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Interstitial cells of Cajal in health and disease: A comparative review in humans and animals

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Abstract

Interstitial Cells of Cajal (ICCs) are critical modulators of gastrointestinal motility due to their dual roles of initiating slow waves and providing the interlinking connections to enteric neurotransmission within the syncytium known as the SIP, consisting of smooth muscle cells, ICCs, and platelet derived growth factor- α (PDGFRα⁺) cells. Experimental and clinical evidence consistently demonstrates that ICC loss or dysfunction, whether due to quantitative depletion or functional impairment, is strongly associated with motility disorders such as gastroparesis, chronic constipation, and pseudo-obstruction, as well as veterinary conditions including equine colic and congenital disorders in young animals. Advances in diagnostic tools, including ANO1 immunostaining, live-cell calcium imaging, and genetic analysis, have significantly improved our ability to characterise ICC networks. Therapeutically, while current management relies mainly on prokinetic drugs and supportive care, future strategies such as stem cell–based regenerative therapies, organoid technology, and molecular targeting of ANO1 channels and c-Kit signalling pathways offer promising avenues for restoring ICC function and improving outcomes in both veterinary and human medicine. This review provides an overview of ICC pathophysiology and highlights recent progress in diagnostic and therapeutic developments, with particular emphasis on comparative evidence across human and veterinary medicine to identify shared mechanisms and translational opportunities.

1. Introduction

Coordinated peristalsis is fundamental to an animal's gastrointestinal (GI) health, ensuring the effective propulsion and mixing of luminal contents. This vital process relies on the sophisticated integration of smooth muscle contractility, enteric neural input, and specialized pacemaker activity. Interstitial Cells of Cajal (ICCs) are now definitively recognized as the principal pacemaker cells of the gut, generating and propagating slow electrical waves that synchronize smooth muscle contractions and facilitate peristalsis (Sanders et al. 2014; Sanders et al. 2023). SIP syncytium (consisting of Smooth muscle cells, ICCs, and PDGFRα⁺ cells), supports the production of multifaceted motor patterns like peristalsis and segmentation (Sanders et al. 2023). Disturbance of this system causes gut motility impairment and can be expressed in disease conditions such as gastroparesis, slow transit constipation, and pseudo-obstruction (Foong et al. 2020; Mostafa et al. 2010; Sanders et al. 2014). Animal models demonstrate that inflammation-mediated ICC damage, such as that induced by *Trichinella spiralis* infection, characterized by structural and functional disruption of ICC networks, leads to desynchronized pacemaker activity and abnormal motor patterns, which is reversible upon resolution of inflammation (Zhang et al. 2025). Even though ICC deficits are invariably observed in a range of motility disorders, it is a key challenge to determine whether ICC loss is a causative factor or only a secondary outcome of a motility disorder (Sanders et al. 2002; Mostafa et al. 2010; Ward and Sanders 2001). This review – a) synthesizes the evidence regarding the physiological actions of ICCs in the generation and coordination of GI motility, b) identifies the role of ICCs in motility disorders in animals and humans, and c) identifies therapeutic approaches targeting the ICCs. The evolution in the area involves enhanced mapping of the motor patterns and clarification of Ca^{2+} dynamics in ICCs (Sanders et al. 2023). The current therapeutic interventions that are being researched, as well as modulation of ICC ion channels by pharmacological interventions and regeneration strategies, reveal that precision-targeted treatment approaches are urgently needed to recover normal GI motility in both animals and humans (Huizinga et al. 1997; Zhang et al. 2025).

2. Interstitial cells of Cajal (ICCs)

2.1 Definition and Background

ICCs are mesenchymal cells and the major pacemakers and regulators of GI motility. ICCs were first described by the Nobel laureate Santiago Ramon y Cajal in 1893 and initially identified as specialized interstitial cells distinct from neurons and smooth muscle cells (Sweet et al. 2024; Mostafa et al. 2010). With the help of electrophysiological and ultrastructural studies they are now well established as pacemakers and regulators which initiate and conduct slow electrical waves,

coordinating contractions of smooth muscle in the GI tract (Sanders et al. 2023; Sanders et al. 2014). c-Kit signaling plays a critical role in the proper maturation and maintenance of these essential networks, and disruptions in these networks is directly involved in a wide array of motility disorders (Ward and Sanders 2001; Mostafa et al. 2010; Iino et al. 2020).

