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Short communication

Histomorphological changes in lumbar sympathetic chain ganglia of the female pig during prenatal development

A. Franke-Radowiecka[1], A. Bossowska[2]

[1] Department of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 13, 10-719 Olsztyn, Poland
[2] Department of Human Physiology and Pathophysiology, School of Medicine, University of Warmia and Mazury in Olsztyn, Warszawska 30, 10-082, Olsztyn, Poland

Correspondence to: A. Franke-Radowiecka e-mail: ameliaf@uwm.edu.pl

Abstract

This study presents, for the first time, a histomorphological analysis of the lumbar sympathetic chain ganglia (L SChG) during prenatal development in 5-, 7-, and 10-week-old female porcine foetuses. Single immunohistochemical staining for protein gene product 9.5 (PGP) as a neural marker has shown that in 5-week-old foetuses, the ganglia appeared as paired, structures adjacent to the dorsolateral aspect of the descending aorta, measuring approximately 170-190 μm in diameter, composed of small oval neurons (6-8 μm) with large nuclei and scant cytoplasm. They formed elongated, oval structures, like a segmented column, with slight variations in shape and size. By seven weeks, the ganglia had grown to 270-200 μm, becoming broader than tall, while the neurons enlarged to 8-11 μm and took on a more rounded shape. At 10 weeks, L SChG showed significant growth and morphological diversity (up to 530-630 μm), with neurons varying in shape (oval, round, triangular) and size (12-16 μm). The progressive enlargement and neuronal differentiation of L SChG suggest functional maturation relevant to autonomic innervation of the abdominal and pelvic viscera. The findings may aid early diagnosis of neurodevelopmental disorders, optimize prenatal care, and support broader veterinary insights into autonomic development across mammals.

Keywords: sympathetic chain ganglia, prenatal development, pig

Introduction

The pig (*Sus scrofa domesticus*) is an important animal for both animal husbandry and biomedical research (Pejsak et al. 2023). Proper prenatal development is crucial for healthy offspring and sustainable breeding. However, the ontogenesis of the peripheral nervous system (PNS), which controls visceral, vascular, secretory, and thermoregulatory functions, remains understudied. Understanding its development is key to elucidating autonomic regulation in health and disease. While embryonic SChG development is well-studied up to 10 weeks post-fertilisation in humans (Kruepunga et al. 2021), later prenatal stages remain poorly understood in both humans and animals (Patten 1948, Young et al. 2011, Kruepunga et al. 2021). This study, therefore, aimed to analyse the histomorphological development of the lumbar segment of the SChG in 5-, 7-, and 10-week-old foetuses.

Materials and Methods

The porcine foetuses were obtained from a slaughterhouse. According to Polish law and EU Directive No. 2010/63/EU, the experiments performed in the present study did not require the approval of the Ethics Committee. In this research, 5-week-old (n=5; 3-4 cm in length), 7-week-old (n=5; 7-8 cm in length), and 10-week-old (n=5; 13-14 cm in length) female foetuses were used. The age of the foetuses was marked according to the crown-rump length (CRL) method. CRL sets the distance from the top of the head of the embryo or foetus to the lower limit of the buttocks (Evans and Sack 1973). Sex identification was determined using the PCR technique (Franke-Radowiecka et al. 2019). The tissue fixation, single immunohistochemical staining procedures, and controls applied in this study were precisely described in previous articles (Franke-Radowiecka et al. 2019, Dudek et al. 2024). The lumbar region of the foetus' back, including the SChG (in 5- and 7-week-old foetuses) or only the lumbar SChG (in 10-week-old foetuses), was dissected and cut into 10 µm-thick cryostat sections (cross section – L SChG from 3 foetuses, longitudinal – L SChG from 2 foetuses). Sections were labelled using a primary antibody against PGP (mouse, monoclonal, dilution 1:400; Biorad, catalogue no. 7863-2004) and a secondary antibody (goat anti-mouse IgG conjugated with AlexaFluor 488, dilution 1:1000; Invitrogen, catalogue no. A-11001). The omission of primary antisera, and preincubation of the primary antisera with an excess amount of appropriate antigen (PGP 14-855, Merck; 10-50 µg of synthetic substance per 1 ml of diluted antiserum) were used to investigate the specificity of immunohistochemical labelling. No fluorescence was observed in any of these control stainings, which confirmed the specificity of the staining. The slides were evaluated by two independent investigators, each analysing ten sections collected at comparable intervals along the entire lumbar segment in every animal examined. Microscopical analyses were conducted on the whole slide. The relative frequency of PGP-positive nerve fibres (Table 1) was evaluated in the field of view and was defined with quantifiable criteria: many fibres +++(>15), few ++ (5-15), single fibres + (<5). For preliminary verification of staining quality, a Zeiss Axiophot fluorescence microscope equipped with epifluorescence and an appropriate Alexa Fluor 488 filter was used. Subsequently, the sections were analysed (AlexaFluor 488 filter) and recorded using a Zeiss LSM 700 confocal laser scanning microscope (Zeiss, Jena, Germany) and ZEN Software 2009. Measurements of neuronal cell bodies and ganglia were also performed using the measurement tools available in this software. The figure set was prepared with CorelDRAW X7 graphical software, version 17.6.0.1021 (Ottawa, ON, Canada).

