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The Use of Ultrasound Diagnostic Method and Histological Technique to Detect Pathologies of the Internal Organs and Skin of Sturgeon Species

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ABSTRACT: To restore the stocks of sturgeon fish species in a depressed state, it is necessary to form broodstocks in hatchery farms. The technology of forming stocks in industrial conditions is far from perfect due to the low quality of breeders. The aim of this work was to identify the frequency of occurrence of anomalies and diseases of sturgeon internal organs using the method of ultrasound scanning and histological study. Two-dimensional black-and-white image, Doppler, elastography, and panoramic scan were used. Regular sonographic studies of abdominal organs, carried out in 2018–2025 at 20 fish farms, revealed a number of common pathologies and diseases in generative tissue: cystosis and polycystosis, fatty degeneration, underdevelopment and torsion of testes, neoplasm, hermaphroditism, sludge in the gallbladder, ascites, and others. The maximal number of individuals with disorders in the development of internal organs reached 67.9%, an average is 24.8%. Two stages of cystic degeneration of the ovaries were characterized by ultrasonic scanning. A significant increase in the number of females over 9 years old, with replacement of generative tissue with connective tissue (13.6%), was noted. The nature of ‘ink spots’ on the skin of Siberian sturgeon natural populations was studied. Malignant skin neoplasms were detected, and treatment routes were outlined.

Keywords: US-method; Diseases; Gonads; Liver; Gall bladder; ‘Ink Spots’

1. Introduction

Due to overfishing, natural populations of sturgeon fish are at risk of extinction, and effective measures are needed to restore their populations and control the quality of their habitat for their conservation [1,2].

The current situation in artificial reproduction and aquaculture of sturgeon is also characterized as a critical one [3]. Fish farms specialized in the artificial reproduction of these particularly valuable species are experiencing a shortage of mature breeders. The fragmentation of regulatory requirements and methodological principles in the formation of broodstocks at fish farming enterprises underscores the urgent need to develop innovative sturgeon reproduction technologies, including the development of non-invasive methods for monitoring their physiological condition. Over the last ten years in Western and Eastern Siberia, old fish breeding facilities have been renovated and new ones built to support artificial reproduction of sturgeon species as compensatory measures, as well as for commercial farming. Most of them are full-system pool complexes with the use of recirculating water supply systems (RAS). Existing technologies for artificial reproduction and the formation of broodstocks, as well as biotechnological standards for cultivating sturgeon in the European part of Russia, the West and East Siberian fishery basins, do not differ significantly, and they are aimed at the most efficient use of available production capacities [4,5].

Despite certain successes in organizing the planning of breeding work at fish hatcheries, annual ultrasound monitoring of broodstocks revealed a number of common problems related to violations in the biotechnology of their formation, maintenance, and operation.

The express method for early determination of sex and gonadal maturity stages in sturgeon using ultrasound scanning was developed by M.S. Chebanov and E.V. Galich [6]. It is now actively used in the study of sturgeons [5–8] and other fish species, such as Atlantic salmon, European eel, Clarias catfish, Channel catfish, and pangasius [9–13].

Due to the insufficient experience and literacy of operators, diagnostic errors are often observed, especially when assessing anomalies in the development of generative tissue and detecting pathologies of internal organs. Modern ultrasound technologies (Dopplerography, elastography, panoramic scanning, tissue harmonic mode, and others) significantly improve imaging quality and the study of animal tissue structures, enabling rapid and accurate diagnosis of abnormalities and diseases [14,15]. The use of new technological techniques in sonography can fill existing gaps and significantly increase the accuracy of diagnosing the condition of the reproductive system, liver, gallbladder, heart, gastrointestinal tract, muscle tissue, and other internal organs.

The use of ultrasound diagnostics (USD) opens up new opportunities for detecting abnormalities in the skin epithelium of sturgeon, for example, when so-called ‘ink spots’ appear on the skin. The etiology of the appearance of ‘ink spots’ on the skin of sturgeons of natural populations, which do not have any visible negative effect on their physiological condition, is still unknown. Various assumptions have been made about their nature, but because the number of individuals with spots is small, a detailed study of this phenomenon has not been conducted before. According to literary sources, in addition to the Siberian sturgeon of the Lena population [16], cases of ‘ink spots’ have also been recorded and described in domesticated breeders of Siberian sturgeon from the Ob-Irtysh basin and Russian sturgeon domesticated at Volga sturgeon breeding farms [16]. A year after ‘ink spots’ were discovered by specialists at the Abalak Sturgeon Hatchery Farm, an increase was noted both in their number on the bodies of the fish and in the number of affected individuals. The occurrence of fish with spots in the sexually broodstock amounted to 10–15% [16]. Our research was aimed at studying the nature of ‘ink spots’ using ultrasonic and histological methods. The spots were detected on the skin epithelium of Siberian sturgeon from the Lena population two years after the start of fish domestication at the Chernyshevsky Fish Farm in the Republic of Sakha (Yakutia). It should be noted that this work was conducted for the first time in Russia, and it is not described in the scientific-methodological literature.

The aim of this study is to estimate the efficiency of a non-invasive express method of visual ultrasound diagnostics together with histological study for the early detection of pathologies and diseases of the internal organs of sturgeon fish raised on industrial aquaculture farms.

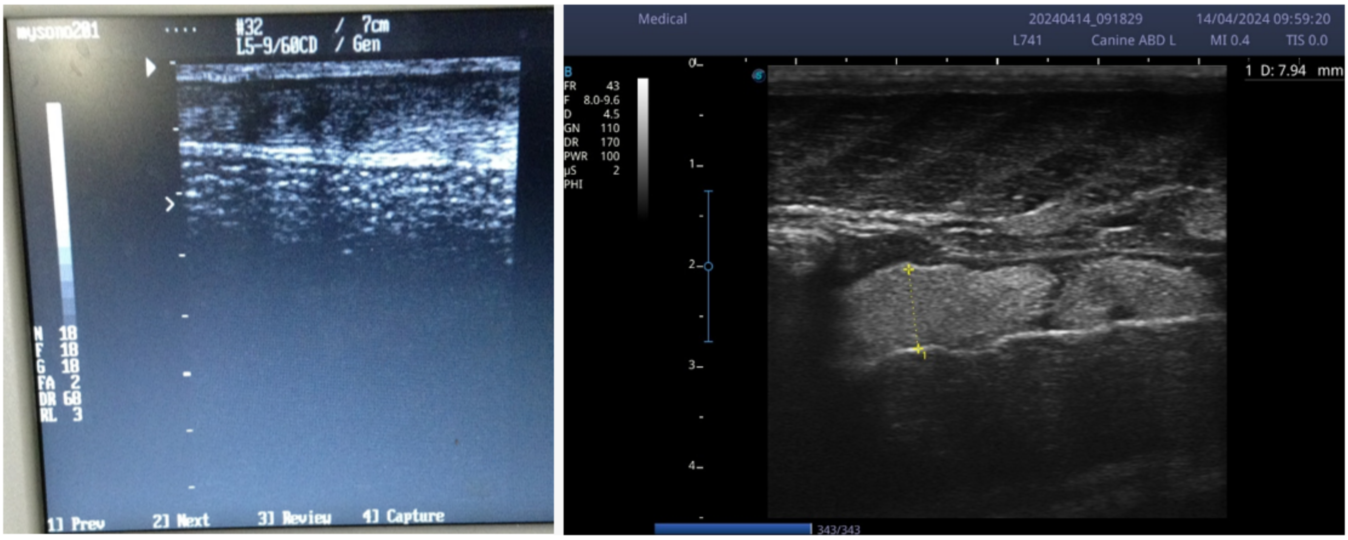
2. Materials and Methods

The study subjects were individuals of Siberian sturgeon (*Acipenser baerii* Brandt, 1869) of different ages from the Ob, Baikal, Yenisei, and Lena populations; European sterlet (*Acipenser ruthenus* Linnaeus, 1758) from the Kama population; Siberian sterlet (*Acipenser ruthenus marsiglii* Brandt, 1833) from the Ob, Irtysh and Yenisei populations, Russian sturgeon (*Acipenser gueldenstaedtii* Brandt, 1833), Beluga (*Huso huso*, Linnaeus, 1758) raised at fish breeding farms both as part of planned fish breeding activities for artificial reproduction and for aquaculture.

Scientific and production works were carried out from 2018 to 2025 at 20 Russian fish farming enterprises: in Eastern and Western Siberia, the Urals, the European part of the country, and the Caucasus. Various water sources were used for fish farming at the enterprises: reservoirs, rivers, artesian and/or geothermal water, employing either flow-through or recirculating systems.

The grading and culling of sturgeon from broodstock were carried out at different periods: before and after artificial wintering, before spawning, and during the period of active fish feeding. During the ultrasound examination of the fish abdominal cavity, the main scanning regime was B-mode (Brightness Mode), which allowed obtaining a two-dimensional outline of the organ in a black-and-white image and visualizing individual structures in a clear format. In some cases, when data from two-dimensional sonography did not provide sufficient information about an anatomical object (for example, a large tumor), the panoramic view option was used by sequentially scanning slowly. Panoramic scanning allowed the spatial arrangement and relationship of anatomical structures to be displayed along their length and depth. For the examination of cavities, cysts, and large vessels, one-dimensional M-mode (Motion) was used. In M-mode, the ultrasound beam penetrated the tissues at a single point, and it was reflected. The study of large vessels (for example, in the liver) was performed using various Doppler techniques: continuous-wave Doppler (CW), pulsed-wave Doppler (PW), color mode, power Doppler, and tissue harmonic imaging [14]. The following portable ultrasound scanners were used for scanning the abdominal organs: with grayscale imaging—My Sono-201 (Country of origin—South Korea, representative office in Moscow, Russia), AcuVista Grace (Assembly country—People's Republic of China, distributor center in Moscow, Russia), SonoScape-A6 (China, SonoScape Co., Ltd., representative office in Moscow, Russia), Mindray models DP-50, 6600, 6900 (China, Shezhen Mindray Bio-Medical Electronics Co., Ltd., representative office in Moscow, Russia); with color Doppler—SonoScape-S2N model (China, SonoScape Medical Corp., distribution center in Yekaterinburg, Russia). For clear visualization of the gonads and muscle tissue, a standard high-frequency linear 2D probe with a 40 mm working surface and frequency of 5.5–12 MHz was used. For ultrasound scanning of the heart, liver, gallbladder, and other internal organs, low-frequency microconvex and convex 2D probes with a smaller aperture and a scanning depth of 2.5–5.0 MHz were used. The following values were used when setting up the My Sono-201 device. The amplification of signals reflected from the surface (N) was set to a value of 18. The amplification of reflected signals from the depth (F) was 18, and the overall gain (G) was also set to 18. The set scanning depth when using a linear transducer was 4–7 cm (Figure 1a). On other devices (for example, Sonoscape S2N for veterinary medicine), presets were used—a pre-configured set of parameters (frequency, depth, gain, focus) that allowed to instantly adapt the scanner for a specific type of study, animal species, or organ. When using the Sonoscape S2N ultrasound device with presets, the frequency (F) was set to 8.0–9.6 or 9.5–12.2, depending on the task and the object of study (Figure 1b).

