Příloha I:

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The use of porcine corrosion casts for teaching human anatomy*

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ARTICLE   INFO

ABSTRACT

Inte.achin.g and learri ng human anatomy, anatomie.al autopsy and prosected. specimens have always been indispensable. However, alternative methods must often be used. to demonstrate particularty delicate structures. Corrosion casting of porcine org.ans wi th Biodur E20• Plus is valuable for teaching and learning both gross anatomy and, uniquely, the micromorphlogy of cardiovascuar. respirato ry, digestive, and urogenital system.s Assessments of c.astswithastereomicroscopeand/ or sanningelectron microscope as well as highlighting cast structures using color coding help students to better unclerstancl how the structures that they have observed. as two-dimens.ional images actually exist in three dimen- sions, and students founcllusin.g the c.asts to be highly effective in their learning Reconstructions of a st hoUow structures from (rnicro-)computed tomography scans and videos facilitate detailed. analyses or branching patterns and s:patial arrangements in c.ast structures, aid in the understanding of d inically relevant structures and provide innovativevisu.al aids. The casting protocol and te.achingmanu.al we offer can beadjustedtod'ifferent technicalcap.a.bili tiesand might alsobe founduseful for veterinary or other biologial science classes.

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1. Introduction

Corrosion castingis an established method for the visu.alization of the morphology of hollow structures. lt is based on the filling the c.avity with solidifying materi.al, a.fter which the surrounding *tissue* is remove d with use of a corrosion agent after its polymer- iz.ation. Altho ug h the firstattempts.areoftenattributed to Leonardo cla Vinci (Hermiz et al.,2011; Oe Sord i et al.. 2014), who filled the ox brain ventricles with wax and scr.ape d off the overlying tissue between 1504 and 1507 (Paluzzi et al,, 200n casting was per-

formed earlier and can be divided into the · pre-corrosion".and · corrosion· periods (Aharine jad and 1.ametschwa ndtn e r, 199 2a). The oldest recorded attempts to fill the human blood vessels date back to 14 th centuryand Alessa ndra Giliani (1307- 1326i the first woman.an.atomist.and prosector , professor of medicine at the Uni- versity of Bologna. Giliani developed a te<hniqu e to make blood vessels visible byfillingthemwithcolored, hardeningliquids. How- ever, the first notes ofinjection ofa solidifyingmaterial followed by pa.renchymal corrosion were recorded by Dutch a.natomist Govert Bidloo (1649-1713). the author of the anatomical atlas Anatomia Humani Corporis, who u.sed mel ted me ta.l to in ject trache.a and bronchi and boiled the specimen to remove connective tissues (Davies, 1973).Since then, c.asting ma teria ls and procedures have been gr.adually impr oved. At present, the c.asting media. available whicha.resuitableforcorrosionrangefromlow-viscosity.   hardand brittJe m.ate ria.ls, such .as methy l me thac rylates, to pli.ab le the r-

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Resins suitable for corrosion casting.

Group	Resin	Citation	Miaowssel permeabi:lity	Radio pacity	Official price per liter
Acrylic resins	Mercox 0-28	Lametschwa nd t ner *et* al. (2004)	**Ves**	**Ves**	Out or product
	Mercoxti kit	Eberlova et a.1.(20 15)	**Ves**	**Ves**	<*nn*
	Bacson·s #17 kit	Oebbautet al. (2014)	**Ves**	**Ves**	€ 329
Epoxyresins	Biodur E2d' Plus	Eberlova et al. (2015)	**Ves**	**Ves**	€40
Polyurethanes	Souda-foam•	OeSordiet al. (2014)	No	**Ves**	€20
	Polyurethane Elastome r (PU4ii) kit	Twt'fves *et* al.(2012)	No	**Ves**	**€954**

• AsOn Aprit, 2017.

mostable polyuretha.nesor epoxy resins to flexible silicone rubber (Haenssgen *et al.*, 2014 : Table 1). Each of the agents has its pros and cons, a.nd choosing the optima.l one is always a balance between the research purpose and the specimen qua.lity. For ex.am p le. for the hig h vol ume va.scula.r corros ion castsa resin with a sufficient processing time must be cha.sen. And if we intend to ex.am ine the ca.st with the useof X-ray, the resi n mustbealsoradiopaque.

Since the 1960s, corrosion casts(((s) have beenstudied using light microscopy. Nowell and Tyler (1971) and Murakam i (1971) first applied sca.nningelectronmicroscopy(SEM)in CCassessment.With recent advances in high-resolution computed tomography (CT) tech nol- ogy, a pixel resolution close to 1 µ..mallows for morphometry of microvessels comparable withcfassicallight microscopy. CCµ.-Cl sca.nning provides new data from the microvascuJar tree that can be used both for the mathematical modeling (Debbaut et al.,2014) or quantitative purposes (Jiřík *et* al. 2016).

CCs enable the study of three-dimensional (3- D) arrangement of (micro)vascular systems. including angiogenesis (Gerlach et al. 2014).ln combination with SEM, vascular *CCs* pro vide information about structures that protrude from the wa.11 a n d cause imprints, e.g... the endothe lia.l cell nuclei, valves. and constrictions. *These* replicates help to differe ntiate arte rial from venous vessels and to identify different parts of the vascular **bed** (Lame tschwandtner *et* al..1990:Aharinejad and Lametschwandtner , 199 2). Human v. s- cular *CCs* are usua.Hy not suita.hle for uJtrastru ctural studies and need to **be** substituted by ether mammals (Cope, 2008; Hermiz *et al.*, 2011) as the fully filled corrosion cast requires a fresh hep- arinized specimen, irrigated vasculature and surgery preventing ga.s e mbo lization.

Ourgoal at the very beginningwas purelyscientific- we needed to obtain three-dimensional data of human liver microvascula- ture for mathematical modeling of the blood perfusion. Due to ethical concems and the practica.1 impossibility of obta.ining *fresh*
and healthy human livers. we focused on the use of porcine liv- ers. Given its size, morphology and function, the porcine liver is frequendy used a.s a large-a.nimal liver model. e.g... for *tes t -* ing surgica l techn iq ues(Bedoya *et* al..2014),liver transplantation (Okada*et* al., 2015). regeneration (Morte nsen and Revhaug , 2011: Avritscher*et* al..2013). mathematical modeling(Fu and Chui,2014), gene therapy (Carreño*et* al.,2013) and pharmacology (Gnutzmann et al., 2015 After testing different resins , we optimized the pro- tocol of he tectomy and high-volume corrosion casting with Biodur E20 Plus(Eberlova *et* al.,2015).Of all resins tested, Biodur E20,, Plus (Biodur products, Heidelberg, Cermany) exhibited the

best handJing qualities: it *passes* with minima.l lea kage through

the capillaries. its processing time enables high volume filling, it is slightly flexible and alcohol resistant after hardening, and it is radio-opaque. Because of the high quality and high teach- ing potential of the resulting preparations *as* well a.s eas y orga.n accessib ility- pigs are bred in ou r biomedi ca.1 center and are sacri- ficed for a variety of research topics- we decided to prepare othe r parenchymal org.ans tha t oth erwise would be wasted for use in

The aims of our studywere(1) toevaluate the suitability of Bio- dur E20• Plusporcinecorrosioncastsforteachinghumananatomy: (2) to enhance students' understanding of macro- and microvascu- lar structures of clinical relevance: (3) to interconnect the macro- with the microanatomy with cast assessment by light microscopy and scanningelectron microscopy;(4)tocreateoriginal,innovative visual aids useful for lecturesand projections.

2. **Methods** and res ults

The expen·mental surgical and anesthesia procedures and theuse ***cf*** *piglets* in *this*study*were certified by the*Commission *for***Work*** with ExperimentalAnimalsat *the*Pi/sen *Medical Facultyof Charles* University, *Prague, and were* under *the* control *of the Ministry of* Agriculture*oftheCzech*Republic.Aliprocedures *were prepared and performed* under*thelawof theCzech Republic,which*is *compatible with the legislation oftheEuropean* Union

2.1. Surgery

The detailed protocols for surgery and casting were published by Eberlova *et* al. (2015). Briefly, healthy Prestice Blac k- Pied pigs (Vrtková, 2015), N=7. age 16-18 weeks, weight 35-45 kg, both sexes. were premedicated, and intravenous anesthesia was administered continuously. For organ casts, see Table 2. After myorela.xation, the pigs were intubated and mechanica.lly venti - lated. Heparin (30.000UI i.v.) and volume-substituting infusions were administered. Before hepa tectomy, the va.scula r bed of the liver w.s flushed with SLof heparinized (10.0001.U.) Hartmann's solutionadministered via.atubeinserted into the suprahepatic por- tion of the caudal caval vein. Then.. the orga.n under consideration was disconnected from blood circulation and the vascular tree of liver. kidney and/or spleen was flushed with heparinized solution (dilution 10,000 Ul of heparin per 1 Lofcold Hartmanní s solution) via the suprahepatic portionof the inferiorcaval vein (liver), renal arteries (kidney) or splenicartery(spleen).The hepatic portalvein (PV) and hepaticartery(HA)or renal veins orsplenicvein remained unligated to d rain the perfused solution. The bronchial tree was harvested together with thelungs.

2.2.Casting procedure

Ali vessels were cannulated while subme d inwater topre ventaspirationof air into vessels. Biodur E20 (Biodur Products, Heidelberg, Cermany) for injection w.s mixed at a ratio of 100g

Biodur E20 Plus/45g of catalyst E2(0). The PV was filled using

teachinganatomy.

manual injection pressure. For injecting the hepatic, splenic and renal arteries, the cannula was held and guided by a micromanipulator(Aharinejad and Lametschw andt ne r. 1992b).Afterfilling, specimens weresubmerged in tap w.ter (20'C: 24 h). For harde n-

ing. they were transferred to 15% potassium hydroxide for tissue remov.l (40' C;2-3 **days),** rinsed in tap wa ter, and either stored in 70%denatu red alcohol, or. for higher resolution imaging, frozen in distilled water to be subsequendy cut into- 1 cm3 ice cubes with a belt saw.

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Porcineorp nsa.st withBiodu r E2•0 Plus.

Organ	Met hodorfilling	Volume of Biod ur E2d' Plus
Livff(H•2)	Porta!vein(MP)	500ml
Livf(H•2)	Porta!vein(M,P) hep•Uic anery(MMP)	500ml. 150 mt
Livf(H•*t*)	Porta!vein(M,P) hepaticanery(MMP). bile duet:(MP)	500ml. 150ml. 70 ml
Spleen(N• 2)	Splenic an ery(MMP)	180ml
Spleen(N• t)	Splenican ery(MMP).splenic vri n (MMP)	180ml. 70ml
Kidney (N•4)	Abclomina l aorta(MP)	300ml
Kidney (N•2)	na.la n ery (MMP). renal w,in(MMP)	130 ml. 70 ml
Kidney (N•t)	na.lan ery (MMP).urtt er(MP)	130 ml. 70 ml
Bronchial tree(N• 2)	Trachea(MP)	800 ml

MP- manu.al pressure: MMP- micromanipulator: total numberoranimals, N• 7 (diffe rent combinations *o(* org.ans re ind ividu.ally haf'W'St ed).

23 . 5<:anning and data promsing

Although even the gross CCcan **be** very ed ucational(Fig.1)-the students ca.n *see* the nature and density of the vasculature *even* with the na.ked eye- various devices ca.n be used to *see* the details ofthevascula.rtree.The liverCC. owingto its size, ca.n be examined with the common multi-slice huma.n CTscanner (Fig. 2A). a.nd a.ny ca.st ca.n be examined witha.stereomicroscope(Fig. 3(. D).

To pro vide the students morphological details in ascending resolution, our casts were analyzed first with the stereomicroscope(Olympus'5ZX7: Tokyo ,Japan). Livercasts were thenscanned in 90% de na.tured a.lcohol (pro vides higher contrast tha.n water) with a. mu lti -s lice huma.n CT scanner (Soma.tom Sensation64, Siemens, Forchheim, Germa.ny: slicethickness: 0.6 mm: voxelsize: 4 x 0.4 x 0.6mm). For high-resolution imaging, a lobe (liver) or lobule (bronchial tree) w.s dissected. whereas whole kidneys or spleens werefrozenin distilled tera.nd ra.ndo ml ycut witha. belt saw into pieces(size - 1 cml).1Yndomlychose n prisms were then immersed in 5%formic acid for 5 min (Lametschwa.ndtner et al. 1990 i rinsed in distilled water. air dried, mounted, sputter oated withgold(l min)andex.am ined usingthe sca.nningelectronmicroscope in a Stereoscan 250 instrument(Cambridge, UK) at 10 kV or in a SU-70 (Hitachi.Japan) at 1 kVacceleratingvoltage(Figs.4, SB).

Liver. sp lee n, kidney a.nd Jung ca.sts were scanned in a.ir with a μ.-CTscanner (Xradia μ.XCT400 , Pleasanton, CA, USA). Samples werefirstscanned usingthe Macrodetector (pixel size:47μrn/px. 1024 x 1024 px resolution) to display the overall liver caststructure.Then, the most interesting parts werescanned again with a. high-resolution detector using the following pa.rameters : source voltage 40 kV, source power 4W. pixel size 4.1 μm. detector *res* lution 1024 x 1024 pixels(binning 2), rotation angle 360-, number of lmages 1500, overa.11 sca.nning time 3 h, a.nd temperature 28°(. After scanning. recorded images were used for 3-D reconstruction using specialized software supplied with the Xradia ma.chine. Then, reconstruct ion scans were filtered a.nd se mi-a.utomatically segmented using the software **Avizo** 7.2 (vsg) and FIJI Image) 1.50f (Nationa l lns titu tes of Health. USA). Salt-a nd-pepper noise was removed by filte ring. lma.ge segmen tation was performed by thresholding and manually controlled flooding. Skeletonization was used to extract topological information(Fig. 28 , D).

For the 3-D reconstructionsand brief data processing. the data fromμ,-CTscannere.in be usedfor the volume renderingtechnique and thin-slab maximum intensity projection., software commonly available in hurnan CT-sca nne rs (Fig. 2 ( ).

2.4 . Useof corrosion *casting in elective anatomical classes- the schedule*

Ba.sicknowledgeof genera la.na.torny and histo logy we re prereq - uisites to beaccepted forelective anatomica.1cla.sses. Theschedu le for the second-year medical students (Table 3) is outlined below: *Week* 1: ln collaboration with surgeons, the instructors obta.in

a fresh organ that is immediately cannulated and injected with resin a.s desc ribed in the Materials a.nd Methods. Students only watch this procedure, which is performed by an instructor, a.nd wear protective gla.sses. The room is ventilated to prevent exposing students and staff to tox.ic fumes. The injected orga.n is left submerged in tap water forapproximately 24 h at 20' Cfor hardening. ln the next 3 days, the organ is macerated (corroded) and rinsed in running ta.p water.*Week* 2: Students inspect casts with the stereomicroscope and select the part of the ca.st the y wa.nt to a.na.lyze in the SEM. This part is frozen in distilled wa.ter a.nd cut into - 1cm^3 ice cubes. lce cubes are allowed to thaw in distilled water. a.nd ca.sts are cleaned from any debris underbinocula.r con - trol (Lame tschwandtner et al., 1990). Clean casts are then placed onto filter paper a.nd a.ir dried.*Week* 3: Students a.re in trod uced to theoretical principles of SEM and pre pare specimens for SEM obse rva.tion; i.e., mounting of dry ca.sts on to specimen stubs a.nd sputt e r-coating specimens with gold. Fina.lly. students together withthe instructor watchimagesofselected area.sof vascu lar ca.sts on the SEM display a.nd discuss the displayed structures in terms of cast qua.lity, va.scula.r pa.ttems. normal versus pathological, and truestructure versusartifact.

25. Feedback questionnaire

To ex.am i ne the contribution of the *CCs* to better understanding of anatomy and histology, students(N= 15) of the elective course Corrosion Casts inAnatomywereaskedina questionna.iretochoose the mostfittingmark on a li kert scale(scale: 5- Strongly Disagree, 4- Disagree. 3- Not Sure, 2- Agree, 1- Strongly Agree) for the fol-lowingstatements:

a Viewing the corrosion ca.sts with use of the following devices (stereomicroscope, SEM. micro-CTa.nd video) helped metolea.m the 3-Danatomy of the liver, spleen, kidneyand Jung.

b Viewing the corrosion ca.ts helped me to interconnect histology withgrossa.na.torny.

c Viewing the corrosion casts helped me to bette r understand 2-D histological specimens I knew from histological cJasses .

d Using the corrosion ca.sts s va.luab le in studyi ng th e clinica l condition of theseorgans.

Following a.dministrationof the questionna.ire. mea.ns and sta n-dard deviations werecalcula.ted for ea.ch res ponse item.

3. Results

Regardingthe questionna.ire.students werevery positive about the use of corrosion casts when learning anatomy a.nd histology. On average. students agreed or strongly agreed that all methods by which theyexperienced the corrosion ca.sts helped them lea.m the 3-D anatomy of that organ (Table4). Comparing the average *results* for each orga.n a.nd device, the best ma.rked was the liver
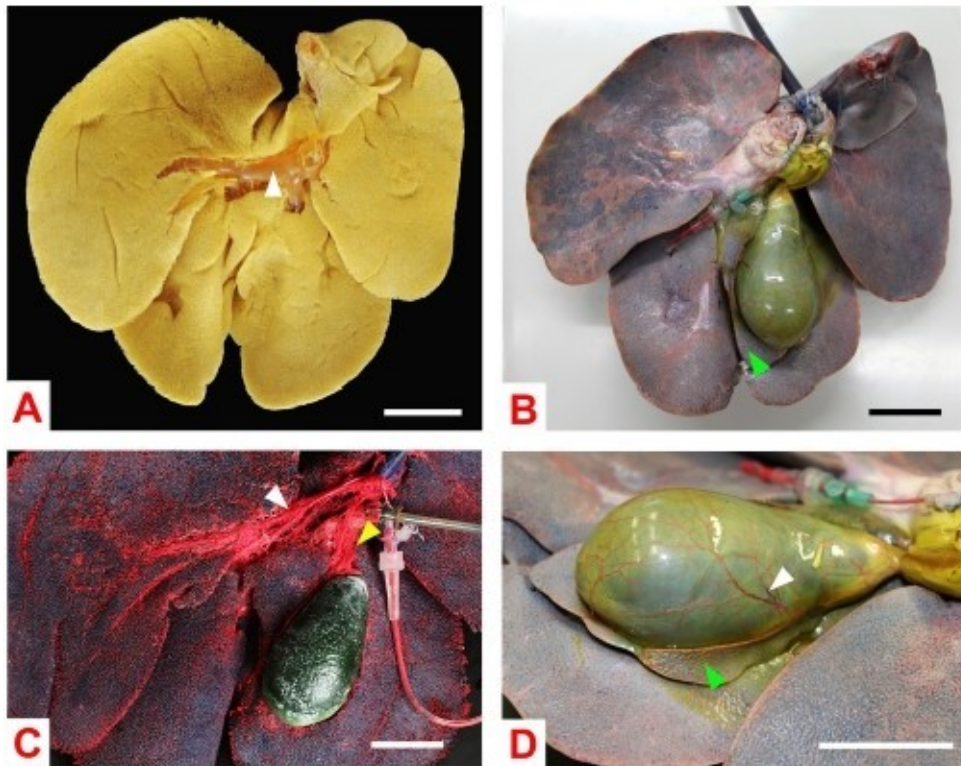
Fig. t. Corrosioncasts of pig liver. (A) Filling with Uow< olore d Biodu r E20" Plus via the hepatic port.al vein (white arrowhead). (B, O) Cau:s orli w r and ga UbLad d er (uncorrod e d) filled via the hepatic anery(red)andporta) w in (blue).Quadrate lobe(g:nenarrowhead.)(C) Live r cast(aftercorrosion.)Note t he vasavasorumof the portal vein (white an-owhead) and vesselsofcysticduet (yeUow arrow he a d). (O) Gall bladder with cyst ic an eryand vein (white arrowhead),quadrate lobe (g:rtt n arrow head). Scalebars4 an . (f.or interp retu ionof the references tocolorinthis figurelegend, the rl!ader is referred to the web venionoí this a.nic" .)