Results and Discussion

In 5-week-old foetuses, the L SChG was located beneath the lumbar vertebral bodies. They formed a paired structure arranged dorsolaterally to the descending aorta. On the cross-section, they were almost circular, and the dimensions of ganglia were about 140-190 µm in height and 130-170 µm in width (Fig. 1). When observed in a longitudinal section, they formed elongated, oval structures that resembled "a column cut into several pieces" but differ slightly in shape and size (Fig. 1). The internal structure of the ganglia appears to be loose. L SChG PGP-positive neurons were mainly oval with a small amount of cytoplasm (Fig. 2A-B) and a large nucleus. The size of these forming nerve cells was 6-11µm, but most of them were 6-8µm. In 7-week-old foetuses, the L SChG appeared more rounded than oval. In cross-section, the ganglia measured approximately 250–270 µm in width and 180–200 µm in height (Fig. 1). Overall, they were broader than they were tall compared to the previous developmental stage. Longitudinal sections revealed greater variation in the length, width, and shape of the ganglia than was observed at the earlier stage (Fig. 1). L SChG neurons were mainly oval, still with a small amount of cytoplasm and a large nucleus (Fig. 2C-D). The size of PGP-positive neurons ranged from 7 to 14 µm, but most of them were 8-11 µm in size. At this developmental stage, few PGP-positive nerve fibres were observed (Fig. 2D). In 10-week-old foetuses,

Table 1. A summary of the results obtained.

		5-week-old	7-week-old	10-week-old
Maximum size of ganglia in µm, cross-section	height	140-190	180-200	230-530
	width	130-170	250-270	450-630
Shape of ganglia	in cross-section	rounded	more rounded than oval	oval
	in longitudinal section	"a column cut into several pieces"	a greater variation in the length, width, and shape	highly differentiated in size and shape
Size of PGP-positive neurons in µm	min-max	6-11	7-14	8-19
	most frequently observed	6-8	8-11	12-16
Shape of neurons		oval	oval	mainly oval, sometimes round, single triangular
Relative frequency of PGP-positive nerve fibres		single +	few ++	many fibres +++

Fig. 1. A schematic view of the developing porcine female L SChG at the examined prenatal developmental stages.

L SChG were mainly oval. On the cross-section, the dimensions of the ganglia were about 450-630 µm by 230-530 µm. Observation of the longitudinal section revealed that ganglia were highly differentiated in size, shape, and number compared to those in the previous stages of development (Fig. 1, 2E). L SChG PGP-positive neurons were mainly oval, sometimes round or triangular, with a large nucleus located in the cell centre (Fig. 2E-F). The size of these nerve cells ranged from 8 to 19 µm, but most of them were 12-16 µm in size. PGP-positive nerve fibres were observed more frequently compared to the previous developmental stage (Fig. 2F).

Previous research on pig foetuses mainly examined the number of ganglia in the post-diaphragmatic SChG (Pospieszny and Brużewicz 1998) between weeks 6 and 15 of prenatal development. It was found that ganglia fuse, and their number decreases, with differences in the number of ganglia between the sides of the body at various periods studied. However, their number remains much higher than the vertebral count in lumbar, sacral, and coccygeal regions to week 15 of prenatal development. Our study, for the first time, has revealed dynamic structural changes in size, shape, and neuronal differentiation in L SChG of porcine foetuses between weeks 5 and 10 of prenatal development. The ganglia enlarged between weeks 5 and 7 and nearly doubled in size between weeks 7 and 10. Variation in neuronal size in each studied period suggests ongoing proliferation, apoptosis, and neuronal maturation. The increasing number and elongation of nerve fibres may indicate the formation of interneuronal connections within the ganglion. Based on the combined findings of previous and current studies, it can be concluded that intense fusion, elongation, and internal structural changes in the sympathetic trunk ganglia are already observed by the 5th week of development and continue throughout the prenatal period, or possibly even beyond. These processes occur concurrently with the progressive and intense development of the organs, along with the