The duration of ultrasound examination ranged from 2 to 10 s per individual. Exceptions were complex cases, such as anomalies of the reproductive system, liver and gallbladder pathologies, polycystic conditions, hydrocele, and other diseases requiring additional time for a more thorough study. For 8th years period more than 250,000 ultrasound examinations were performed (Table 1). Of this number 700 ultrasound examinations (red color) were conducted on domesticated Siberian sturgeon from the Lena (150 echograms) and Ob (150 echograms) populations and sterlet from the Irtysh population (400 echograms).



(a) **(b)**

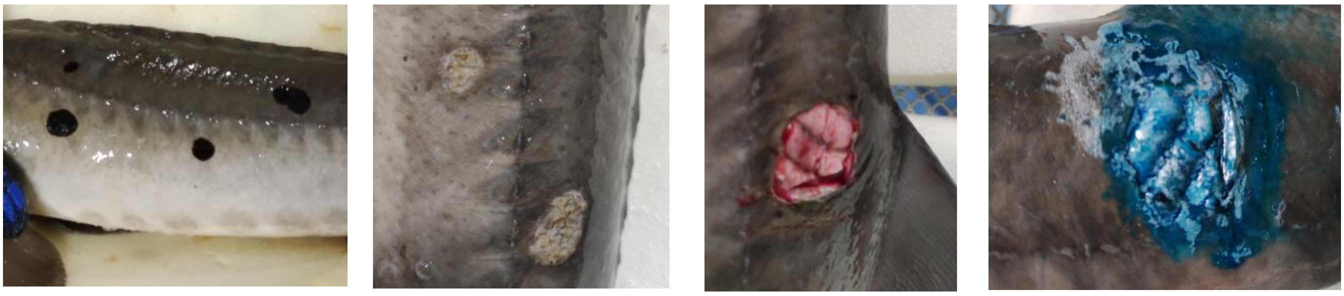
Figure 1. Visualization of Siberian sturgeon gonads using various ultrasound devices: (**a**) My Sono-201; (**b**) Sonoscape S2N (vet); on the left side of the echogram, the parameters necessary for grayscale ultrasound examination are displayed: scanning depth, gain of reflected signals from depth and surface, overall gain, frequency, dynamic range, and other characteristics.

Table 1. Ultrasound examinations of sturgeon species were performed at 20 Russian fish hatchery farms from 2018 to 2025; the total number of ultrasound examinations—over 250,000, and the number of US-examinations for domesticated fish—700 (beyond the line, red color).

Russian Fish Hatchery Farms	**Sturgeon Species**							
	Acipenser baerii				***A. gueldenstaedtii***	***Huso huso***	***A. ruthenus***	
	Ob-Irtysh Basin	**River Lena**	**River Yenisei**	**Lake Baikal**			**River Kama**	**Siberian Rivers**
SUE Chernyshevsky Fish Farm (Yakutia)	-	9850/150	-	-	-	-	-	-
LLC Irkutsk Trout (Irkutsk Region)	-	1000	-	1000	-	-	1000	1000
LLC Belorechensky Fish Farm (Irkutsk region)	-	-	500	500	-	-	-	-
LLC Maltat Krasnoyarsk Region	1000	1000	1000	-	-	-	-	1000
IE Prokudin (Kemerovo Region)	-	-	1000	-	-	-	1000	5000
LLC Novosibirsk Fish Farm (Novosibirsk Region)	30,000	-	-	-	-	-	2000	-
LLC Siberian Sturgeon (Omsk Region)	500	500	-	-	-	-	1000	2000
LLC Yugorsky Fish Hatchery (Khanty-Mansi Autonomous Okrug)	20,980/20	-	-	-	-	-	5000	30,000
LLC SPA Sobsky Fish Hatchery (Yamalo-Nenets Autonomous Okrug)	-	-	-	-	-	-	-	3000
LLC Uvatsky Aquaculture Center (Tyumen Region)	500	-	-	-	-	-	-	200
LLC Siberian Sturgeon (Tyumen Region)	2000	-	-	-	-	-	-	-
FSBE Tyumen fish hatchery (Tyumen Region)	29,900/100	-	-	-	-	-	-	24,800/200
LLC Pyshma-96 (Tyumen Region)	19,970/30	1000	-	-	100	50	5000	25,000
LLC Fish Farm (Tyumen Region)	100	-	-	-	-	-	2000	1000
LLC New aquaculture (Tyumen)	1000	-	-	-	-	-	-	1000
LLC Reftinsky Fish Hatchery Farm (Sverdlovsk Region)	1000	1000	-	-	-	50	-	-
LLC Dobryanskoye Fish Farm (Perm Region)	400	400	-	-	-	-	-	-

IE Sosunov (Perm)	500	500	-	-	400	-	1000	1000
LLC Levzhensky (Mordovian Republic)	-	1000	-	-	500	-	-	-
LLC Tyrnyauzsky Sturgeon Farm (Kabardino-Balkarian Republic)	-	1000	-	-	1000	-	-	-

During the procedure to remove the epithelium of one of 'ink spots' in Siberian sturgeon of natural origin, a clove oil solution was used as an anesthetic: 10 mL of the solution per 100 L of water at a water temperature of 10.7 °C. The skin epithelium under 'the ink spot' was removed with a scalpel, after first scraping the spot. A biopsy of 'the ink spot' and a removed section of skin measuring 1.1 × 1.3 cm were fixed in 10% formalin solution. After the operation, the sturgeon was given an intramuscular injection of vicasol (vitamin K). The wound surface was treated with 0.05% chlorhexidine digluconate, Terramycin (spray), and the antiseptic preparation 'Second Hide Super' with silver for 30 days (Figure 2).



(**a**) (**b**) (**c**) (**d**)

Figure 2. Removing the epithelial 'ink spot' in Siberian sturgeon, wild Lena population: (**a**) 'ink sport' before surgery; (**b**) skin epithelium after removal of 'ink spots'; (**c**) exposed muscle tissue after surgery; (**d**) wound treated with the 'Second Skin' preparation.

Biopsies were processed through alcohols of various concentrations and embedded in paraffin using the standard method [17]. Sections 5 μm thick were stained with hematoxylin and eosin according to the standard Van Gieson technique. The histological preparations were examined using Micros light microscope (magnifications of 100, 200, and 400), assessing the condition of cells and other structures.

During ultrasound examinations, the frequency of occurrence of various diseases of the internal organs of sturgeon was assessed, which allowed for determining the statistical probability of the occurrence of pathologies. The formula used to calculate the statistical probability was: $p = f/n$, where f is the frequency of occurrence and n is the sample size [18].

3. Results

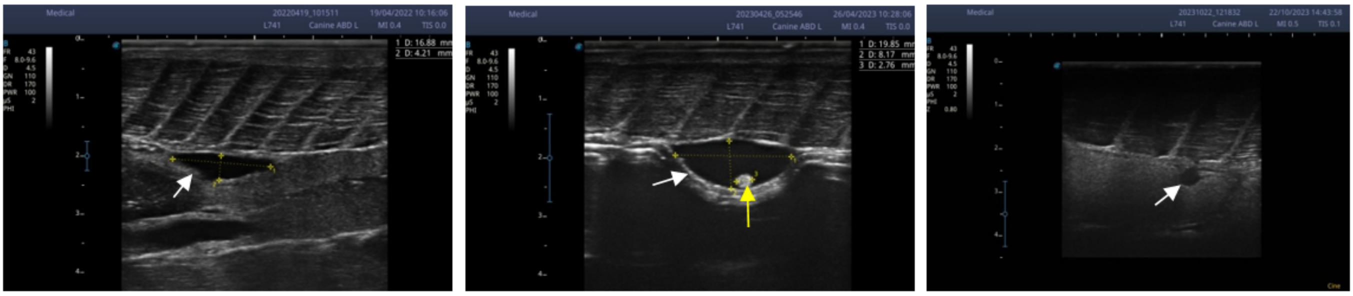
During the assessment of Siberian sturgeons using the visual analysis method, the following pathologies were identified: abnormalities of the olfactory organs (AOO), damage to (or absence of) fins, scoliosis (curvature of the cartilaginous structure of the notochord), emaciation, skin defects (growths, papillomas, pigmented 'ink spots'), and others.

The use of linear and microconvex probes, continuous-wave and pulsed Doppler and color Doppler made it possible to visualize a range of internal organ pathologies: cystic (polycystic) changes in reproductive tissue, fatty degeneration of the gonads, underdevelopment and lobulation of the testes, neoplasms in reproductive tissue, cystic formations in the liver, cholecystitis, foreign bodies in the gastrointestinal tract, swim bladder inflammation, changes in the heart muscle, and others.

3.1. Ultrasound Image in Pathologies of the Generative Tissue

Cystic (polycystic) testes and ovaries. During ultrasound examination of the testes and ovaries at various stages of maturation, cystic and polycystic conditions were visualized in 1.2–1.4% of Siberian sturgeon individuals from different populations, with ovarian cysts observed in 62% of cases.

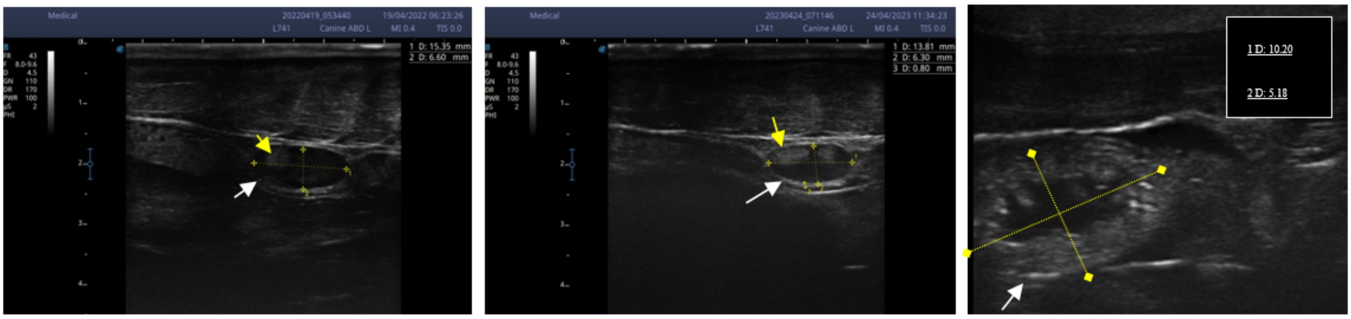
The cysts on the ultrasound appeared as single or multiple oval or round-shaped formations containing anechoic fluid (Figure 3, cysts indicated by white arrows on the ultrasounds). The cyst walls were thickened to varying degrees; in some cases, they might become mineralized (Figure 3b, Figure 4a,b, yellow arrows).