Fig;.2. Com pu ted tom og:raphy o{ porcine livercorrosion c.ast . (A) Macr1rCT, VRT.Syntop ic view of porta) and hepatic wnoussystnns: hepaticwin(HV).port a.I vei n (_grttn an-owhead). (B) µ-CT,3-0 recons tructio, n castfilted via the ponalvein. (C)µ.-Cf,volume rendering technique. Demm stration of ponir systemic a n.istomosis (nod eUipse). tributaryoíhepaticporta.I win (g:reen ,u rowhead) , a nd tribut.aryor hepatic win (HV).(O) 1,1,-CT. 3 -0 rKo n st ru ction,castfiUed viathe porta!vein(PV)a.nd the he!paticartery (black arrowhead).(Forinterpretation oí the reíerencesto colorin this figure legen, d the reader i.sreferre d to the we b versio n of t his a rti cle.)

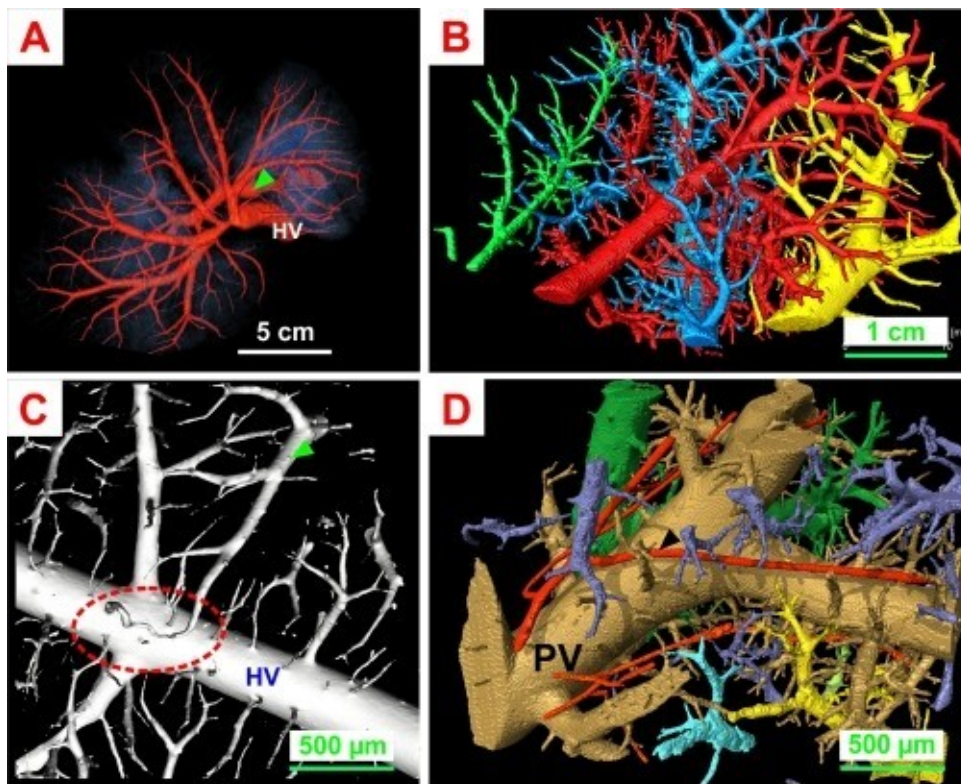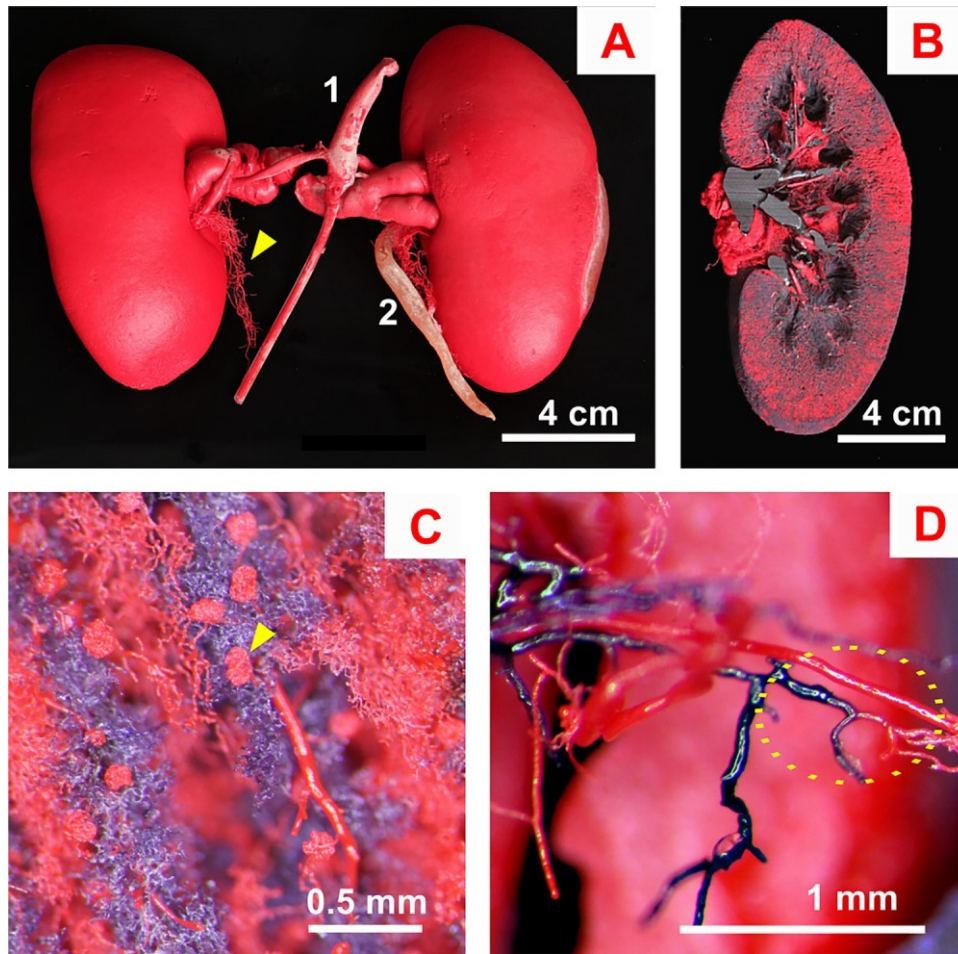**Fig. 3.** Pig kidney cast with Biodur E20® Plus. (A) Cast filled via the abdominal aorta (1), left kidney also via the ureter (2), ureteric branches (yellow arrowhead). (B, C, D) Filling via the renal artery (red) and the renal vein (blue). (B) Frontal section. (C) Renal cortex with glomerulus (yellow arrowhead). (D) Arterio-venous anastomosis (yellow ellipse). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Schedule of elective anatomical classes.

	Practical session (150 min)	Subsequent week activities	Essential student aids
Week 1	Organ cannulating, resin injecting	Cast hardening, corrosion, rinsing	Protective glasses, lab coat, surgical gloves, respiratory mask
Week 2	Stereomicroscopy, cast freezing	Specimen cutting and cleaning for SEM	Stereomicroscope
Week 3	Scanning electron microscopy		Scanning electron microscope

Table 4
Results of the questionnaire, statement 1: "Viewing the corrosion casts with use of the following devices helped me learn the 3-D anatomy of the kidney, liver, lung and spleen."

Organ	Stereomicroscope	SEM	MicroCT	Video
Liver	1.5, SD 0.7	1.3, SD 0.7	1.5, SD 0.9	1.4, SD 0.8
Spleen	2.1, SD 1.1	1.4, SD 0.8	1.3, SD 0.7	1.7, SD 1.0
Kidney	1.5, SD 0.9	1.4, SD 0.8	1.4, SD 0.8	1.8, SD 1.0
Lung	2.0, SD 1.0	1.4, SD 0.7	1.3, SD 0.7	–

SD—standard deviation.

Table 5
Results of the questionnaire, statements 2–4:

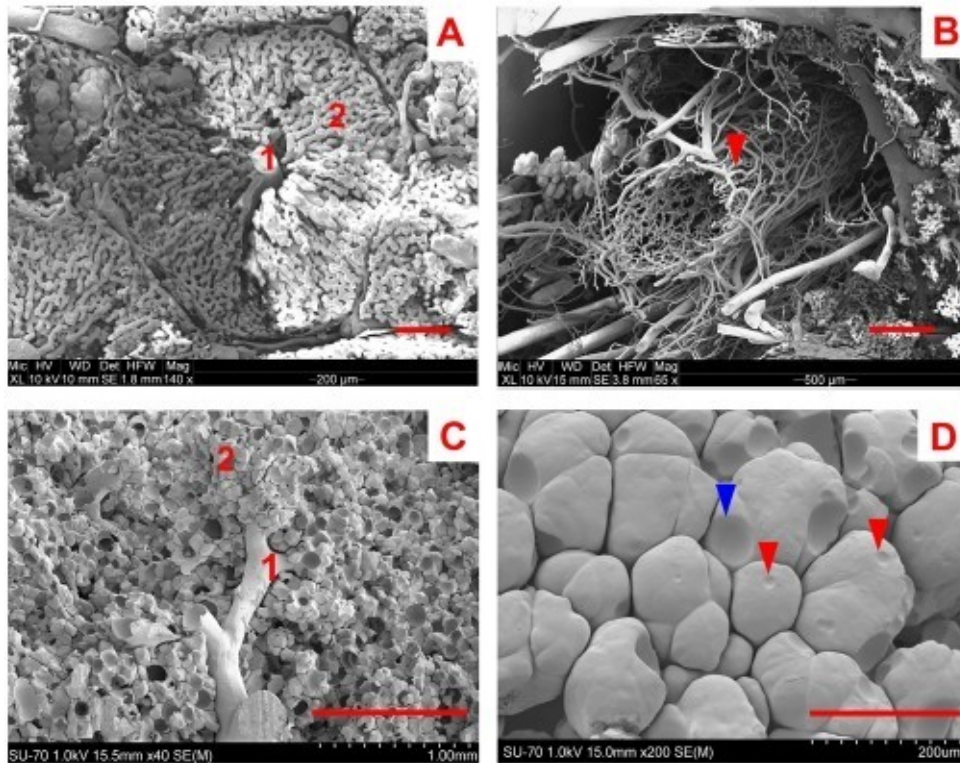| Statement	Mean	SD
Viewing the corrosion casts helped me to interconnect histology with gross anatomy	1.5	0.7
Viewing the corrosion casts helped me to better understand 2-D histological specimens I knew from histological classes	1.4	0.7
Using the corrosion casts was useful when studying the clinical condition of these organs	1.8	0.8

Fig.4. Vascular corrosioncastsof porcirw:live r a.nd lung. SEM. (A) l iw:rklbule. Centra.I vein (I), sinusoids (2).(B) Peribilia ry plu us(arrowhead). (C) Term inal bronchiole (I) and alveoli (2) oíl ung. (O) Pulmonaty alveoli. Notenuclear imprin ts of p neum ocytes (ffclarrowheads)ndait buble(blueanowhead)arcle 200μm.(A). SOCIμm.(B).   l mm (()). 200 μ.m.(0). (For i nterpreta t io n o f the references to color in th i.s figure legend. the readeri.sre Jerr t'CI to t h e we .b w:r s ion of t h i s att icl e .)
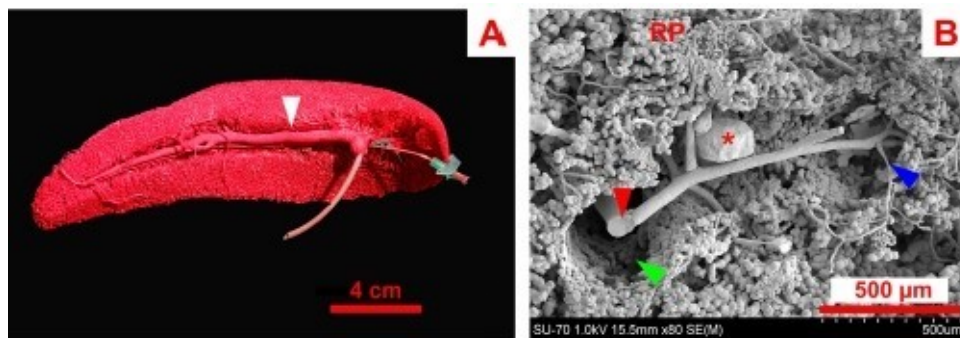

Fig.S. Vascular corrosioncast of the porcine spleen. (A) Fillingwit h red--<olored Biodur E2d' via the splenic annyandw in(white an-owhad).(8)SEM. Note centra)artery (reda1TOwhead). m iss ing pe rian eriolar lym phatic sheath(grttnarrowhead). penicitlarartery(blue arrowh!ad)and nod pulp (RP). Asterisk marks extravasated Biodur. (For i.nterpret a tion oíthereíuencesto color in this figure legend, the re:ader is reíerred to tbe web version oí this article.)

(Mean 1 A, 50 0.7)a nd μ.-CT(Mean 1.4, 50 0.8). 5tudents also posi- lively viewed the role of the castsinhelpingthemconnect histology withanatomy. understa.nd the previously seen2-0 specimens, and understandclinical conditions of those organs (Table 5).

4. Oiscussion

ln teaching a.na.t o rny, the importance a.nd merit of anatomi- cal autopsy ha.ve not been and probably will not be overcome in the near future. Nevert heless. prosected specimens do not always provide the student with a clea.r picture of particular, delicate anatomicalstructures. Thisgap can be pa.rtly bridged by corrosion casts, which represent a 3-0 replica. of hollow structures. As CCs represe nt real specimens, theysupport the learning process con-

siderably more thantextbooksorevencomputer-ba.sed 3- 0 models (Preeceet al.,2013).

Theidea.of engagingstudents in activelearningby lettingthem work with CCs is no t new: *CCs* of bronc h ial trees ha.ve been used in classes previously (Cope, 2008; Herm iz et al., 2011: De Sordi et al., 2014). However, given that casting media were too viscous a.nd did no t fill tiny spaces , the cas ts only exhibited gross struc- tures, such as the basicbranchingpattem, and they lacked detailed information on the termin al tree (Klaws and Till mann , 1993). ln ce ntr a.st, BiodurE2•0 Plus pa.ssesth rough capilla ry-sized tubesand can fill (cas t) large-sized spaces(high volume filling). This resin is a two-component, temperature-tolerant a.nd chemica Uy resis ta.nt resin that issuitable both for freezingand corrosion. It is relatively cheap(Table 1) and suitable for macro- and μ.-CTscanning(Fig. 2). lts slightJy flexible consistency is also advantageous for comfort-

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Differencesberwttn porcine:a nd hu man orga ns rele van t for vascu lar corrosioncasting.

Organ(Pig)	Macroanatomy	Microanatomy
	Rightand lefthemitiverdividt'd bya deep fissure. S lobes (quadratelobe is variable), caudalcava.l veinembedded in the right lateraland the caudatelobe.hiJum moredorsally	Each lobuleisenveloped in fibrousconnea ive rissue: fibrous tissue intercon nect adjace nt pon.al spaces
Spleen	Elo ngated, stra like: 2 stgmmts.,c.J,, 4- 5 segment.sin bJ:man spleen(Christo and DiOio, 1997)	
Kidn e y	Upper and lower polar an eries at the hi lum: 4 st;g:men ts and4 segment.al an tries	
Bronchial tteeandJung	4 lobesin the right Jung, right cranial lobar bronchusarises directly fromthe lateral wall of trachea: completieconrw:ctive tis sue se pta border the secondary lobules: monopodial branchingof brm chial *trtt*	

ab le handling a.nd de monst rat ion purposes compared withbrittle. quickly hardeningcasting materials(Haenssge nn et al., 20 1 4). Afte r

so lidifying.casts madefrom Biodur E2•0 can also be stored inalco-

hol to prolong the precorrosion period. Alcohol preservation is also useful forvoluminous corroded CCs to preventthem from molding.

An additional advantageof using this method of corrosion cast- ing is that highlighting ca.st structures by color-coding. e.g., using different ly colored Biodur E2•0 Plus for arteries (e.g., red Biodur) and veins(e.g.., b lue Biodur).enables distinguishing the two vascu- lar bedsand castsassessment usingthe dissecting microscope and allows the visualization of arteriovenous ana.stomosis (AVshunts , fjg.3DJ.

4.1. Porcine Uver corrosion c.asts

When teaching human anatomy with porcine corrosion ca.sts, the morp hologica l simi la.rities a.nd dissimilaritiesin the castorgans compa.red to their hu.man counterpa.rts mustbe takenintoconsid- eration(Tables 6 and 7 Rega rding e xterna l morphology. the right and left hemilivers in pigsa.reseparated bya deepfissure. a.nd onl y five lobes are invariably present: the left la teral. left media (, right lateral. right medial and the caudate lobes(Court et al,, 2003). The quadrate lobe is present occasionally (Fig. 1). Neverthe less. given the similarity of the vascula.r and bilia.ry tree. the eigh t segmen ts in the parenchyma are present a.nd are ro ughly simila.r to those described in the human by Couinaud(1954) and Courtet al.(2003 Microscopically,porcine liver lobules a.re clearlydemarcated by the interlobular connective tissuesepta that meet in the porta.I a.rea. ln hea lthy huma ns, liver lobules normally Jack connective tissue: the presence of connective tissue outside of the porta.I areas indicates liver fibrosis or cirrhosis (Hytiroglou. 2011).Similar to the human liver, the interlobula.rspacein the porcine liver is occupied by the porta) triad. The interlobular hepatic a.rtery a.nd porta.I vein open into sinusoids. which empty into the centra.I vein(Fig. 4A

Using the CCs e nab les the demonstration of the difference between porta! vein and hepatic artery perfusion (Fig. 1A and () . The hepatic a.rtery exclusively supplies three compa.rtme nts : the peribiliary vascular plexus (Ohtani et al., 1982: Fig. 4 8), the porta! tract interstitium and the porta! vein wall (Elias and Petty, 1953: fjg. 1(Detailed knowledge of the anatomy of the biliary path- ways bloodsu pply is crucial not only to cholecystectomies but also to liver transplantations, as the bile ischemia is believed to be the major factor in biliary complications (Wang et al.,2015).

Our liver CCs also cJear ly demonstrate the vasa vasorum (Fig. 1 ( ). The vasa vasorum play a crucial role in vascular patholo- gies (Mulligan- Kehoe and Simons. 2014: Tonar et al. 2016), especially in atherosclerosis(Xu et al., 2015).

The macro-CT of liver vascula.r CC applies clinical imaging to the teaching of anatomy (Fig. 2A). CTscansenable the different ia - tion of vessels with a lumen diameter as small as 3 mm. Similarly. J.L-CT and 3 - D reconstruction visualize the bra.nching and spatia.l arrangement

vessels may reveal the presence of intrahepatic porto-systemic anastomoses (PSshunts: Fig. 2( ). Although the PSshuntsare only

sporadically noted within the human liver parenchyma(Palvanov

of the microvascular tree (Fig. 2B-D). Tracing the

et al. 2016), the extrahepatic porto-cavalanastomoses (PCshunts)

are relevant structures in huma.n medicine and play an extremely importa nt role in ca.se of porta) hypertension. PCshun ts are found in the rectum, esophagus. abdominal wall. and retroperitoneum. They are va.lve less a.nd serve *as* bypasses that open in ca.se that the blood cannot flow through the liver. When a large volume of blood circumvents the liver. varicose veinsdevelop at the sites of porto-syste mic a.nastomoses . They protrude, may ruptureand, in

combination with the lack of coagulation proteins caused by the accompanied hepatopathy. cause life-threatening bleeding.

