PHYSIOLOGY

The role of stem cell potential in the regeneration of the liver in *Cyprinus carpio* during postembryogenesis

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— the Helsinki Declaration of 2000. "On the humane treatment of animals";
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Abstract

The polyfunctionality of the liver and the high level of regeneration explain the enormous interest in the study of regeneration mechanisms, which have been largely studied in mammals. At the same time, the study of regeneration mechanisms in lower vertebrates, such as fish, provides important information regarding the conserved mechanisms also present in higher vertebrates. The present study focuses on the role of stem potential in liver regeneration of fish species *Cyprinus carpio* under physiological normal conditions during post-embryogenesis. From the first to the third year of postembryogenesis, a significant decrease in the number of haematopoietic stem CD34+CD45+ cells (haematopoietic progenitor cell population) was detected, whereas the number of CD34+CD45– cells (haemangioblast population) remains relatively constant. From the first to the third year of postembryogenesis, the number of intrahepatic stem cell precursors CK19+ cells (intrahepatic progenitor cells) increases.

Keywords: oval cells, liver progenitor cells, regeneration, fish, postembryogenesis, stem cells, immunophenotyping, immunohistochemistry, hepatocyte, hepatic acinus.

Introduction

The liver, a polyfunctional and structurally heterogeneous organ, is a central regulator of metabolism, detoxification, functions as a 'peripheral integrator' of the energy needs of the body in this regard, ensures the maintenance of homeostasis of the body as a whole (Gernhöfer et al., 2001; Young, Woodford, and O'Dowd, 2013; López-Luque and Fabregat, 2018; Akulenko et al., 2019; Gardner, Laurin, and Organ, 2020; So, Kim, Lee, and Shin, 2020; Leão et al., 2021; Gao and Peng, 2021).

A distinctive feature of the organ is its high ability to regenerate. Regeneration of the liver, on the one hand, is a long-standing and widely studied process, but on the other hand, it is still unknown. In this regard, the regenerative potential of the liver is an inexhaustible source for scientific research, which finds its application in fundamental and applied aspects (Choi, Ninov, Stainier, and Shin, 2014; He, Lu, Zou, and Luo, 2014; Lemaigre, 2015; King et al., 2017; Siapati, Roubelakis, and Vassilopoulos, 2022; Oderberg and Goessling, 2023).

The first studies of liver regeneration are directly related to the introduction of resection of part of the organ into medical practice at the turn of the 19th and 20th centuries (Kordes and Haussinger, 2013). In the course of numerous experiments in 1931 G. Higgins and R. Anderson developed a classic surgical model — partial (68–75 %) hepatectomy — which is still widely used in studies of liver

regeneration mechanisms (Castorina et al., 2008; Zhang, Theise, Chua, and Reid, 2008; Onishchenko et al., 2011; Vestentoft, 2013). Removal of two-thirds of the liver results in compensatory hyperplasia and hypertrophy of the remaining lobes to restore the original liver mass in rodents; this proliferative response is maintained in fish (Hong, Li, and Hong, 2011; López-Luque and Fabregat, 2018; Bram et al., 2021).

The mechanism of liver regeneration in lower vertebrate fish and amphibians (cold-blooded/ectothermic animals), in contrast to higher vertebrate mammals (warm-blooded/endothermic animals), has not been studied in such detail (Akiyoshi and Inoue, 2004; Antonova, Omarova, Firsova, and Krasnikova, 2024). Whereas the analysis of the stem regenerative potential of fish liver will largely expand the significance of the evolutionary factors responsible for the formation of high regenerative capacity of the liver as an example of the complexity of structural and functional organisation of organs. It will also expand the understanding of such a fundamental problem of biology as the analysis of regularities of formation of reactivity and plasticity of functionally similar tissues in evolution from the position of the theory of parallelism (Pritchard, 2002; Scholz, 2002).

In the evolution of various species, temperature is one of the factors that ensures the development, growth, proliferation, function and cell death in the whole organism system in ecto- and endothermic animals (Shahbazov, 2001; Bayne, 2004; Vinogradov, 2005; Frampton et al., 2006). Studies on lower vertebrates provide important information regarding the conserved mechanisms of liver regeneration also present in higher vertebrates.

Most of the works are focused on modelling liver injuries and studying the regenerative potential on the example of various diseases of higher vertebrates (Wang et al., 2015). The activation of both hepatocytes of different subtypes and liver progenitor cells (LPCs) has been observed in various models of liver injury. At the same time there are not enough studies on the analysis of mechanisms and sources of liver regeneration in lower vertebrates under physiological normal conditions. In this regard, it is relevant to study the role of LPCs in the processes of physiological regeneration (So, Kim, Lee, and Shin, 2020; Miyajima, Tanaka, and Itoh, 2014; Duncan, Dorrell, and Grompe, 2009).

Regeneration proceeds in several phases — priming, progression, termination, remodelling of liver parenchyma (Yanger et al., 2014; López-Luque and Fabregat, 2018). Two pathways can be distinguished to trigger liver regeneration. The first one — through hepatocyte proliferation (considered the main one). The second pathway involves both intrahepatic and haematopoietic LPCs, which includes triggering mechanisms such as dedifferentiation of biliary epithelial cells (BECs) or hepatocytes into LPCs; proliferation of LPCs and subsequent differentiation of LPCs into hepatocytes. The second pathway is usually triggered when the first pathway fails to work efficiently (Mancino et al., 2007; Chen et al., 2012; Shin and Monga, 2013; Font-Burgada at el., 2015; Wang et al., 2015; Lopez-Luque and Fabregat, 2018; So at el., 2020; Bruno et al., 2021; Oderberg and Goessling, 2023).