(a) (b) (c)

Figure 3. Echograms of cysts located in the generative tissue of the ovaries and testicles in Siberian sturgeon cultivated Lena population (indicated by white arrows on the ultrasounds): (**a**) 3-yr-old, cyst 15.88 × 4.21 mm; (**b**) 4-yr-old, cyst 19.85 × 8.17 mm (yellow arrow indicates a solid formation 2.76 mm); (**c**) 4-yr-old, round cyst.

In polycystic cases, small cysts had indistinct, irregular boundaries. When visualizing larger cysts, an effect of distal pseudo-enhancement and lateral shadows was observed (Figure 4a–c, white arrows).



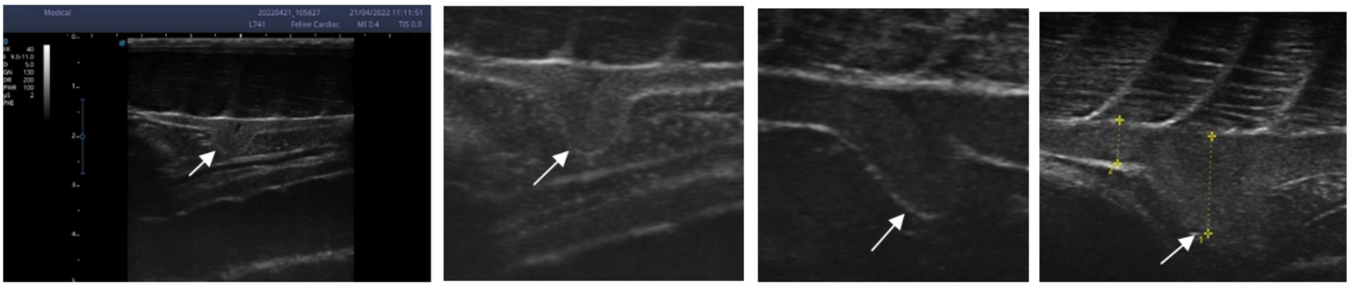
(a) (b) (c)

Figure 4. Echograms of an ovary cyst (indicated by white arrows on the ultrasounds) in female Siberian sturgeon, cultivated Lena population: (**a**) 3-yr-old fish, cyst 15.35 × 6.60 mm (yellow arrow indicates a solid formation); (**b**) 4-yr-old fish, cyst 13.81 × 6.30 × 0.80 mm (yellow arrow indicates a solid formation); (**c**) 5-yr-old fish, cyst 10.20 × 5.18 mm.

A year later, visualization of the cyst showed a slight decrease in its size and thickening of the walls (13.81 × 6.30 × 0.80 mm). Inside the cyst, instead of one hypoechoic formation, two rounded formations were found (Figure 4b). In a 5-year-old sturgeon, the cyst has shrunk by 1.5 times from its original size (10.2 × 5.18 mm). At the same time, the cyst wall increased almost 3 times (2.3 mm), and solid formations filled almost the entire anechoic area of the cyst (Figure 4c). If the sizes of large cysts do not decrease but increase (more than 2 cm), then an investigation using contrast agents (for example, SonoVue, ethyl iodide-dipamide), an aspiration biopsy under ultrasound guidance to detect pathogenic microorganisms, clarify the diagnosis of the disease, and determine treatment methods should be conducted.

3.2. Torsion, Testicular Underdevelopment

In underdevelopment and torsion, the testicle decreases in size after its normal development due to impaired blood circulation. On an echogram, the testicle in torsion appeared as a hypoechoic loop surrounded by a bright hyperechoic shell. Underdevelopment might be associated with a neoplasm in the opposite testicle. Underdeveloped testicles were generally small in size, but their structure remained normal. There were hypoechoic inclusions with areas of reduced echogenicity. The number of males with gonadal underdevelopment and torsion in different populations of Siberian sturgeon ranged from 0.25% (the Ob river) to 2.19% (the Lena river). During ultrasound examinations of males, the shape of the torsion and underdeveloped testes generally persisted, and the stages of testis maturity did not change over several years (Figure 5). Males with testicular torsion and underdevelopment are needed to take away from the brood stock.



(a) **(b)** **(c)** **(d)**

Figure 5. Echograms of testis underdevelopment and kinking (white arrow) in male Siberian sturgeon, cultivated Lena population; maturity stage M2: (**a**) 2-yr-old; (**b**) 3-yr-old; (**c**) 4-yr-old; (**d**) 5-yr-old.

3.3. Fatty Degeneration of the Germinal Tissue

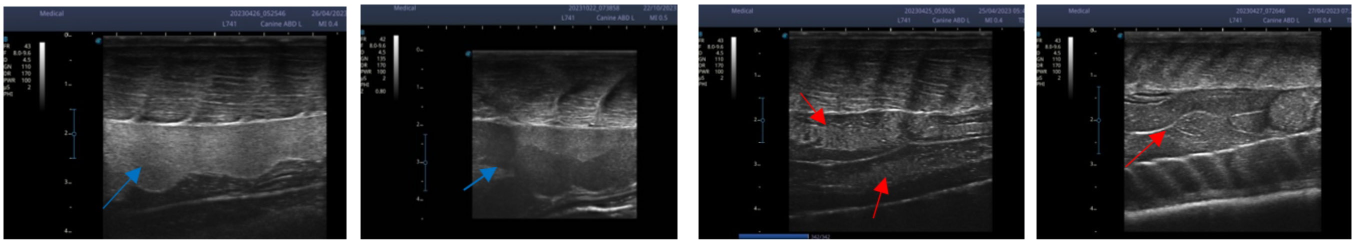
When the biotechnological maintenance regime of sturgeon brood stock is not followed, cases of fatty degeneration of the generative tissue of testes and ovaries are not uncommon and, without regular ultrasound screening, can reach up to 23.6%.

3.3.1. Males

Normally developed testes, starting from stage M2, were visualized on ultrasound as a fine-grained homogeneous structure of increased echogenicity, with a hyperechoic light-gray or almost white capsule (Figure 6a). In fatty degeneration, the germinal tissue of the testicle did not develop, and fatty tissue covered the germinal tissue from the medial and lateral sides. On the ultrasound, the testicles appeared as a heterogeneous structure of mixed echogenicity, with short hyperechoic discontinuous bridges visible. Sometimes, individual lobes of the testicles had a heterogeneous coloration—from dark gray, almost black, to light (Figure 6b). In some cases, when the cultivation biotechnology was violated, a sharp increase in gonads was observed due to the growth of adipose tissue. On echograms, the testes appeared as large, homogeneous, lobulated anechoic structures. During ultrasound scanning, such gonads visually resembled stage M2. The development of spermatogenesis stages was disrupted, and the testes did not develop for several years.

3.3.2. Females

Normally, the generative tissue of the ovary appears on ultrasound images as an area of mixed echogenicity, does not adjoin the muscles, and has no clear boundaries (Figure 6c); at early stages, it is separated from the muscle tissue by hypoechoic fatty tissue. Oogenesis is disrupted during ovarian fatty degeneration. On the ultrasound, the gonads appear as heterogeneous structures of mixed echogenicity with separate hyperechoic inclusions in the form of short septa; the generative tissue is not pronounced and has no boundaries (Figure 6d). Individuals with fatty degeneration of either the testes or the ovaries are to be removed from the brood stock.

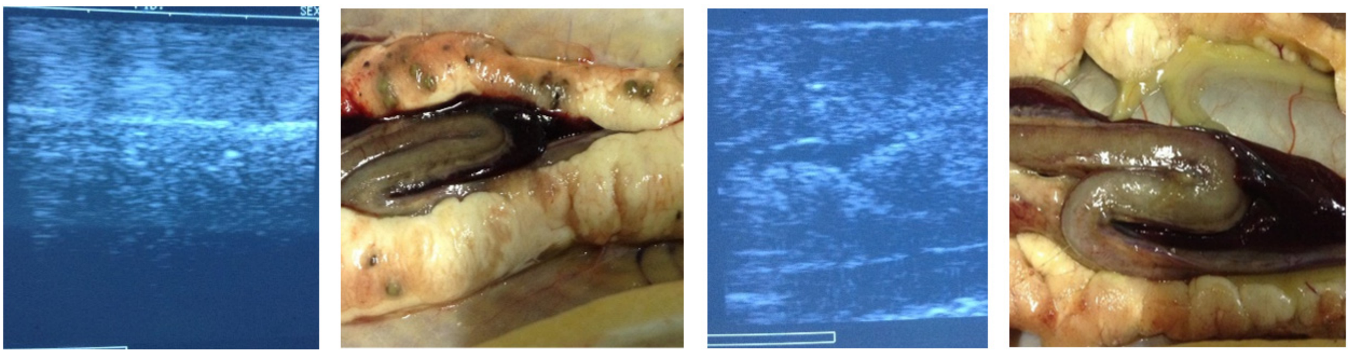


(a) **(b)** **(c)** **(d)**

Figure 6. Echograms of longitudinal cross-sections of testes (**a**,**b**: blue arrows) and ovaries (**c**,**d**: red arrows) of Siberian sturgeon, aquaculture: (**a**) 4 y-r-old, Lena population, *normal*; (**b**) 10-yr-old, Ob population, *pathology*; (**c**) 4-yr-old, Lena population, maturity stage 2, semi-fatty ovaries (F2sf), *normal*; (**d**) 4-yr-old, Lena population, *pathology*.

3.4. The Phenomenon of Hermaphroditism

Failure to comply with the technological regime for breeding sturgeon fish to obtain reproductive products of high aquaculture quality leads to disruption of gametogenesis. The annual absence of hibernation combined with food deprivation led to synchronous and asynchronous hermaphroditism in 12% of eight-year-old sterlet individuals from the Kama population out of 300 fish studied (Figure 7). On echograms, the ovotestes of such fish appeared as structures of mixed echogenicity with blurred edges, exhibiting features similar to both testes and ovaries.