2.2 Classification and Anatomy

ICCs are not a monolithic population; they are classified based on their anatomical location, and corresponding functional properties are distributed throughout the GI tract in distinct anatomical locations (Sanders et al. 2023; Komuro 2006):

- ICC-MY (Myenteric Plexus): These cells are between the longitudinal and circular muscle layers, forming extensive networks. They are principally responsible for generating and propagating slow waves.
- ICC-IM (Intramuscular): They are distributed in the circular and longitudinal muscles and mainly engaged in neurotransmission of enteric motor neurons to the smooth muscle.
- Other Subtypes: Other ICC populations exist deeper in the muscular plexus (ICC-DMP) as well as in sub-serosal regions, in which there are often region-specific motor modulatory functions.

These ICCs are consistently located in networks, and these networks influence coordination bouts of peristalsis (Sanders et al. 2014; Komuro 2006; Hirst and Ward 2003). Ultra-structurally, ICCs are highly differentiated with abundant mitochondria, intermediate filaments, and most importantly, numerous gap junctions. These gap junctions are involved in strong electrical coupling with smooth muscle cells and other ICCs to facilitate the synchrony of muscle contraction within the GI musculature (Komuro 2006).

2.3. ICCs as Pacemaker and Signal Transducers

ICCs play their biological role as the main rhythmic drivers and the key mediators of neuronal regulation of smooth muscle function in the gastrointestinal tract. The slow waves that control phasic contractions in GI smooth muscle are generated by a process that relies essentially on Ca^{2+} dynamics in ICCs. This is initiated by de-novo, localized release of Ca^{2+} into the endoplasmic reticulum, activating Ca^{2+}-dependent chloride channels (ANO1), which triggers an inward depolarizing current to start the slow-wave upstroke (Zhu et al. 2015; Sanders et al. 2023; Sanders et al. 2024; Majeed et al. 2024a). The resulting depolarization activates T-type voltage-gated Ca^{2+} channels to further increase Ca^{2+} influx. This is supplemented with a plateau phase ensured by clusters of Ca^{2+} transients, ensuring the ANO1 channels remain active (Drumm et al. 2017; Sanders et al. 2024). This plateau phase continues until the Ca^{2+} stores are exhausted and ANO1 channels close and the membrane repolarizes, thus, completing the cycle (Sanders 2019).

In addition to their primary function, ICCs also play a crucial role as signal transducers by integrating excitatory and inhibitory signals from the enteric nervous system. Neuronal activation modulates the quantity of intracellular Ca^{2+} transients in ICCs. For example, the activation of excitatory neurotransmitters causes an increase in intracellular Ca^{2+} transients, promoting slow wave activity, whereas inhibitory neurotransmitters decrease intracellular Ca^{2+} transients to suppress slow wave generation (Sanders et al. 2024). Anatomically, ICCs are strategically located to play this two-fold role. They mainly form localizations in the myenteric plexus (ICC-MY) between the longitudinal and circular layer of the musculature and the musculature itself (ICC-IM), which are interconnected, forming a large network that transmit slow wave propagation and mediates neurotransmission to smooth muscle cells (Sanders et al. 2014; Baker et al. 2021; Hwang et al. 2022). Such a specific localization allows this structure to be not only the rhythm generator, but also a critical integrator of neural signals, ensuring efficient GI motility.