LPCs are variously called oval cells because of the large ratio of nuclei to cytoplasm and the oval shape of the nuclei (Farber, 1956; Tatematsu et al., 1984), hepatic progenitor cells, hepatic stem cells, and ductal cells because they are located in Goring's canals. When the first regeneration mode is ineffective, activation of the second regeneration mode triggers dedifferentiation of progenitor cells into hepatocytes. It is also known that CK19-positive portal cells migrate along connective tissue septa and differentiate into cells of two lineages: cholangiocytes forming interval bile ducts and hepatocytes (Kowalik et al., 2015; Lebedeva, 2021). More recent studies have shown that "oval cells" are EpCAM+ (Suzuki et al., 2008; Okabe et al., 2009), marked expression of marker genes TROP2 and Folx1, which were almost undetectable in intact liver (Sackett et al., 2009). EpCAM+–positive cells isolated from damaged liver can proliferate with colony formation and differentiate into both hepatocytes (Alb+, Afp+) and Ck7+– and Ck19+–positive BECs. The origin of LPCs from BECs has been confirmed in various models of liver injury (Yanger et al., 2014; Raven et al., 2017). It has been shown that BECs dedifferentiate into LPCs, and LPCs can differentiate into hepatocytes, a population of which, at the end of liver regeneration, differentiates again into LPCs. In severe liver injury, dedifferentiation of BECs-to-LPCs is noted, and LPCs then differentiate into either hepatocytes or BECs. These mechanisms identified in human liver are supported by studies in mice and *Danio rerio* (Choi, Ninov, Stainier, and Shin, 2014; He, Lu, Zou, and Luo, 2014; Huang et al., 2014; Stueck and Wanless, 2015; Russell et al., 2019; Manco et al., 2019).

Despite the lack of LPCs-specific markers that are expressed in LPCs but not in BECs, BECs are thought to be the source of LPCs because of their phenotypic similarity and topographic proximity in the acinus (Fausto and Campbell, 2003; Tarlow et al., 2014; Raven et al., 2017). The fact that the activated LPCs differentiated into hepatocytes indicates that hepatocytes may be an additional source of LPCs (Yanger et al., 2014), which is supported by overexpression of constitutively active YAP1 (Yimlamai, 2014) or Notch (Yanger et al., 2013; Tarlow, Finegold, and Grompe, 2014). Inhibition of YAP or Notch signalling in hepatocytes suppresses their dedifferentiation into LPCs, which was shown on the example of *Danio rerio* (So, Kim, Lee, and Shin, 2020).

LPCs express both hepatocyte markers — KRT8, KRT18 and albumin Ov6 (Li et al., 2014; Ma et al., 2019)

and BECs — KRT7, KRT19, EpCAM and SOX9 (Furuyama et al., 2011). Thus, in particular, Sox9-positive hepatocytes are localised in the portal tract region (periportal hepatocyte population, hybrid) (Font-Burgada et al., 2015; Li et al., 2019). Axin2-positive cells are localised in the central vein region and represent a universal transcriptional target of β-catenin-dependent Wnt signal transduction, as evidenced by the exclusive expression of Wnt2 and Wnt9b in central vein endothelial cells (Wang et al., 2015).

This result suggests that Axin2-positive pericentral vein hepatocytes are not highly capable of regeneration (Sun et al., 2020; Chen et al., 2020).

Depending on the nature of the injury, LPCs can express hepatoblast marker α-fetoprotein (AFP), haematopoietic markers such as CD34, CD90, CD133, c-Kit, CXCR4 and Sca1 (Cardinale et al., 2011). Mesenchymal stem/stromal cell markers include CD29, CD44, CD73, CD90, HLA class I (Kholodenko et al., 2019), hematopoietic/endothelial cell markers include CD11b, CD14, CD19, CD31, CD34, CD45, CD79β, CD117, CD133, CD144, and HLA-DR (Bruno et al., 2021). Depending on the damage conditions, LPCs can also express the hepatoblast marker α-fetoprotein and the haematopoietic markers CD34, CD90, CD133, c-Kit, CXCR4 and Sca1 (Durnez et al., 2006). This broad spectrum of marker expression in LPCs reflects their heterogeneous nature (Cardinale et al., 2011; Dorrell et al., 2011; Furuyama et al., 2011; Li et al., 2014; Pepe-Mooney et al., 2019; Planas-Paz et al., 2019).

The interactions of LPCs with the liver microenvironment during regeneration are provided by paracrine mechanisms. In particular, the possibility that liver Ito stellate cells (HSCs) are a source of LPCs and regenerated hepatocytes has been considered; other sources do not support this possibility, which increases the uncertainty about HSCs as a source of LPCs (Mederacke et al., 2013; Kordes et al., 2014; Schaub, Malato, Gormond, and Willenbring, 2014; Swiderska-Syn et al., 2014). In addition, organ-specific liver macrophages (Kupffer cells) are known to participate in the activation of LPCs during regeneration by overexpressing tumour necrosis factor-like TWEAK in hepatocytes, which stimulates the proliferation of LPCs through the nuclear factor-κB (NF-κB) signalling pathway (Tirnitz-Parker et al., 2010).