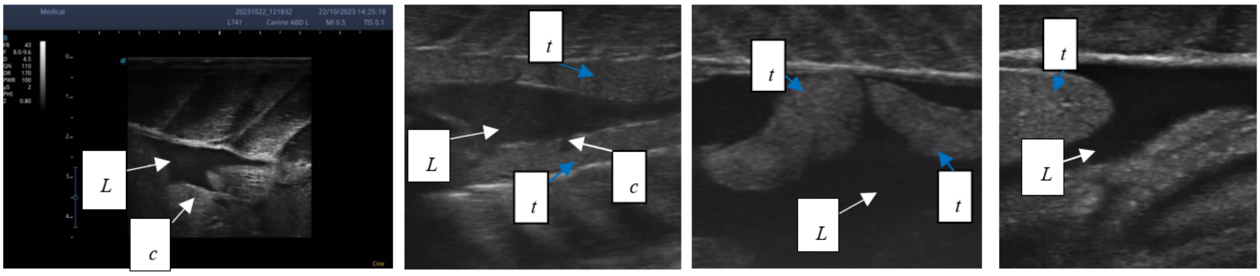
(a) **(b)** **(c)** **(d)**

Figure 7. Ovotestes of sterlet (cultivated Kama population, 8-yr-old) with asynchronous (**a**,**b**) and synchronous (**c**,**d**) hermaphroditism: (**a**,**c**) echograms; (**b**,**d**) ovotestes after autopsy.

3.5. Anechoic Fluid

During the ultrasound examination of the gonads, mainly of male Siberian sturgeon, local serous anechoic intratesticular fluid was visualized between the paired testes (Figure 8).

Cases of liquid detection were quite rare and accounted for no more than 0.2% of all pathologies of the reproductive tissue. They were normally caused by bacterial infections, injuries, blows, and falls. In case it occurred, the fluid did not disappear on its own and tended to progress. Such specimens of sturgeon have to be culled from the brood stock. If it was necessary to preserve individuals for reproduction (especially concerning domesticated sturgeons from wild populations), a minimally invasive treatment method was used, such as sclerotherapy. The serous fluid was aspirated with a syringe under ultrasound guidance, for which a puncture was performed. After the fluid was removed, sclerosing agents were introduced into the cavity. Their mechanism of action is based on stimulating an inflammatory cellular response to the introduction of a chemical substance [19]. Various substances were used for sclerotherapy, such as tetracycline, Betadine (povidone-iodine), polidocanol, ethanolamine, phenol, and 96% ethyl alcohol.

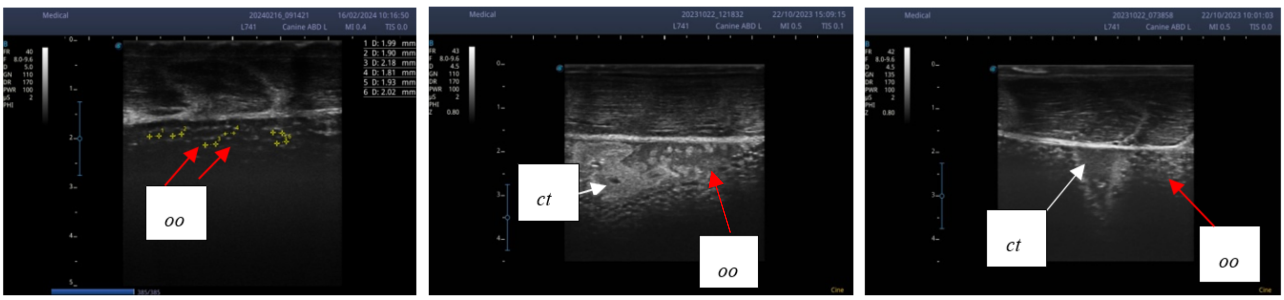


(a) **(b)** **(c)** **(d)**

Figure 8. Echograms of testes (*t*; blue arrows) in males Siberian sturgeon from the wild Lena population (**a**,**b**) and cultivated Ob population, 6-yr-old (**c**,**d**): anechoic liquid (*L,* white arrows) accumulates between the paired testes; several small cysts (*c,* white arrows) are visualized in the testes.

3.6. Connective Tissue Replacements of the Ovaries During the Spawning Period

Normally at the IV completed stage of maturity, the ovaries of females occupy almost the entire body cavity, fat is absent, and fully pigmented oocytes (black in color) reach their definitive size (diameter—1.8–2.5 mm). The echogram shows individual eggs in the form of granular inclusions of the same size, and the ovary has a granular heterogeneous structure. The rows of oocytes are well defined. Ultrasound is almost completely absorbed in the upper (up to 1 cm) layer of the ovary, so visualization of the lower (medial) part of the ovary is absent (Figure 9a). Due to the accumulation of a significant amount of fluid in the follicles beneath the oocytes, a distal enhancement effect is observed, appearing on the monitor screen as hyperechoic (white) dotted lines, more contrastive than in stage IV echograms [6]. When the lateral part of the ovary is replaced by connective tissue (pathology), the ovulated oocytes on the ultrasound are of varying sizes, and the regularity of the rows is disturbed. Islands of connective tissue are visualized in front of individual oocytes as hyperechoic, irregularly shaped structures with anechoic inclusions. The disrupted rows with oocytes are closely adjacent to this structure (Figure 9b,c).



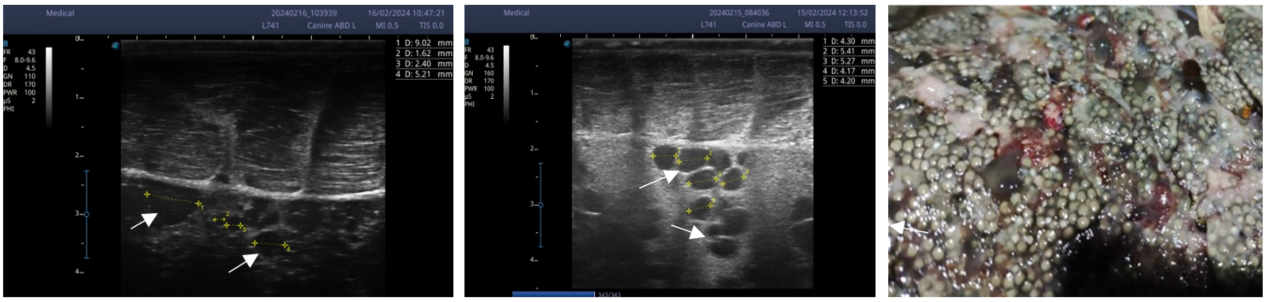
(a) **(b)** **(c)**

Figure 9. Echograms of longitudinal ovary cross-sections in female Siberian sturgeon (aquaculture), maturity stage F4: (**a**) Yenisei population, 10-yr-old, *normal*; (**b**,**c**) Ob population, 11-yr-old, partial replacement of generative tissue, *pathology*; *ct*—connective tissue, white arrows; *oo*—oocytes, red arrows.

3.7. Ovarian Cystic Degeneration, Sclerosis

In some cases (3.6%), when the biotechnology of rearing female sturgeons in industrial conditions was violated (lack of regular artificial wintering with a period of food deprivation, absence of factory spawning with mandatory selection of ovulated eggs), the ovaries retained resorbed eggs, in which cystic 'hydrogel' formations of various shapes and sizes later develop. On echograms, cystic degeneration of the ovaries was visualized as anechoic structures resembling polycystic ovaries. Three main degrees of ovarian involvement by 'hydrogel' plaques could be distinguished, with subsequent formation of cartilaginous neoplasms.

The initial stage. In the echogram field of view during longitudinal scanning, a small number of plaques were visualized in the ovaries (less than 30%). On the echogram, cystic formations with thin capsules and liquid content were visualized as solitary structures of irregular shape, larger (2–3 times bigger) than oocytes. The eggs varied in size with anechoic content, and the rows of oocytes were disrupted. On the echogram, they were visualized along the entire length of the ovary as anechoic and hypoechoic structures with blurred boundaries. Individual anechoic eggs with distal enhancement were present (Figure 10, arrows).

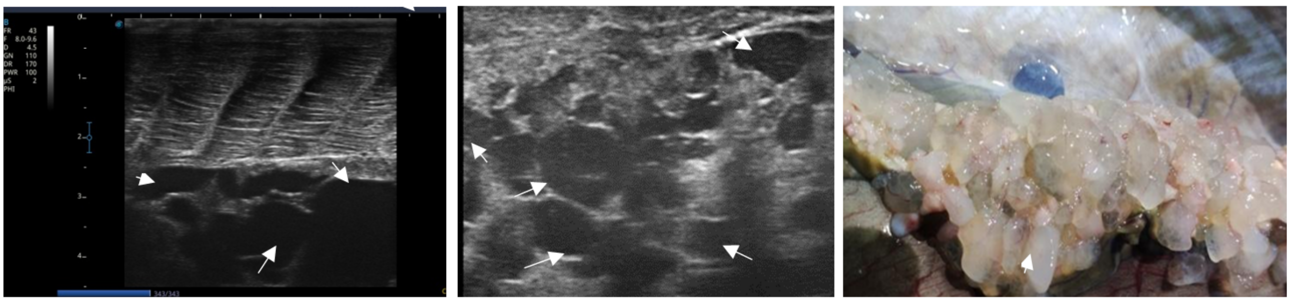


(a) (b) (c)

Figure 10. Visualization of 'hydrogel' plaques (initial stage of cystic degeneration in the ovary); Siberian sturgeon, cultured Yenisei population, 9-yr-old: (**a**,**b**) echograms of the ovary; (**c**) ovary after autopsy; cystic formations are marked with white arrows.

Severe stage of damage. On the echogram during longitudinal scanning, isoechoic and anechoic round formations of irregular shape with hyperechoic shells occupied the entire area of the ovaries. Individual oocytes were extremely rare. Upon dissection, cystic formations in the fish appeared as clusters with a denser consistency than in the first and second stages. The generative part of the gonadal tissue was replaced by connective tissue with formed cartilaginous neoplasms. Sclerosis, or hardening, of areas of generative tissue occurred due to impaired blood circulation (Figure 11). To determine the degree of tumor benignity, clarify the disease diagnosis, and decide on treatment methods, it is necessary to perform an aspiration biopsy under ultrasound guidance.

Timely ultrasound diagnostics of cystic degeneration of generative tissue allows avoiding its transition to an irreversible stage and preventing the occurrence of this serious disorder in other individuals.

