

Anatomical study of pericardioperitoneal canal in immature beluga (*Huso huso*) with ultrasonography

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Abstract

Background: One of the special anatomical structures in sturgeons is the pericardioperitoneal canal (PPC), which has not been studied much. Considering that the presence and absence of this channel and its morphometry have not been investigated in this type of fish, this study was conducted.

Objective: The purpose of present study was to obtain a complete understanding about anatomical features and ultrasonography of PPC in the heart of immature beluga species in order to provide standard approaches for performing sonography and echocardiography on this sturgeon species.

Methods: Ten immature belugas (*Huso huso*) were used to perform ultrasonography with Sonosite MicroMaxx ultrasonography machine of ventral approach between two pectoral fins. After performing the steps of ultrasonographic study, gross anatomical studies were also performed, and the morphometric measurement of the canal was also performed.

Results: A small PPC was observed, which communicated between the pericardial cavity, and the peritoneal (coelomic) cavity. The cranial part of this channel, which was located immediately after the transverse septum, was on the midline of the body and was seen in midsagittal ultrasonograms. On average, the length of this canal was 3.23 ± 0.05 cm, and its diameter (cranial part) was 0.24 ± 0.04 cm.

Conclusions: In the present study on immature beluga, it was observed that the canal is located behind the sinus venosus and is caudoventral to it, though behind the transverse septum. It has been mentioned that this PPC establishes a connection between the pericardial cavity and the peritoneal cavity, so this connection is between the peritoneal cavity and the space between the two layers of the pericardium and has no connection with the pericardial sac (the space where the heart is located). In this study, the anatomical structure and morphometry of the PPC in immature beluga were investigated, and some approaches were presented to observe the canal in ultrasonography.

KEYWORDS

anatomy, heart, immature beluga, pericardioperitoneal canal (PPC), ultrasonography

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1 | INTRODUCTION

Due to the ever-increasing growth of the human population and the increase in the demand for food consumption, the reserves and natural resources of the planet are rapidly decreasing, and at the same time, the gradual use and encirclement of nature by humans has caused the destruction and extinction of its resources, from the air pure in the skies to fish in the depths of the oceans.

Sturgeons are one of the rarest and oldest species of sea creatures, in which many efforts have been made to preserve their species for years. But it is clear that the ever-increasing demand of humans to consume meat and caviar is much more than the natural capacity of these fish. On the other hand, diagnostic ultrasound has become a common and widely used method in medical and veterinary sciences today. This tool is efficient, low-cost, non-invasive and easily accessible.

In the new classification of fishes, the Acipenseridae family is in the class Osteichthyes, subclass Actinopterygii, class Acipenseriformes and family Acipenseridae-Bonaparte. The subfamily Acipenseridae includes two very important genera that include most of today's valuable sea turtles: the genus *Huso* Brandt and *Acipenser*, Linnaeus. One of the characteristics of the *Huso* Brandt genus is its large mouth and its crescent shape. The whiskers are compressed on both sides. Branchial membranes are connected and form the free fold. As the fish ages, the snout becomes relatively sharp and short. There are two very famous species in this genus: the Beluga fish species or *Huso huso* and the Kaluga species or *Huso dauricus* (Hastings et al., 2014). Studies on the anatomy of sturgeon are also important from an evolutionary point of view, because these fish are Chondrostei (bony cartilaginous or primary bony fish), and in terms of evolution, they are placed between bony fish and cartilaginous fish (Zehtabvar et al., 2019).

Beluga fish is one of the most valuable fish in fisheries, whose caviar has always been known as the best, largest and most expensive type of caviar in the world. The mouth of this fish is large and semi-crescent, and the first bony projection of the back row is the smallest. The bony ridges of the back row are oval shaped and consist of comb teeth that are covered by a soft shell in mature fish. The bony ridges in this fish are worn and flattened with age (Vajhi et al., 2019).

The heart includes a ventricle, a sinus venosus, a part of the atrium and the pericardial wall, and behind them is the liver. In terms of echo, the liver has a uniform texture and is hypoechoic compared to the posterior part of the pericardium, which is located in front of the liver. The ventricle is in the lower position, and the atrium and sinus venosus are in the upper position. Moreover, the sinus venosus is behind the atrium. Behind the heart, there are two vessels in the vicinity of the liver, which, after traveling a distance, connect to each other and form a common vessel structure and end in the heart. The right vein is bigger than the left vein. These two vessels are known as anecho in the uniform structure of the liver (Zehtabvar et al., 2019).