2.4. The SIP Syncytium

A major characteristic of ICCs is their involvement in electricity conduction and synchronization of the GI tract, via the SIP syncytium- a functional network of interconnections between the smooth muscle cells (SMCs), ICCs, and PDGFRα cells. ICCs are electrically connected to SMCs and PDGFRα cells through gap junctions thus mediating the transmission of excitatory and inhibitory regulatory signals and their aggregation into the syncytium (Sanders et al. 2014; Sanders et al. 2024). Ca^{2+} activated K^+ channels (encoded by Kcnn3) are also included in another SIP component, PDGFRα cells, which cause outward currents and hyperpolarization effects that constrain the overall excitability of the syncytium. It is known that neurotransmission originating in enteric motor neurons can either cause Ca^{2+} transients to surge or decline in ICCs and PDGFRα cells, with the ensuing depolarizing or hyperpolarizing outputs relayed throughout the SIP network (Sanders et al. 2024; Ward and Sanders 2006). This concerted electrical network enables accurate synchronization of GI motility including peristalsis and segmentation and allows GI to rapidly adapt to both neural and mechanical stimuli. Perturbation of synchronization of the SIP syncytium results in abnormal motility and clinical symptoms in animals (Klein et al. 2013; Majeed et al. 2024b).

3. ICCs in Animal Health and Disease

3.1 Functional Role Across Species

The ICCs are involved in coordinated peristalsis and segmental contractions of GI tract (Huizinga et al. 2022; Mostafa et al. 2010). However, their actual physiological requirements in different species, their distribution, and role vary anatomically:

Ruminants

Recent comparative studies have broadened our understanding of ICCs in the veterinary species and highlighted a structural diversity and physiological specialization between ruminants and carnivores. ICCs are morphologically tailored to the slow and rhythmic contractions of the stomachs of multi-chamber ruminants like cattle, sheep, and goats. These cells play a crucial role in regulating slow electrical waves that coordinate ruminal and omasum movements and hence maintain the effective fermentation and ruminal digestion flow. One of the key studies by Marquez et al. (2014) was able to identify c-kit positive ICCs in fetal, neonatal, and adult bovine forestomaches with significant ontogenetic and regional differences. ICCs in the fetus were smaller and more densely clustered, suggesting early pacemaker activity, whereas ICCs in the adult had an elongated morphology between bundles of smooth muscle. These observations are supported by studies that map age-related changes in the populations of ICCs, which revealed decline of density of ICCs with maturation in the ruminant stomach. Specifically, experiments carried out on pre-weaning goats demonstrate a significant decrease in the number of ICC and related neural components with the age of the animal, which reflects the developmental trend of functional specialization and maturation of the

ruminant gastric organ. This trend corresponds to the development of the orchestrated patterns of motility that are key to the digestive functioning of the adult organism (Liang et al. 2019). With respect to ICC plasticity under microbial and metabolic stress, the literature suggests an adaptive morphological changes in ICCs in response to chronic inflammatory or metabolic challenges rather than widespread degeneration of the cells. Across different models of gastrointestinal pathology, ICCs may undergo ultrastructural alterations and reorganization of their network, but the overall loss of these cells is rare with the exception of more severe or chronic disease condition (Komuro 2006; Mikkelsen 2010).

Carnivorous

ICCs have a clear distribution pattern in carnivorous species, especially dogs and cats, with intramuscular ICCs (ICC-IM) in the pyloric and colonic areas playing a central role in region-specific gastrointestinal motility. In feline pylorus, ICC-IM show a significant concentration in the inner border of the circular muscle layer where they form a synapse type association with enteric afferent fibers with cholinergic, nitrergic, and substance-P markers. This dedicated architecture allows tight control of the pyloric sphincter function and makes a distinction between the activities of antral and duodenal pacemakers, thus facilitating the coordination of gastrointestinal emptying and intestinal transit (Wang et al. 2007; Komuro 2006; Sanders et al. 2024; Ward and Sanders 2001). ICC-IM play an important role as critical mediators of autonomic motility control due to their close liaison with vagal afferents. They transmit vagal motor signals, predominantly nitrergic inhibitory signals, to smooth muscle cells through gap junctions, causing relaxation and regulating contractile rhythmicity of gut. Reduction or inhibition of ICC-IM impairs vagally mediated inhibition, which leads to aberrations in motility including antral hypomotility or pyloric dysfunction (Beckett et al. 2017). This is the pathway in which ICC-IM supports excitatory and inhibitory neuro-effector processes in the gastrointestinal tract in general (Mostafa et al. 2010; Klein et al. 2013).