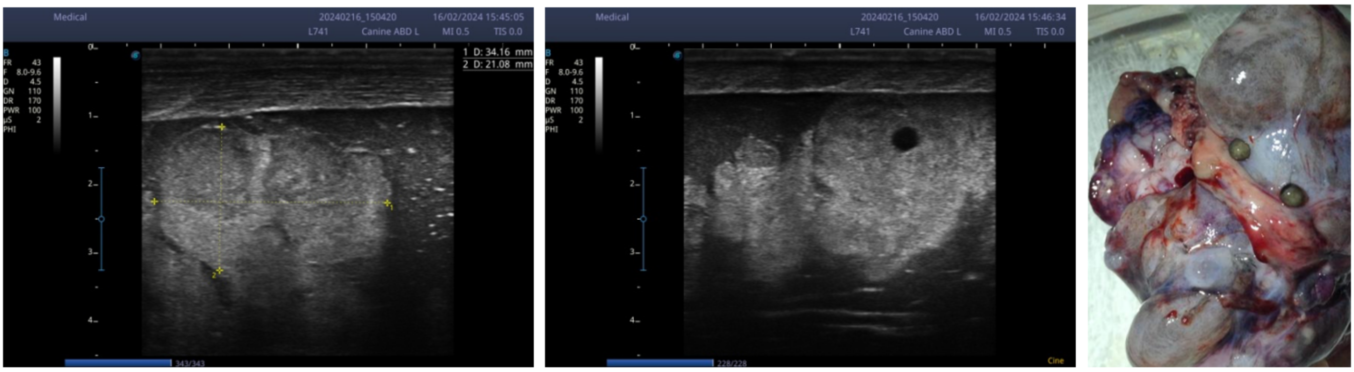


(a) (b) (c)

Figure 11. Visualization of 'hydrogel' plaques (total cystic degeneration in the ovary), Siberian sturgeon Yenisei population farmed in aquaculture: (**a**) echogram of the ovary (9-yr-old fish); (**b**) echogram of the ovary (10-yr-old fish); (**c**) ovary after autopsy; plaques are marked with white arrows.

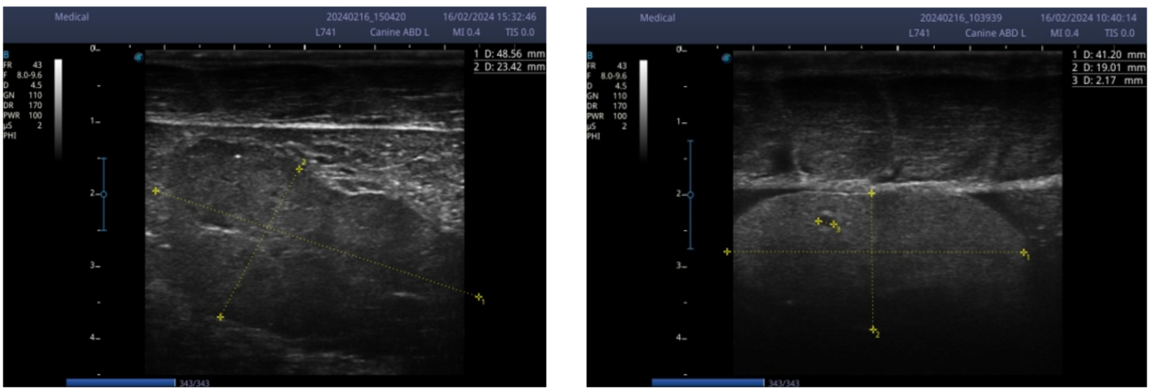
3.8. Neoplasms

Ultrasound diagnostics allowed for quickly and fairly accurately determining the location and size of a tumor in the fish body. On echograms, large tumors appeared as homogeneous hyper- or hypoechoic structures with inclusions of varying echogenicity, with irregular edges that prevented visualization of the underlying organs and tissues. The tumor sizes ranged from 34.16 × 21.08 mm to 48.56 × 23.42 mm. (Figures 12 and 13). The frequency of individuals with neoplasms in the generative tissue made up, on average 1.4%. Histological studies of tumors in the generative tissue confirmed the presence of neoplasms (adenoma, adenocarcinoma, *etc*.). Sturgeon individuals with large tumors must be immediately removed from the brood stock.



(a) **(b)** **(c)**

Figure 12. Tumours of the ovary (*pathology*) in Siberian sturgeon cultivated Lena population, 8-yr-old: (**a**,**b**) echograms of longitudinal cross-sections of tumours; (**c**) tumour after fish autopsy.

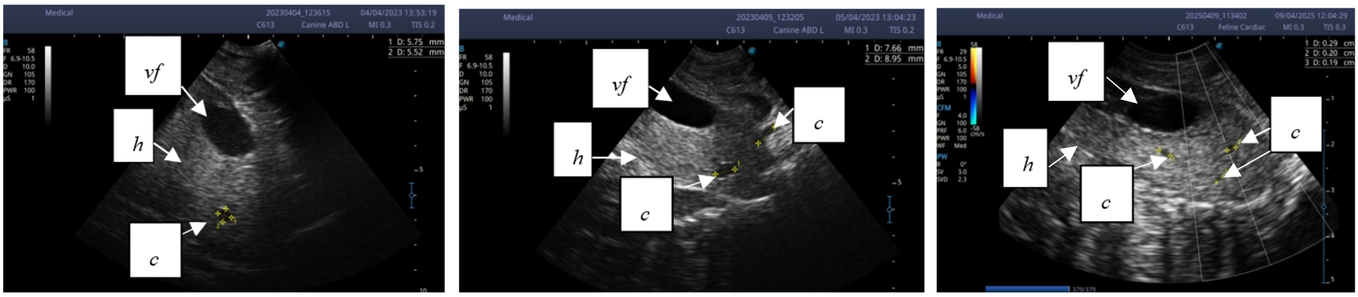


(a) **(b)**

Figure 13. Echograms of longitudinal cross-sections of tumours of the ovary (*pathology*) in Siberian sturgeon cultivated Enisey population, 9-yr-old: (**a**) tumour 48.56 × 23.42 mm; (**b**) tumour 41.20 × 19.01 mm.

3.9. Liver and Gallbladder Disorders

Normally, the liver parenchyma (*hepar*) is homogeneous, hypoechoic, of medium granularity, with smooth, pointed edges, and moderately expressed vascularization. The liver index was 8–10% of the fish body weight. The walls of the hepatic veins and the portal vein (vena portae) were echogenic. Among pathologies in the liver, solitary and multiple cysts were visualized in 0.82–1.9% of individuals (random sample). Most often, cysts were filled with a clear fluid that had no color or odor. In cases of bleeding into the cyst cavity, the contents became hemorrhagic. With infection, they got creamy or purulent [19]. In polycystic disease, cysts compress the liver parenchyma and vessels and could become reinfected. Large cysts on ultrasound were characterized by clear contours, a round shape, anechoic contents, and distal enhancement. Small cystic formations had indistinct contours, septations, and anechoic internal contents (Figure 14).

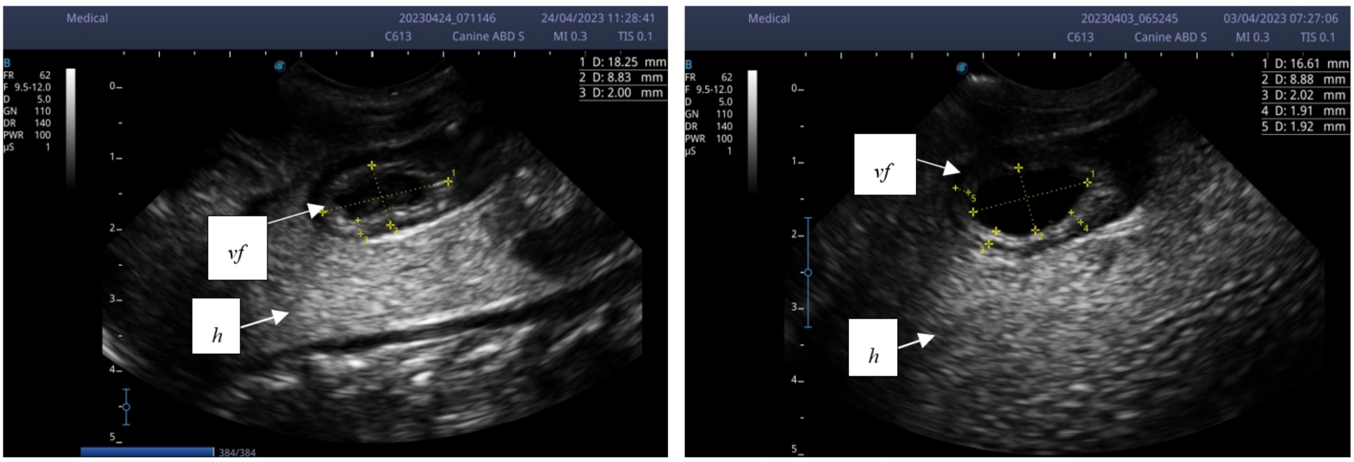


(a) **(b)** **(c)**

Figure 14. Echograms of the liver (*h*, white arrow) with cysts (*c*, white arrow) and gall bladder (*vf*, white arrow) in Siberian sturgeon, cultivated Lena population: (**a**) 3-yr-old; (**b**,**c**) 4-yr-old.

Cysts do not pose a serious threat to the health and life of fish; however, if large cysts rupture, it can lead to hepatomegaly, infection, suppuration in the cyst cavity, peritonitis, liver abscess, massive bleeding in the abdominal cavity, liver failure, malignant transformation, and death [19]. Most often, cysts in sturgeon appeared after injuries, falls, or inflammatory processes, infections, and antibiotic treatments. The highest number of individuals with polycystosis (up to 30%) was observed on fish farms where plastic containers without water were used for transplanting and transporting broodstock.

The bile duct was not *normally* visualized; the size of the gallbladder (*vesica fellea*) depended on feeding. The shape of the gallbladder on ultrasound could vary—pear-shaped, oval, semicircular, or round. When the gallbladder was fully filled with bile, the edges were smooth; when partially filled, the edges were serrated. The contents were anechoic. Normally, the gallbladder wall appeared as a homogeneous thin border with moderately increased echogenicity, less than 0.5 mm thick. Thickening of the gallbladder walls beyond the normal range by 4–5 times was observed in 1.48% of the total number of fish examined; the wall thickness was about 2 mm (Figure 15).



(a) **(b)**

Figure 15. Echograms of liver (*h*, white arrow) and gallbladder (*vf,* white arrow) in Siberian sturgeon, cultivated Yenisei population, 9-yr-old, the walls of the gallbladder are thickened: (**a**) the gallbladder 18.25 × 8.83 × 2.00 mm; (**b**) the gallbladder 16.61 × 8.88 × 2.02 mm.

In order to determine whether the increased accumulation of bile with subsequent stretching and thickening of the walls of this organ is a pathology and the cause of mucocele (hydrops) or other gallbladder diseases, it is necessary to conduct a repeat ultrasound examination of this organ during the active feeding period of the fish. An increase in the diameter of the common bile duct by more than 1 mm may be one of the signs of obstruction (blockage) of the common bile duct. One of the first signs of biliary obstruction was an enlargement of the gallbladder. The wall was thickened and became more convoluted than normal. An increase in gallbladder volume was observed in 4.19% of individuals; the dimensions ranged from 24.41 to 36.86 mm in length and from 12.39 to 25.12 mm at maximum width (Figure 16).