42 . Spleen, kidney and *bronchial tree* corrosion*casts*

With respect to shape and position.. the kidney is one of the mostva.riable organs in humans. The prevalence of vascular varia-tions is high (mar and Tilrkvatan. 2016). Neverthe less , thereare manysimilaritiesbetween the humanand pigkidney(Table 7), **e.g.,** in parenchymastratilication.microa.ngioarch itecture . and nephron morphology (Friis. 1980). The porcine kidney is a widely used ani- mal model ine xperimental medicine(Tonar et al.,2009: Nath et al.• 2014; Yang et al., 2015). Theadult organ hasa similar weight, size and numberof nephronsas that inhumans(Rytand. 1938).Ofnote,

the re are 4 rena l segmen ts in pigs. ln 91% of pigs, the renal artery bifurcates at or before the hilus intoupperand lower polar arteries, eachofwhichbranchesoffinto an anteriorand a posteriorsegmen-tal artery. The avascular plane is transverse in swine(Evan et al.. 1996) but longitudinal in humans(Dyeret al.,2002 Pig kidneyvas-cula.rCCsclearlydemonstrate thedifference betweenthe textureof the cortex and meduJla. Thedotted cortexconta.ins the glomeruli. wherea.s the striped medulla contains the straight a.rterioles. The outlinesof renal pyramids are alsodistinct(Fig. 3B.C).The ureteric branches supplying the renal pelvis and upper ureter a.re clea.rly visible in the castsfilled via the renal artery(fjg. 3A).

Simila.r size a.nd anatom y to the human lung ma.ke the pig lung a beneficial model for biomedical research (Nish ikawa et a•l.2013: Judge et al. 2014). The lobar anatomy in pigs is similar to that of the human left lung. which consists of cranial a.nd cauda. l lobes. The right lung is divided into four lobes: the cra.nial. mid- dle. accessory and caudal. lnterlobar fissures a.re incomplete . The right cranial loba r bronchus arises directly from the latera l wall of the trachea. whereas the otherloba.rbronchi ramifyfromthe ma.in

bronchi at the hilum. ln the pig. the segmentation and bronchia.l ra.milication is moreindentedcompared with huma.ns, e.g.• the left caudal lobar bronchus d ivides into4 ventral and 4 dor sa.l segme nt a.l bronchi (Nakakuki, 1994). Porcine airways are morecartilaginous

but exhibit a simila.r number of bronchial genera.tions (N=23) as humans (Maina and van Gils, 2001). ln contrast to humans, the porcine branching pattem is monopodial (Neble et al,, 2010). ln addition . the secondary lobules are completely separated by con-

Table 7
Similarities between the porcine and human organs relevant for vascular corrosion casting.

Organ (Pig)	Macroanatomy	Microanatomy
Liver	Similar size, hilum with PV, HA proper and right and left hepatic ducts; 8 segments	Hepatic lobule, portal triad in portal spaces, sinusoids, central vein
Spleen	Segmental arteries are end arteries	Red pulp (vascular sinuses) and white pulp (central arteriole, periarteriolar lymphoid sheath, lymphoid follicles)
Kidney	Similar size, smooth surface, medulla and cortex; renal pyramids, minor (approximately 10) and major (2–3) calices end in renal pelvis; segmental arteries are end arteries	Renal corpuscle and convoluted tubules in cortex, straight tubules, loop of Henle and collecting ducts in medulla
Bronchial tree and lung	Similar size; 2 lobes in the left lung	Similar number of bronchial generations

nective tissue septa; i.e., they are clearly demarcated in the CC after soft tissue digestion. Toward the periphery, the respiratory bronchioles give rise to alveolar ducts that end in alveoli (Fig. 4C, D).

The shape of the porcine spleen is significantly different from the human spleen. Spleens obtained from our Prestice Black-Pied pigs aged 16–18 weeks were approximately 20 cm long and 6 cm wide (Fig. 5A). The splenic artery divides close to the hilum into 2 segmental branches, which supply two independent splenic segments (Pereira-Sampaio and Marques-Sampaio, 2006). According to a Web of Science search (April 2017, key words "pig spleen histology"); the ultrastructure of the porcine spleen has been studied mainly in connection with experiments and histopathology (Palzer et al., 2015; Suzuki et al., 2016). To understand the spleen's functions; it is important to have knowledge of the 3-D structure of the microvasculature; the tissue compartments and the cell distribution. Given that mammalian spleens have an open circulation; corrosion casting is not an ideal method for studying the red pulp. Nevertheless; in the white pulp Biodur E20® CCs clearly demonstrate the central and penicillar arteries and indicate the presence of the periarteriolar lymphoid sheaths (Fig. 5B).

4.3. Useful tips for easy teaching

- Highlight cast structures by color-coding, e.g., using differently colored Biodur E20® Plus for arteries (e.g., red Biodur) and veins (e.g., blue Biodur). It enables the ability to distinguish the two vascular beds (Figs. 1, 3). For this purpose, either the colored Biodur E20® dye can be used, or the clear Biodur E20® can be dyed with different dye pastes.
- As the fresh organs are not always available, an organ filled with Biodur E20® can be stored in 50% alcohol and corroded later.
- It is also useful for the anatomy classes for the instructor to demonstrate the vascular density showing the students the organ before and after corrosion (Fig. 1A, B).
- Looking at the CCs, students do not often understand what they see. The instructor should ask questions dealing with the structures on the cast to train the students' imagination; point out that there is not any soft tissue present and that the students are seeing the filling of the former vessels (cavities); and relate the corrosion casts to clinical conditions (for example, the instructor can ask students how the cast would look different in a cirrhotic liver).

5. Conclusions

Pigs are frequently used as large-animal models. Corrosion casts of porcine organs with Biodur E20® Plus appeared to be valu- able for teaching human anatomy of cardiovascular, respiratory, digestive, and urogenital systems. We present a manual for the corrosion casting and the cast analysis in the elective anatomi- cal classes. Our casting protocol and the range of CC assessment offer different variations adjustable to the particular technical demands. Biodur E20® passes through capillary-sized tubes and

enables both gross anatomy and microstructural research. Highlighting structures using different color-coded Biodur allows for a clear demonstration of gross arterial supply and venous drainage as well as different kinds of anastomosis and microvascular patterns. Assessments of casts enable instructors to demonstrate particular structures of clinical importance, using a stereomicroscope and/or scanning electron microscope together with 3-D reconstructions based on CT scans. They also enable the creation of original visual aids, and involve the students directly in the active learning process, helping them to better understand 2-D images they know from textbooks or histological specimens. Students find the use of these corrosion casts useful for their learning, across a variety of tissues and visualization methods. Finally, Biodur E20® Plus corrosion casting might also be a valuable teaching tool in veterinary anatomy or other biological science classes.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aanat.2017.05.005.

References

Aharinejad, S.H., Lametschwandtner, A., 1992. Microvascular Corrosion Casting in Scanning Electrone Microscopy, first edition. Springer, Berlin-Heidelberg-Wien, pp. 3–9 (a), p. 58 (b).

Avritscher, R., Abdelsalam, M.E., Javadi, S., Ensor, J., Wallace, M.J., Alt, E., Madoff, D.C., Vykoukal, J.V., 2013. Percutaneous intraportal application of adipose tissue-derived mesenchymal stem cells using a balloon occlusion catheter in a porcine model of liver fibrosis. J. Vasc. Interv. Radiol. 24, 1871–1878.

Bedoya, M., del Rio, A.M., Chiang, J., Brace, C.L., 2014. Microwave ablation energy delivery: influence of power pulsing on ablation results in an ex vivo and in vivo liver model. Med. Phys. 41, 123301.

Carreño, O., Sendra, L., Montalvá, E., Miguel, A., Orbis, F., Herrero, M.J., Noguera, I., Aliño, S.F., Lopez-Andujar, R., 2013. A surgical model for isolating the pig liver in vivo for gene therapy. Eur. Surg. Res. 51, 47–57.

Christo, M.C., DiDio, L.J., 1997. Anatomical and surgical aspects of splenic segmentectomies. Ann. Anat. 179, 461–474.

Çınar, C., Türkvatan, A., 2016. Prevalence of renal vascular variations: evaluation with MDCT angiography. Diagn. Interv. Imaging 97, 891–897.

Cope, L.A., 2008. Tracheobronchial cast production and use in an undergraduate human anatomy course. Anat. Sci. Educ. 1, 23–26.

Couinaud, C., 1954. Liver lobes and segments: notes on the anatomical architecture and surgery of the liver. Presse Med. 62, 709–712.

Court, F.G., Wemyss-Holden, S.A., Morrison, C.P., Teague, B.D., Laws, P.E., Kew, J., Dennison, A.R., Maddern, G.J., 2003. Segmental nature of the porcine liver and its potential as a model for experimental partial hepatectomy. Br. J. Surg. 90, 440–444.

Davies, A., 1973. The evolution of bronchial casts. Med. Hist. 17, 386–391.

De Sordi, N., Bombardi, C., Chiocchetti, R., Clavenzani, P., Trerè, C., Canova, M., Grandis, A., 2014. A new method of producing casts for anatomical studies. Anat. Sci. Int. 89, 255–265.

Debbaut, C., Segers, P., Cornillie, P., Casteleyn, C., Dierick, M., Laleman, W., Monbaliu, D., 2014. Analyzing the human liver vascular architecture by combining vascular corrosion casting and micro-CT scanning: a feasibility study. J. Anat. 224, 509–517.

Dyer, R.B., Regan, J.D., Kavanagh, P.V., Khatod, E.G., Chen, M.Y., Zagoria, R.J., 2002. Percutaneous nephrostomy with extensions of the technique: step by step. Radiographics 22, 503–525.

Eberlova, L., Liska, V., Mirka, H., Gregor, T., Tonar, Z., Palek, R., Skala, M., Bruha, J., Vycital, O., Kalusova, K., Haviar, S., Kralickova, M., Lametschwandtner, A., 2015. Porcine liver vascular bed in Biodur E20 corrosion casts. Folia Morphol. (Warsz) 75, 154–161.

Elias, H., Petty, D., 1953. Terminal distribution of the hepatic artery. Anat. Rec. 116, 9–17.

Evan, A.P., Connors, B.A., Lingeman, J.E., Blomgren, P., Willis, L.R., 1996. Branching patterns of the renal artery of the pig. Anat. Rec. 246, 217–223.

Friis, C., 1980. Postnatal development of the pig kidney: ultrastucure of the glomerulus and the proximal tubule. J. Anat. 130, 513–526.

Fu, Y.B., Chui, C.K., 2014. Modelling and simulation of porcine liver tissue indentation using finite element method and uniaxial stress-strain data. J. Biomech. 47, 2430–2435.

Gerlach, N., Minnich, B., Lametschwandtner, A., 2014. Microvascularization and histomorphology of lateral line organs in adult Xenopus laevis. J. Morphol. 275, 497–503.

Gnutzmann, D.M., Mechel, J., Schmitz, A., Köhler, K., Krone, D., Bellemann, N., Gockner, T.L., Mokry, T., Kortes, N., Sommer, C.M., Kauczor, H.U., Radeleff, B.A., Stampfl, U., 2015. Evaluation of the plasmatic and parenchymal elution kinetics of two different irinotecan-loaded drug-eluting embolics in a pig model. J. Vasc. Interv. Radiol. 26, 746–754.

Haenssgen, K., Makanya, A.N., Djonov, V., 2014. Casting materials and their application in research and teaching. Microsc. Microanal. 20, 493–513.

Hermiz, D.J., O'Sullivan, D.J., Lujan, H.L., DiCarlo, S.E., 2011. Constructivist learning of anatomy: gaining knowledge by creating anatomical casts. Anat. Sci. Educ. 4, 98–104.

Hytiroglou, P., 2011. Hepatitis C. In: Saxena, R. (Ed.), Practical Hepatic Pathology, A Diagnostic Approach. Elsevier Saunders, Philadephia, pp. 229–231.

Jiřík, M., Tonar, Z., Králíčková, A., Eberlová, L., Mírka, H., Kochová, P., Gregor, T., Hošek, P., Svobodová, M., Rohan, E., Králíčková, M., Liška, V., 2016. Stereological quantification of microvessels using semiautomated evaluation of X-ray microtomography of hepatic vascular corrosion casts. Int. J. Comput. Assist. Radiol. Surg. 11, 1803–1819.

Judge, E.P., Hughes, J.M., Egan, J.J., Maguire, M., Molloy, E.L., O'Dea, S., 2014. Anatomy and bronchoscopy of the porcine lung. A model for translational respiratory medicine. Am. J. Respir. Cell Mol. Biol. 51, 334–343.

Klaws, G.R., Tillmann, B., 1993. From the specimen workstation. Injection of synthetic materials of arteries using a four color technique—with an example of head and neck blood vessels. Ann. Anat. 175, 221–224.

Lametschwandtner, A., Lametschwandtner, U., Weiger, T., 1990. Scanning electron microscopy of vascular corrosion casts—technique and applications: updated review. Scanning Microsc. 4, 889–940.

Maina, J.N., van Gils, P., 2001. Morphometric characterization of the airway and vascular systems of the lung of the domestic pig, *Sus scrofa*: comparison of the airway, arterial and venous systems. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 130, 781–798.

Mortensen, K.E., Revhaug, A., 2011. Liver regeneration in surgical animal models—a historical perspective and clinical implications. Eur. Surg. Res. 46, 1–18.

Mulligan-Kehoe, M.J., Simons, M., 2014. Vasa vasorum in normal and diseased arteries. Circulation 129, 2557–2566.

Murakami, T., 1971. Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. Arch. Histol. Jpn. 32, 445–454.

Nakakuki, S., 1994. Bronchial tree, lobular division and blood vessels of the pig lung. J. Vet. Med. Sci. 56, 685–689.

Nath, J., Guy, A., Smith, T.B., Cobbold, M., Inston, N.G., Hodson, J., Tennant, D.A., Ludwig, C., Ready, A.R., 2014. Metabolomic perfusate analysis during kidney machine perfusion: the pig provides an appropriate model for human studies. PLoS One 9, e114818.

Nishikawa, H., Oto, T., Otani, S., Harada, M., Iga, N., Miyoshi, K., Miyoshi, S., 2013. Unilateral lung transplantation using right and left upper lobes: an experimental study. J. Thorac. Cardiovasc. Surg. 146, 1534–1537.

Noble, P.B., McLaughlin, R.A., West, A.R., Becker, S., Armstrong, J.J., McFawn, P.K., Eastwood, P.R., Hillman, D.R., Sampson, D.D., Mitchell, H.W., 2010. Distribution of airway narrowing responses across generations and at branching points, assessed in vitro by anatomical optical coherence tomography. Respir. Res. 11, 9.

Nowell, J.A., Tyler, W.S., 1971. Scanning electron microscopy of the surface morphology of mammalian lungs. Am. Rev. Respir. Dis. 103, 313–328.

Ohtani, O., Murakami, T., Jones, A.L., 1982. Microcirculation of the liver, with special reference to the peribiliary portal system. In: Motta, P.M., DiDio, L.J.A. (Eds.), Basic and Clinical Hepatology. Martinus Nijhoff Publishers, Hague/Boston/London, pp. 85–96.

Okada, N., Mizuta, K., Oshima, M., Yamada, N., Sanada, Y., Ihara, Y., Urahashi, T., Ishikawa, J., Tsuji, T., Hishikawa, S., Teratani, T., Kobayashi, E., 2015. A novel split liver protocol using the subnormothermic oxygenated circuit system in a porcine model of a marginal donor procedure. Transplant. Proc. 47, 419–426.

Paluzzi, A., Belli, A., Bain, P., Viva, L., 2007. Brain 'imaging' in the Renaissance. J. R. Soc. Med. 100, 540–543.

Palvanov, A., Marder, R.L., Siegel, D., 2016. Asymptomatic intrahepatic portosystemic venous shunt: to treat or not to treat? Int. J. Angiol. 25, 193–198.

Palzer, A., Austin-Busse, R.L., Ladinig, A., Balka, G., Zoels, S., Ritzmann, M., 2015. Histopathologic lesions in conventional pigs experimentally infected with Haemophilus parasuis serovar 5. Tierarztl. Prax. Ausg. G Grosstiere Nutztiere 43, 91–96.

Pereira-Sampaio, M.A., Marques-Sampaio, B.P., 2006. Anatomical study and proportional analysis of the pig spleen arterial segments. Cells Tissues Organs 182, 32–34.

Preece, D., Williams, S.B., Lam, R., Weller, R., 2013. Let's get physical: advantages of a physical model over 3-D computer models and textbooks in learning imaging anatomy. Anat. Sci. Educ. 6, 216–224.

Rytand, D.A., 1938. The number and size of mammalian glomeruli as related to kidney and to body weight, with methods for their enumeration and measurement. Am. J. Anat. 62, 507–520.

Suzuki, R., Shin, D., Richards-Kortum, R., Coghlan, L., Bhutani, M.S., 2016. In vivo cytological observation of liver and spleen by using high-resolution microendoscopy system under endoscopic ultrasound guidance: a preliminary study using a swine model. Endosc. Ultrasound 5, 239–242.

Tonar, Z., Janácek, J., Nedorost, L., Grill, R., Báca, V., Zátura, F., 2009. Analysis of microcracks caused by drop shatter testing of porcine kidneys. Ann. Anat. 191, 294–308.

Tonar, Z., Tomášek, P., Loskot, P., Janáček, J., Králíčková, M., Witter, K., 2016. Vasa vasorum in the tunica media and tunica adventitia of the porcine aorta. Ann. Anat. 205, 22–36.

Twelves, D., Nerurkar, A., Osin, P., Ward, A., Isacke, C.M., Gui, G.P., 2012. The anatomy of fluid-yielding ducts in breast cancer. Breast Cancer Res. Treat. 132, 555–564.

Vrtková, I., 2015. Genetic admixture analysis in Prestice Black-Pied pigs. Arch. Anim. Breed. 58, 115–151.

Wang, J.Z., Liu, Y., Wang, J.L., Lu, L., Zhang, Y.F., Lu, H.W., Li, Y.M., 2015. Sequential vs simultaneous revascularization in patients undergoing liver transplantation: a meta-analysis. World J. Gastroenterol. 21, 7036–7046.

Xu, J., Lu, X., Shi, G.P., 2015. Vasa vasorum in atherosclerosis and clinical significance. Int. J. Mol. Sci. 16, 11574–11608.

Yang, H., Weng, G., Yao, X., Tang, C., Shan, Y., 2015. Arterial injury during percutaneous nephrostomy: angiography findings from an Isolated Porcine Kidney Model. Urol. J. 12, 2396–2399.