Icardo et al. (2009, 2002) have conducted studies on the anatomy and development of the heart in *Acipenser naccarii* and pointed out that the atrium is adjacent to the conus arteriosus from above. Therefore, the adjacent points of these two structures can be seen in transversal ultrasound. When the probe moves backward, a part of the ventricle

that is inclined to the left side is added to this collection, and when the probe moves forward, the atrium gradually disappears.

Ultrasound devices are used to image human and animal bodies. Devices used to image animals are no different from medical devices, and only sometimes do they have probes or software specific to animals more than medical devices (Thrall, 2018). Ultrasound, as a valuable technique, has many applications in aquatic animals, including fish and marine mammals. This technique is biologically safe. Perhaps the shark is one of the best subjects for conducting ultrasound experiments, because it has no mineralized bones and no gas-filled swim bladder that interferes with ultrasound (Walsh et al., 1993). Moreover, ultrasound technique is one of the best methods for anatomical studies. In general, it is mentioned that diagnostic imaging techniques are one of the best methods for performing anatomical studies. Especially as there is no need to kill the animal in the anatomical study using these techniques, the animal remains alive after the study. Therefore, it is one of the best ways to study endangered and noble animals (Zehtabvar et al., 2023).

Fish do not need to prepare their skin for ultrasound because they do not have hair on their body surface. Ultrasound in water is simple, because water is a carrier of sound waves and the attenuation of sound while passing through water is very low, so no other medium such as ultrasound gel is needed. Performing ultrasound while the transducer is placed a few centimetres away from the body surface is better than sticking it to the body surface, because the animal is calmer in this state. In some cases, it may be necessary to remove the fish from the water for ultrasound. In these cases, the fish is periodically taken out of the water and placed on the ultrasound table, but in this case, the period of rest and relaxation of the fish is short, and the ultrasound will not be easy in such a situation. It is useful to use sedatives and anaesthetics in the container containing the fish, a few minutes before the ultrasound, especially when the ultrasound is done outside the water. In ultrasound outside of water, the mucous moisture of the body surface acts as a sound interface, and in rare cases, using gel or pouring water on the fish may be useful. It is important to be familiar with the anatomy of the examined organs and to systematically perform ultrasound based on a specific and defined method. In order to prepare ultrasound images, it is recommended to give the fish abstinence from food. The quality of the images is due to the reduction of food in the intestines. Some applications of ultrasound in fish include determining the gender of fish, examining the evolution of reproductive organs, examining the quality of body tissues, diagnosis of diseases of different organs, and anatomical research (Vajhi et al., 2019).

Iwashita et al. (2018) mentioned that in rat foetuses, the sign of pleuroperitoneal folds projection was noted in the sidewall of the pericardioperitoneal canal (PPC) at embryonic Day 12, and they have pointed out the existence of the PPC in the developmental stages of the rat embryo.

Pericardioperitoneal shunt (PPS) is also used for the treatment of some diseases in the form of surgery in humans. Pericardial effusion is a common finding in clinical practice, whether incidental or as a manifestation of a systemic or cardiac disease. Various surgical approaches for pericardial window creation for drainage have been proposed, but these radical procedures remain controversial. In Masaru et al.

study, they report the case of a 74-year-old man treated for refractory non-malignant pericardial effusion using a PPS. After the failure of conventional pericardiocentesis, a PPS using a Denver shunt was inserted to drain the pericardial effusion into the peritoneal cavity. At 3-year follow-up, the effusion was well controlled and the shunt remained patent (Masaru et al., 2022).

The pericardial and peritoneal spaces of elasmobranch fishes are connected by the PPC, which allows pericardial fluid to escape when pressures exceed 0.1–0.3 kPa (Abel et al., 1994). Sturgeons also have this channel, a one-way conduit into the peritoneum (Gregory et al., 2004). Considering that the presence and absence of this channel and its morphometry have not been investigated in *H. huso*, this study was conducted. The purpose of present study was to obtain a complete understanding about anatomical features and ultrasonography of PPC in the heart of immature beluga (*H. huso*) species in order to provide standard approaches for performing sonography and echocardiography on this sturgeon species. Of course, it is mentioned that this channel also exists in hagfish (Gregory et al., 2004).

So far, ultrasound has been used in sturgeon fish for different purposes such as anatomical studies (Zehtabvar et al., 2017, 2019) or sex detection or sexual maturity stage by different researchers (Masoudifard et al., 2011). In this study, ultrasound technique was used to examine the anatomy of the PPC.