3.2 Pathophysiology of ICC Dysfunction

ICC dysfunction can trigger a whole range or spectrum of GI motility disorders, and its pathophysiology includes quantitative loss (cell depletion) and functional impairment (physiological failure). The knowledge of the difference between the two is essential to appropriate diagnosis and prognosis described below and summarized in Table 1.

Quantitative loss (Cell depletion)

Quantitative loss is the reduction in absolute cell number of ICCs, or ICCs c-Kit and ANO1 immunoreactivity. This type of loss is usually attributed to chronic and long-standing insults. Meta-analysis illustrates severe depletion of ICC in chronic gastroduodenal lesions, where the degree of such loss is correlated with the delay in gastric emptying and level of symptoms (Varghese et al. 2025). Mechanical obstruction and chronic inflammation in different animal models gradually lower ICC numbers and destroy the network architecture, and these effects correlate with insult duration and intensity (Li et al. 2019; Wu et al. 2013). The result of this longstanding loss is a direct contribution to the poor generation of slow waves, as shown in Fig. 1.

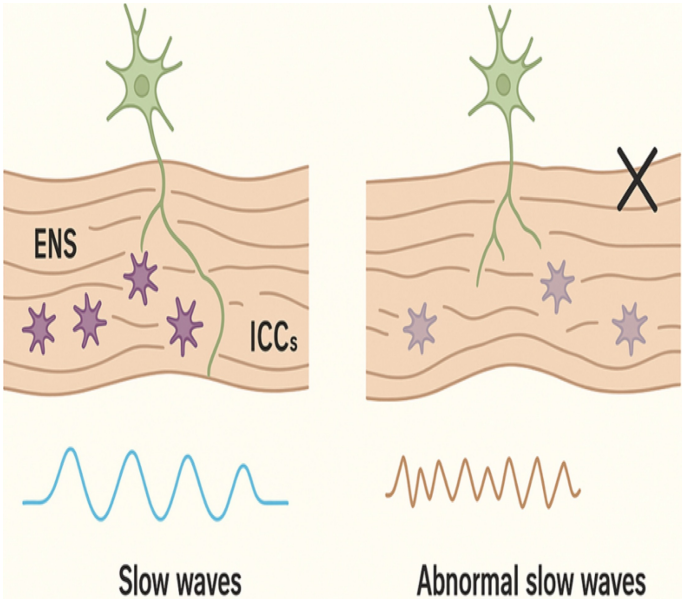

Fig. 1. *Role of ICCs in coordinating gut motility; (A) Healthy ICCs, together with the Enteric Nervous System (ENS), produce a periodic series of slow waves which control gastrointestinal motility; (B) ICCs loss or impairment interferes with this process leading to the occurrence of abnormal slow waves and motility disorders*

Table 1. Interstitial Cells of Cajal and associated gastrointestinal motility disorders in different species

Species	Associated motility disorder	Pathological defect	Pathophysiological mechanism	References
Human	Gastroparesis	Severe quantitative loss or functional impairment	Failure in autonomous slow wave generation and propagation	(Varghese et al. 2025)
	Chronic Constipation / CIPO	Depletion of ICC-MY and ICC-IM networks	Impaired neuromuscular signal transmission; loss of SIP synchronization	(Huizinga et al. 2021; Sanders et al. 2024)
Equine (Horses)	Large Intestinal Obstructive Colic	Marked depletion in ICC-MY and circular muscle	Disruption of GI pacemaker activity and motility patterns	(Hudson et al. 2001; Koenig and Cote 2006)
	Equine Grass Sickness (Dysautonomia)	Loss associated with autonomic neuropathy	Failure of vagally-mediated inhibitory neurotransmission to smooth muscle	(Pirie et al. 2014)
Ruminants (Cattle, Goats)	Ruminal/ Abomasal motility	Ontogenetic changes and regional structural diversity	Disrupted coordination of slow, rhythmic movements crucial for fermentation	(Marquez et al. 2014; Liang et al. 2019; Wang et al. 2018).
Carnivores (Dogs, Cats)	Pyloric dysfunction	High concentration of ICC-IM in the pylorus; functional impairment.	Defective transmission of inhibitory signals (e.g., Nitric Oxide) to the sphincter.	(Wang et al. 2007; Beckett et al. 2017)