Příloha II:

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Res earch review

Porcine Liver Anatomy Applied to Biomedicine

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ARTICLE INFO

ABSTRACT

Currently, there are altleas:t *70* pure domes:tic pig b reeds , buton.ly ttrtain breeds are used in biomedi<:al research . The domes:tic pig liver i:s suita b le for predinical research because its size, physiology, and anatomy are similar to that of the hu.man liver; in addit ion, there i:s a high degree of genetic similarity between the two species. For planning e.xperiments and identifying improvements in both invasive and noninvasive methods of liver di:sease management, the morphological similarities and dissimilar· ities of the pig liver to, its hu.man counterpart must be taken intoconsideration along with sexu.al dimorphism and interindividua.l and interspecióc: variability. Rettnt his· tological eval uat ions ba.s ed on stereo logical methods enable precise quantitative morphological estimates and guarantee their unbiased accuracy. The res:ults thereof are crucial for revea.ling and assessing histological changes and can contribute to the optimization of study designs. New trends in computed tomography data processing have also been introcluced. This review art i,c:le swnmarizes the newes:t trends and findings in th• field of porcin• liver anatomy and histology as a pplic abl• to prttlinical researc:h.

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lntrodu ction

The domestic pig (Sus *scrofa* domestirus) is an even -toedungu·
late mammal. Genetic evidence indicates that this species

developedfromthewildboarin SoutheastAsia approximately
5 million years ago, and domestication began in Eurasia
approximately 10,000 years ago.[1] **Hence , there is a general**
oonsensus that al! variations of pigs (induding mini/micrcv
miniature) descend úom the Eurasian wild boar. Swine were
6rst usedin biomedical research (BMR) in the 19 40s, and their
use has increased since the 1960s.[2] **More recently, molecular**
biology and geneticshavebeenappliedtothebreeding process
toenhanceselectionforvatuabletraits. Atpresent, the number
of pig breeds is uncertain, but a review of more than 70 pure
domestic pigbreedsfromallover the world is maintained on

The Pig Site.[3] Most of thesebreedsare primarily used for con-
sumption, but certain small breeds are also used for BMR[4]**.**
Because the porcine genome, which consists of18 autosomes
and2sexchromosomes,hasbeensequencedandits extensive

homology *to* humans has been demonstrated,[5] genetically

modi6ed pigs have played an important role in translational
studies focused on numerous ctise ases, including canc:er, dia
betes mellitus, Alzheimer's disease, cystic 6brosis, and
Duchenne muscular dystrophy.[•] ln addition, because the

structure and function of the porcine cardiovascular, renal,

and gastrointestinal organs are similar to those of humans.[']
theseorgans are extensivety used in experimental surgery to
testsurgical techniques and experimental therapies.,. before
theiruseinhumans(rable1). ln addition, the swine can live up
to l Oyea rs, therebyallowingtheresearcherslong-term foilow-
up after experimental procedures.

The small **pigs** used in BMR. from an anatomical and

physiological perspective, are similar *to* the domestic swine.
The advantages of their smail sizeare obvious: these pigsare
easy *to* handleand house, and they require Jess food and test
substances than larger pigs. Therefore, certain smail breeds are
predominantly used in pharmacology and toncology studies
for the testing of new drugs and medical devices4[2]
(rable 1). Nevertheless, the availability of small breeds is

limited.

Porcine liver gross anatomy

ln term.s of gross anatomy, from four to six liver lobes have

been described in pigs (Table 2), and the right and Jeft he mi-
livers are separated by a deep notch for the round ligament
(Fig. 1A and **B).** The number and size of the lobes (segments)
reported varies and thus may be breed-specmc. Only the

Table 1 e Pordne liverapplications in biomedica research

Objects of research	Refere.nc:es	Breed
Ablation, resecti.on	•	OJ.angxi e.ama mi.nipig
	'''	Wuzlŭshan pig
Biomecha.rŭc:s	"	Not mentioned
	u	Not mentioned
	''	Ecol ogicallybred a.rŭmals of variousoriw.ns
Gene**therapy**	','''	HSDllBl-tran.sgerŭc pig
tmagingtech.rŭques , diagnostic:s	u	Duroc, Pietrain,Landrace, large white pig
	"	e.ama miniature pig
	',· ···''	
tnnovation *d.* surgical techniques		Lan dracepig
	', ·	Landrace pig
	'''	e.ama miniature pig
J.iver transplantatioo	2?	Micromi.ni pigs{FujiMicra Ine., Slŭzuoka, Japan)
	• •	L an dracepig
	• •	GalT-KO pig
J.iver traumatology	2S	Yorblŭre-1.andracepig
	" !	Cermandomestic pig
	'',·'	Not mentioned
Material testing	', ·	Olinese experimental hybrid pigs
	••	Cerman Landrace pigs
Mathematical modeling,	" ,	Not mentioned
quanti.6.cation		
		Prestice Black:-Pied pig
	",	Prestice Black:-Pied pig
Morphology, physiology	••	Prestice Black:-Pied pig
	ss	White pig
	••	
		Prestice Black:-Pied pig
	·	Prestice Black:-Pied pig
Pathogenesis,liver íailure	••	SpeciJic pathogen-free piglet
	'	Sama experimental mi.niature pig
	••	
	••	
		Sama experimental mi.niature pig
Pharmacology, toxicology	"	Prestice Black:-Pied pig
	••	

following 6ve Jobes are invariably present the Jeft lateral, Jeft media rightlater right media!, and the caudate Jobe. From

our experiencewiththe Prestice Black-Pied pigbreed,.. which

is in agreement with the veterinary anatomical text'books[49] the quadrate lobe is present occasionaily (Fig. 1B and D). There also seems to be interbreed variability in the Jobe pro- portions. Court *et* al.[50] found the left lateral lobe to be the

largest, whereas Bekheit *et al.,*[35] based on computed tomog· raphy (Cl) volumetry, assessed the right media! Jobe to be the **mostvoluminous.**

		Minipig. review
	'''	**Landrace** x **largewlŭte pig**
Regeneration	''	**Not mentioned**
	''	**Polishwlŭte pig**
	,'''_'	**Prestice Black:-Pied pig**
	,	

ln the pig, eight segments roughly similar to those in the human liver havebeen desmbesd ..s, (Tables 2 and 3; Fig.1A and B). However, both the differences between pigs and

Tahle 2. Uve:r segmental anatomy, literarure review .

Breed	Noof lobes	Name of the lobes	Noof segments	Reference
Not menti.oned	S	Rightlateral, rightmedial, leftlateral, left med.ial, caudate	8	SS
Not men ti.oned	S	Right , left, right media!, left media!, ca udate	8	!!!
Prestice Black:-Pi ed pig	S (6)	Rightlateral, right med.ial, l eftlateral, left med.ial, caudate, quadrateoccasionally	Not mentioned	• •
Not mentioned	4	Right, left, right paramedian, leftparamedian	8	!!
Prestice Black:-Pi ed pig	6	Rightlateral, rightmed.ial, leftleteral, left med.ial, caudate, quadrate		• •
Not men tioned	3	Notnamed	8	S2
Not mentioned	S	Right, left , rightmiddle, left middle, caudate	8	S3

humans andamongbreedsof pigs mustbetaken intoaccount in extrapolating from porcine to human liver surge,y. Unlike in the human liver, the so-called Rex-<:antlie linethat divides the embryological and morphological right and left hemiliver is weil marked in pigs by the deep notch for the round ligament, flssura ligament teretis (Fig. 1A and B). This linecan be seen afterligation of theright orleft lobarbranch ofthe porta!

vein (PV)'. However, owing to the intraparenchyrnal thin·

wailed caudal vena cava (Fig. 10), resection of the right lateral lobe in the pig liver is extremely difficult. The liver hilum is located dorsallyon the visceral surface, and the PV ram.ines a few centimeters outside the parenchyma out to the right lobe.⁵⁰ ³ The hepatic artery, the porta! vein, and the common hepatic duet branch are accompanied by consider able connective tissue (Fig. 18) that derives from the

perivascular flbrous capsule (of Glisson) and forms pedicles very similar to the ones found in the human l.ivers. s.⁵⁵ The gailbladder lies within the substance of the right media! lobe, if present, and it separates thislobefromthequadrate lobe⁴⁹ (Fig. 18 80).

Although the PV s upp lies up to 90% of the blood volume and up to 2/3 of the oxygen supply to the liver, the hepatic

artery proper is irreplaceable for its supply of the biliary tree

(Fig.1 q . 1n experimental studies, embolization or ligation ofa PVbranchwas used to stop the blood flowthroughout a part of the liver. ln this procedure, thepart ofthe liverwith restricted blood flow atrophies, whereas the rest of the tissue hyper· trophies.a This method is also used in human subjects, for example, in patients withcolorectal carcinoma metastasis in the right liverlobe; afterintestinalsurge,yand alsomostoften
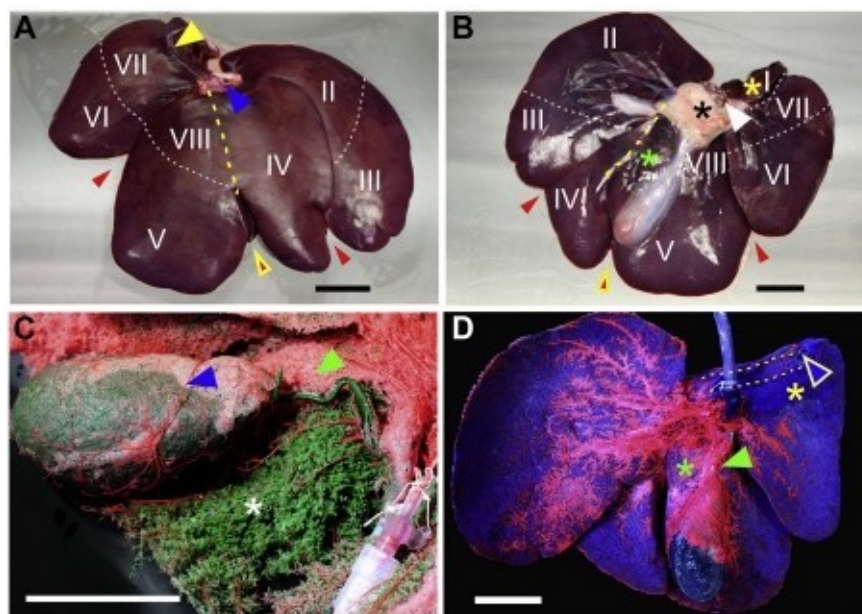

Fig. 1 e Pcrcineliwrgrossanatomy andcorrosic:o casts,Prestia, Black-Pied breed. (A,B)Segmental anatomy, cliapbragmatic (A)and visaoral surface(8). lntedcbal notthes(reciarmwheacls;), notch fcr the ligamenrum reres(yellow-red armwheads), Rex-Candieline (yellow Jines), corc:oary ligament (yellow arrowhead), caudalvena cava (blue armwhead), porta) win (white arrowhead), hepatoduodenalligament (black asterisk), caudatalobe (yeDow asterisk), and quadrare Jobe (greenasterisk). (C, D) Vascular corrosion cast, fillingwith colored Biodur E20 Plus (Heidelberg, Cermany) via the hepatic portalvein (blue), hepatic ane,y (red) and bDe duet (green). Caudare Jobe (yellow asrerisk), quadrare Jobe(green asrerisk), cystic arrery (blue arrowhead), capiDary network of the cystic duet (green arrowheads), bDeduetules(whiteasteriskj, caudal wna cavacoune

(yellowline), and c:riflce (yellow-blue armwhead.) Scale barsS an. (Colorversion of 6gure is available c:oline.)

Tahle 3 e Pordne liverlcbes and segments according to Court *et* al..*se* and Zancbet and Monteiro[53]

Corresponding parenchyma	Segment
Caudatelobe	S I
Left:laterallobe	sn, m
Left media!lobe	S IV
Right media!lobe	sv, vm
Rightlaterallobe	SV!, VII

of the human liver.[7] The parenchymal architecture is organized into well-defmed, polygonal lobules centered around thecentralveinwithportaltriadsat itscomers (Figs. 2A and C, 3 AeC). The porta! lobules are demarcated by 6brous septa containing variable amounts of connective tissue(Fig.3Ae E). Thr ee - dimensional (3·0) reconstruction of the porcine liver has revealed that the morphological lobule is pentagonal rather than hexagonal as it is described in humans.[58] How· ever, the exact sizeand shapeof porta!lobules in the porcine liver and their spatial organization remain unknown.

Thefunctionalacinarconcept,frrstdescnbedinthehuman liver by Rappaport,[59] divides the parenchyma into three concent:ric zones based on the perfusion of the liver: the dassical lobule, porta! lobuleand a<inus . As per thisscheme,

„metabolic zonation refers to thedifferencesinexpression of

the keymetabotic genesin hepatocytes (HEPs)withindifferent zones of the liver lobule; this zonation corresponds, to some degree, to the zonesof the hepatic ad nus.[60] However, this set of concepts is not sufficient to explain al! the physiologkal and pathologic-al processes in the liver.[61]

The morphometry and distnbution of HEPs within the por<ine tiverwas assessedby)unatas etal.[36] ln healthy pigsof the Prestice Black-Pied breed,.. each lobe contained HEPsofa comparable si.ze, nuclearity, and density. However, the size and density were not unifonnly distributed when comparing the ctifferent regions of the l.iver in relation to the hepatic vasculature (periphera paracaval, and periportal regions.) The reported fraction of binudear HEPs, possibly tinked to liverrege.nera tion, was 4%.lnter estingly, the numberofnudei
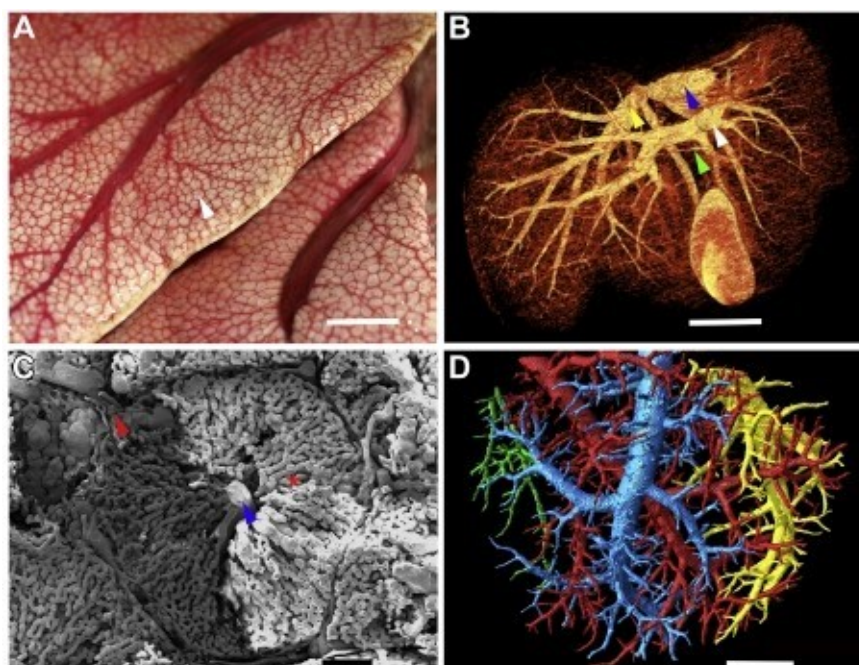
after chemotherapy, embotization oftherightPVisperformed (the arterial supply is preserved to avoid necrosis.) The lobe with metastasis atrophies, whereas the contralateral lobe

undergoes compensatory hypertrophy, which, after several

weeks (provkled that the parenchyma is functionally suffi-<ient) , ailows for the resection of the partwith the malignant deposit. Unfortunately, the growthof the nonoccluded tiver is not atways sufficient to avoid liver failure. Therefore, experi· mental studies using the porcine liver model for testing various ocdusive methods and/or pharmacologic-al treat · ments have been designed.a.[46, 57]

Porcine liver microanatomy

For studies of human liver diseases using the porcine liver model, detailed knowledge of tiver microanatomy is neces-sary. The tissuestructure of the porcine liverissimilar to that

Fig. 2 a **Vascular oorrmion csa m ofpigtiver, Preslice Black-Pied breed. Fillingwitb oolored Biodur E20 via tbe bepatic artery (A-C) and ponal vein (B-0). (Aj Qassical bepatic lobule (white arrowbead) before oorrosion. (B) Macm-CT,volume rendering teclmique. Syntopic vino ofportal and bepatic ven ous sys tems ; bepatic vein (yellow arrowhead), pon al vein (wbite arrowbead), hepatic duet(green arrowbead), and caudalven a cava (blue arrowbe ad). (C) Qass ical hepaliclobule in scanning electron microscopy. Pona! lliad in porta) spaa, (red arrowbead), sin uso ids (redas terisk), and centra)vein(blue arrowhead).**
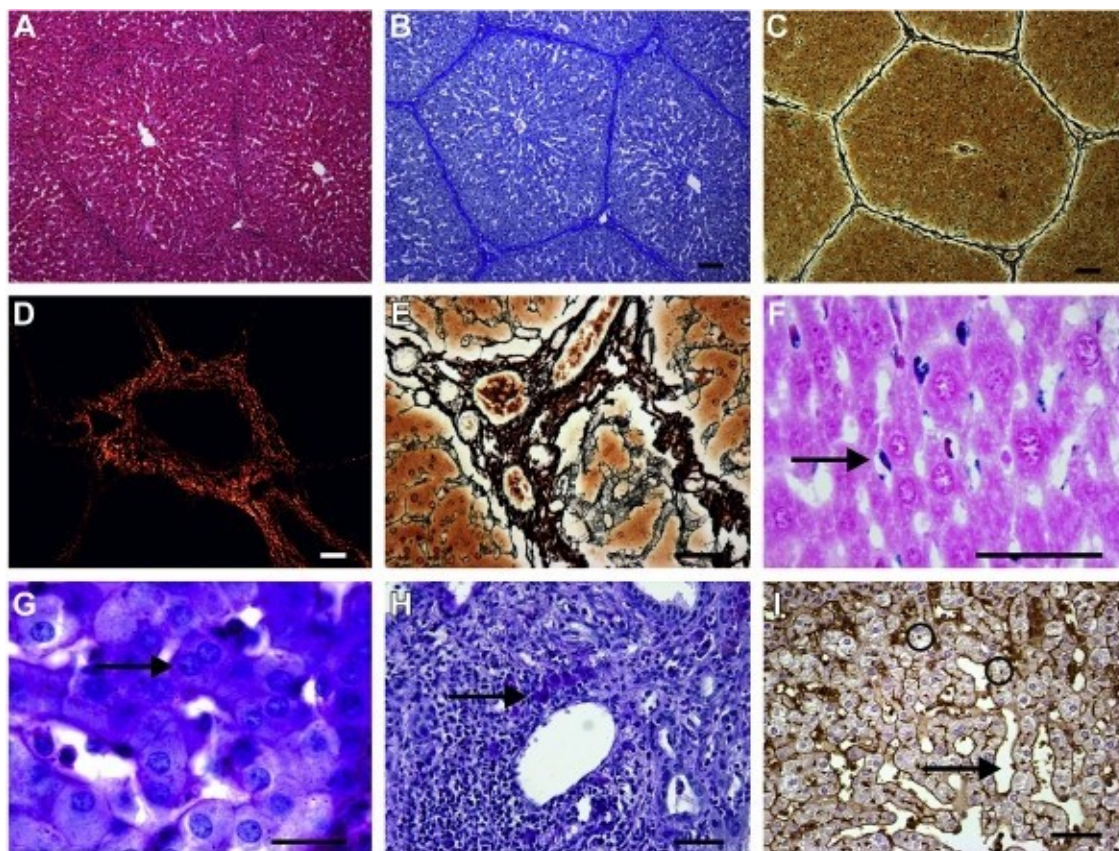
(O) Micm-CT, 3· 0 recc:sn truction, cas t 6Dedvi a tbe pc:rtal vein. Scalebars: 1 cm (A, O), S cm (B), 200 mm (C). (Cdorversion of 6gure ıs **available online.)**