2 | MATERIALS AND METHODS

2.1 | Individuals

Ten immature belugas (*H. huso*) with an average weight of 3.5 ± 0.54 kg and an approximate age of 2.5 years were purchased from a private fish breeding centre (for food, meat and caviar) located in Sari, Iran and transported to the Faculty of Veterinary Medicine of University of Tehran using plastic barrels. It should be noted that before purchase, the species were examined through ultrasound based on the standard method presented in the articles, and all the samples were selected from males (Masoudifard et al., 2011; Moghim et al., 2001). After being transferred to the faculty of veterinary medicine, the samples were kept in special plastic cylindrical containers in the aquarium for 24 h to return to their normal physiological conditions.

2.2 | Ultrasonographic study

A Sonosite MicroMaxx ultrasonography machine and a linear probe with a frequency of 6–12 MHz were used. In a rectangular plastic tub filled with water, each specimen was held by hand with its ventral surface facing up. As the topographical area of the heart in sturgeon is located between the pectoral fin (Zehtabvar et al., 2017, 2019), the probe was placed in this area (ventral approach) to perform ultrasound. In the longitudinal mode, the probe was moved by moving to the left and right for more detailed investigations. To examine the anterior and posterior parts more, the probe was moved anteriorly and posteri-

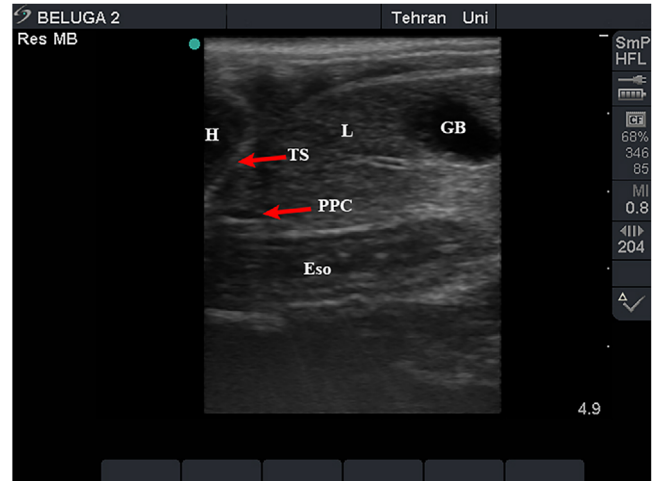


FIGURE 1 Midsagittal ultrasonogram of the immature beluga. Eso: oesophagus; GB, gall bladder; H, heart (ventricle); L, liver; PPC, pericardioperitoneal canal; TS, transvers septum (pericardium).

orly in both longitudinal and transverse modes. In the present study, a hypothetical triangle was considered to explain the placement of the probe for ultrasound; so that this hypothetical triangle was created on the ventral surface between the pectoral fins and two hard cartilaginous structures, which indicates the topographical position of the pericardial cavity.

2.3 | Gross anatomical and morphometric study

All 10 fishes of the previous stage were studied in this stage. Cuts were made in the hypothetical triangle of the pericardial cavity, and then its caudal part, which is the location of the liver, was cut. At this stage, after morphological study of the structures and determining the topography of the channel, the length and diameter of the channel were measured using a digital caliper. The diameter of the canal was measured in three locations: cranial, middle and caudal. Parameters of the PPC were compared by running a paired sample *t* test in SPSS software version 16 ($p > 0.05$).

3 | RESULTS

3.1 | Ultrasonographic study

A small PPC was observed, which communicated between the pericardial cavity and the peritoneal (coelomic) cavity. The cranial part of this channel, which was located immediately after the transverse septum, was on the midline of the body and was seen in midsagittal ultrasonograms. The middle and caudal parts were tilted to the right and were not seen in midsagittal ultrasonograms (Figure 1). The channel was located in the vicinity of the ventral surface of the oesophagus and was adjacent to the liver from the ventral side (Figure 1). The channel had a larger diameter than the vessels observed inside the liver, and its wall