Functional impairment (Physiological failure)

Functional impairment refers to the loss of peacemaking ability or capacity of neurotransmission even though there is a preserved or near conserved cell number (Sarna 2008). ICCs integrate and inhibitory signals in the nervous system to align smooth muscles in the nervous system (Klein et al. 2013); in this case, when these activities fail to perform, they end up having abnormal motility despite a relatively good number of ICCs. For example, in postoperative ileus inflammation and nitric-oxide mediated signaling temporarily impairs ICC pacemaker signaling and organization, which may be restored due to decrease in inflammations or cell death occurs in chronic diseases (Kaji et al. 2018).

The Clinical Significance of ICC Loss in Intestinal Motility Disorders

The absence of ICCs is a common pathological feature irrespective of the types of motility disorders, which indicates a shared underlying mechanism of impaired neuromuscular coordination.

Inflammatory Diseases: Inflammatory bowel diseases, such as Crohn's disease and comparable disease conditions in animals, exhibit near-complete elimination of ICCs in involved intestinal segments and the loss of these cells contributes to the motility impairment and clinical manifestations (Porcher et al. 2002). Similar ICC deficits are noted in other inflammatory and neuromuscular conditions (Burns 2007; Mostafa et al. 2010).

Obstructive Syndromes: ICC loss is contributing to the chronic pseudo-obstruction and slow transit constipation (Friedmacher and Rolle 2023; Huizinga et al. 2021). Both functional and mechanical obstructions induce stress and sustained inflammation, disrupting ICC networks with consequent impaired slow wave generation (Wu et al. 2013; Hwang et al. 2025). The resulting cell loss and dysfunction is an important pathophysiological process at the heart of many GI motility disorders in both humans and animals (Sanders et al. 2002; Mostafa et al. 2010; Huizinga and Chen 2014).

4. Therapeutic and Clinical Implications

4.1 Susceptible Animal Populations

The animal populations at high risk of experiencing ICC dysfunction are horses and young animals with congenital motility disorders. ICC depletion was observed in horses with large intestinal obstructive colic and equine grass sickness (dysautonomia) by c-Kit immunohistochemistry (Fintl and Hudson 2010; Fintl et al. 2004; Pavone et al. 2012; Hudson et al. 2001), particularly in the myenteric plexus and circular muscle tissue. Similarly, small animals and infants with congenital motility dysfunction express severe deficiency or absent ICCs, such as Hirschsprung disease and other syndromes, characterized by a ganglionosis or hypoganglionosis (Friedmacher and Rolle 2023; Rolle et al. 2007; Okamura et al. 2009; Chen et al. 2014; Burns 2007). Collectively, the literature suggests that in horses with life-threatening motility problems and young animals with congenital defects, the lack of or defects in ICC is a central pathologic mechanism that directly contributes to clinical symptoms of gastrointestinal dysmotility in both groups.

4.2 Advanced Diagnostic and Research Techniques

To effectively evaluate ICCs, it is necessary to combine morphological, functional, and molecular methods since none can individually give complete information on cell integrity and activity (Fig. 2).

A. Morphological Diagnostics (Immunohistochemistry)

Immunohistochemistry is the main clinical technique in the detection of ICC networks in tissue. The c-Kit (CD117) and ANO1 immunostaining are the common methods to visualize ICC networks in both human and animal GI tissues (Fintl et al. 2004; Pavone et al. 2012; Fintl et al. 2020). However the limitation of c-Kit is that it is also expressed by mast cells and further both the markers can produce false negative results due to artefacts or disease induced losses of antigen or markers (Friedmacher and Rolle 2023; Huizinga and Chen 2014).

B. Functional Diagnostics

Functional diagnostics are essential for clarifying ICC physiology and confirming motility dysfunction caused by functional impairment.