In addition to the liver injury model in higher vertebrates, various models of liver injury in *Danio rerio* have been established, including partial hepatectomy of one third (Goessling et al., 2008; Oderberg and Goessling, 2021), ethanol treatment (Huang et al., 2014), oncogene-induced liver cancer (Wang et al., 2017), and hepatocyte ablation to study regeneration mechanisms (Wei et al., 2021; Oderberg and Goessling, 2023; Cox et al., 2014).

In a model of severe hepatocyte injury in adult *Danio rerio*, BECs act as facultative liver stem cells in an EGFR-PI3K-mTOR-dependent manner (Clevers and Watt, 2018; Oderberg and Goessling, 2023), activating hepatocyte proliferation (He, Lu, Zou, and Luo, 2014; He et al., 2019; Michalopoulos and Bhushan, 2021). BECs of two types are identified in *Danio rerio*: intrahepatic, in close proximity to hepatocytes (Ellis et al., 2018), and extrahepatic, located in the ducts draining bile from the liver. Due to this, intrahepatic BECs may be the equivalent of oval cells in *Danio rerio*, they express transcription factors associated with hepatoblast identity (Oderberg and Goessling, 2023). At the same time, inhibition of the differentiation process of LPCs negatively affected the processes of liver regeneration and repair (Ko et al., 2019).

The aim of this study is to investigate the role of liver stem cells in *Cyprinus carpio* fish as a source of regeneration during post-embryonic development under normal physiological conditions.

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Materials and methods

The study was conducted on 90 male individuals of the *Cyprinus carpio* species, aged one (0+), two (1+), and three (2+) years post-embryogenesis, with 30 individuals per period. The fish were raised under standard conditions at the "Fish place" fish farm (Omsk, Russia). For each post-embryogenesis period, 30 liver samples were collected. The fish weighed 23.3 g at one year, 710.5 g at two years, and 1107.4 g at three years. Studies were carried out after anaesthesia with lidocaine ($C_{14}H_{22}N_2O$) (0.4 g/L) with a dose of 0.08 g, by applying the anaesthetic in a bath of water, to irrigate the gills.

The liver for research was fixed in 10 % neutral buffered formalin (LLC "Ergo production", Russia), and serial sections were made with hematoxylin-eosin staining using the standard methodology (Mishhenko, Petrova, and Medvedeva, 2017) for further morphological analysis of liver histotopography.

The analysis of intrahepatic and haematopoietic markers was conducted using immunohistochemistry and immunophenotyping methods. To investigate the expression of markers for liver progenitor cells and hematopoietic stem cells, antibodies against CK19 were used as a marker for oval cells (intrahepatic ductular structures in the area of the canal of Hering) and CD34 and CD45 were used as markers for stem cells/precursors of hematopoietic precursor cells, according to the laboratory-optimized protocol.

Immunohistochemical analysis of liver sections was performed. The CD34 antigen clone RAM34 (Invitrogen, USA) and CK19 antigen clone RCK108 (Abcam, UK) were detected after HIAR demasking. Liver histological sections were deparaffinized and dehydrated, then incubated with primary and subsequently with biotinylated secondary antibodies (Link, PrimeBioMed LSAB+Kit Peroxidase). The sample was washed and incubated

with streptavidin conjugated to horseradish peroxidase (Streptavidin, PraimBioMed, LSAB+Kit Peroxidase). A solution of aminoethylcarbazole and hydrogen peroxide was used as the substrate for the peroxidase reaction.

Morphological analysis of histological specimens was performed using the AxioImagerA1 light microscope (Carl Zeiss, Germany). Histological sections were digitized using the Pannoramic SCAN scanning microscope (3DHISTECH, Hungary) and processed using the ZEN software (Carl Zeiss, Germany).

Immunophenotyping markers study

Immunophenotyping was performed on a flow cytometer (Partec CyFlow space, Germany) according to the recommendations and protocols of the EuroFlow consortium (Kalina et al., 2012; Beznos et al., 2017). Monoclonal antibodies against CK19 (Beckman Coulter, USA), CD34, and CD45 (Beckman Coulter, USA) were used to study the expression of markers of liver progenitor cells and hematopoietic stem cells by immunophenotyping. The histograms were analysed using FloMax software (Germany). Two sources of radiation were used simultaneously — a blue laser at 488 nm and a red laser at 638 nm; 4 colours (FL1–FL3+FL4).

Statistical analysis of the obtained data was performed using Prism 8.0.1 software package (Graphpad, USA). The main statistical characteristics of the studied parameters (mean, median, variance) were determined in the first stage of data analysis. Normality of data distribution was assessed according to the Shapiro — Wilk hypothesis. The significance of differences between independent samples was evaluated using the Mann — Whitney U test. Differences were considered significant at a level of $p < 0.05$ (Kremer, 2004).

Results and discussion

The liver parenchyma consists of parenchymal cells (hepatocytes) and non-parenchymal cells. Hepatocytes are irregularly shaped, pentagonal or hexagonal, with an expanded basal portion and a narrowed apical portion, resembling truncated pyramids (Fig. 1). Hepatocytes are arranged radially around the perifocal central vein. The parenchymal hepatocytes are represented by trabeculae, which form tubular structures surrounded by sinusoidal capillaries, consistent with the literature (Akoul and AL-Jowari, 2019). The sinusoids form tubular-loop structures, with the central lumen being surrounded by more than six hepatocytes.