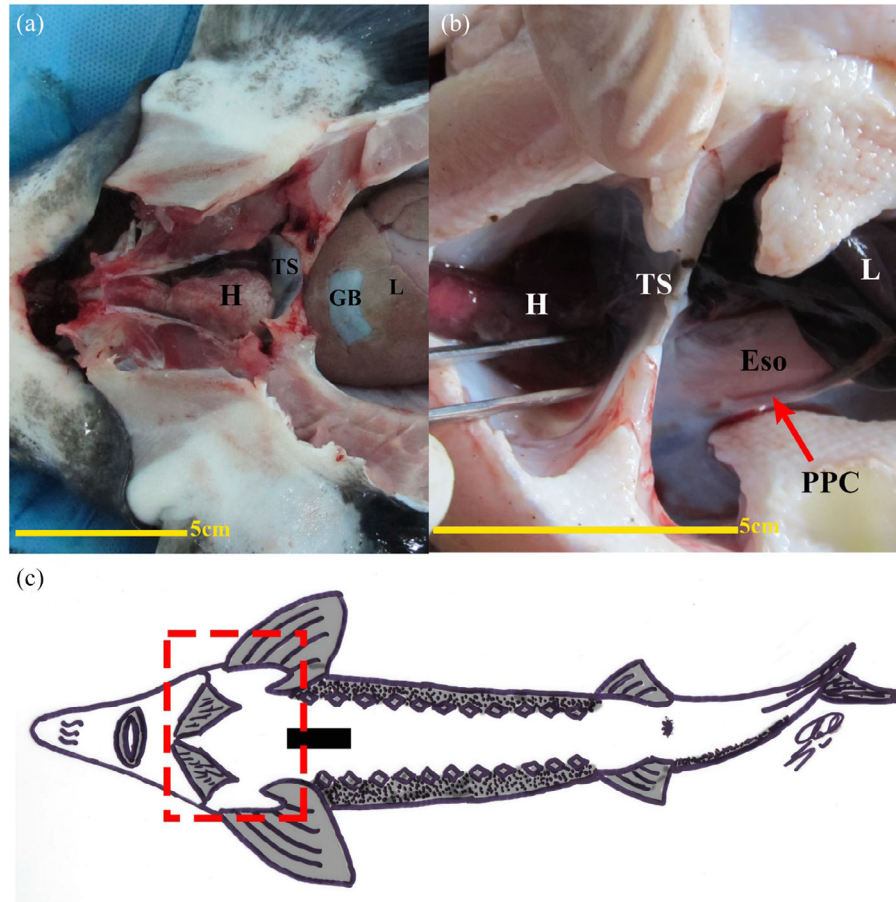


FIGURE 2 Ventral view of the immature beluga (a and b). In image B, the liver and gallbladder are moved aside to show pericardioperitoneal canal (PPC), ventral schematic figure of the immature beluga (c): The range of image A is shown by the red rectangle, and the black rectangle shows the placement of the ultrasound probe in Figure 1. Eso, oesophagus; GB, gall bladder; H, heart (ventricle); L, liver; TS, transvers septum (pericardium).

thickness was greater than the vessels of the liver (Figure 1). The canal was placed caudoventral to the sinus venosus.

3.2 | Gross anatomical and morphometric study

The pericardium was almost separated from the peritoneal cavity and was in a cranial and ventral position. Different parts of the heart were located in the triangular pericardial cavity. The apex of this triangle was on the cranial side, and its base was on the caudal side. In the cranial part (base of hypothetical triangle), the pericardium was thicker than other parts and created a transverse septum that separated the pericardial cavity from the peritoneal cavity (Figure 2).

The anatomical structure and placement of the PPC can be seen in Figure 2. As can be seen, this canal has a small size and is located between the liver and the oesophagus, and its function is to establish communication between the inner space of the pericardium and the peritoneal area. In part B of Figure 2, it can be seen that the cranial part of the canal was located on the midline. Of course, due to moving the right lobe of the liver to make it possible to see the canal, the caudal and

TABLE 1 Morphometric results of pericardioperitoneal canal (PPC) (unit of measurement is cm) in the Immature male beluga (cm).^a

Parameter	PPC length	PPC diameter (cranial part)	PPC diameter (middle part)	PPC diameter (caudal part)
Mean ± SD	3.23 ± 0.05	0.24 ± 0.04	0.22 ± 0.06	0.22 ± 0.04

^aThe difference in diameter size in three parts was not statistically significant ($p > 0.05$).

middle parts of the canal have moved out of their original place, but it is clear that they are not placed on the midline.

Table 1 shows the results of morphometric measurements related to the channel. As mentioned in the table, the difference in the diameter of the cranial, middle and caudal parts was not statistically significant ($p > 0.05$).

4 | DISCUSSION

According to the observations of the present study, it can be said that the pericardial cavity in beluga is a triangular cavity surrounded by

the pericardial membrane, and the structures of the heart are located inside it. This cavity is located in the cranioventral position compared to the peritoneal (coelomic) cavity. It is also adjacent to the oesophagus from the dorsal and the structures of the pectoral fins from the sides. The pericardium is thicker on the posterior side adjacent to the coelomic cavity, which creates a diaphragm-like structure (transverse septum). In the existing sources, such a structure is also mentioned for the species of sturgeon and shark (De Luliis & Pulerà, 2011; Kapoor & Khanna, 2014; Zehtabvar et al., 2017). In bony fish, the pericardial cavity is a part of the coelomic cavity that is separated from other parts by the pericardium. On the contrary, this structure has been separated from the coelomic cavity in sturgeon species (De Luliis & Pulerà, 2011; Moghim et al., 2000). In fact, it can be said that in this group of fish, due to the thickness of the pericardium, especially in the caudal part, the pericardial cavity is more independent than the coelomic cavity.