- Electrophysiological Recordings: Techniques like patch clamp allow direct measurement of ICC pacemaker activity and slow wave generation, which provides knowledge about the electrical properties of ICCs and their coupling to smooth muscle (Sanders et al. 2002; Sanders et al. 2024).
- Live Cell Calcium Imaging: This method enables the real-time visualization of spontaneous and evoked Ca^{2+} transients in ICCs, directly underlying slow wave activity and neurotransmission (Sanders et al. 2024; Baker et al. 2016). The researchers have revealed the stochastic and regionally variable nature of Ca^{2+} signaling in ICC subtypes and their dependence on intracellular stores and specific ion channels such as ANO1 (Drumm et al. 2019; Sanders et al. 2024).

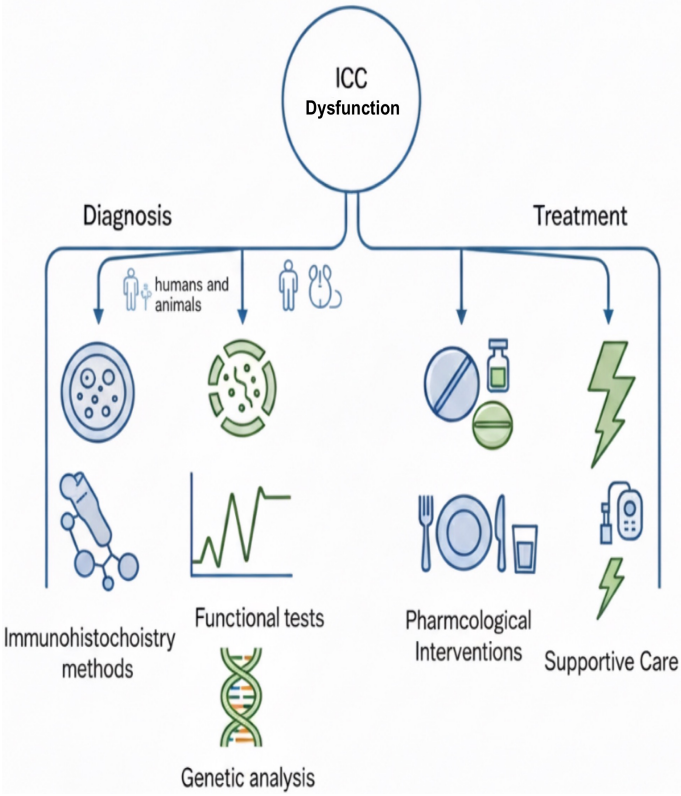

Fig. 2. *Diagnosis and management of ICCs related motility disorders. The scheme details the three main diagnostic modalities (Immunohistochemistry like c-Kit, Functional Testing such as Electrogastrography, and Genetic Analysis) and the two primary treatment modalities (Pharmacological interventions and Supportive care) used to manage ICC-associated motility disorders*

C. Molecular Analysis

The molecular or genetic studies also help explain ICC functioning and malfunctioning. It has been demonstrated that c-Kit mutant animals (W/Wv, Ws/Ws, etc.) reveal the causal connection of Kit signaling in ICC development, maintenance, and motor disorders (Sanders et al. 2002; Hwang et al. 2025). ICCs can be isolated and characterized via genetic labelling (e.g., Kit+/copGFP mice) and flow cytometry to perform transcriptomic and proteomic analyses (Ro et al. 2010). Cross-breeding of diseased models (e.g. diabetic mice) enables the possibility of investigating the ICC responses to pathophysiological insults. Finally, to achieve adequate diagnosis and mechanistic studies in gastrointestinal motility disorders, a combination and integration of immunohistochemical, functional, and molecular methods is essential as shown in Fig. 2.

4.3 Therapeutic Strategies

Treatment of gastrointestinal motility disorders related to ICC dysfunction involves a multi-modal approach using pharmacological and non-pharmacological interventions and supportive care along with emerging interventions as shown in Fig. 2.