The bile duct is present both within the portal tract (portal-tract type) and separately in the parenchyma — isolated type (Fig. 2).

The liver parenchyma contains the pancreas in the form of an acinar structure, separated from the liver parenchyma by a thin layer of connective tissue. The pancreatic islets are located around blood vessels of varying sizes and shapes, sometimes associated with the bile duct (Fig. 1).

The immunohistochemical study revealed the presence of CD34-positive hematopoietic stem cells and CK19-positive cells in all zones of the acinus (periportal, perivenular, and centrilobular) as well as in the area of the bile duct, both in the portal tract and in the isolated type of the bile duct. The localization of these cells does not exhibit zonality.

Based on the results of the immunophenotyping of liver stem cells (Figs 3–5), the following was identified:

— during the first three years of post-embryonic development, there is a statistically significant decrease in the number of **CD34+ CD45+** cells. Specifically, there is a 5 % decrease ($p = 0.39$) from the first to the second year, and a 37 % decrease (779 ± 61, $p < 0.0001$) from the second to the third year. Overall, there is a 40 % decrease (881 ± 104, $p < 0.0001$) in the number of CD34+ CD45+ cells from the first to the third year of post-embryonic development;

— during the first three years of post-embryogenesis, there is an increase in the number of **CD34+ CD45–** cells by 6 % ($p = 0.52$), with a slight decrease in the second year compared to the first year by 23 % (783 ± 326, $p = 0.02$). By the third year, there is an increase of 37 % (985 ± 123, $p < 0.0001$) compared to the second year;

— from the first to the third year of post-embryonic development, there is a statistically significant increase in the number of **CK19+** cells: in the second year, it increases by 15 % ($1{,}451 \pm 616$, $p = 0.02$) compared to the first year, and by the third year, it increases by 14 % ($1{,}374 \pm 570$, $p = 0.02$) compared to the second year. The overall increase in the number of CK19+ cells from year 1 to year 3 of post-embryonic development is 25 % ($2{,}824 \pm 659$, $p = 0.0001$).

It is widely recognized that the liver mass is maintained within a very narrow range relative to the total body mass, known as the hepatic index, which is maintained by tissue homeostasis mechanisms — cell death and proliferation (Cienfuegos et al., 2014). This unique interrelation is called the “hepatostat” (Delgado-Coello, 2021). Our study highlights the involvement of stem cell potential in maintaining this ratio under conditions of physiological normality in fish.

Liver progenitor cells create a niche within the zones of the hepatic acinus (Dollé et al., 2010). This niche is a specialized microenvironment consisting of parenchymal and non-parenchymal cells that release growth factors and cytokines, receive signals, and interact through the extracellular matrix (ECM), growth factors (GFs), and signaling pathways (Chen et al.,