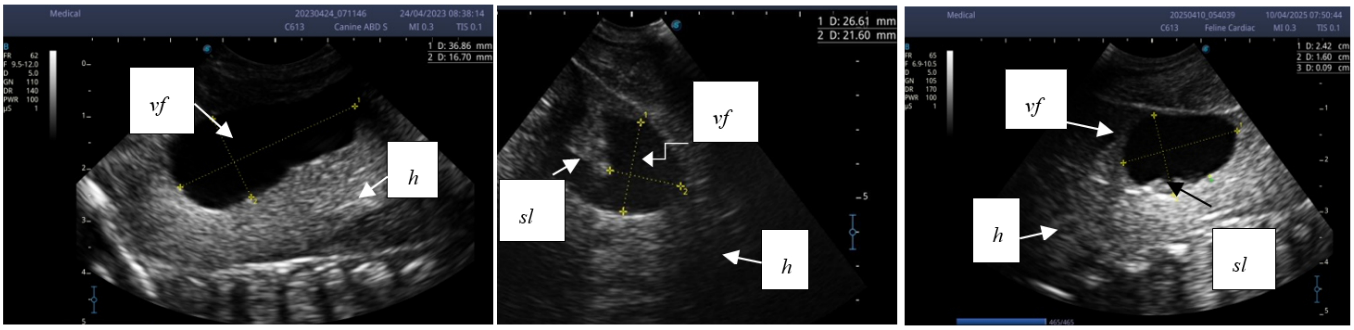


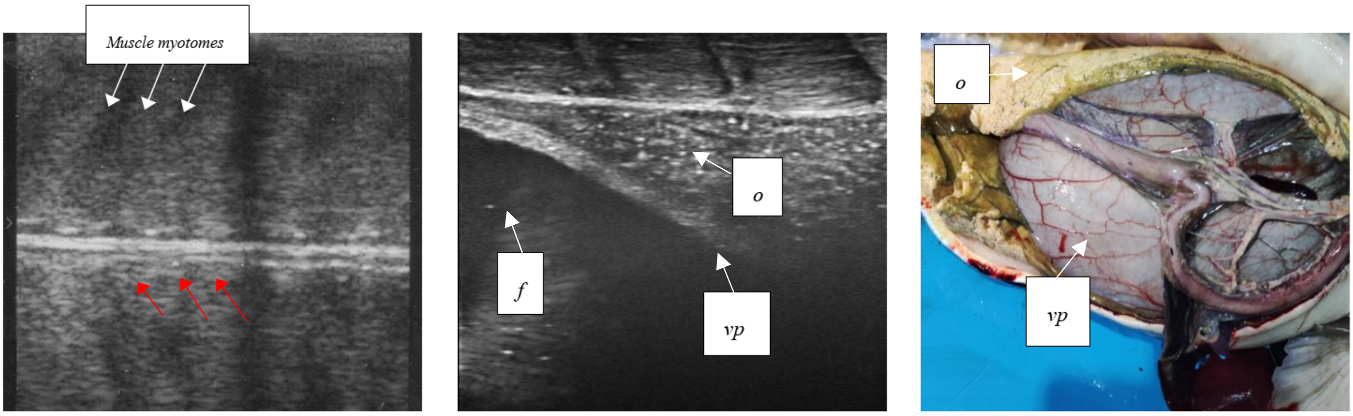
Figure 16. Echograms of the liver (*h*, white arrow) and the gallbladder (*vf*, white arrow) in Siberian sturgeon (aquaculture): (**a**) enlarged gallbladder (*vf,* white arrow), 36.86 × 16.70 mm, Lena population; (**b**,**c**) gallbladder (*vf,* white arrow) with sludge (*sl,* white arrow (**b**)*,* black arrow (**c**)), Yenisei population, 8-yr-old.

In 1.2% of sturgeons, a significant reduction in the size of the gallbladder with thickened walls was noted—length no more than 11.74 mm, and wall thickness was up to 2.10 mm. The formation of biliary sludge may be the first stage of cholelithiasis or gallstone disease. If an individual sturgeon is large, with well-developed gonads, and represents particular value for artificial reproduction (for example, a breeder from the natural habitat), then it is recommended to treat the sludge to prevent the formation of large, dangerous stones [19].

3.10. Pathological Changes of the Swim Bladder on Ultrasound

The swim bladder (*vesica pneumatica*) topographically occupies the entire dorsal region of the abdominal cavity, firmly adhering with its upper wall to the peritoneum. Normally, the swim bladder appears on an echogram as a mirror image artifact or mirage of the real structure occurring behind a highly reflective boundary between media [6].

The gas in the swim bladder shields the tissues and organs located behind it due to signal refraction and the acoustic shadow of the gas (Figure 17a). One of the pathologies of the swim bladder was its filling with fluid (in no more than 0.01% of individuals). On the echogram, such a bladder was visualized as an anechoic structure with thickened hyperechoic walls (Figure 17b). Individuals with such a swim bladder are needed to selection from the broodstock.

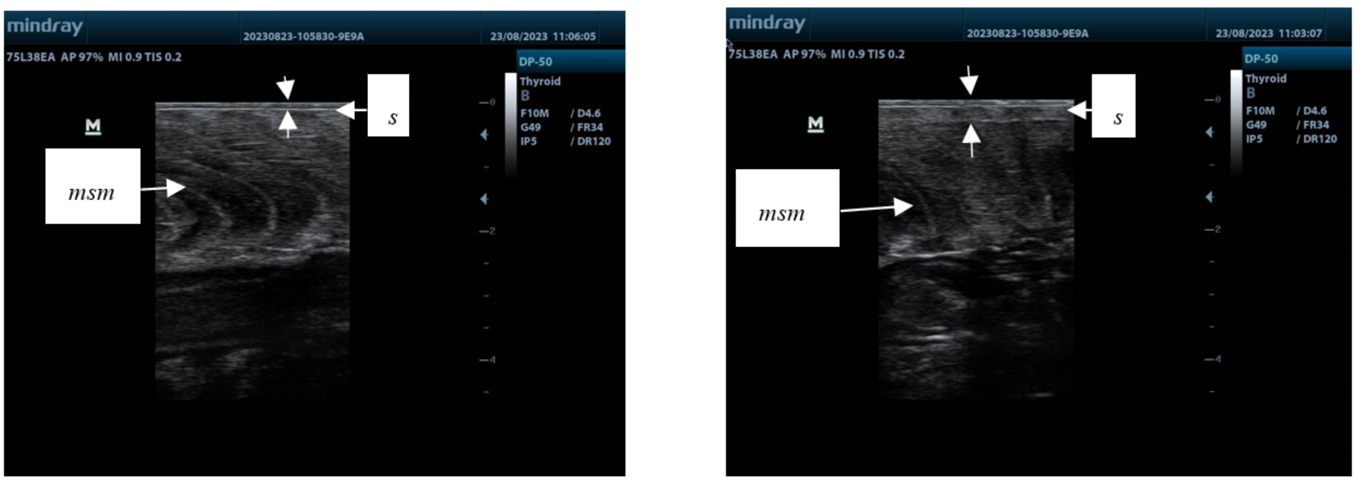


(a) **(b)** **(c)**

Figure 17. Echograms of swim bladder in Siberian sturgeon, cultivated Yenisei population; female, 9-yr-old: (**a**) mirror reflection artefact of muscle myotomes (red arrows), *normal*; (**b**) fluid (*f*, white arrow) visualized in the swim bladder (*vp*, white arrow), ovary (*o*, white arrow), *pathology*; (**c**) fluid-filled swim bladder (*vp*) and ovary (*o*) after fish autopsy.

3.11. Study of Siberian Sturgeons of the Lena Population with 'Ink Spots'

During the domestication of the Siberian sturgeon of Lena population from their natural habitat, two individuals, after a year of artificial keeping in the pools of Chernyshevsky fish hatchery with a recirculating water supply system, were found to have 'ink spots' appear on their skin. After a year, the number of spots in one individual increased more than threefold. Ultrasound revealed a thickening of the skin epithelium in the area of 'ink spot' by 2.0–2.5 times compared to the skin of a healthy sturgeon. On the echogram, the thickening appeared as a band of mixed echogenicity between the skin and muscle tissue, bordered on both sides by a bright hyperechoic line (Figure 18).



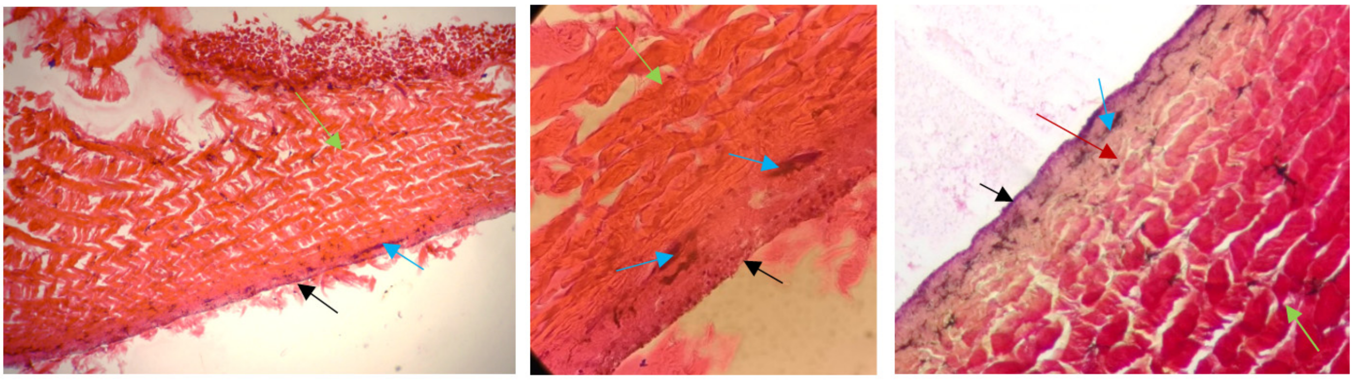
(a) **(b)**

Figure 18. Echograms of the skin (*s*) epithelium (arrows), Siberian sturgeon, wild Lena population: (**a**) a healthy specimen, (**b**) a specimen with 'an ink spot'; myotomes of the spinal muscles (*msm*).