Fig. 3 - PordneJivu miaoanatomy invarioos bistological1111ining metbods. Hematmylin and eosin smining (A), anilin blue staining oounterstainedwitb nuclear fast red:deieclion of coDagen (B), Gomori reliculin staining:deteclion of relicular 6.bers (C, E), piaosirius redstaining in polarizedlight:deteclion of ooDagen I and m (D), Periodic acid-Shiff smining (G, H), leclin bistocbemisny staining using Ricinus communis Agglutinin leclin Ϛ, (A-C) Demc:nstration of bepaticlcbules dearly demarcsted by oonneclive tissu e. (D, f.) Detail cfportalspacecontainingbiledueta and brancbes ofhepatic ane,yandporta! vein surmunded by oonnective tissue. (1) Hepatic stellate oells (armwj. (G) Binuclear hepatocyte (arrow). (H) Cluster cf Kuppfer oeDa after pbagocytoaing oeDular debria (arrowj, **a** typical hallmark of hepatitis. (l) Diaoontinuoua endotbelium cf hepatic sinusoida (armwj and bile canaliculi formed by apicsl membranea of adjacent hepatocytm (drdes). Scale bars: 100 µm.(A-C), 50 µm. (D-1). (Colorveraion of 6.gure is **available**, c:nline.)

does not always correspond with the ploidy of HEPs.[62] However, the morphometry and dist:ribution of HEPs was not mapped with respect to their position within the classical morphological hepatic lobules and liver acini [63] Data on lobular and acinar distnbution would provide valuable information ooncerning the roleof HEPs in pathological processes, which predominantly involve the acinar zones. ln addition, an estimation of the total number of HEPs within the different lobes[64] would serve as a powed'ul tool for comparing the functional capacity and regenerative poten tial of the hepatic lobes after a partial hepatectomy.[47 "]

The exocrine function of the liver, bi te production, is morphologically represented by a delicate intralobular netwodc of chan nels, bile canaliculi (Fig. 31), which drain the bileintotheinterlobularbileducts. Hepaticsinusoids (Figs. 2C and 39 are low- pressur e vessels that tie between plates of HEPs and are tined by speciatized endothelia! cells and fuced macrophages called Kupffer cells (KCs) (Fig. 3H). The integrity of KCs has been tinked to hepatoceilular protiferation and

thus presumably plays a role in liver rege.nera tion.[66] A recent study on the rat liver revealed that female rats havea greater number of HEPs and KCs and a largerfraction of binuclear cells than males[63] These fmdings indicate a higher regenerative potential of the female tiver. The stellate cells (Fig. 3f), whichare alsocalled Itocellsorfat-storingceils, are located in the perisinusoidalspace(of Disse) between the HEPs and the sinus oids. They contain lipids that are invotved in vitamin A metabolism and also play a leading rolein 6brogenesis.[6,]

ln the pig. design-based stereology[64,...] (Fig.4) has been demonstrated to be a powerful tool for assessing numerous morphological charac teristics, namely, the number of HEPs and the amount of connective tissue within the porcine tiver.[36.3'] Generating data on the morphometry and distnbution of liver ceils, together with the 3-D architecture of the vascular and biliary systems, could be the next step in understanding ofliver *function* and pathophysiology and could increase the potential of succ:essful xenotran splanta tion.[69] The evidence indicating that the liver is a sexuaUy
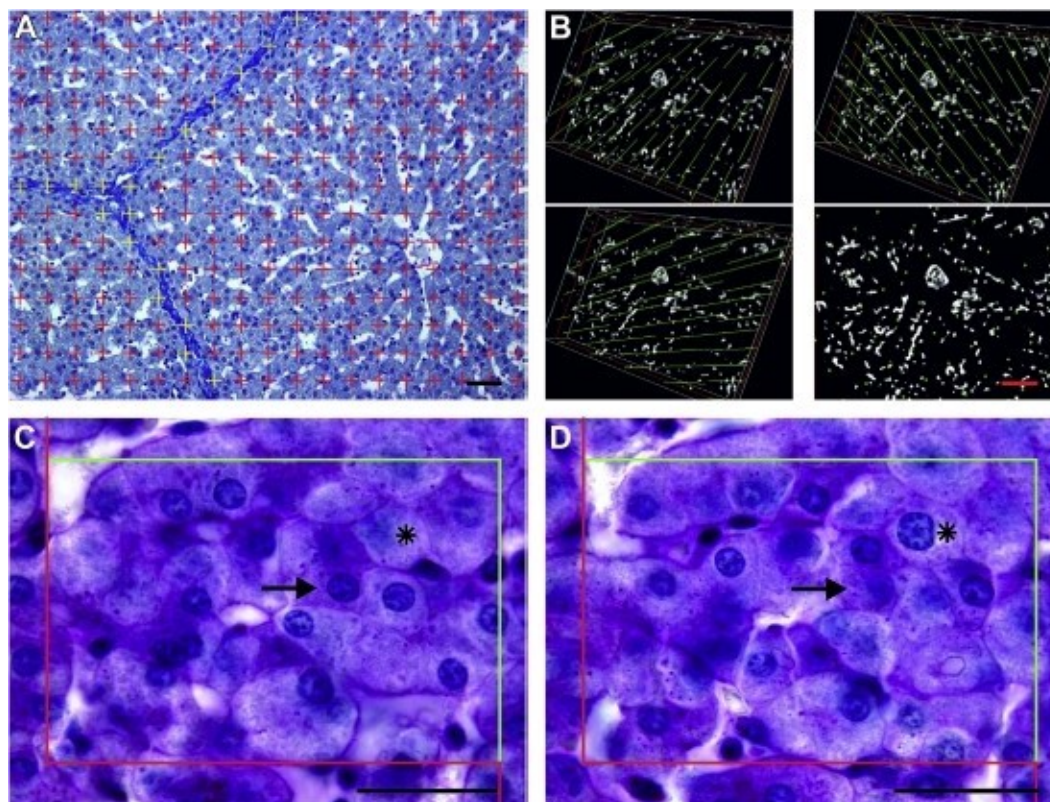
Fig.4 - Demon.stra.tion of Sb!l'l!o k,gical metbods for quantitative asaeument inporcine tiver. Point gtid[68] used for tbe quanti.6cationof tbe conneClive tisaue &acticn, anilinebluewitb nudearfa.st l'l!d s tain.Ďl.g . A:>int .s over tbe cormective tisau e witbin tbe predetermined **area are** bigbligbted in yellow (A). (B) Eslimating tbe surt'aced mia ovesselain a seriea of µ·CT **scans of vascular corrosion ca.st usinc tbe fakir prcbe ccn.sisting of an isottopic pid witb tbree differendy oriented, perpendirular sem of pmbea witb random initial orientalion. Lengtb oftbe probe intenectiona witb tbe microveaael p mfilea** ia **proponional to tbeil'suJface (B). Demonatralion of opiicaldi.asec:tor for eatim aling tbe numerical denairy of hepatocytes** in **two opiicalsectiona(C,D). 1be ceDprofileawirbnuclei**in focuain tbe bottom referenceplane (C)an d absent**oroutoffocuai**n **tbelook·upplane** (D) tbatJie inaide tbe counting frame *ac* toucb tbe green allowance botdem but **do not a oaa** tbe reci **fcrbidden bordenare a:>unted (black armwj . Tbe hepatocyte** in focua in tbe look· up plane ia **not counted (marked witb asteriak.) Scale bars: 50** µm **(A), 100** µm **(B); 2 0** µm (C, D). **(Colorvenic:o d 6gure** ia **available c:oline.)**

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dimoiphic organ warrants cons ideration in the planning of experiments." ·[63] SUf6cient and systematicsamp ling is cru<ial **for de tec ting the biologie-al di fferences in live r ti ss ue when** studying liver regeneration, the pathophysiological mecha· nisms of liverdiseases or the toxicityof drugs.[64]

Liver conn ective tissue

Most typesof chronic liverdiseasesleadto liver6brosis and/or **eventuaUy to dnh osis, that is, to the excessive accumulation** of extraceDular matrix, including 6briilar ooilagen. Staging and grading of liver 6brosis is a signi6cant histopathological **evalua tio n.**⟨ᵥ ⁰ **ln the human , six spec:iftc foci of liver 6bro-** genesis have been proposed for scoring. Namely, the porta!, periceDular (perisinusoidal), pericentral (perivenulalj, cen- trilobular, dueta! (periductal), and ductular 6brosis can be dislinguished.[61] The typeandextent of 6brosis descnbesboth **the architecture of connective tissue and its amount and**

measures how far the liver disease has progressed to its end stage- liver cirrhosis. ln addition, the grade of the liver dis- easereflects the speedat which the diseasewill progress to its **end stage. The am ount and the architecture of connective tissue in the human liveris usuaily estimated during routine analysis of liverbiopsies, usually as perone of the threemost** widely used scoring systems." However, the subjectivity and hence the lacle of reproducible results of this routine biopsy **scoring have been articulatedn.**

For the study of the mechanisms of origin and spread of **6brosis, and also to assess the liver regenerative capad ty,** bothsmailand largeanimalsare widely used. However, sma il **animal models have limitations due to their si.ze and differ e ntial su sceptib ility to toxic agents or pathogens.**[1]₋[15] A chal lengeinevalualing6brosis using this model is that thenormal **pordne livermicroscop caily resembles 6brosis in the human;** por<ine liver lobules are clearly demarcated by the interlob· **ular connective tissue septa that meet in the portal area** (Fig. 3A- D). Moreover, according to the qua ntitative studies,

thedistnbution of intralobular and interlobular oonnec tive **tissue in the healthy domestic pig varies with respect to sex and location wi thi n the l.iver .[37] ln addition, the mean area fraction of the interlobular connective tissue is greater in male pigs: signi6cant differences have alsobee.n found among in-** dividuals and liver lobes. OWing to the qua ntitative methods that provide us with objective and reproduoble data, when both the sex of specimen and the position of the harvested **tissuebloc.kare taken into account. more precise quantitative estimates canbe achieved[31]"4.""**

Corrosion casting

Perfusion is a key elemen t to understand the physiology and **rege.neration of any organ. Bec-ause of having two blood in-** puts, tiver perfusion is very complicated, and research is based on the analysis of the geometrical model of thevascular tree. To obtain the highest authenti<ity model of the 3-0 **vascular bed, vascular corrosion casts (VCCs) have bee.n**

usedfor many dec-ades[9] [52,5][h]   Cwren t casting materials are

abletofill the entire vascularbed,whichneedstobepreserved free from gas and blood d ots. As it is almost impossible to **obtain a human liverwith these charactristics.'[8] the porcine** tiver model is irreplaceable.[79] The gap between 2·0 histolog-kal studiesand 3-0 microarchitecture can bebridged usingX· ray microtomographyd micro-CT,[3] Micro-CT data of tiver **VCC repr ese.nt another source to provide microvascular** morphometry.[33] Using differently colored resins in the VCC (Figs. lC and O, 2A) is also useful for teaching anatomy, as it enables the studen t to distinguish the vascular (biliary) beds **and differentiates 6.ne structures that cannot otherwise be demonstrated.34-,at**

Computer vision algorithms in pig liver research

To model liver anatomy and the processes in the liver pa-renchym.a, it is necessary to create macroscopic and micro- scopic models of the vascular bed. Obtaining imaging data **with the required resolution from human patie.nts are not possible for tec.hnic-al reasons , so the pordne liver model is** often used. Many kinds of software have been designed to

analyze images from 3-0 imagingdevices; most of them were developed with regard to dinical practice. For the specme re-**quirements of pig liver anatomy research, however, spedal** software needed to be developed[37]·

To extract the shape of the tiver parenchyma and the **macroscopic model of the vascular system, macro..CTwith a resotution of „1 mm was perfonned using pig livercorrosion** casts (Fig. 28). Segmentation of the tiver parenchyma by the macro-CTwas performed interactively by themodifled graph·**rut algorithm[43] available in the Lisa app lic-ation.[37][112]The pre·processing was carried out by resampling to a voxel size of** 2 mm. Voxels representing the subject of interest (e.g., liver parenchyma)and background (othertissues) wereselected. Of **these, an intense Gaussian mixture model with three fore·ground and background components was created Vascular** bed segmentation was performed by a modifled connected threshold algorithm. ln postprocessing.binary morphological operations were apptied. Extraction of the microvascular bed geometry was performed using the image data acquired from micro-CT with a resolution of · 2 11m (Fig. 20). For the flow

modeling and the vascular bed quantitative description, the

softwareQuantancanbe used.[33] to es timate the radius, le.ngth, tortuosity, and branching angles.The apptication alsoenables **the evatuation of the number of microvessel segments, the volume fraction, as well as the surface and length densities. Semiautomatic subtraction facilitates the evaluatot's work andailows formore precise estimates.**

Challenges and pilfalls of pig liver utilization in biomedical research

The domestic pig as an experimental animal was critical to many of the key shifts inresearchon serious human diseases. Althoughthe detailed knowledgeof(micro)anatomyis cru<ial **both for designing and evaluation of most experiments, it is** interesting that very few breed-related and/or gender-sensitive studies have been pubtished on the anatomy of **porcine organs yet. For this reason, we found use.fu! to sum ·** marize the current lmowledge of (micro) anatomy of the **porcine liver to point out critical points in planning and asses smen t of studies conducted in this animal model** (rable4 }. Given the sizeof porcine organs and vast biological

Table4e Morphological differencesbetween thenorma}porcineandbumanlive:r,basedon Prestice Black-Pied pig breed.[48]	
Gross anatomy	a.od sometimes IV.[85]
Pis Deep 6ssures markingwell-mark.ed lobation, right and left hemiliverd.ividedby the notch forligamentum teres.'[79] Hurnan: Extemallobationdoesnotcorrespond to the functional{i.e. surgical) anatomy: three main 6ssuresarenotvisibleon thesurface a.odcontain three mainhepatic veins; theyd.ivide theliver intofour porta! sec tors; righta.odleft:hemiliver d.ividedbyCantlie'sline.	
Pig; Superúcial, extraparenchymal portalvein (PV)lobar branclú ng visible fromthevi.sceralsur-face {Fig. 10) , thecaudalcavalvei.n runs through the leftlaterallobe.so. Hurnan:PVd.ividesinto therightandleftbranchesatthelůlum;only theleftPVinvariablygivesextraparenchym.albranches to segment I	

Pis Inegular, rather pentagonal hepaticlobules {Fig. 2A) clearlydema.rcated by connective tissue septa {Fig. 3 AeC). Caution, norma! porcineliver microscopicallyresem.bles 6.brosis in the hum.an; more connective tissue in males.[37]

Hum.an: Roughlyhex.agonalhepaticlobules,[61] no interlobular septa, connective tissue fou.od in interlobular space.[85]

variability, it is dear that morphological studies cannot be **carried out without the use of stereologic-al procedures.**[68] Unbiased stereology also allows for setting up sarnpling opti· **mization-the latest studies revealed different tissue struc**

ture depending on the sampling location ,з l.M These

procedures *can*be **advantageouslyusedalsoforevaluation in** imaging methods such as (micro)CT or magnetic resonance imaging."'

ln experimental medicine, the pig liver is preferably used **as a large animal model of acute liver disease and/or liver failureas it is suitable forextracorporealcin:ulation, repetitive** bloodsarnpling. and trainingof surgical techniquesapplicable **to humans, for example, partial hepatectomies, orthotopic liver transplantation, or PV arterialization.**[87] **Unlike smail** animals, experiments on pigs and other large animal models

are limited because oftechnicaily demanding treatment and higherfmancialcoststhatresult insmaileroohorts,whichdue to considerable interindividual variability makes statistka! **evaluation and interpretation of results dif6rult.**

Condusions

A variety of modem pigbreeds are available today, and they **have been increasingly used in biomedic-al research. Despite** recent efforts to obtain detailed knowledge of **pig** liver mac- **roanatomy and mic:roanatomy, very little is stiil known in accordance withbiologie-alvariability, andcertain issuesneed** to be addressed for the appropriate translation of animal ob- **servations to clinic-al testing. Quantitative results based on stereology guarantee unbiased estimates that are able to** reveal even subtle histological changes, and it is also possible **to optimi.ze the algorithm for study designs in accordance** with sex, breed, or position of the harvested tissue block. Newly developed software is able to process data obtained from (micro}CT for 3-D reoonstructions, quantitative estima- tions, and morphometrics, which is highly oonsistent with **manual counting techniques. Volumetrie data extracted from** oomputer imaging methods can **be** used to model liver **perfusion and optimal liverresection.**

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ped shape the manuscript; and Liska Vaclav provided critical revision and helped shape the manuscript

Disd osu re

REFERENCES

conceptual idea and wrote most of the article; Malec.kova Anna cowrote the article and provided histological ógures;

1.

Larson C, Dobney K, Albarena u, et al. Worldw phylogeo aphy of wild boar n,v•als multipl• centers of pig domecstic:ation. science. 2005; 3'.>7:1618-1621.

2. Bustad LK, McCleilan RO. Swine in bio cal recsearch. Sáena.. 1966;152:1526-15'1>.

3. Bn,eds · I Th• Pig Sit•. Availabl• at: https://th• pigsite. com/. Accessed March 21, 2019.

4. Swindle MM. Swine In the IA.bora.1nry: surgery, Anesthesia., ima9in9, a.nd experimental t« hn.iques. 3rd . Boca Raton, n.: CRC press ; 2016.

5. Groen•n MAM. An:lubald AL, U•nishi H, et al. Analyses of pig genome,s provi insight into porcine mography and evotution. Nature. 2012;491:393-398.

6. P@rleberg C, Kind A. Schni@b A. G@neticaily @nginf!en,d pigs as models for human disease. DisModel Mech. 2018;11 . https:// doi or9'10.1241/dmn030783

7. Lossi L, D'Angelo L, O. Girolamo P, M... ighi A. Anatomical fearures for an ad ate cho of .xp!.ritnental animal m l in bio .icine: ll Small laboratory ro nts, rabbit, and pigAnn Anat. 2016;204:11-28.

8. Liska V, ed. E,perimental Surgery. Plzen: Nava Publishing; 2016.

9.)iang K, Chen J, Liu Y, et al. H•at-irrigate •ffect' of radiofrequMcy ablation on re vant regional hepatocyte in living swine liver-initial study on pathology. Cell Biochtm Biophys 2015;72:37-41.

10. Dong J. Geng X, Yang Y, et al. Dynamic imaging and pathological change,s in pig liv« after MR - microwave ablation. BMC C<tntt r. 2018;18:397.

11. Smolock AR Crist•scu MM, Vlaisavljevich E, et al Robotically assist sonk: the.rapy as a noninvasive nonth«mal ablation modality: proof of concept in a porcin• liver model Radiology. 2018;287485-493.

12. W•x C. Ston A. Frohlich M, Arndt s. Lippert H. How precseNation time change,s the linear viscoelastic prope.rties of portine liver. Biorheology. 2013;50:ll S-131.

13. W•x C. Ston A. Frohlich M, Arndt s. Lippert H. Me<:hanics of fresh. frozen.thawed and heated porcine liver tissue. tntJ Hypt rthermia. 2014;30271-283.

14. Cam,ňo O, S.ndra L, MontalvA E, et al. A swgical model lbr isolating the pig liver in vivo for gene therapy. tur Surg Res. 2013;51:47-57.

15. Kang HY, Choi Y-K. Je<>ng Yl, et al lmmortalization of portin• 11beta-hydroxyst...oid dehydrogenase typ•1- transgenic liverceDs using SV40 largeT antigen. !nt/ Mol Sá. 2017;18:2625

16. X.iberta P, Boada I, Bardera A, Font·i·Furnols M. A se.mi• automatic: and an automatic: segmentation algorithm to re.move the interna! organs from live pig CT image,s. Comput Ele<eb'on Agric. 20V;140290-302

V . L--T, Xu D·X,Tian C,et al. Va1ueof two-d.imensiona.lshear wave elastography for asse,ssing acuteli\ll!r conge,stion in a bama mini-pig modol. Di9 Ois Sá. 2018;63:1851- 1859.