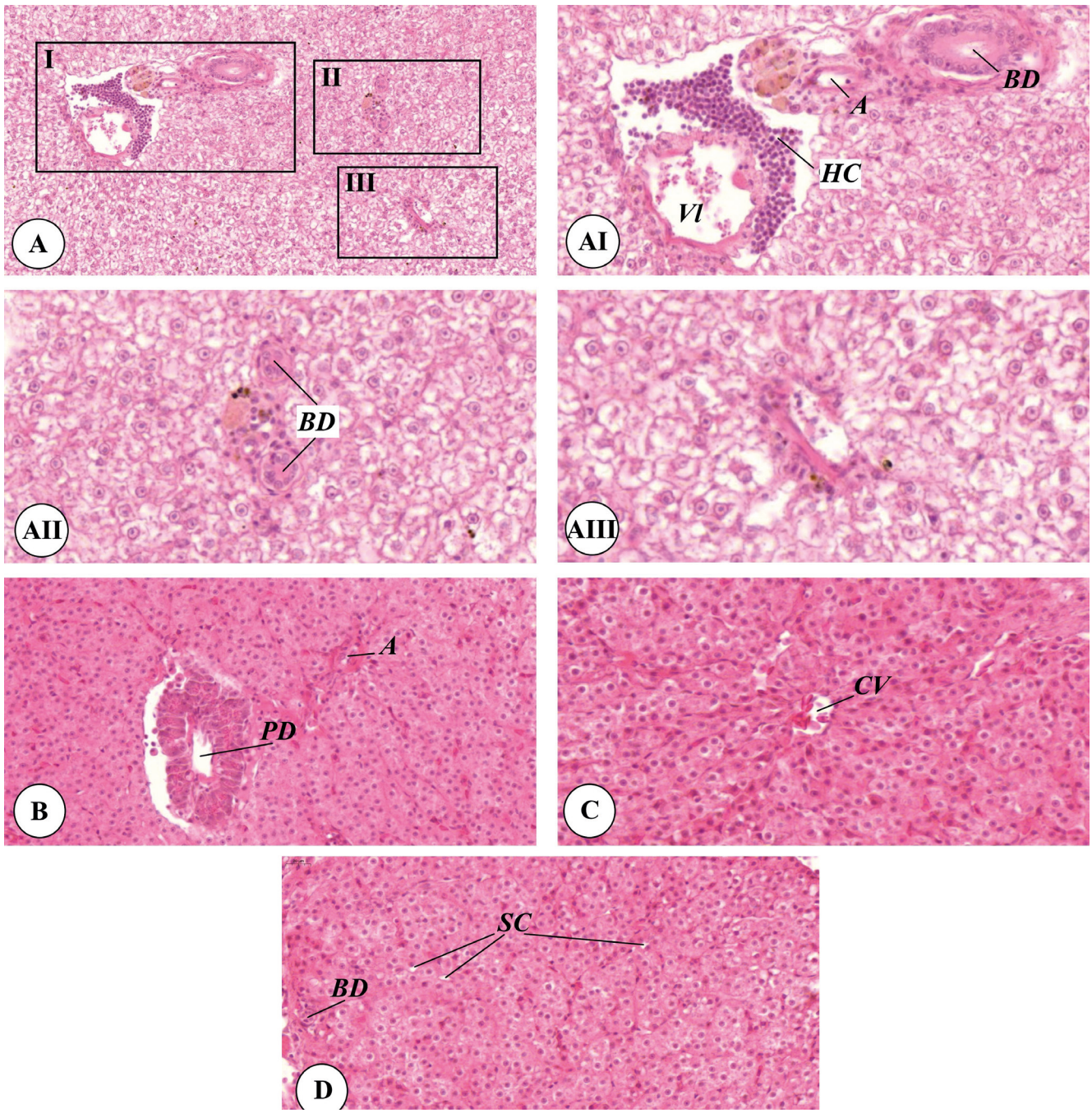

Fig. 1. Section of the fish liver of the species *Cyprinus carpio.* Haematoxylineosin staining. Magnification of figures: A, B, C, D — 50 μm; AI, AII, AIII — 20 μm. A — liver acinus: I — periportal zone; II — centrilobular zone; III — perivenular zone. AI — periportal zone of hepatic acinus (BD — portal-tract type of bile duct; A — arteriole; HC — haematopoietic cells; Vl — venule. AII — bile duct group — BD; AIII — portal tract of hepatic acinus; B — centrilobular zone with pancreatic duct; C — perivenular zone of the hepatic acinus (CV — central vein); D — centrilobular zone of hepatic acinus (BD — isolated type of bile duct; SC — sinusoid capillaries).

2012; Vestentoft, 2013; Chen et al., 2017; López-Luque and Fabregat, 2018). In our study, we examined several niches, including the canals of Hering, intrahepatic bile ducts as a portal-tract type of bile duct, as well as isolated perivenular and centrilobular zones of the hepatic acinus. Thus, within the hepatic acinus of *Cyprinus carpio* fish, all zones serve as niches for both hematopoietic and intrahepatic stem precursor cells. The niches of liver precursor cells support and regulate stem cells to ensure organ homeostasis and regeneration (Dollé et al., 2010).

Cytokeratin 19 is considered one of the molecular markers of oval cells, which provide oval cell proliferation (Jensen et al., 2004; Duret et al., 2007; Castorina et al., 2008; Dollé et al., 2010; Miyajima, Tanaka, and Itoh, 2014; Szücs et al., 2020). The expression of both hepatocyte and biliary markers reflects the bipotential nature