3.11.1. Results of Histological Studies

Sturgeon skin normally has an epidermis (black arrow), dermis (blue arrow), and hypodermis (green arrow) (Figure 19a). In our histological research, the epidermis consisted of three rows of cuboidal cells in the non-keratinized squamous epithelium (black arrow), with oval nuclei located at the center of the cell

(Figure 19b). The epidermis contained melanocytes, in the cytoplasm of which the pigment melanin was found (blue arrow). They were well organized and grouped into dense clusters throughout the dermis under the basal membrane of the epidermis. At the base of the epidermis were elastic fibers (Figure 19c). The dermis was composed of collagen fibers that ran in different directions across several rows and alternated with one another.

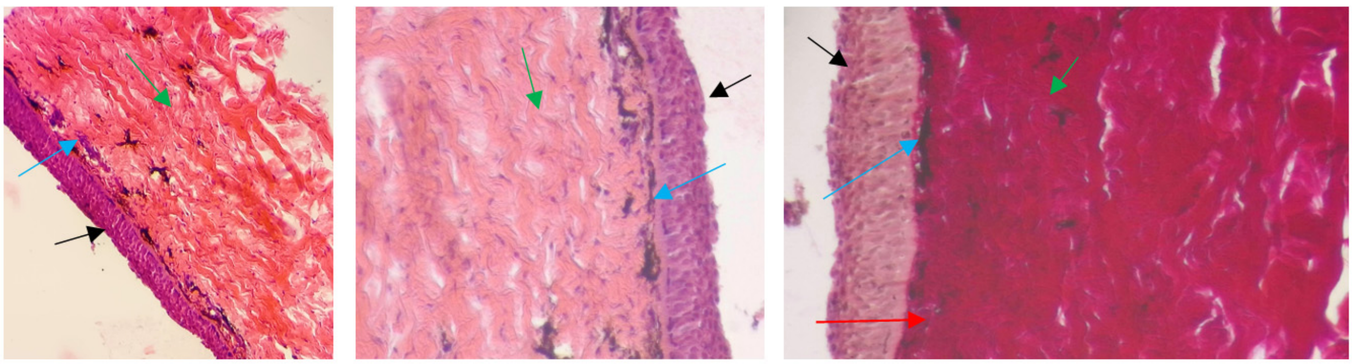


(a) (b) (c)

Figure 19. The histological pattern of the skin, Siberian sturgeon, wild Lena population: (**a**–**c**) epidermis (black arrow), dermis (blue arrow), hypodermis (green arrow); hematoxylin-eosin staining, 100× (**a**), 400× (**b**); (**c**) elastic fibers of the epidermis (claret arrow), collagen fibers of the dermis (red color); Van Gieson staining, 100×.

3.11.2. Sturgeon Skin in Pathology

In the skin of Siberian sturgeon, when 'ink spots' appeared (Figure 20), there was an overgrowth of epidermal cells, initially elongated and rectangular in shape, with nuclei displaced to the periphery, while the other layers were composed of cuboidal cells with small areas of erosion. The number of melanocytes with melanin increased in both the epidermis and dermis; they were arranged in clusters and contained pigment in the lesions. At the same time, elastic fibers in the epidermis were absent when stained with Van Gieson's stain (Figure 20c).



(a) (b) (c)

Figure 20. Histological pattern of the skin with 'ink spot' in Siberian sturgeon wild Lena population: (**a**) epidermal thickening, haematoxylin-eosin staining, 100×; (**b**) epidermal thickening, haematoxylin-eosin staining, 400×; (**c**) collagen dermis fibres (red); Van-Giezon staining, 400×; (**a**–**c**) epidermis (black arrow), dermis (blue arrow), hypodermis (green arrow).

3.11.3. Histological Study of 'Ink Spot'

Histological examination of 'ink spot' showed the following. 'Ink spot' (Figure 21a) consisted along the outer edge of round cells with light cytoplasm, nuclei displaced to the periphery (having a 'ring-like' shape), and the cells were arranged in 2–3 rows. When stained with Van Gieson (Figure 21b) and hematoxylin-eosin (Figure 21c), it was found that the central part consisted of collagen fibers and clumps of melanin ranging from light blue to purple. These changes indicated melanoma. Usually, there is a small difference between precancerous melanosis and melanoma: in the former case, the number of pigment cells (melanophores) is increased but confined to the dermis (most lesions in this study). In the second case, the number of pigment cells (melanophores) also increased, but the melanophores penetrated the underlying tissues.

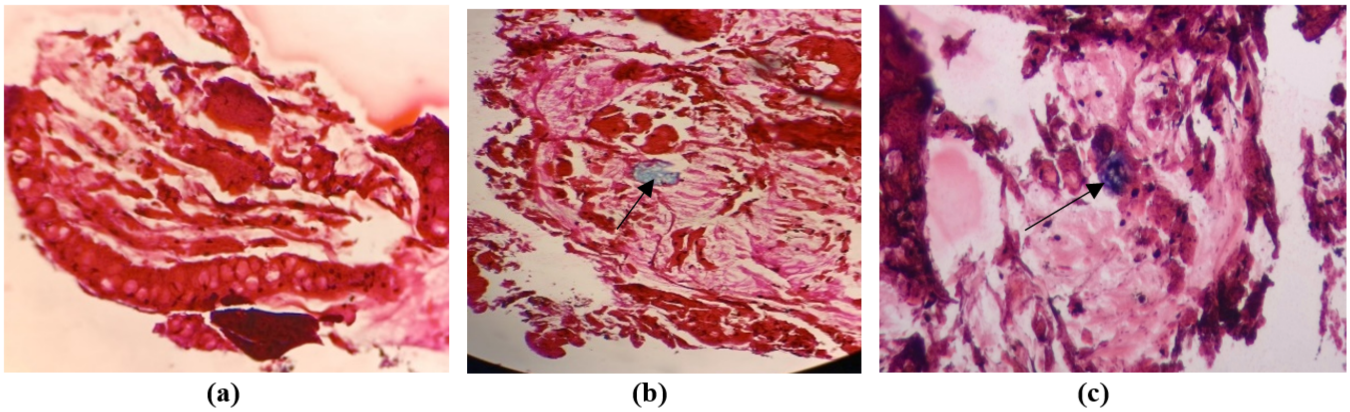


Figure 21. Histological pattern of 'ink-spot' in Siberian sturgeon, wild Lena population: (**a**) hematoxylin-eosin staining, 100×; (**b**) melanocytes with melanin (blue formation, arrow), Van Gieson staining, 200×; (**c**) melanocytes with melanin (arrow), hematoxylin-eosin staining, 200×.

It should be noted that a year after the surgery, visual inspection of the specimen revealed that all the spots had blurred edges, indistinct contours and merged with the natural color of the skin epithelium (Figure 22a–c). Two years after the surgery all 'ink spots' disappeared (Figure 22d).

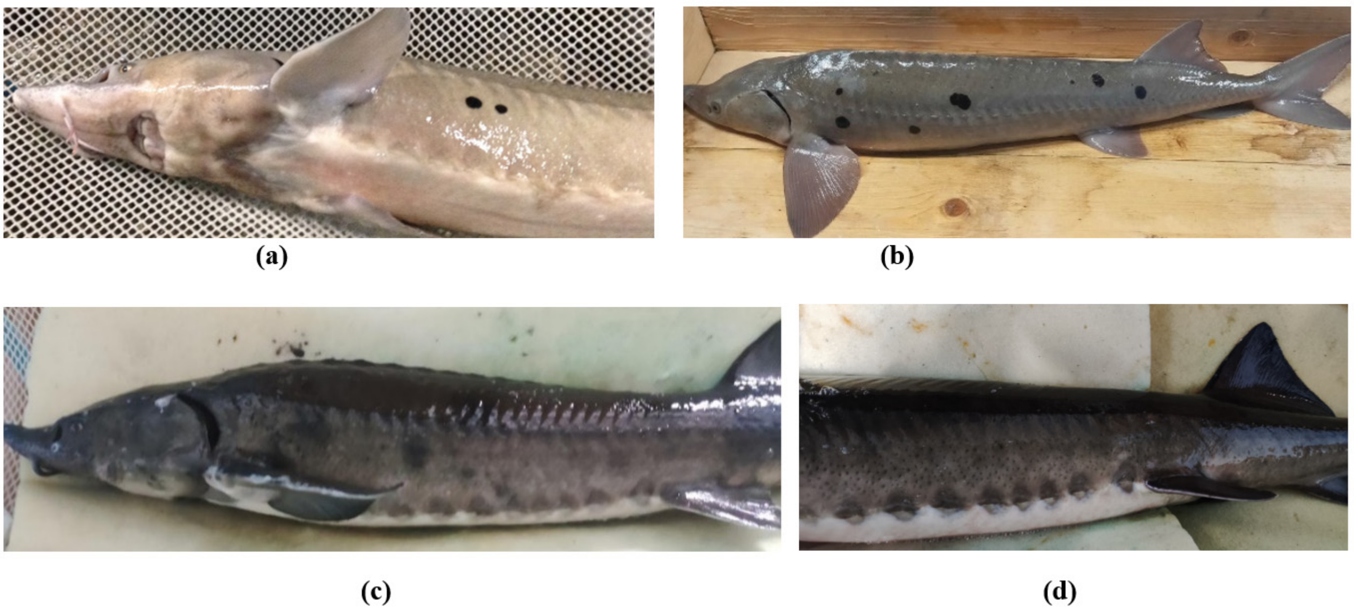


Figure 22. A female individual of the Siberian sturgeon wild Lena population with 'ink spots': (**a**) two years after domestication; (**b**) five years after domestication, before 'ink spot' removal surgery; (**c**) one year after surgery, outlines of the spots are blurred; (**d**) two years after the surgery, all 'ink spots' disappeared.

4. Discussion

Comprehensive studies using various ultrasonography methods have, for the first time, provided statistical probability data on the occurrence of certain pathologies and diseases of the internal organs of sturgeon fish raised at a number of fish farming enterprises in Siberia and the Urals (Table 2).

Table 2. Statistical probability (%) of internal organ pathologies and diseases in sturgeon grown in fish farms of Siberia and the Urals (Russia) revealed by the ultrasonic examination.