18. Court R:;, LawsPa Morrison CP,et al. Subtota.l hepatectomy : a porcine model for the study of 1.iverrege,ne.ration. J Sllr9 Res. 2004;116:181- 186.

19. Clowka TR,Standop J, Paschenda P, Czaplik M, Kalff JC. Tolba RH. Argon and helium plasma coagulation of porcine liver tissue.J !nt Mal Res. 2017; 45:1505- 1517.

20. Vrochides D,Kardassis D, Ntinas A,et al. A novel liver parMchyma transection technique using locking straight rigid ti<,s. M experimental study in pigs. J lnuest Sur9. 2014;271 06- 113.

21. Zhang Z-B, Gao W, Liu L,Shi Y, Ma N,Shen Z-Y. Development and assecssrnent of nonnothennic machin!! p!.rfu.sion preservation for e.xtracorporea.l splitting of pig liVl!r. Ann 'l)-4nspldnt. 20V ;22:507-5 17.

22. Okada N, Mlzuta K. Oshima M. et: al A novel split liver protocol using the subnonnothttmk:oxygi!nat circuit system in a porcine model of a marginal donor pr ure. 'l)-4nspldnt Proc. 2015;47:419-4 26.

23. VogelT, Broclanann JG. Pigott O, et al. Suca,ssful transplantation of pordne liver grafts fi:>llowing 48.hour nonnothttm.ic precseNation. PLoS One. 2017;12:e<>188494.

24. Louras N, PatelM, Shah J,Sachs O, VageJiP. Biochemistry profile,s following pig.to.nonhumanprimate liv« xe.notransplanta tion. AmJ Tra.nspla.nt 2018;18:763- 764.

25. Detennan C, And«son R. Beck« A, et al. F state prior to h orrhagicshod: andpolytrauma in a porcine model recsults in altered l.ivtt transcriptom.ic response. PLoS Orte. **2014 :e10008&**

26. Frohlich M, Hildebrand F, Weuster M, et al lnduced hypothennia re,ducecs the hepatic inflammatory response in a @ multipl@ tr.1uma mod@l. J n-auma kutt Cdrt SUr9. 2014;76:1 425- 1432.

27. E:schbach D,Horst K.Sassen M, et al. Hypothermia does not intfluen liv« damagi!and function in a porci.ne polytrauma **modol.** Te<hnol Health Cdre. 2018;26:209- 221.

28. Mylonas Al, Orfanos NF, Karmaniolou O, et al. Th• effects of h orrhagicshod: secondary to hepatectomy in a swine modol.J SUr9 Res. 2015;195:228- 234.

29. Tao L,Li Q, RenH, et al. Repairof extrahepaticbíleductdefe<:t using a collagen patch in a Swine model Arti/ Orga.ns. 2015;39352- 360.

D. Tana.ta H, Fuku.shima K, Srinivasan PK, et al. Dňcacy of the novel med.kal adhesi\11!, MAR·VIV0·107, in an acuteporcine liver recsection model Surg tnnou 2017;24423-431.

31. FU VB, Chul CK. Mod.Oing and simulation of pon:in• liver tissue indentation using finiteelement method and uniaxial stre,ss-strain data.J Biomedt. 2014;47:2430- 2435.

32. Krlillčl:ová A, Eberlová L, Kalusová K, et al. Q.uantification of l.iver microci.rculation using x.ray microtomography of vascular corrosion casts. Key tn9 Mater. 2014;592:505- 508

33. Jifll: M, Tonar Z, Krállčl:ová A, et al. Ste,eological quantification of microvessels usingse.m.iautomat evaluation of x.ray microtomogra.phyof hepatic vascular corrosion casts. lnt)Comput Assist R4di.ol SUrg. 2016;1 119:>3- 1819 .

34. Eberlova L, Liska V, Mirka H, et al Th• use of pon:in• corrosion casts fi:>rteactůng human anatomy. Ann Anat. 2017;21369- 77.

35. Bekheit M, Bucur PO, Wartenbe,g M, Víbert E. Computerittd tomography.based anatomie description of the porcine liv«. JSur9 Res. 2017;210:223-23:l.

36. Junatas KL, Tonar Z, Kubll:ová T, et al. Ste,eological analysis ofsizeand den.sity of hepatocytes in the porcine liver.J Ana.t. 2017;2300575- 588.

37.

Mik P,Tonar Z, Malečková A, et al. Distribution of coMect.ive tissue in the male and female porcine live.r: histological mapping and re<:ommendations fi:>r sa mpling . J Comp Pathol. 2018;162:1- 11

38. Rogée S, Le Cali M, Chafey P, et al. Quantitative proteom.ics identine,s host factors modulat du.ring acute hepatitis E: virus infection in the swine model J Vlrol. 2015;891 29- 143.

39. WangJ, Sun Z.JiangJ, et al. Proteomic signarureof acute liver failure: f.rom discove.ry and veri.6cation in a pig model to confi:rmation in humans. MolCeD Prota:>mics. 2017;161 188- 1199.

40. tiska V,fuska V, Mirka H,et al. lnhlbition of transfonning growthfactor beta •l augments liverre,generation afterpartial porta!vein ligation in a porcine e.xperimental model Hepat09astroentero09y. 201259.235- 240

41. Yu R, Zhang Y, Lu Q, et al Differentially expn,ssed genes in n,spons•to cyadox in swine liveranalyzed by OORT-PCR. Res Vet Sá. 2018;118:72- 78.

42. Swindle MM, Malcin A, Herron AJ, Clubb FJ, Frazier KS. Swine as models in biomed.ical recsearch and toxicology testing. Vet Pnthol. 2012;49:344- 356.

43. 1H KS,Santagostino SF, Li o, et al. Cathet:er-<lirected intr. 1portal delivery of endothelia! cen therapy for liver re,genera.tion: a feasibility study in a large.animal vodel of clrrhosis. R<tdiol09y. 2017;28514- 123.

44. Bartas M, CeNenJ, OppeltJ, et al. liv« regen«ation du.ring the associating liv« partition and porta! vein ligation for stagi!d hepatectomy procedure in Sus scrofa is positivoly modulated by stem c.Os. Onool Lett 2018;156309- 6 321.

45. m.ipponi F, Leoncini C, Campatelli A, et al segmental organization of the pig liver: anatom.ical basis of controUed partition for@xperimental gmlting. rurSurg Rl!S. 1995;27 :151 - 157 .

46 . Liska V,Tn>ska V, Mirka H, et al. lnterleulcin-6au ents activation of l.iverregi!nera.tion in porcinemodel of partial porta! ve.in ligation Antica.ncer Res. 2009;9:2371 2377.

47. Bruha/, Vycital O, Tonar Z, Mirka H, Haidingerova L. Monodonal antibodyagainst transforming growthfactorbeta 1 does not influenceliv«íecgi!nera.tionafterre,section in large animal experiments. tn Vluo. 2015;340:327- 340.

48. Vrtková I. C.netic admlxture analysis in pn,stic•black-pied pigs. Arch Anim Breed. 2015;58:115- 121.

49. KOnig Ha Liebich HC,eds. Anatomie doma.Och sauc-u, 2. díl. Bratislava : H & H; 2013:70- 76.

50. Court FC, Wemyss-Holden SA, Morrison CP, et al. S.gmental naru..re of the porcineliv«anditspotential as a model fi:>rexperimental partial hepatectomy. BrJ Sur9. 2003;90:440-444.

51. Witter K,Tonar Z,SChOpp« H. How many la)'l!.rs has the adventitia? Structu.re of the arttial runica e.xterna recvisited. Mdt Hi.stol Embryo!. 2017;461 10- 120.

52. Lehmann KS, Ritz J·P, Valdeig S, et al. Porta! vein segmentation ofa 30.planning sy-stem fi:>r1.iver sur gery-in vivo evaluation in a porcine model Ann SUrg Onc-ol. 2008.15:1899- 1907.

S3. Za.nehet OJ, Montero E:F. Pigliv«secrorization and segmentation and virtual recality depiction. Arta Cir Bras. 2002;17:381- 387.

54. Couinaud C. (Liver lobes and segments: notes on the anatom.ical architectu.re and surgi!ry of the live.rJ. Presse Med. 1954;62 :709- 712.

SS. Martins AC, Machado MA, Fena.z M . Porcine live.r: experimental model for the intra,hepatic glissonian approach . kf!lCir BrdS. 200!;23:204- 207.

56. Nykonenko A,Vavra. P,Zonca P. Anatomie peculiariatiecsof pig and human liver. Clin Trd ldnt 2017;:25- 26

57. D!nys A. Prior J, Bize P, et al. Porta! veinembolization: what do we know? Cardi.oua.sc Interuent R4diol. 2012;35:999- 1008.

58. E'kata.ksin W, Wa.ke K. Llver units in thrtt d.imensions: I. Organization of argyrophilic: connective tissue skeleton in porcine 1.iver with partic:ular ref«ence to the" compound hepatic lobule". AmJ Anat. 1991;191:113- 153.

59. Rappaport AM, Wilson WD. The stuctural and functional unit in the human liver (liver acinus). Anat Re<.:1958;130:673 -689.

60. I.Amers WH, Hilberts A, FUrt E, et al. Hepatic enzymic zonatiort a ruva1uation of the con ptof th! liver acinus. Hepatology. 1989;10:72-76.

61. Saxe.na R. Practi<nl Hepatic Pathology: A Diagnostic Approach 2nd ed. Philadelphia, PA: Elsevier; 2018.

62. Tanami S, een. Moshe S, Elkayam A, Mayo A, Sahar Halpern K, ltzkovitz S. Dynamic zonation of liver polyploidy. Cell Ti.ssue Res. 2017;368 : 405-4 10 .

63. Marcos R, Lopes C, MalhMo F, et al. Stereological assessment of sexu.al dimorphism in th! rat liver reveals diffe.ren s in hepatocyt.s and Kupffer ceDs but not hepatic steDate cells. J Anat. 2016;228:996- 1005.

64. Marcos R, Mont@i.ro RAF, Rocha E.. The use of design.based ste.reo\ogy to eva1uate volu..me,s and nu.mb«s in the liv«: a review with practical guidelines. J Anat. 2012;220:303- 317.

65. liska V, Treska V, Mirka H, et al. Preop«ative use of biological th«apy does not influence liver regen«ation after large resection . porcine e.xpe.rimental model Tumor Biol. 2012;33 :102 - 103 .

66. Yang K, DuC, Cheng Y, Li Y, CongJ, Liu Z. Augmenter of liver regi!ne.ration promotes hepatic: regene.ration depend.ing on the.integrity of Kupffer n .in rat smaD.for..size liver tra nsplantation . J Surg Res. 2013;183:922-92 8.

67. Puche JE.. Saiman Y, Friedman SL Hepatic: steilate IIs and liver fibrosis. Compr Physiol. 2013; 3:1473- 1492.

68. Mouton PR Principles and PractiCI? of Unibia.s,d 511,...logy: An Introduction for Bioscientists. Baltimore, MD:}Ohns Hopkin.s Univ«sity Press; 2001.

69. Cooper DKC, Ayares D. The immense potential of xenotransplantation in su..rge.ry. lntJ SUrg. 2011;9:12:2-129.

70. Toosi A.tK. Llver fibrosis: causes and rnethods of assessrnent, a review. Rom J Intem Med. 2015;53:304-314

71. 8atts KP, Ludwig). Chronic hepatitis. An update on terminology and reporting. Am J Surg Pathol. 1995;19:1409 - 1 417.

n. a.dossa P, Dargére D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology. 2003;38:1449- 1457.

73.

D'Angelo L, Lossi L, Merighi A, de Cirolamo P. Anatomical fearu..res for the adequate choo of. xpe.rirnental animal models in biomedicine: I. Fishes . Ann Anat. 2016;205:75- 84.

74. O.Jin, B,Starke!P, Leden:q I. Animal models for fibrotic liver diseases: what we have, what we need, and what is under development. J Clin Tran.sl Ilepa!)l. 2015;3:53-.6 .

75. Starke!P, U!clen:q IA. Animal models for the study of hepatic fibrosis. Se.st Prac-t Res Clin Gastroenterol. 2011;25:319- 333.

76. Tschanz S, SChneider JP. Knudsen L Design-based ster eology: plann.ing, volumetry and sampling are crucial steps for a suc ssful study. AnnAnat 2014;I96.:3-11.

77. Lametschwandtner A, Lametschwandtnl!r U. Historka! review and technical su.rvey of vascular casting and scanning electron microscopy. In: SCannlng Eltttron Mic-rostopy o/ Vascular Ca.st.s: Met.hods and Applications. Eltttron Microstopy ln Bi>logy and Medicine. 8oston, MA: Springer;1992:1- 11.

78. Debbaut C, S.gers P, Comillie P, et al Analyzing the human l.iver vascular architecrure by combin.ing vascular corrosion casting and micro.cr scanning: a fioasibility stu dy. J Anat 2014;224:509-5 17 .

79. Eberlova L, list.a V, Mirka H, et al. Porcine liver vascularbed in Biodur E20 corrosion casts. lblia Morphol. 2016;75 :154 - 161 .

80 . Schladitz K. Q.uantitative mk:ro.cr . J Microsc. 2011;243:111- 117.

81 . Pálek R, Liška V, Eberlová L, et al. [Experimental processing of corrosion casts of large animal organs). Rozh.l Oifr. 2018;97 :222 - 228 .

82 . Lisa JM. Coputer-assisted liver surgery. 2018 . Available at: http s:// github.com/ m jirik/lisa Accessed July 31, 2018.

83. Boykov VY,)olly MP. lnteracti"" s,aph cuts for optima! boundaryamp; region segmentation of objects in N-Dimages . ln: Proctaiings Ei9hth IEEE International Conference on Computer Vision. !CCV 2001 . Vol. 1 . 2001 :105 - 112 . http s:// doi.org/ 10.1109/°ICCV.Dl l.937505.

84. Tomái.el:P, Tonar Z. Grajciarová M, et al. Histological mapp.ing of porc.ine carotid arte.ries· an animal model fi:>rthe assessrnent of arti.&ial conduits suitable for coronary bypass grafting in humans. Ann Anat. 2019;228:151434.

85. Bech), Clud AN, SangiD R, et al. The porcine corticospinal decussation: a combined neu.ronal tracing and tractography s tud y. Brain Res Bull. 2018;142:253 - 262 .

86. Stand.ring S. Cray's Anainmy: The Anainmi:al Basiso/ Clinic-al Prac-tic-e. 41st ed. China: Elsevier Llmited; 2016:1166- 1170.

87. Malea:ová A, Tonar Z, Mil: P, et al. Animal models of liver diseases and their application in e.xpe.rirnental Surgi!ry. Rozhl Chir . 2019 8:100- 109.

Příloha III:

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Stereological analysis of size and density of hepatocytes in the porcine liver

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Abstract

The porcíne liver is frequently used as a large animal model for verífication of surgk.al technique as well as experimental therapies. Often. a hístological evaluation ís required that include measurements of the size, nudearity or de nsity of hepatocytes. Our aims were to assess the mean number-weighted volume of hepatocytes, the numerical density of hepatocytes, and the fraction of, binuclear hepatocytes (BnHEP) in the pordne liver, and compare the dístribution of these parameters among hepatic lobes and macroscopic regions of interest (ROls) wíth different posítions related to the liver vasculature. Using d isector and nucleator as design-based stereologk.al meth ods, the morphometry of hepatocytes was quantified ín seven healthy píglets. The samples were obtaíned from all sÍlC hepatic lobes and three ROls (peripheral, paracaval and para portal) wíthin each lobe. Histo logk .a l sections (thickness 16 μm) of formalin-fixed paraffin. embedded material were stained with the periodk acid- Schíff reaction to indicate the cell out lines and were assessed in a seriesof 3-μm-thick optical sections. The mean number-weighted volume of mononuclear hepatocytes (MnHEP) in a ll samp les was 3670 ± 805 μm³ (mean ± SD). The mean number-weighted volume of BnHEP was 7050 ± 2550 μm³ The fraction of BnHEP was 4 ± 2%. The numerical density of all hepatocytes was 146 997 ± 15 7'38 cells mm ³ of liver parenchyma. The porcine he patic lobes contained hepatocytes of a comparable size, nudearity and density. No significant dífferences were identífied between the lobes. The periphera l ROls of the hepatk lobes contained the largest MnHEP wíth the smallest numerk .al de nsity. Thedistribution of a larger MnHEP was correlated with a larger volume of BnHEP and a sma ller numerk .al de nsity of all hepatocytes. Practk.a l recommendations for designing studies that involve stereo logk .al evaluations of the síze, nuclearity and density of hepatocytes in porcine liver are provided.

Key words: animal model; disector; li ver surgery; morphometry; nucleator; pig; stereology; swine .

Introduction

Pordne liver in expe rimenta l surgery

Human hepatobiliary surgery has achieved substantial pro-gress in previous decades (Kwon et al. 2015; Song, 2015;

Parie et al. 2016). Operations such as extended resections. stage procedures and transplant ations would not be possi· blewithout previous pr-ed inical research. Procedures neces-sary for experimental verification of newly developed surgical techniques cannot be evaluate d in smaU anima ls because they lack anatomical and physiological simil arities to the human organism The porcine liver is currently the best described and most suitable large animal model of the human liver in terms of organ size as \'\lell as gross and microscopic morphology and physiology (Court et al. 2003;

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Arkadopoulos et al. 2011). The major difference betv...en the microscopic morpho logy of porcine and human liver is that porcine liver has much more defined interlobular con

nective tissue septa. T he use of pigs as the last step of pre-

dinical research prior to designing dini cal tr ials is done not only for new ly suggested surgical procedures but also in pharmacological research on liver excretory, detoxification and synth etic functio ns import ant for liver regeneration fol· lowing a partial hepatectomy (Liska et al. 2012; Bruha et al. 2015). Similarty, the porcine liver is used in surgical experi· ments regarding therapy of smalJ.for-size injuries that occur after partial Iivertransplantati on or extended hepatectomy (Kelly et al. 2009; Hessheimer et al. 2014). The porcine model has l:e en demonstrate d to be useful w hen examin in g histological alterations of the l iver during carbon diox· ide pneum operitoneum (Alexak is et al. 2008). M oreover, the porci n e liver is used as a model for drug-in duced liver failure (Newsome et al. 20101 a mode l for hepatectomy with portocaval shunt (Iida et al. 2007), or for hepaticelec- t rolyti c ablati on (Gravante et al. 2012.)

The porcine liver has been suggested as a source of hepa-, tocytes for bi oarti'ficial liver devices (Nicolas et al. 2016) and xenografts for transplantati on (Cooper et al. 2015); how- ever, these applications are cont roversial (\Nang & Yang, 2012). Neverthe·less. t h ere is promising research on decellu larized porcin e hepaticlobes used for liver bioengin eerin g (Hussein et al. 2015) in which porcine tissue scaffolds are supposed to be recellularized by human hepatocytes (W u et al. 2015). Recently, Bahr et al. (2016) evaluated samples of liver and oth er organs in tra n.sgenic pigs t hat i:r oduced a recombinant prote in that may be capable of modifyi ng the T<el1-mediated rejection process, th us contr olJin g cellul ar rejection in xenotran.spl ant ation .