Gregory et al., in 2004, pointed out the presence of a canal between the pericardial cavity and the coelomic cavity in *Acipenser transmontanus*, which is important for the regulation of pericardial cavity pressure. It is placed in the posterior direction, and then it is placed in the midline of the body from the transverse septum (Gregory et al., 2004). Zehtabvar et al., in 2019, have stated in their study on the tissue structure of the sinus venosus that this structure is often composed of connective tissue and a small amount of muscle fibres. In the present study, the sinus venosus was also noted as a thin wall without contraction and echogenicity (Zehtabvar et al., 2019). In the study of Abel et al. in 1994, the existence of this canal was mentioned in the horned shark (*Heterodontus francisci*), and its function is to prevent the increase of fluid pressure inside the pericardial cavity to more than 0.3 kPa (Abel et al., 1994). In the present study on immature beluga, it was observed that the canal is located behind the sinus venosus and is caudoventral to it, though behind the transverse septum. In the immature beluga, the canal starts on the midline, and then it inclines to the right. In fact, the cranial part of the canal is on the midline, and its middle and caudal parts are inclined to the right and are adjacent to the right lobe of the liver. Of course, all parts of the canal are adjacent to the oesophagus from the dorsal side and from the ventral side to the liver. As in the study of Gregory et al. in 2004, it has been mentioned that this PPC establishes a connection between the pericardial cavity and the peritoneal cavity, so this connection is between the peritoneal cavity and the space between the two layers of the pericardium and has no connection with the pericardial sac (the space where the heart is located). Therefore, in the dissection process, when we enter the pericardial sac, we do not expect to see the hole corresponding to the canal. As in the present study, it was not observed on the immature beluga. Farrell (1991) has mentioned that the PPC is adjacent to the sinus venosus, and this proximity was also observed in the present study on immature beluga.

In the case of *A. transmontanus*, it has been mentioned that the structure and placement of the PPC are similar to that of elasmobranchs, a funnel-like structure (Gregory et al., 2004). Of course, in the case of beluga, it was observed in our study that this structure is completely tubular, and its diameter is from the beginning to the end. Gregory et al. (2004) have mentioned that in *A. transmontanus*, the flac-

id and collapsed PPC extends across the transverse septum and ends on the ventral side wall of the oesophagus. In two white sturgeons (3.7 ± 0.5 kg), the PPC is 3.03 ± 0.04 cm long and has a 0.52 ± 0.03 cm diameter. In the current study on immature beluga, we observed that the canal length is 3.23 ± 0.05 cm and the diameter of the cranial part is 0.24 ± 0.04 cm, the middle part is 0.22 ± 0.06 cm, and the caudal part is 0.22 ± 0.04 cm. It should be noted that the average weight of the immature beluga was 3.5 ± 0.54 kg.

Gupta (1996) has mentioned about the PPC in dogfish (*Squalus acanthias*) that this structure is located on the ventral surface of the oesophagus like beluga, and there is no valve in it. In our study on immature beluga, no valve was observed inside the channel. In addition, they pointed out that the ultrastructure of the ventral wall of the PPC was devoid of smooth muscle cells. Relative to collagen, elastin was minimally present. In our study on the canal in beluga, the external structure of the canal wall was different from the thin wall of veins and had a structure similar to the wall of arteries.

In this study, the anatomical structure and morphometry of the PPC in immature beluga were investigated, and some approaches were presented to observe the canal in ultrasonography. According to the limited information on this structure in all types of fish and sturgeon, it is suggested to carry out additional studies, such as histological in this field.

AUTHOR CONTRIBUTIONS

Omid Zehtabvar: Methodology; project administration; supervision; writing – review and editing. **AliReza Vajhi:** Funding acquisition; validation; writing – review and editing. **Majid Masoudifard:** Funding acquisition; writing – review and editing. **Somaye Davudypoor:** Conceptualization; data curation; software; writing – original draft preparation. **Seyyed Hossein Modarres Tonekabony:** Formal analysis; resources; software; writing – original draft preparation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The experiment was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, university of Tehran, Iran.

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