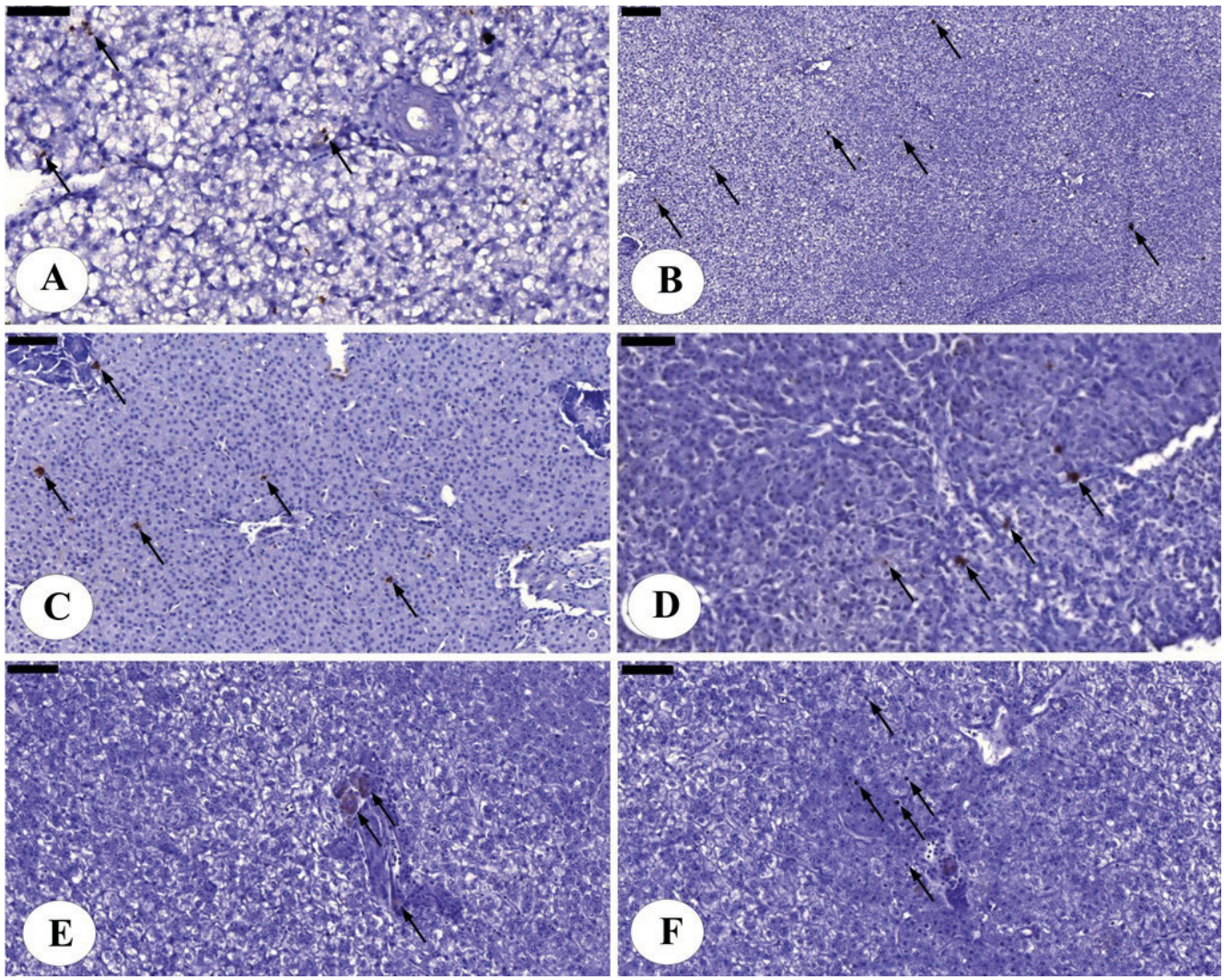

Fig. 2. Section of the liver of fish of the species *Cyprinus carpio.* A, C, D, E, F — 50 μm; B — 100 μm. A — Isolated type of bile duct in the centrolobular zone of the hepatic acinus. CK19-positive cells; B — Parenchyma of the hepatic acinus. CK19-positive cells; C — Parenchyma of the hepatic acinus. CD34-positive cells; D — Parenchyma of the hepatic acinus. CD34-positive cells; E — Portal tract area. CD34-positive cells; F — Central vein area. CD34-positive cells.

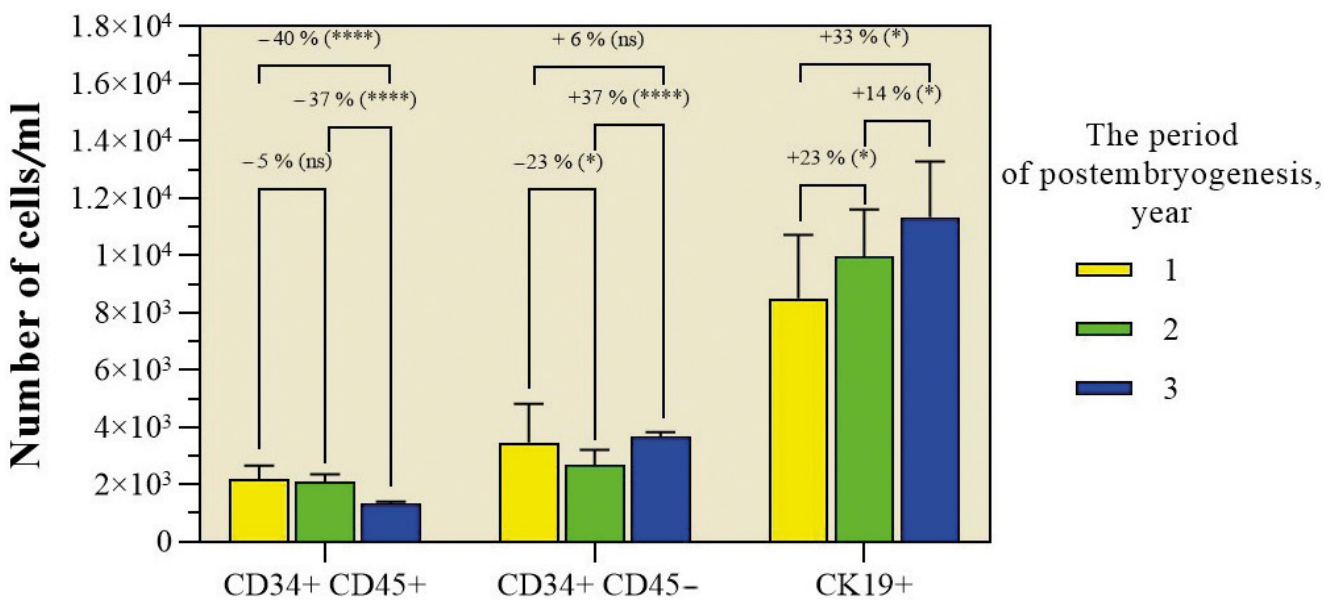

Fig. 3. Immunophenotyping of the liver stem potential of fish species *Cyprinus carpio*.
Note: the differences were considered significant at the significance level: * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$, **** — $p < 0.0001$.

