ICC-IM Long. muscle		present	present
ICC-DMP			present
DMP		present	present
ICL		present	present

of age and they too continue to differentiate after birth. The ICC-DMP differentiate after birth, together with the DMP and the ICL. Interestingly, the differentiation of these three populations of ICC occurs in parallel with that of the related enteric plexuses and muscle layers. This information may help when evaluating the intestinal motile behaviour of foetuses and of either normal or pathological pre-term and full-term neonates.

At the earliest prenatal ages we studied, the ICC-MP were seen in between the two muscle layers forming a single layer of cells and, interestingly, most of them were circularly orientated. At the level of the nerve elements of the myenteric plexus the ICC, still forming an uninterrupted layer, partially or completely enveloped the plexus. Noteworthy, the ICC-MP that had the major axis parallel to the longitudinal muscle cells were apposed to the longitudinal side of the myenteric plexus, and those having the major axis parallel to the circular muscle cells were apposed to the circular side of the plexus. The presence of ICC-MP in the foetuses aged 17 weeks is not surprising since the literature refers to the appearance of these cells in the ileum of human foetuses by 7–12 weeks [9–12]. According to some authors [9], at 7 weeks these cells, or some of them, are located within the myenteric plexus and move out of the ganglia at 14 weeks. We observed in foetuses aged 17–19 weeks that some ICC were crossing the myenteric plexus for its entire thickness. At this age the large groups of enteric nerve cells are beginning to fragment and to give raise to the ganglia; therefore, it is reasonably to hypothesize that ICC might be not located within the plexus, but in the septa that are subdividing the large groups of myenteric cells in the future ganglia, thus contributing to the maintenance of the continuity of the ICC-MP network surrounding each ganglion. By 17–19 weeks up to at term, the ICC-MP increase in

size and change their shape giving raise to processes. Initially, the processes are one and short; then increase in number and each of them becomes rich in secondary processes. The increase in ICC size and processes reasonably contributes to the maintenance of the ICC network during ileal lengthening and enlargement. The presence of the ICC-MP network by the early foetal ages should ensure the occurrence of peristaltic movements. The human foetus is known to swallow amniotic fluid from at least 16 weeks' gestation, although coordinated sucking movements do not occur until about 28 weeks. At 25–30 weeks' gestation irregular contractile activity can be recorded in the foetal small intestine, by 33 weeks the activity can be detected as rhythmic clusters, and by 36 weeks a mature migrating motor complex can be identified [27]. It is a common experience of many neonatal intensive care units that even extremely low birth weight infants (birth weight <1000 gr, gestational age < 28 weeks) can be fed, but a paralytic ileus is expected in this subgroup of neonates.

According to the present data, ICC-IM are absent in the human ileum at 27 weeks but present at 36 weeks. Some authors [11] report their presence in foetuses aged 26 weeks, but information is vague and no images are provided. According to others [12], these cells are not present during foetal life. However, all these data agree that ICC-IM differentiate later than the ICC-MP, presumably in the near-term foetuses, and that their differentiation continues after birth. Moreover, we observed that the ICC-IM located within the circular muscle layer appear before those located within the longitudinal muscle layer. According to our data, both of them seem to differentiate from a pool of ICC precursors located at the myenteric plexus region that progressively enter and colonize the muscle layers. The presence of ICC-IM should ensure the neurotransmission and guarantee a link with the ICC-MP. Therefore, in the presence of both these cell types the ileal motility should be similar to that of the adult, although not identical due to the lack of the ICC-DMP. Few cells, likely precursors of the ICC-DMP, were seen at-term, but our images, although suggestive, give no certainty on the identity of these cells. Some authors [11] report about the presence of ICC-DMP in newborns but images are not provided. In the mouse, these cells were seen at birth, but having immature features, and to further develop during the suckling period [28]. In mammals and birds, all the types of ICC are considered as

differentiating from mesenchymal cells [28–31]. In mice, the ICC-DMP are presumed to originate from the mesenchymal cells located close to the submucosal border of the circular muscle layer, *i.e.* from the same cells that give rise to smooth muscle cells of the ICL [28]. In humans the source of ICC-DMP could not be clearly identified, but according to some of the images obtained in the pre-term babies, these cells seem to migrate from the myenteric plexus area, closely apposed to the nerve fibers directed towards the submucosa. Moreover, neither DMP nor ICL could be recognized in the pre-term babies. Huizinga *et al.* (2001) are the only authors reporting on their presence in full-term neonates [32].

By 12 weeks, the two muscle layers are already present in human beings [9]. We presently noticed that the  $\alpha$ SMA-IR is less intense in the fetuses aged 17 weeks than in at-term babies. Moreover, we identified the ICL only in the pre-term babies; this layer, however, was very thin at this age and thicker in at-term babies. Briefly, we can conclude that either the smooth muscle cells or the muscle layers are not completely differentiated before birth.

The invasion of the gut by the neural crest cells occurs very early in all mammals [33]. In human beings, these cells arrive in the gut by the 4<sup>th</sup> week [34] and the organization in myenteric ganglia initiates by 7 weeks [10]. However, we could notice that at 17 weeks the organization in myenteric ganglia was not definitively accomplished and neuronal cells were not completely differentiated, as some of them were NSE-negative and some others had a faint NSE-IR. From this age to birth, neurons were seen to progressively mature, but in the full-term neonates some of them were still NSE-negative. Nerve fibers were seen to gradually colonize the muscle layers but the DMP could be identified only at term, together with the ICL. Briefly, ICC-DMP, DMP and ICL initiate their differentiation just before birth and continue after birth. Since these structures are considered the intestinal stretch receptor [5, 7], their absence is not surprising when babies do not eat, as well as the fact they continue to develop during the suckling period. These data are new for man, although something similar has been reported for the mouse [28]. We consider this information of extreme interest as it indicates at which paediatric age the intestinal stretch receptor is present and, possibly, able to work. Moreover, its absence at the expected age should be considered responsible for important motile disorders of the neonates.

The maturative steps of the ICC during the fetal life and the degree of cyto-differentiation in the pre-term and at-term newborns is of particular interest in order to understand the ileal motile behaviour of these babies, especially of those prematurely born and artificially fed. The lack or the delay in the differentiation of each ICC type can cause important functional disturbances in gut motility, as it has been demonstrated in mutant mice [35–37]. Moreover, the key role of the ICC has been raised in a series of articles describing the altered distribution of these cells in different neonatal gastrointestinal diseases [16–26]. Most of these papers simply describe the reduced number of ICC, mainly based on the immunoreactivity to the c-kit antibody, without reporting detailed microscopic changes. Just one paper [12] gave important insights on the ontogenesis of ICC in human beings, but it did not consider the other components of muscle layers and enteric nervous system.

In conclusion, in human beings ICC differentiation occurs gradually during the prenatal life and in concomitance with that of neurons and muscle layers, and it continues into postnatal life. The identification of the morphology of the immature ICC, their cytological changes and their organization during differentiation might help in interpreting the significance of abnormalities in the ICC distribution, density and morphology at birth and in the early paediatric age, as well as in understanding the pathophysiology of intestinal motile disorders in neonates and young children.

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