A. Pharmacological Interventions

Medical management is based on kinetics of prokinetic agents. These medications increase the activity of remaining neural and muscular pathways in an attempt to adjust the loss of the ICC controlled pacemaker activity. However, there is currently no agent that directly reinstates ICC functional activity. A good example is prucalopride, a selective 5-HT4 agonist, which provides increased intestinal contractile activity, primarily in duodenum, cecum, and colon without major adverse effects in horses with hypomotility (Laus et al. 2017). Another 5-HT4 agonist promotes enteric neurotransmission with an improved peristalsis in young animal species with chronic idiopathic constipation (Chang et al. 2023). The clinical response differs considerably across species and underlying pathology, but requires further validation in veterinary use, as well as an individualized dosing regimen.

Besides traditional prokinetics, following newer agents aim at the fundamental processes of ICCs:

- *ANO1 Modulators*: ANO1 is the Ca^{2+}-activated chloride-channel fundamental to ICC pacemaker activity, and modulators of ANO1 are promising agents (Eact, prostratin, and tannic acid) for direct restoration or augmentation of residual ICC activity in motility disorders (Huizinga and Chen 2014).
- *Signaling Pathways*: They are influenced by the c-Kit signaling such as the PI3K/Akt and MAPK/ERK pathways are being explored as the targets of certain agents which play an important role in the maintenance and survival of the ICC (Choi et al. 2023).
- *Traditional Chinese Medicine (TCM)*: TCM products, such as the herbal formula Rikkunshito or compounds like ginsenosides, with multi-pathway regulation have been found in experimental models, regulating the autophagy-apoptosis process and promoting ICC homeostasis in alterations of GI motility (Zhang et al. 2025). This provides a promising direction of supporting pharmacological intervention.

Determining how ICC is regulated at a molecular level is very important. By unraveling the mechanisms involved in ICC damage and regeneration (c-Kit/SCF, ANO1, and PDGFRα signaling), researchers can work out accuracy-based methods to avoid the loss of said cells. These comprehensive plans constitute a translational pipeline to revive the functional ICC networks and long-term improvement in motility of both veterinary and pediatric patients (Friedmacher and Rolle 2023; Choi et al. 2023).

B. Supportive and Non-Pharmacological Measures

Supportive care consisting of nutritional support and fluid management is vital in acute and chronic motility disorders and may constitute the most defining frontier of management. These interventions ensure the maintenance of hydration, electrolyte, and energy levels, which are important in avoiding the occurrence of any life-threatening complications due to ileus, obstruction or excessive dysmotility (Fintl et al. 2010; Fintl et al. 2004). In severe congenital ICC disorders (e.g. Hirschsprung disease or intestinal neuronal dysplasia, hypoganglionosis), the pharmacological response is usually inadequate in pediatric patients (Friedmacher and Rolle 2023; Burns 2007; Jackman et al. 2024). In such instances, first-line therapy consists of supportive treatment, enteral or parenteral nutrition, to guarantee sufficient caloric uptake and fluid balance (Rolle et al. 2007; Hudson et al. 2001).

C. Neuromodulation Techniques

Neuromodulation, which concerns various electrical stimulation applications, is also being studied in the hope that it will be able to restore or enhance ICC-mediated pacemaker activity (Huizinga and Chen 2014; Choi et al. 2023). Functional studies indicate that electrical stimulation may serve to stimulate motility through the action upon ICC and enteric neural networks. This provides a potentially encouraging adjuvant treatment, particularly in resistant patients in whom conventional medicine and supportive care has failed (Choi et al. 2023). However, evidence of the robust clinical efficacy is still lacking, meaning that future studies have to focus on species-specific responses.

4.4 Regenerative therapy and Future Directions

The current review highlights the future and emerging regenerative therapeutic approaches in restoring ICC networks that hold much promise in terms of improving the outcomes in vulnerable animal populations, including horses with colic and patients with severe congenital motility disorders. Such options include treatment of such a damaged ICC network through repairing and replacing, or pharmacological assistance.