H i stological quantifkation of hepatocytes

Studies on path ophysiological mechanisms of liver di.seases, liver rege n erati on, efficacy and toxicity of drugs, and evalu ati on of surgical techniques oft en requir e quanti fication. Num erous morphol<>gcal characteristics may be estimate d, preferab ly using design-based histo logi cal t echniques (Mou- t ai , 2002; How ard & Reed, 2005) that make no further assumpt io ns rega rd in g t he orie ntation or shape of liver cells and other structures under investigation (Marcos et al. 2012). The quantitative parameters of the liver t hat have been assessed in t h e frame of different studies includ e the w hole liver volume. the volume den.sities of orga n con sútu ents (particularly of connective tissu e), the mean vol· ume of hepatocytes and other liver cell.s. the t ot al nu mbe rs of liver cells and t heir numerical density per volume unit (Jack et al. 1990; Karbalay-Doust & Noorafshan, 2009; M ar- cos et al. 2016). Knowle dge regarding the variability of t hese microscopic parameters wit hin t he porcine liver is required for correct histological samplin g.

The vo lume fractions of hepatocytes w ithi n t he liver and the nu merical den.sity of m ononu clear hepatocytes (M nHEP) or binuclear hepatocytes (BnHEP) assessed by stereologi cal methods have been published for mice, rats and humans (Altunkaynak & Ozbek, 2009; Karbalay-Doust

& Noorafshan, 2009; Odaciet al. 2009). Marcos et al. (2016) qu antifi ed hepatocyte,, Kupffer cells and hepatic stellate cells in t he rat liver, thereby combin ing the advanta ges of stereology **with** cytometric analysis of cell ploidy. **However,** other methods and techniques have also been used for the quantifi cation of hepatocyte,, namely, automated image analysis , ceU pro lif erati on assays, prot ein concentrati on measurements. DNA content methods and cytochrome P4SO co n te n t methods (Carlile et al. 1997; Stegemann et al. 2000; Haga et al. 2005; Schlenius-Sten beck, 2006; Oeng et al. 2009; Miyaoka et al. 2012; Garrido et al. 2013; Best et al. 2015; Asaoka et al. 2016). Many of these techniques are based on the use of single ceU suspen.sions or liver homogenates. An unbiased measurement of t he fraction of MnHEP and BnHEP is important for correcting methods that estimate the hepatocellularity using DNA or protein contents (Marcos et al. 2007).

Aims of th e study

To t he best of our knowledge, no published data exist regarding quant itative histological parameters that demon strate the normal interindividual variabil ity in the size and density of hepatocytes in various macroscopic regions of porcine liver lobes, e.g. peripheral regions vs. the parench yma located near t he porta hepatis or adjacent to t he cau- dal **venacava.**

Therefore.. our aim was to assess the number ·'INeighted volume of hepatocytes, t he numeri cal density of hepato- cyte and the fraction of binuclear hepatocytes (subse- quently abbreviated as ·nucl earit ý) in the po rcine liver. These data '\Nere compared bet'\Neen the anatomi cal hepatic lobes as 'INell as bet\Neen three macroscopic regions with different positions related to the liver vascul atu re (region of interes!, ROI): the peripheral regions of the liver lobe the regions near the porta hepatis and the regions adjacent to the caudal vena cava.

M ateria ls and methods

AnImals

Uve r sam ples were obtained from ieven healthy Prestlce Bladc·Pied pg s (Vrtkova, 2015) aged 12 week:s and weghng 2S-a5 kg (35.7 ± 6.1 kg. rrean ± SD). F piglets we<e male and two female. The nuIT'ber of an imals co rrespond s t o t he ty pical number of individuals in other studies using pig as an animal m::,del in sur. ge<y (Darnis et al 2015; Hazelton et: al. 201S). The animals rece d humanec.are in co mpllance wlth1:he European Conventlon on Ani· mal Care, and 1:he project No. 27374/2011·30 was approved byt he Faculty Committee for t he Prevention Of Cruelty t\:> An imals. The piglets were pre-medcated intran USOllarly wlth a trop ine 1.0 mg, ketamine 200 mg (S-10 mg kg· ') **and** azaperon 160mg (2- 8 mg kg-'). For gene ral anerthesia propofol and fentanyl were mntinuously admin in e red t hrough a periphe ral or central venous cathete< in 1:he following t ota l aYerage doses: propofol (1% mixture

5-10 mg kg-' h- 'J **and fentanyl** (1- 2 µg kg- • h- 'J. 1he piglets receiYed infusion and volume substltution when necessary {Plasma. lyte; Baxter, Vienna,Austria, and Gelofusine;B-Braul\ Maria Enzers-dorf, Austr1a, respe<tlvely). Ali su rg ic.al procedures were performed un der aseptic and antlseptic oondltions. The anima lsw ere saa lficed during deep geoeral anerthesla via an intraveous administ ration of ac.ardiop leglc solution(potassium chlorid e).

Immediately after sac:rlfice, 1:hewhole fiverwas removed. The vol. ume of each freshly oollected fiver was measu red us ing the wa te r d is p laceme nt IT'!!!thod . A large graduated <ylinder wlth a total vol. ume of 3 LWM fill edwith water predsely to the 1·1 mar'l<ing, and the liver was fu ll y su b me rged in the wate r. The lh.er volume was c.alru lated by subtracting the water level before and after submer. sion. The liYer volumes ranged from 640 to 910 ml and the mean ± SD values were 813 ± 85 ml (see Supporting lnfonnation Table S1 for details). The li ver volulT'l!! did not oorrelate withthe bodyweight.

Tissue sample collection

Each liver was sectioned into 1< m-thick slabs. The slabs were fixed wlth 10% buffered formalin. From each liver, 36 tlssue probes(each approximateJy 25 x 25 x 25 mm) were sampled. Each of 1:he slx hepati: lobes(left lateral, right lateral, r1ght latera l right medial, c.aud'ate and quad rate lobes)WM re presented by slx probes. Within each lobe, three ROIs w1th different posltions relatlve to the llver va.sculatu re were sampled: two probes represented the per1phe r a l r egio ns, two probes represented the parac.aval reg ion, and two pro b es rep resented the paraportal region aa:o rding to Fig. 1. The pe riphera l reg ionsw ere def ined as located no more than 1onfrom the surface and peripheiy of each hepatielobe. The paracaval local·iza tion WM the region of each lobe immediately adjacent to the openings of the hepatieveins intn the c.aud'al vena cava. The para.portal (hilar) localiza tion was immediate Jy adjacent to the main branc:hes of 1:he po rtal vein follO\N'ed from the hilum w1thin each anal:>mic.al lobe . ln t ota l, 251 t iS5UE! pr o bes were mllected (one sample was Ion du ring processing).

The orientation of each tissue block WM random ized using the orien tato r sc:heme (Mattfeldt et al. 1990). The procedu re was done aa:ording to Nyengaard & Gunderten (2006) and MOhlfe ld et al. (2010). ln the first 5tep, each tissue block wM placed on a cirde divided into 100 equal din nces wi1:h one edge paralle l to 1:he 1- 1 direction . A random number between 1 and 100 was generated using the Mi:rosoft Exa RAND function and 1he t i.sue blodc was eut at 1:hls ang le. ln the ie<X>nd m! p, 1:he freshlycut 51.Jrface ofthe block wM laid on a ieoondd rde wl1:h cos ine-weighted angular divi·slons. Anat.her random numbe r WM generated. and the block WM cut pa rallel to 1:hls numbe r. Thus. isot ropie unifonn rancb m (IUR) hist olog ical iKf ions were produced. The newly generated artting plane wMc.arefully maintained during hist ologie.al pr oc essing and wM used M the hist ological sectlon plane. All:houg h hepatocytes c.an be ViE!lft(ed as i.i:>tro p i: parti:les and sim ple cutting Vt10uld be suffiden t for producing isot ropie se<tions (Manda r1m.de·Lacerda, 200.3), we dec:lded to rand omlze1:he orientation of eachtiWJe blodc wlth respectl:> furt.her use of the same embedded blodcs for analy-sls of hepatiemia ol.essels in a future study.

Histological processin g

The tlssue blockswere embedded in paraffin. From each blodc, two consecutlve hi5tologlc.al se <tions randomly posltioned w1thin the
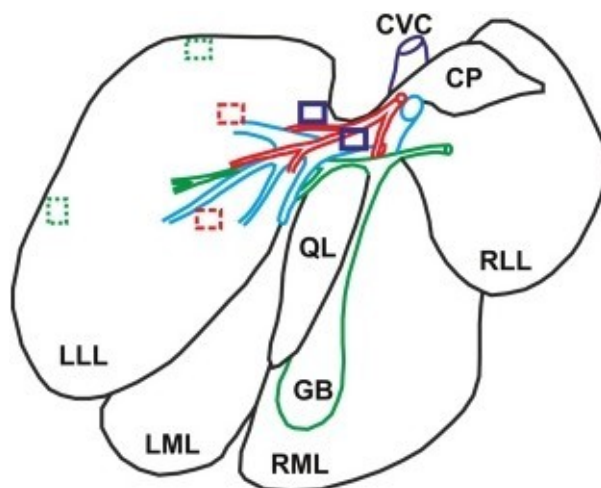

Fig. 1 Cdlectioo of dssue samples (rectangles) of the pordne llver for quantitatlve histology. Schem atlcdrawlng ol the ladesvlsa,ralkd thepordne llver shONlng theoudlnesof the left lateral lobe 0..LQ, left me<llal l obe (LML), right me<llal l obe (RML), right lateralb be(RLL), **quadrate bbe** (QL) **and** caudate bbe (rnos11y hiclden behlnd theves-sels, thescheme shov.,s ïtscaudate process, CP,ooty). The gall adder (GB) and the caudal venac.wa f: VC)arealsoshown. Brand'lingof hepat lc arterles k:shov.in asred, brandi ngof portal vein asblue and t:randling of t::ile dJCl'S asgreen The LLL k:used asan exampled coi. lection of hk:tologlcal i:robes from three regiortS of lnterest: parac.aval region(dal'lcblue rectanglesdrav.inwlth acontinuousline),paraportal region(red rectanges witha dashéd line), and penpheral region (greenrectangleswlth adotted line). Samp'esof the other lobeswere m llectedacccrdng y.

blodc we re prep ared at a thi:kness of 16 µm and mounted on sila·nlzed Super'Fron:B>Plus slides (Menze Gl&SE!r, Braunsc:hwelg. Ger. many). Periodi: add- SChiff staining {PAS) was peifonned aa:ording l:> Rome ls (1989) using a m mmerdal PAS staining kit (Morphi5to, Frankfurt/Mail\ Germany) to highlight the contours of the individ- ua! hepatocytes.

Mia oscopi c sampling an d qu ant if i cation

FollO\N'ing the productlon oftwo ions for each of the 251 t issue block.s.the ana tysis w as based on 502 randomly orientated histolog-ie.al sections. ln each sectiOI\ fou r fields of view(FOV)were ielected bysystemati:unlfonn random sampling (9.JRS) M follO\N'S: The use of a Lelc.a DM2000mia ot::ope {Lelc.a, Wetz lar, Germany) equipped w1th a Lelc.a DFC42SCcamera and an oil immeision obje<th.e with 100x magnification (numerical aperture 1.25) resulted in a field of view {FOV) of a phol:>g raphed slze of 94.86 x 63.2 µm. The sam. plin g started on the top left of the first se<tion using two random numbers generated wlth the Mi:ro50ft Exm. RAND functlon as *X*- and y.o::,ordinates, whi:h represented the distances ofthe fint FOi from the left and upper borders in µm. The distance interval between the FOVS ina eased or dec:reased proport iona lly, i.e. the sampling m!p ranged between 1 and 3 mm. The him loglc.al sampling strategy is illustrated in Fig. 2A.B.

The next step required focusing of optic.al sectlons. The Z..<direc-tlon feed rate of the fine focusof the Leic.a DM2000mierot::ope WM verified using a Zeiss lmage r.Z2 mia oscope equipped with an Axio-Cam MRCS camera (Zeiss, Vienna, Austria) and the ZEN PR0201 2
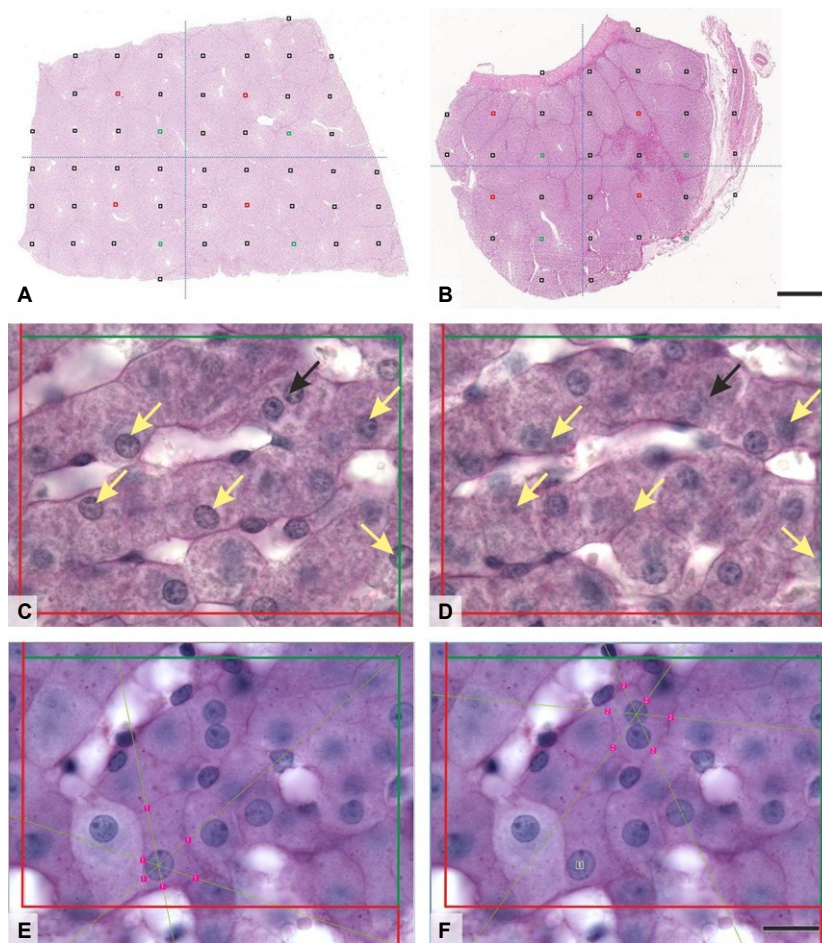
Fig. 2 Sampling of fields of view (FOV) in micrographs of liver tissue, the disector and the nucleator method. (A,B) Systematic uniform random sampling of FOVs based on a randomly positioned first FOV and equidistant placement of further FOVs, four of which were photographed for the first section (red squares) of each tissue block and four further photographed for the adjacent section (green squares). Each quadrant of the section profile was represented by one FOV. Micrographs (A) and (B) represent different shapes of tissue profiles and the necessary adaptation of distances between the FOVs. (C,D) To estimate the numerical density of hepatocytes and the fraction of binuclear hepatocytes, two optical sections from the same FOV illustrate the disector principle as follows: Whole cell profiles that were inside the counting frame or that touched the green allowance borders but not the red forbidden borders were counted, provided that their nuclear profiles were in focus with clearly visible chromatin in the bottom reference plane (C) or two subsequent planes of the disector volume probe and absent or clearly out of focus on the fourth plane (D, the look-up plane of the disector). The nuclei of the counted mononuclear hepatocytes are marked with yellow arrows, whereas the nuclei of counted binuclear hepatocytes are marked with black arrows. (E,F) To estimate the number-weighted mean volume, the cell borders of the hepatocytes previously selected with the disector method were marked with six intersecting isotropically oriented rays of the nucleator probes. This analysis was performed separately for mononuclear (E) and binuclear hepatocytes (F). Periodic acid-Schiff staining: note that cell borders are easy to identify notwithstanding the natural staining variability as shown in (C–D) vs. (E–F). The counting frame size in (C–D) was tested in a pilot study; however, the definitive size shown in (E–F) (95 9 63 1m) was used consistently throughout the main study. Scale bars: 1 mm (A,B) and 10 1m (C–F).

program, allowing precise computed Z-stack measurement. With the 1009 immersion objective of the Leica microscope, the distance between two scale lines indicating 1 1m in the fine focus of Leica DM2000 corresponded to the verified slice thickness of 0.97 1m.

Within each FOV, a series of four optical sections was captured at a resolution of 2592 9 1944 pixels. The Z-step between the adjacent optical sections was set to 3 1m, which was sufficiently small to not lose the readability of the sequence of the optical sections. This made it possible to follow the continuity between the corresponding cell and nuclear profiles on adjacent optical sections. Starting from the bottom, the first sharp plane of the physical section was focused using the nuclear chromatin and outline of the nucleus and cell borders as reference points. In total, 8032 optical sections that documented 2008 FOVs were recorded. Using ELLIPSE software (ViDiTo, Kosice, Slovak Republic), the four stereological parameters described in Table 1 were quantified in the optical sections.

Numerical density of hepatocytes

Within each stack of micrographs that documented one FOV, the hepatocytes were counted using the optical disector method as shown in Fig. 2C,D. The nucleus with one or more nucleoli clearly in focus was selected as the decisive counting event (Gardella et al. 2003; Marcos et al. 2016). For the proper identification of the borders of individual hepatocytes and the chromatin of the hepatocyte

Table 1 Stereologcal parametersassessed fcr pord ne he patocytes: ln thé framéd thé study.

Name and unit	Abbreviation and unit	Definition	Stereologic.al t ech nique used for quantific.atlon
NumbeMNe ighted mean volume of mononudea r hepatocytes	V,'AMnHEP) (µmi	Average volume of hepatocytes tha1o::,nta ined one n uc:leus	Optical disector (Gundersen et al. 1988; Mara>s et al. 2012)
Numbe r.we ighted mean volume of binudea r hepatocytes	ii,ABnHEP)(µm3)	Ave r age volume of hepatocytes tha1o::,nta ined two nude i	Opt ical disector
Fract ion of binudea r hepatocytes	f(BnHEP) (- . parts of1)	Rela1 1ve a.mo unt of hepatocytes tha1o::,nta ined mo re than one nudeus among all hepatocytes witíhin the same reference volume	Optical disector
Nume rical denslty of hepatocytes	N.,(HEP) (mm -3)	Average number of hepatocytes per volume unit of liver t issue	Optical disector and nudeator (Gundersel\ 19Ba Gundersen et al. 1988; Marcos et al. 2012)

nudei, aswell as for reliab le mea9.Jrements in the Z·dire<tiOl\ an oil immersion oij m ive wlt.h 100 x magnific.ation and a hlgh numerlc.al apertu re (1.25) was Uied . The opt ical dlse<tor o::,unt ing rules (Gun. derser\ 1988) were used. wlt.h o::,unt ing in whole cell profiles loc.ated inside the co unt ing f rarne or touching 1:he a llowan ce bor. ders. but not touchingthe forbidden borders. The cell and nudea r profiles were tradced 5tarting from the fint (reference) o ptical plane towards1:he last (look..up) opt lc.al p lane 4. Cells with nudei visible at any ofthe planes 1- 3 and lon before reaching the level of the look· UP p lane were o::,unted . The nu merical density of the hep. atcxytes NoA.HEP) was c.alculated as follOW'S:

$$Oi\ (IIEP)$$

$$\frac{\blacksquare}{!;\ P1(re\,f)} \cdot a_{:,,,}^{p}$$

w he re Q, (HEP) represents the number of hepatocytes

with a clearly visible nucleus counted w it hin t he i•thdisec· t or, P,(r ef) represents t he nu mber of po ints of t he auxil· iary grid t hat hitt he *i-th* reference space, *p* represents the t otal number of points of t he auxiliary grid, *a* represents t he area of t he count ing frame. *h* denote s t he height of t he di secto r and *n* denote st he tota l num ber of additions wit hin t he mathematical summat ion of all disectors and ref eren ce space points. At least 100 cells \'\lere count ed in eight disecto rs appli ed to the two ti ssu e sectio ns of each sam pl e. The reference space com pris ed all components of t he liver t issue; how ever, the peri po rt al areas compo sed of conn ective ti ssue w ere avoided.