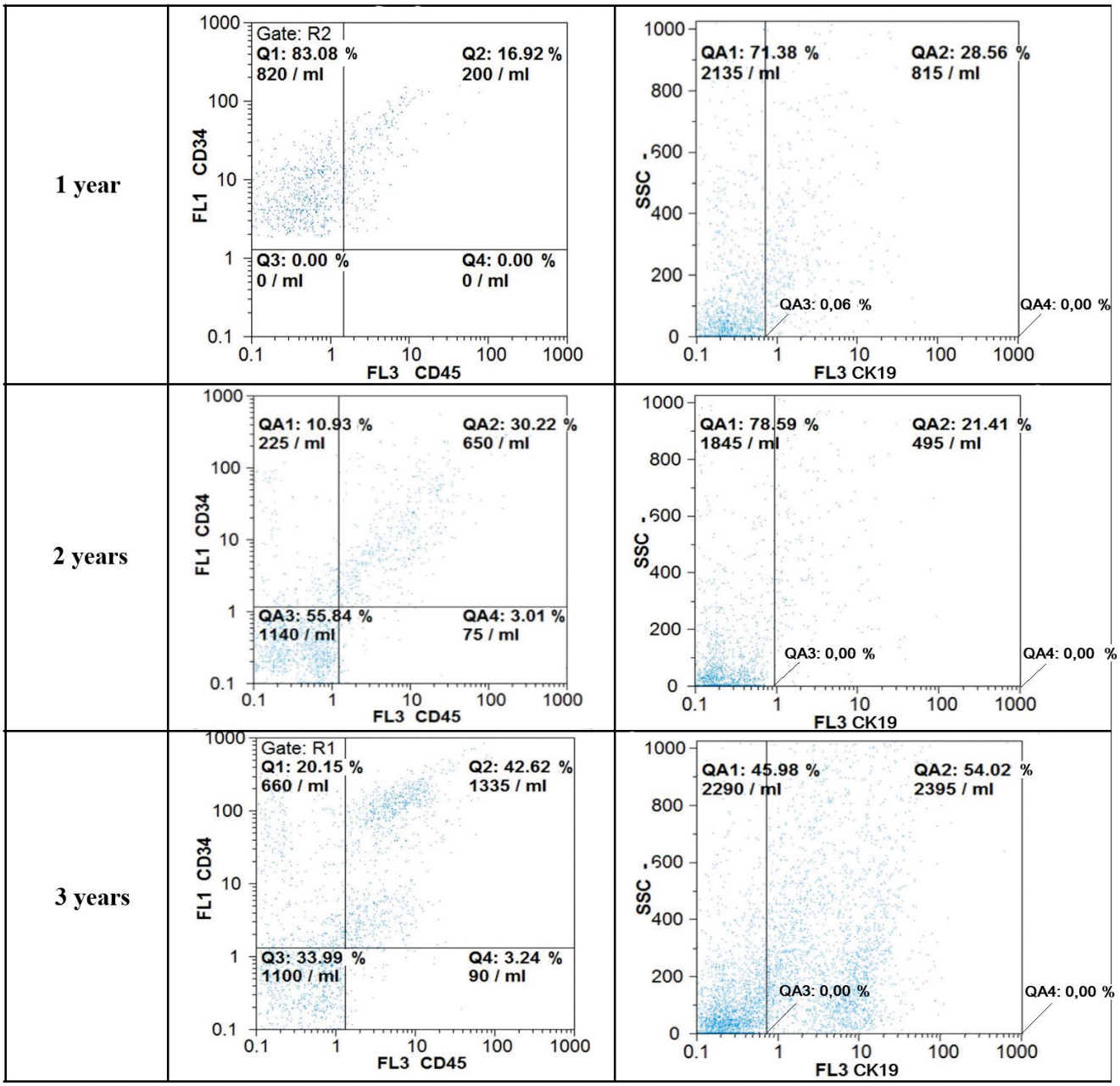

Fig. 4. Flow cytofluorimetry. Dynamics of the number of cells with $CD34^{+}CD45^{+}$, $CD34^{+}CD45^{-}$, $CD19^{+}$ — positive immunophenotype cells in the first year of postembryogenesis in the liver of fish species *Cyprinus carpio*.

of liver stem cells with respect to both the hepatic and biliary lineages, according to their immature and bipotential phenotype (Gaudio et al., 2009). The acquisition of an intermediate transamplifying phenotype involves the progressive loss of biliary cytokeratins, particularly during differentiation towards the hepatocytic lineage. Finally, complete maturation into hepatocytes is characterized by the complete disappearance of biliary markers. In contrast, immature cholangiocytes maintain CK-19 expression until full differentiation into mature cells. Our research has shown that during the three years of post-embryonic liver development in fish, a high level of CK19-positive cells is maintained from the first to the third year, and by the third year, they become the main precursor cells compared to hematopoietic sources. Additionally, it is currently believed that in the adult liver, CK19+ liver progenitor cells represent remnants of the fetal ductal plate. The hepatic progenitor cells arise during the process of liver development as a part of angiogenesis/vasculogenesis. They are formed from primitive hepatoblasts that are adjacent to the mesenchyme around the vessels of the portal zone. The ductal plate, which is detected in the liver during embryonic development and the first year of post-embryonic development,