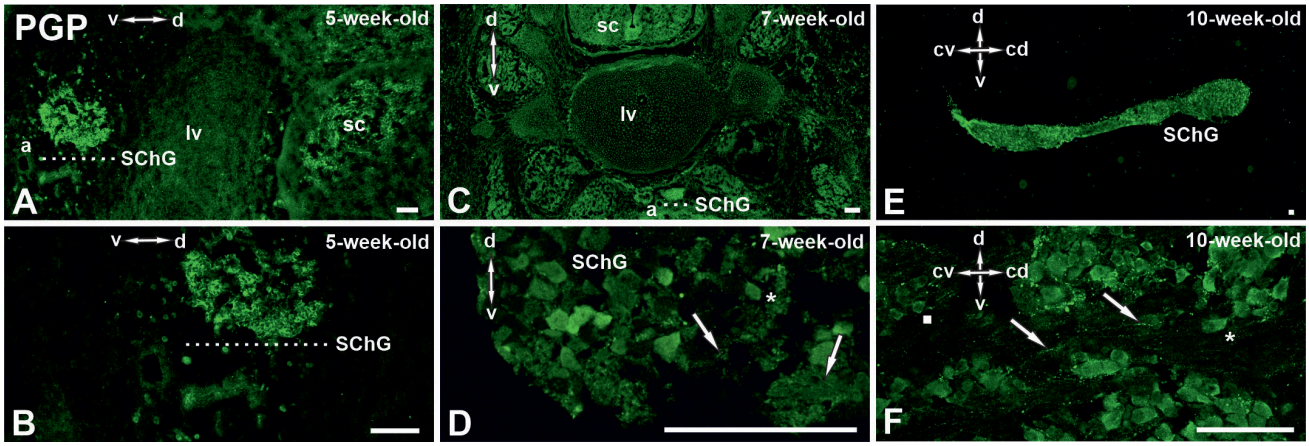

Fig. 2. A cross-section (A, dorsal-ventral orientation; C, dorsal-ventral orientation) and a longitudinal section (E, cranial-caudal orientation) of the L-SChG in general view. In 5- (B, cross-section) and 7-week-old (D, cross-section) porcine foetuses, neurons were mainly oval with scant cytoplasm and a large nucleus. In 10-week-old foetuses (F, cross-section), PGP-positive neurons were oval, sometimes round or triangular, with a centrally located large nucleus. Few PGP-positive nerve fibres (arrows) were observed in 7-week-old fetuses (D), while they were more frequent in 10-week-old foetuses (F). d-v - dorsal-ventral orientation, cv-cd cranial-caudal orientation (the arrows next to d and v, as well as cv-cd indicate the orientation of the sections), the dashed line marks the SChG, sc – spinal cord, lv – lumbar vertebra, a – aorta. Scale bar = 50 µm.

maturation and structural remodeling of their innervating neural components, as observed in the development of reproductive system innervation in porcine foetuses (Franke-Radowiecka et. al. 2019, Franke-Radowiecka 2020, Franke-Radowiecka et al. 2024). The studies indicate that dynamic changes in innervation begin around the 7th week of prenatal development, marked by the appearance of nerve fibres in the mesenchyme of the uterovaginal canal and the formation of a peripheral cell cluster, suggesting the development of the paracervical ganglion. Between weeks 7-10 and again 10-12, a significant increase in innervation density and structural changes in the organs are observed. These developments correlate with the maturation of the sympathetic chain ganglia, including the lumbar region, which also provides neuronal supply to the urogenital system. These findings enhance understanding of sympathetic nervous system development and offer a reference for assessing autonomic maturation in porcine foetuses. However, research conducted on such a unique material as foetal specimens involves certain constraints. The limited number of animals precludes extensive, multifactorial analyses and the parallel application of additional approaches, such as ultrastructural or functional studies, which would likely broaden the scope of the results. Nonetheless, each new piece of evidence may contribute to advancing early diagnosis of neurodevelopmental disorders, enhancing prenatal care and breeding practices, and further reinforcing the value of the pig as an important model in translational neurobiology and regenerative medicine.

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