Stem Cell-Based Replacement Therapies

One of the leading forms of regenerative research is stem cell therapy, whose target is to repopulate lost gut ICCs (Yoshimaru et al. 2024). The exploration of the potential of various stem cell sources are actively underway and includes:

- Enteric nervous system progenitor cells
- Embryonic stem cells
- Induced pluripotent stem cells (iPSCs)
- Mesenchymal stem cells (MSCs)

These cells have shown the potential to regenerate ICCs and related neuromuscular components in injured portions of the gut and is currently under investigation in conditions such as Hirschsprung disease and chronic intestinal pseudo-obstruction (Yoshimaru et al. 2024; Zhou and OConnor 2017). Moreover, an improved technique in the field of animal organoid has allowed the development of functional

ICCs *in vitro* with a future potential to develop a cell-based replacement therapy targeting ICCs (Zhou and O'Connor 2017; Huizinga et al. 2021).

5. Limitations and Controversies

In spite of the fact that significant advances in understanding the biology of ICCs and their importance in GI motility have been made, several limitations and controversies remain unresolved.

5.1. Cause or Consequence

There has always been a lingering debate as to whether ICC loss is directly the primary factor leading to motility disturbances or the secondary effect of another underlying cause, such as inflammation, obstruction, or neuropathy. According to some studies, ICC depletion occurs before motility impairment, whereas others report depletion occurring during significant and prolonged disease progression (Kishi et al. 2020; Rybak et al. 2020), so it is hard to determine a cause and effect relationship.

5.2. Species-Specific Variability

Although ICC dysfunction was observed in all species, there are striking differences in the level of defects and related clinical effects (e.g., in horses, carnivores, and ruminants). This inconsistency serves to complicate translational research and questions what degree of validity can be achieved in characterizing animal versus human disease processes (Sander et al. 2014; Galiazzo 2020).

5.3. Diagnostic Challenges

ICC identification by immunohistochemistry, using c- Kit and ANO1 markers, is a gold standard. However, both these markers suffer from certain limitations. Mast cells also express c-Kit, which may lead to misinterpretation. Furthermore, a loss in marker expression may happen in ICCs without cell loss, generating false-negatives results. These limitations make predictability of ICC quantification in both experimental and clinical applications problematic (Hawes et al. 2009; Al-Ahmadi et al. 2023).

5.4. Functional and Structural Loss

Another controversy is whether functional impairment (loss of pacemaker activity despite preserved ICC density) is equally important clinically as structural depletion. Indicatively, acute inflammations can impair ICC activity without causing cell loss, and still, manifest with severe dysmotility. Such distinction is not perfectly well-resolved in existing literature (Huizinga et al. 2009; Li et al. 2019). Accordingly, the question of whether ICC loss is a primary or secondary effect is still a significant gap in the way of targeted therapies.

5.5. Heterogeneity of ICC Populations

The three ICC subtypes (ICC-MY, ICC-IM and ICC-DMP) exhibit different physiological functions, whereas the majority of studies lack differentiation between the subtypes in clinical tests. Such simplification obscures a subtype-specific susceptibility that can lead to focused treatment (Drumm et al. 2019; Sanders et al. 2024).

5.6. Therapeutic Uncertainty

Although emerging therapeutic approaches, such as regenerative therapies and ion channel modulators (e.g., ANO1-targeted drugs) are intriguing, their clinical implementation remains limited (Friedmacher and Rolle 2023; Choi et al. 2023). Long-term safety, efficacy, and whether true SIP syncytial functionality can fully be restored have been the subjects of controversies about these drugs. In addition, neuromodulation is an interventional procedure that produces different results across species, which further complicates validation and standardization (Choi et al. 2023).

6. Conclusions

Interstitial Cells of Cajal (ICCs) are essential as modulators of gastrointestinal motility. Their malfunction, which is either quantitative depletion or impaired functioning, is always linked to devastating motility disorders in both humans and animal species, with equine colic as a typical case of such disorders. The evaluative precision is improved with diagnostic advancements like ANO1 immunohistochemistry and therapeutic potential with approaches such stem cell-mediated regeneration and ANO1/c-Kit targeted inhibition. ICCs manipulation is a significant translational field. The further development of the field requires more accurate and integrative methods of research implementation to be able to apply the basic findings to clinical practice in both human and veterinary medicine.

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