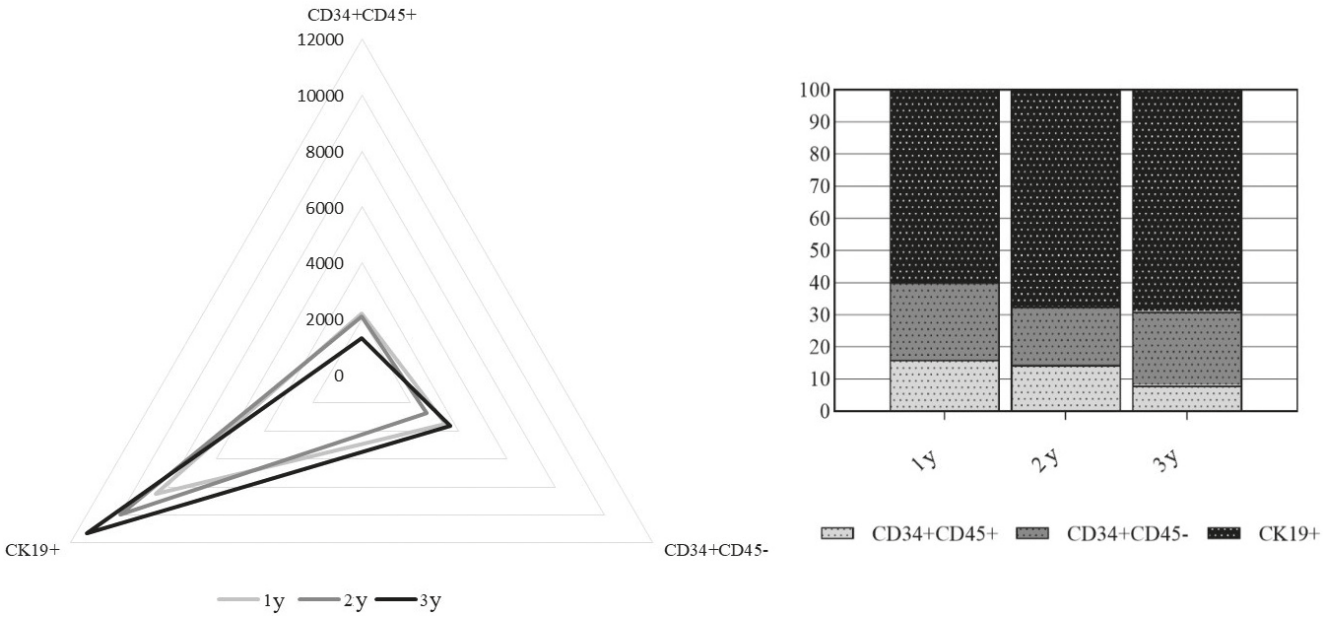

Fig. 5. Dynamics of liver stem potential indicators of fish species *Cyprinus carpio*.

transforms into the canals of Hering and is observed in the liver during later stages of post-embryonic development. Therefore, the canals of Hering can be considered as remnants of the ductal plate. The text describes the origin of bipotent liver precursor cells, known as CK19+ cells, which are pluripotent stem cells and hepatoblasts. These cells are the source of mature hepatocytes (Castorina et al., 2008; Gaudio et al., 2009; Dollé et al., 2010; Kang, Mars, and Michalopoulos, 2012; Yin et al., 2014; Itoh, 2016; Chen et al., 2017; López-Luque and Fabregat, 2018; Huang, Zhang, Gracia-Sancho, and Xie, 2022).

Currently, there is an opinion that stem cells from extraliver sites, particularly the bone marrow, may contribute to liver regeneration (Theise et al., 2000). Since the liver is itself a hematopoietic organ during embryogenesis, hematopoietic (mesenchymal) stemness markers — CD34 and CD45, which are selectively expressed in hematopoietic precursor cells, play an important role in maintaining the organ's cellular homeostasis (Castorina et al., 2008). In our research, the number of CD34+CD45+ cells decreases from the first to the third year of post-embryogenesis, while the number of CD34+CD45– cells remains relatively constant (Omori et al., 1997; Duret et al., 2007; Gaudio et al., 2009; Dollé et.al., 2010; Vestentoft, 2013; Yin et al., 2014; Hsieh et al., 2020; Bram et al., 2021; Choi et al., 2021).

The identified characteristics of the dynamics of the analyzed indicators may be related to the living conditions of fish and the development of "hypoxic stress" — when hypoxic conditions are replaced by hyperoxic ones, a functional load on mitochondria increases due to reoxygenation, which can be a source of hepatocyte damage (Voejkov, 2001; Danielson, 2002; Hahn and Wray, 2002; Baker, 2003; Nelson et al., 2003; Jayatri, 2006), a mechanism of thermoregulation (Budd and Jensen, 2000; Conway, 2000; Rome and Swank, 2001; Fischer, Koenig, Eckhart, and Tschachler, 2002; Schleucher and Withers, 2002; Angilletta, Wilson, Navas, and James, 2003; Litzgus and Hopkins, 2003; Angilletta, Steury, and Sears, 2004).

Conclusions

The study found that the liver parenchyma is composed of hepatocyte trabeculae, which form tubular structures surrounded by sinusoidal capillaries. The sinusoids form tubular-loop structures, with a central lumen bounded by more than six hepatocytes. The bile duct is present both within the portal tract (the portal-tract type) and separately in the parenchyma — the isolated type. The liver parenchyma contains the pancreas in the form of an acinar structure, separated from the liver parenchyma by a thin layer of connective tissue.

According to the results of the immunohistochemical study, it was determined that intrahepatic and hematopoietic stem cell precursors are not zonally localized and are present in all acinus zones (periportal, perivenular, centrilobular), in the area of the bile duct as a part of the portal tract, as well as in the area of the isolated type of bile duct.

Immunophenotyping revealed that the number of CD34+CD45+ cells decreases from the first to the third year of post-embryogenesis, while the number of CD34+CD45– cells remains relatively constant. The number of intrahepatic CK19+ precursor stem cells increases from the first to the third year of post-embryo-

genesis. Thus, the participation of both hematopoietic and intrahepatic sources of hepatocytes in liver parenchyma regeneration is observed during post-embryogenesis.

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