Disease, Pathology (Number of Fish—over 100,000)	Age, Year					
	<3	3–5	6–8	9–11	>11	Average
Gonadal fat degeneration	-	1.7	13.8	15.2	7.8	9.6 ± 4.9
Cyst, gonadal polycystosis	-	0.6	1.2	1.4	1.8	1.3 ± 0.4
Ovarian cystic degeneration	-	-	-	3.4	3.8	3.6 ± 0.2
Tumors in the generative tissue	-	-	-	1.6	1.2	1.4 ± 0.1
Hermaphroditism	-	-	0.3	0.1	0.1	0.2 ± 0.1
Underdevelopment, testicular torsion	0.4	1.3	1.4	1.6	1.3	1.2 ± 0.3
Liver cyst, polycystic liver	-	-	0.82	1.44	1.90	1.39 ± 0.09
Gallbladder diseases	-	0.3	1.7	3.5	3.7	2.3 ± 1.3
Swim bladder inflammation	-	-	-	0.01	0.02	0.02 ± 0.01
Hydrops	-	0.05	0.2	0.4	-	0.2 ± 0.1

Our research has shown that in the absence of regular inspections of broodstock using ultrasound, the number of individuals with developmental pathologies of internal organs (fatty degeneration of gonads, testicular atrophy, neoplasms in reproductive tissue, cystic or polycystic gonads and liver, gallbladder diseases, and others) in certain sturgeon farms with severe violations of the biotechnology reached up to 67.9%. On average, across the entire studied region, it was 24.8%. Most often, a single sturgeon individual exhibited not one, but several pathologies. For example, fish with gallbladder pathologies showed changes in the parenchymal tissue of the liver. Excessive fat accumulation in the gonads led to pathological changes in the structure of the ovaries or testes. Two consecutive stages of imaging of ovarian cystic degeneration are described for the first time. It should be noted that the number of females with this condition in the broodstocks was not previously recorded, but in recent years it has increased to 13.6% of individuals, on average, 3.6 ± 0.2%. This pathology is primarily associated with the absence or incomplete selection of fish breeding eggs after artificial overwintering. The occurrence of cystic degeneration of the ovarian germinal tissue can be completely eliminated in a fish farm through the use of timely ultrasound monitoring of sexually mature females in the broodstock, especially before and after obtaining eggs. Ignoring the data from ultrasound studies leads to a decrease in gameto-somatic indices (normally for Siberian sturgeons—20–25%, in pathology—less than 5–8%), an increase in the duration of egg maturation, oocyte resorption, and the formation of malignant tumors. It was found that the number of females with this disease was 1.4 ± 0.1%.

In some fish (2.29 ± 1.31%), various gallbladder pathologies were diagnosed—the presence of sludge, significant wall thickening, shape changes, and others. If the number of individuals with these pathological changes in the gallbladder increases, the daily intake of feed high in cholesterol and animal-derived fatty acids should be limited. In addition, the following medications may be used: Nicodin, Allochol, Festal, Oxafenamide, Digestal, Heptral [19]; the dosages of the drugs should be determined in each specific case. Based on our experience, we recommend using Heptral (active ingredient—ademetionine) for this purpose. Heptral is used to restore liver cells and to treat intrahepatic cholestasis (bile stasis), cirrhosis, and fatty liver degeneration. The drug is administered in the feed at a dosage of 25 mg/kg of fish body weight per day. The therapy is carried out for 7–14 days.

The main reason for the presence of serious internal organ pathologies in older fish (more than 7–9 years old) is the lack of, or infrequent, use of ultrasound diagnostics on fish farms. Often, ultrasound diagnostics is only used for selecting mature sturgeons, ignoring pathological changes in the gonads and not examining other internal organs. The first assessment of Siberian sturgeons should be conducted no later than a year and a half of rearing, following the recommendations below. Minimum weight for ultrasound examination: Siberian sturgeon of the Ob river, the Baikal Lake, and the Yenisei river populations—2.0–2.5 kg, the Lena river population of natural origin—0.8–1.0 kg, from formed brood stock—1.0–1.5 kg; sterlet (rivers Ob, Yenisei)—0.5–0.7 kg, Irtysh River—0.3–0.5 kg. It is necessary to identify individuals with underdeveloped reproductive tissue and adjust their feeding. During subsequent ultrasound examinations (before the fish mature), it is necessary to predict female maturation in the upcoming spawning season, determine egg production, identify diseases and abnormalities in internal organs, determine treatment methods if necessary, and adjust aquaculture techniques.

One of the serious reasons for pathological changes in internal organs is the violation of the biotechnics of sturgeon breeding: the absence or irregularity of artificial wintering with mandatory food deprivation. Often, farms conduct only one short-term (2–3 weeks) artificial wintering before obtaining sexual products. For Siberian sturgeon, the number of wintering grounds should be at least 3–4, with each lasting 2–4 months. The most important aspect of internal organ damage is the use of artificial feed that does not meet the standard requirements for raising animals:

- the energy balance of the feed is disrupted;
- the energy value of the feed does not correspond to the species and fish age;
- daily rations are overestimated or underestimated, *etc*.

There were several other reasons that contributed to the poor quality of sex products when growing fish in RAS, including excessive levels of certain hydro chemical parameters, overestimated fish density, and inbreeding depression.

In some farms, when using underground artesian waters, a constant oxygen deficiency (saturation should be at least 80–100%), an increased content of ammonium ($NH_4^+ > 0.5$ mg/L) and nitrites ($NO_2^- >$ 0.1 mg/L) caused chronic ammonia toxicosis. It led to systemic hypoxia and metabolic disorders, which caused direct and indirect complications of the reproductive system of sturgeon fish. With endocrine disorders, the synthesis of gonadotropic hormones of the pituitary gland was blocked, which disrupted the process of maturation of sexual products [20]. Accumulation of toxins and asphyxia caused calf resorption or delayed ovulation in sturgeon females. During spawning, sturgeon with toxicosis produced non-viable larvae with morphological defects (for example, problems with the formation of the gastrointestinal tract, spinal curvature, underdevelopment of the circulatory system, *etc*.) (Figure 23a).

In some farms, the high fish density of the broodstocks (more than 50 kg/m^2) was the strongest stress factor limiting the formation of high-quality producers (Figure 23b). It is known [20] that the level of cortisol in the blood increases due to over-crowding of individuals in the BS. Cortisol blocks the synthesis of sex hormones. Reserving producers in small pools with high fish densities during the spawning period led to the development of a chronic stress response (Figure 23c). Chronic stress caused a decrease in the level of sex steroids, which, in turn, led to an increase in the number of immature fish and a decrease in the quality of sex products in mature ones. According to materials from different years [20], the normal cortisol content in beluga female whales in the plant ranged from 50 to 80 ng/mL and in isolated individuals with injuries that die at the onset of spawning temperatures, the cortisol level was ~200 ng/mL.

Figure 23. (**a**) Larva of Siberian sturgeon of the Yenisei population with an undeveloped gastrointestinal tract, the age—32 days; (**b**) maintenance of the broodstock of Siberian sturgeon of the Yenisei population at an overestimated fish density; (**c**) spawning of Siberian sturgeon in small cramped pools.

One of the reasons for the appearance of sturgeon individuals with low-quality sexual products in fish farms was inbred depression. Closely related interbreeding in RAS led to deformities, decreased larval survival, and decreased female fertility.

On some farms, sturgeon fish were selected for broodstocks only based on their growth rate, while the fastest-growing individuals often had defects in their reproductive system. Ultrasound diagnostics was an excellent aid in assessing the quality of the testes and ovaries, despite the external similarity among sturgeon individuals.

Regular ultrasound examinations of the gonads, liver, gallbladder, and heart (at least twice a year) will help to adjust the feeding rate and composition of the feed in a timely manner, thereby preventing the development of serious and irreversible pathologies. The use of Dopplerography in polycystic ovary syndrome made it possible to assess qualitative changes in intra organ blood flow, since conventional ultrasound showed only the shape and structure of the organ, while Doppler examined the vascular network. In the study of cysts in other organs (testes, liver), color Doppler mapping was used to assess blood flow within and around the structures, which was a key criterion for differential diagnosis. The classical ultrasound examination of sturgeon fish, described in detail by Chebanov M.S. in B-mode [5–7], made it possible to visualize the cavity, but it was the Doppler that helped to instantly distinguish a small cyst from an enlarged blood vessel or aneurysm, which in normal grey scale mode may look the same (as anechoic rounded structures). There was no blood flow inside the cavity and along the walls of a simple benign cyst (avascular formation).

Histological studies of the skin epithelium of naturally occurring Siberian sturgeon, following the first-ever *in vivo* ultrasound-assisted procedure to remove 'the ink spot', revealed the presence of melanoma on the skin. It is necessary to note the promise of performing this surgical procedure for wild sturgeon breeders, which, after a year of rearing, leads to the complete disappearance of spots on the fish body. The etiology of the appearance of 'the ink spots' is not yet known, but it is quite likely related to the stress experienced by individuals during the domestication period—a significant weight loss (up to 25.6%) and a considerable slowdown in gametogenesis.

5. Conclusions

The systematic use of ultrasound monitoring, combined with histological studies, to assess the condition of the internal organs of sturgeon fish allows early stages in the formation of sturgeon broodstocks to be identified and adopted fish farming techniques adjusted. The use of ultrasound screening opens new opportunities to determine the optimal temperature regime, feeding standards, and feeding rations, as well as to detect reproductive maturation disorders and internal organ pathologies at an early stage. This ensures food security for commercially farmed fish raised under industrial conditions and contributes to the sustainable development of Russia's fisheries. Unfortunately, there are no published data on the economic efficiency of using ultrasound monitoring in the formation of sturgeon broodstocks, but it should be noted that in those farms where ultrasound screening of fish internal organs is regularly performed and the biotechnological cultivation scheme is adjusted, the number of individuals with pathologies decreases by 2–5 times.

Through comprehensive studies of fish pathologies using the highly informative diagnostic power of ultrasound and laboratory histological analysis, it is possible to detect dangerous diseases such as fish skin cancer at early stages and outline ways to treat it. The likely cause of the appearance of 'the ink spots' is the stressful condition of sturgeon from their natural habitat when they are transited in RAS for care and feeding.

Statement of the Use of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used Google Gemini service in order to obtain information on the potential use of various portable ultrasound scanners in fish farming. After using this service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Author Contributions

Conceptualization: M.A.K. and A.I.L.; Methodology: M.A.K.; Software: A.G.E. and M.A.K.; Validation: A.G.E. and M.A.K.; Formal Analysis: M.A.K. and A.I.L.; Investigation: M.A.K., A.I.L. and A.G.E.; Resources: M.A.K. and A.G.E.; Data Curation: M.A.K. and A.I.L.; Writing—Original Draft Preparation: M.A.K.; Writing—Review & Editing: M.A.K.; Visualization: M.A.K.; Supervision: M.A.K. and A.I.L.; Project Administration: M.A.K. and A.I.L.; Funding Acquisition: A.I.L., A.G.E. and M.A.K.

Ethics Statement

All animal experiments were approved by the Research Ethics Committee of State Agrarian University of Northern Trans-Urals (Tyumen, Russian Federation) (Protocol No. 16 dated 15 February 2018) and were carried out in strict accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123) and the National Standard of the Russian Federation GOST 33215-2014 (Guidelines for accommodation and care of animals).

Informed Consent Statement

Informed consent was not applicable.

Data Availability Statement

All research data described in the article can be obtained from online sources.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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