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RESEARCH ARTICLE

CONTRIBUTION OF THE INTERCOSTAL NERVES TO THE DIAPHRAGMATIC INNERVATION IN MIXED-BREED SHEEP (OVIS ARIES LINNAEUS, 1758): AN ANATOMICAL STUDY IN FETUSES

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Abstract

The costal grooves house vascular and nervous structures known as intercostal nerves, which are responsible for innervating the intercostal muscles and, occasionally, supplying syntopic structures such as the diaphragm. This study aimed to verify the existence of collateral diaphragmatic innervation via the intercostal nerves in fetal sheep (Ovis aries Linnaeus,1758),of both sexes and at different stages of developme nt. Thirty specimens, previously fixed in 10% aqueous formaldehyde solution for over 72 hours, were analyzed. After thoracotomy through the costochondral joints, the intercostal nerves were dissected along their entire course, preserving their branches, especially those directed to the diaphragm. The findings revealed that, on the right antimere, the 6th intercostal nerve contributed in 11.11% of cases, the 7th to 12th pairs in 100% of cases, and the 13th in 66.66%. On the left antimere, the 5th intercostal nerve was present in 11.11% of cases, the 7th to 12th pairs in 100%, and the 13th in 66.66%. The results demonstrate the recurrent participation of the intercostal nerves—particularly the 7th to 12th pairs—in the collateral innervation of the diaphragm, with significant anatomical variation. These findings provide relevant information for understanding the respiratory physiology of sheep and for planning thoracic surgical procedures in this species.

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Introduction:-

The diaphragm is the primary muscle involved in inspiration, functioning as a broad, cranially arched musculotendinous structure. It serves as an anatomical septum between the thoracic and abdominal cavities, being covered by the pleura on its thoracic surface and by the peritoneum on its abdominal surface. Structurally, it consists of a central tendinous core from which peripheral muscle fibers extend, organized into three portions: lumbar, costal,

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and sternal [1].The phrenic nerves constitute the main motor pathway of the diaphragm in several animal species. In humans, in addition to the predominance of the phrenic nerve, contributions from the last six intercostal nerves have been reported, particularly in the juxtacostal region of the diaphragm [9,10,11].Experimental studies conducted in dogs, rabbits, and primates demonstrated that, after sectioning of the phrenic nerve and subsequent electrical stimulation, the diaphragm also receives nerve impulses from the fourth to ninth pairs of intercostal nerves, with the eighth pair showing the greatest contribution [9,10]. The intercostal fibers involved in this process are described as being associated with reflex mechanisms, without directly contributing to spontaneous respiratory activity. Thus, the motor innervation of the diaphragm comprises fibers originating from both the cervical (C4–C7, via the phrenic nerves) and thoracic (T5–T13, via the intercostal nerves) vertebrae [3].

In horses, the second to sixth intercostal nerves emerge from the intercartilaginous spaces, sending muscular branches to the transverse thoracic muscle, while the second to eighth pairs contribute branches to the costal portion of the diaphragm. These nerves are also primarily responsible for innervating the internal and external intercostal muscles. In dogs, studies have shown that in 93.54% of cases the diaphragm is innervated by the eighth to twelfth intercostal nerves, with the seventh intercostal nerve contributing in only 3.22% of cases [1]. In fetal zebu cattle, the intercostal innervation of the diaphragm varied: 50% of cases showed contributions from the seventh to twelfth intercostal nerves; 33.3% from the sixth to twelfth; 13.3% exclusively from the sixth intercostal nerve in the left antimere; and 3.3% from the seventh intercostal nerve in the right antimere [12].Taken together, these findings indicate that, in addition to their established role in innervating the intercostal muscles, the intercostal nerves also play a relevant role in diaphragmatic innervation [9,10]. In this context, the present study aimed to describe the intercostal innervation of the diaphragm in mixed-breed sheep fetuses (Ovis aries Linnaeus, 1758), providing anatomical support applicable to clinical and thoracic surgical practices in this species [5,6,7,8].

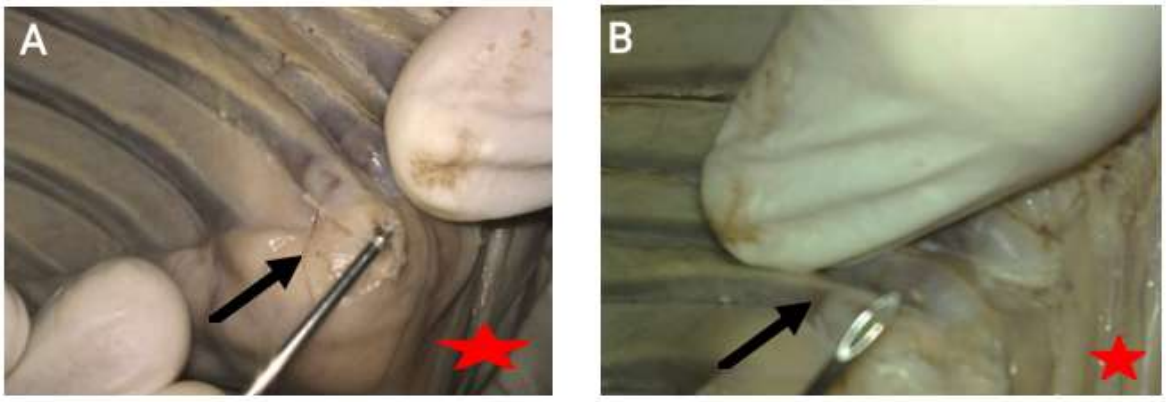
Methodology:-

Thirty mixed-breed sheep fetuses (Ovis aries Linnaeus, 1758), consisting of 15 males and 15 females at different gestational stages, were obtained from the Petrolina Municipal Slaughterhouse, Pernambuco [8].After collection, the specimens were transported to the Laboratory of Anatomy of Domestic and Wild Animals at the Federal University of the São Francisco Valley (UNIVASF), where anatomical fixation was performed. For this procedure, the umbilical vein was cannulated and perfused with 10% aqueous formaldehyde solution. Subsequently, the fetuses were completely immersed in the same solution and kept submerged for at least 72 hours to ensure adequate tissue preservation [7].Following fixation, the skin was removed from the ventrolateral regions of the thorax. The thoracic cavity was opened at the level of the costochondral joints, beginning at the third rib of both antimeres, allowing the sternum to be retracted. On the intrathoracic surface, the intercostal spaces starting at the fifth rib were dissected to identify the presence of intercostal nerves leading to the diaphragm. The intercostal nerves were then carefully dissected along their entire course, preserving their main branches.All structures were photographically documented, and the data obtained were tabulated for comparative analysis [8].

Results and Discussion:-

The analysis of diaphragmatic innervation in mixed-breed sheep fetuses revealed significant participation of the intercostal nerves in both antimeres. Figure 1 illustrates the emission of intercostal branches to the diaphragm, emphasizing the anatomical relationship between these structures.

Figure 1 – In A and B, photographs of the internal aspect of the rib cage of fetal sheep are shown, in which one of the intercostal nerves (arrow) sends branches to the diaphragm (red star) – Brazil, Pernambuco, Petrolina, 2025.



Source: Own collection.

The distribution of intercostal innervation of the diaphragm is summarized in Table 1. On the right antimere, the 6th intercostal nerve contributed to diaphragmatic innervation in 11.11% of specimens. The 7th to 12th pairs of intercostal nerves were consistently present in 100% of cases, demonstrating a uniform pattern of participation. The 13th intercostal nerve was involved in 66.66% of individuals.On the left antimere, the 5th intercostal nerve supplied branches to the diaphragm in 11.11% of specimens. As on the right side, the 7th to 12th pairs of nerves were observed in 100% of the cases. The left 13th intercostal nerve also contributed in 66.66% of specimens, mirroring the frequency recorded on the contralateral side [12].

Table 1 – Frequency of diaphragm innervation by intercostal nerves in fetal sheep (n = 30) – Brazil, Pernambuco, Petrolina, 2025.

Intercostal Nerve	Right antimere (%)	Left antimere (%)
5º	–	11,11
6º	11.11	–
7º	100.00	100.00
8º	100.00	100.00
9º	100.00	100.00
10º	100.00	100.00
11º	100.00	100.00
12º	100.00	100.00
13º	66.66	66.66

Source: Author's own

The results obtained in this study demonstrate the recurrent involvement of the intercostal nerves—particularly the 7th to 12th thoracic pairs—in the collateral innervation of the diaphragm in fetal mixed-breed sheep [8]. This reinforces the hypothesis that, although the phrenic nerve is recognized as the primary motor pathway of the diaphragm, the intercostal nerves play a relevant complementary role, especially in the costal portions, as previously reported in other domestic species.In domestic cats (Felis catus), Faria et al. demonstrated that the intercostal nerves also contribute to diaphragmatic innervation, particularly the 6th to 13th pairs, corroborating the present findings regarding anatomical variability and the extent of intercostal participation. In zebu cattle, Scheffer et al. identified a similar pattern, with contributions from the 6th to 12th pairs, suggesting a shared neuroanatomical organization among ruminants. In dogs, the 7th to 12th intercostal nerves contribute to diaphragmatic innervation in over 90% of cases, reinforcing the functional relevance of these structures across domestic species [2,12].

Studies in wild species such as collared peccaries (Tayassu tajacu) and crab-eating foxes (Cerdocyon thous) have also revealed variations in the origin and distribution of the phrenic nerve and its connections with intercostal nerves, expanding knowledge of the anatomical plasticity involved in diaphragmatic innervation.The recurrent presence of the 7th to 12th intercostal nerves in all fetuses analyzed suggests a stable anatomical pattern between antimeres, while the 13th intercostal nerve, present in approximately two-thirds of cases, indicates individual variability. The occasional contribution of the 5th and 6th pairs reinforces the hypothesis that intercostal innervation of the diaphragm may present specific morphological nuances, even within the same species [1]. Therefore, the present findings corroborate anatomical trends reported in other domestic species, highlighting the functional importance of intercostal nerves as secondary sources of diaphragmatic innervation [8,13].

Conclusion:-

This study demonstrated that intercostal nerves play a significant role in innervating the diaphragm in fetal mixed-breed sheep, with particular emphasis on the 7th to 12th thoracic pairs, consistently observed in 100% of specimens. The participation of the 13th intercostal nerve in two-thirds of cases and the sporadic contribution of the 5th and 6th pairs indicate notable anatomical variations that should be considered in clinical and surgical thoracic approaches in this species.These findings corroborate previous reports in other domestic species, such as dogs and cattle, reinforcing the existence of a shared anatomical pattern in diaphragmatic innervation by intercostal nerves. Precise identification of these nerve pathways enhances applied anatomical knowledge, especially in procedures involving rib cage manipulation, thoracic interventions, and regional anesthesia.Thus, the data presented here provide valuable insights into comparative anatomy and veterinary practice. Moreover, integrating these findings with comparative

literature advances the understanding of mammalian functional anatomy and opens perspectives for future studies on neuromuscular development and morphological variations in diaphragmatic innervation in sheep and other species.

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