Fraetion of binuclear hepatocytes

ln each sample, the relative amount of hepatocytesthato::,nta ined two visib le nudei {BnHE was expressed as a ratio of these cells to the t otal number of all hepatcxyteso::,unl!d w lt.hin the same refer. ence volume. This asses:sment involved predominately binudear hepatcxytes. Trinuclear hepatocytes were observed very rarely and none of thesewascounted aa:ordingt\:>the   d ised:or rules.

Number -weighted mean volume of hepato cytes

The nudea l:>r met.hod was used as a local SU!reo log lc.al pr obe to

tNery live r ce ll pr ov ided the se<tions are isotropie uniform or verti· cal uniform (Gundersen 1gga Gundersen et al. 1gga Mara>s et al 2012). Briefly, in every stack of optlc.al sectionsinvestigated. hepato- q,te profiles were selroed using the prevlously desaibed dise<tor met.hod. The volume of tNery ie lected hepal:>cyte was lT'l!asu red in 1:he cen tra l region, whe re 1:he nudeus with nudeoli was visible. USing the Nucleato r module of E1.1.PSt software. nudeated cell pro- files sam pled b ythe dise<tor in the previous stepwere measured by mar'l<ing the i nt ersectlonsof slx i.sotro pically oriented ra yswith the cell bo rders {Fig. 2E,F). The number of rays was ba.sed on a p ilot 5tudy showing that usingfive, slx or se"\len nudeato r ra ys do not reduce the variabilit}' of mea9.1re ments when compared with four nudeator' ltfS· An average value independen!ly calculated for the

MnHEPand BnHEPresulted in numbe r..welghl!d mean volumes V_{11} of these cells (Table 1). ln t ota l 29 054 hepatcxyteswere o::,unted andevaluated in thi.sstudy.

Corrections for shrinkage and z•axi s com pr ession

estimate the volume of hepatcxytes. This met.hod i.s b ased on the use of a unique ar'bitrary point in tNery objed: under study, Le. in

Formalin fixatiOl\ dehydration and paraffin ioning are steps that leadt\:> volume shrinkage (Oorph-Petersen et al 2001; Gardella et: al. 2003). Marcos et al. (2004) reported 38% shrinkage from fresh

rat li'\ler to mounted sections. As a result of shrinkage, cell volumes appear smalle r and nu mericalcell densities ap pear larger compared with fresh organs prior t\:> pr o cessing. Aax, rding to Mara>s et: al (2012), two main g:,urces of shrinkage are expe<ted to contribute bias t\:> o ur study: {i) the volume shrinkage caused by the formalin flxatlon and paraffin e!T'bedd ing ; and (ii) a deaease inthe ion 1:hickness (compres:slon) in 1:hez ..axis in the t hidc **lons asar9**.Jlt of the 5tretrh ing of it!<itons in 1:he warm t issue flotatlon water bath. To compensate for the shrinkage, two cor ions we re applied to the results.

◊ The volume shrinkage caused by the formalin fi:xat ion and pa raffin proces:sing was est imated as follows: 11 blodcs were sectioned from the fresh liver, avoiding the he patic capsule. Each block had a shape of a prec:ise ly shaped cuboid

bounded with six quadrilateral faces. The $x.y.z$ dimens io ns of each t issue block were pred sely measured using a c.alipe r with a reading error of O.S mm and the volume of each

block was calculated . ln all samples. the $x.y.z$ direct ions were preserved o::,nsist en t ly to a llo w fo r eva luat ion of the isot ro py of the shrinkage as follows: the x..<fir ect i on was ta ng en t l a l to the li ve r surface from the periphery of liver lobe towards the hilum of the lobe; the y..<fir ect i on was ta ng en t i a l to the su r . face and perpendlcular to the X·dire<tion; the z directlon was perpendicular to the liver surface, thus running fromthe

liver surface into the deeper parts of the lobe. Follo wing routi ne fi:xatiOI\ de hydration and embedding. the t i.ssue blodcs were exhaustively cut into series of consecutive 5-μm-thidc histological sections. and every 10thsection wM stained with hematoxylin and eosin. The *,t,y,r* dimensions of each processed block were measured using the histologie.al sec-t ions and the distance between the first and the la.st se<tion and the volume of the blodc wasc.alrulated. The mean vo ume ratio after/before processing (± SD) was 0.675 ± 0.058. The cell volumes and dise<tor volumes we re a::,rrected b y multiplying the valu.es measured in the paraffin sections by a correction factor of 1/0.675. Moreover, the mean ratio ± SO of dimensions after/before processing was calculated M fo lows: *XIX*= 0.897 ± 0.071, *ýly* = 0.938 ± 0.043, and *:ti* z = 0.803 ± 0.033. When a::,mparing the linear shrinkages in the *x-, y-.* and the z-directions. the Friedman AHOVA revealed differences (P = 0.002), suggesting an anisotropie shrinkage. The *post-hoc* Wilcoxon matched pairs tests showed that the shrinkage in the z.di rection (perpendirular to the liver sur-face) WM more prominent than the shrinkage in the x-direc-tion (P = 0.003) or the y.direction (P = 0.003). No difference was found when comparing the shrinkage in the *x-* and the y.directions parallel to the liver surface.

ⓦ The actual thidcness of our final sections ah! r stretching in the water bath and staining was meMured using the z-stack too l of the *AYIO* IMAGat *Z2* light microscope (Zeiss. Vienna. Austria) by manually forusing the first and la.st sharp optic.al planes of the sections. The mean distance between these planes was 13 μm. The mlc:roto me produced 16-μm-thick sections; thus. it demonrtrated a compression fa<tor 13/16 = 0.8125. The cell volumes and disector volumes *were* increased b y multiplying the values measured in the thidc paraffin se<tions by a correc-t ion factior o f 1/0.8125.

Statistlcs

The **software** STA>SKA **S...** 11 package (Stat:Soft, lnc, TUlsa, O **K,** USA) was used for the statistical anattsls. Part of the data did not pa.ss the Shapiro-Wilk's W-ten for normality; thus. nonparametrlc: methods were Uied for furt.her analysis. The Kruska Walli.s AHOtA and the Man Whltney *U.ten* were uied *to* assess the differences between the slx lobe\ 1:he diffe rences between the ROt defined wlthin 1he lobes. and the differences among the individua! animals investigated. The a::,rrelations between the four 5tereologic.al parameters were evaluated using 1he Spearman rank order o::,eff cient. The meffic:ients of e rror (CE) enimating the sall'1)1ing error were calrulated aa:ordingt\:> Gundersen et al (1999) ieparately on the level of ti.ssue bloclcsand on1:he level of individua! animals.

Results

The mean number•'\Neight ed volume of mononuclear hepa-, tocytes after shrinkage correction in au samples investigated **was** 3670 ± 805 μm^3 (mean ± SOJ. The mean number-w eighted volume of BnHEPwas 7050 ± 2550 μm^3·The frac-t ion of BnHEP was 4 ± 2%. The numerical density of all

hepatocytes was 146 997 ± 15 738 cells mm '''. No patho-

logical findin gs, such as in fl ammation, necrosis, fi brosisor exten.sive steatosi.s. '\Nere identified in the animals investi·

of tissue blocks and between 0.014 and 0.01B on the level of individual animals. The complete stereological resutts for all samples are provided in Table Sl.

Size, nud earity and density of hepalDcytes In the hepatlc lobes of 1he pig

No differences between t he hepatic lobes were identified whencomparing t he values of au four quanútative pararne,. ters investigated (Fig. 3A-D).

Size, nud ea rity and density of hepatocytes in paraportal, peripheral and paracaval ROIs

The greatest number-weig hte d mean volume of mononu clear hepat ocyte, iiN (MnHEP)was identified in the periph-eral regions of the liver lobes (P < O.OS); however, there was no difference between the paraportal and paracaval regions (Fig. 4A). The number-weight ed mean volume of binuclear hepatocyte, iiN(BnHEP) (Fig. 48) and the fraction of binuclear hepatocyte, among all hepatocyte, f(BnHEP) (Fig. 4Q were not different among the ROIs The numerical density of t he hepatocytes Nv(HEP) was smaller in t he peripheral region than in the paracaval region *fJ'* < 0. 01) or the paraportal region (**P** < 0.001, Fig. 4D).

Size, nud earity and density of hepatocytes compared among 1he individua! animals

In all four parameters investigated (Fig. SA-0) , significant interindividual diff erencesw ere identifi ed.

Correlatio n between the size, nuclearity and density of hepatocytes

The number•weighted mean volume of mononuclear hepa-, tocytes iiN(MnHEP) was moderately correlated (Spearman *R •* O.SS) with the number-weighted mean volume of binu-clear hepat ocyte, iiN(BnHEP) identifi ed in the same samples. The numerical density of hepatocytes Nv(HEP) exhibited a moderate negative correlation with the number'•\Neigh t ed mean volume of mononuclear hepatocyte, iiN(M nHEP) *(/l •* - 0.52) as w ell as with the number-weighted mean vol- umeof binuclear hepatocytes iiN (BnHEP) (R • - 0.33).

Oiscussion

The mean number-weighted volume of MnHEP (Table 2) partially corresponds with the number-weighted mean cell volume of MnHEP published in ot her mammalian species. Using model-based stereology, Rohr et al. (1976) reported

the volume of human hepatocyte, as 11 305 μm ^{3} For a

gated. The CE ranged between 0.084 and 0.125 on the level

direct comparison 'tl'Jith studies t hat used similar design based stereological methods applied in histological sections, Jack et al. (1990) reported t he number-weight ed mean cell

Fig. 3 Estimatesof siz.e, nud ear-lty and dél'ISlty of patoc:ytes In slxpa dne hepatlc lobes. (A} Numbér•weighted mean \Olumeof mooonuclěar hepatocytes: VN(MHEP) (B) Numbér..wěighted mean \Olumeof binuděar patocyteS VN(B,EP) (C) Fractlon of t::fo ..c léar hepatocytes: amoog all hepatocytes: f(BnHEP) (D) Numerical densltyd hepatocytes: N..(HEP). Kruska Wallis 1mvA lndkat ed that t rě wéi'ě no dlfférences amoog thé lobes in t parameters. Dataaredk pla)«l asmedlanvalueswrth boe.es éhat span t limits of thé first and ttird quaritlesandwhk:kěrs that span t minimll'll and maximum values for ead't'g o .

volume as 4740 μm^1 in MnHEPand 6930 μm^1 in BnHEPin female rats. Karbalay-Ooust & Noorafshan (2009) deter- mi ned the volume,..'INeighted mean ceU vo lu me of mice to be 5300 μm^3 Marcos et al. (2016) determined the number- weighted mean ceU volum e of Mn HEPto be 6044 μm^1 in male rats and 4789 μm^1 in female rats. whereas the nurrr ber•'\Neight ed meanceU vo lume of BnHEPwas 7530 μm^1 in male **vs. 6565** μm^3 in female rats. **Hammad** et al. **(20 14)** de termi n ed the mean volume of hepatocytes in miceto be 5128 μm^3 Moreover t he porcine BnHEPin our study had volumes comparable wit h BnHEPin the rat liver (Jack et al. 1990; Marcos et al. 2016). We foundthat BnHEPhad almost twice the volume of MnHEP. According to Watanabe & Tanaka(1982) and Peinado et al. (1990), the size of hepato- cytes corresponds to the sum of ploidy of au the nucl ei and

is alsolinkedto the volumeof stored lipid droplets. To date, t hereis no information published regarding the volume of BnHEP wit hin a ti.ssuecontext (l.e. not in single ceU suspen sion)in species oth er th an ratsand pigs.

Pordne hepatic lobes contain hepatocytes of comparable size, nuclearity and density

Our results (Fig. 3) demonstrated that the sixhepaticlobes of the porcine liver have a similar distribution of hepato-,

cytes of various sizes. numbe rs of nuclei and den.sities. This finding suggests that individua! lobes may be chosen as

t echni caUy appropriate for experiments on liver regenercr tion such as partial lobectomyor hepatectomy (Bruha et al. 20151 even if quantitative histology is applied. The mor- phomet,y of hepatocytes wou ld remain unbiased by the finding t hat some tissue probes w ould origin ate from vari oushepaticlobes, provided the sampling of ti.ssue probes is not substantially lowerthan in the present study.

Peripheral regions of hepatic lobes contain the largest MnHEP with the smallest numerical density

The finding of intralobar differer.:es in t he hepat ocyte size and density (Fig. 4) clearly demonstrates the

Fig. 4 Estimates of siz.e, nud ear-ity and dĕnsity of hépatoc:ytes In three m.ac:l'OSQ)pically dĕfinéd reglons wlth respect to théir prc.:imlty to thé 111.er vasclJatur e in thé plg (A) Thé largest numbér..wélg'lt ed mean volumĕ of monooudear hépatOOytes \N(Mri-IP}was identified in thé perii:tléral region. (B) Nll'I'l'bér Wéighted lllĕan volume *d* binud ear hépatoc:ytes VM BnHEP)exhiblt ed no dlfferences bĕtwéén thé reglons. (C) Fr.Ktioo *d* binu-clĕar héJ)atOOytes: amoog all hépatoc:ytes f(BrHEP) e.xlIblted no dlfferences bétwéen thé reglons (D) Thésmallest l'IJmerical densityd héJ)atOOytes: N ...,(HEP)was ldĕntlfied in thé periphéral ré9oos *d* thé lil.Er. S9'1lftant d fferĕnces (•p <QOS .. *P<0.o, , •••*p*<0.001) identlfied using thé Mann-Whitney CJ..test arĕ presented. Data are dkpla asmed:l.ln \QlUĕs wlth bo)QĕS that span thé limil'So f thé firstand ttirdquartilĕsandwhL'S--kers that span thé mirimum and max:imll'I'I\QlUĕsfcread'Igroup.

importance of systematic samplin g w hen obt ain in g hi sto-, logi cal probes from hepatic lobes that exhibit a promi-nent anatomical hierarchy of blood vessels. HcrtNew r, t h e bio logica l i nterpr etation of t hese resutts is not straightf or· w ard. The mammalian ceU si.ze is regulated in correlation with the cell cycl e prog ression (e.g. Shen et al. 2000); how ever, it has been demo nstrat ed that cell growth and ceU cyding are separable and th us distinct processes (Fn gar et al. 2002). The coordination of cell growth with cell repli-cation has al.so bee n suggested for liver regeneration (Fausto & Campbell, 2003). How ever, it is not known how the hepatocyte volume changes du ring the cell cycle and

whether the proliferation rate and duration or dynamics of the cell cycle diffe r with distance from t he large br anches of the portal vein and hepatic proper artery.

Proteins such as membrane transporters. volum e-sensitiw io n chann els. and stretch...activate d ch ann e ls, as '\Nell as

molecules that function as cellular osmolytes play a crucial role in cell size regulation (Hoffmann & Dunham, 1995). It is not known whether the expression of these proteins differs within the liver lobe, which would provide an explanation for the cell size variability. A specific lobular zonality as described for some hepatic enzymes (Spear et al. 2006) *may* be expected with differences between periportal vs. pericentral regions.

Polyploidization of hepatocytes increases with age (as 'INe:11 as after stress) and leads to an increased cell volume (Pandit et al. 2013). It *may* be hypothesized that the peripheral parts of the liver lobes represent the developmentally oldest hepatic regions and thus contain more large polyploid cells. Tanami et al. (2016) mapped the zonation of liver polyploidy in rat, revealing that liver polyploidy proceeds in spatial waves. advancing more rapidly in the mid-lobule zone than the periportal aOO perivenous zones. The
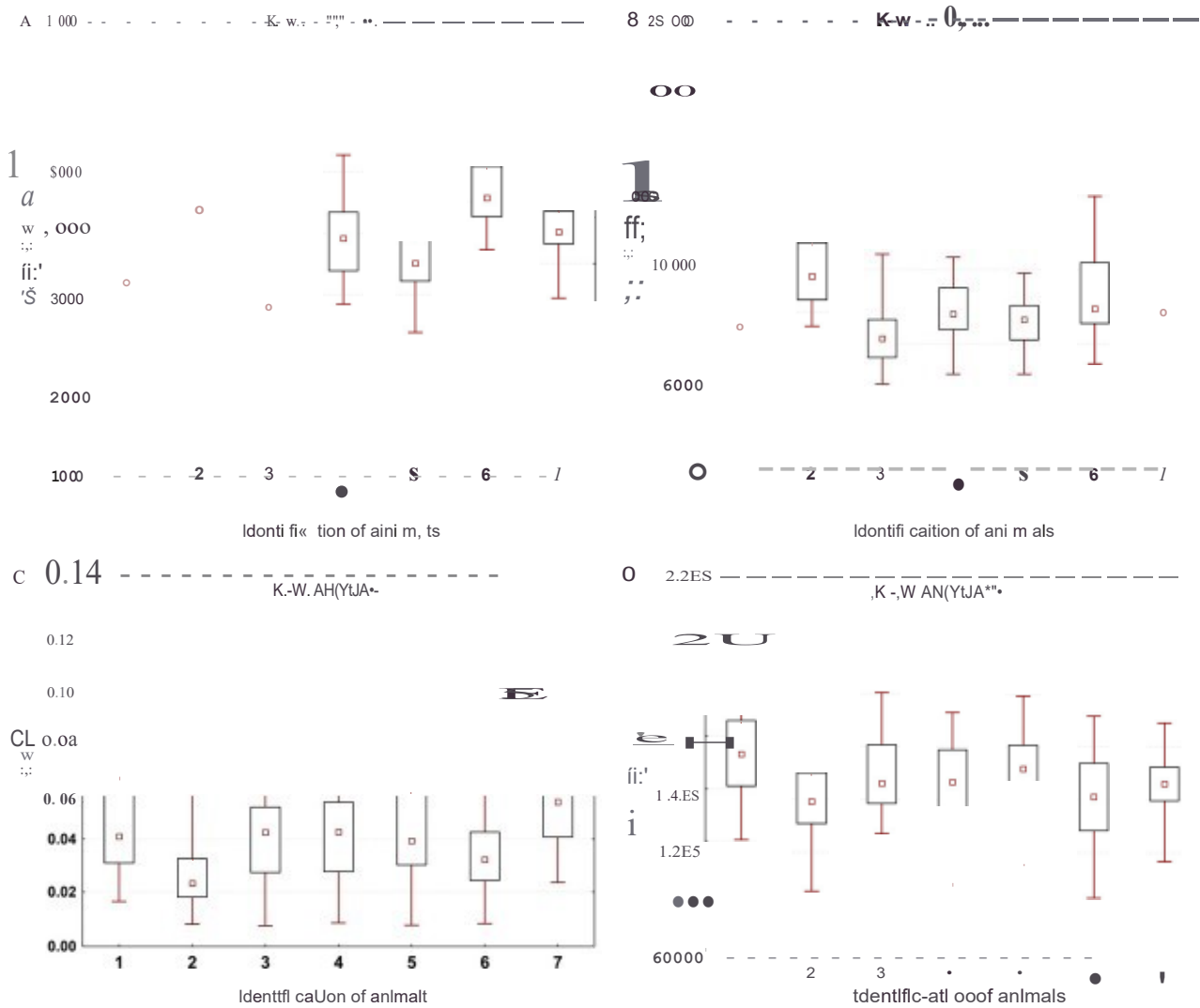
Fig. 5 Estimates of size, nuclear-ity and děn.s:ity of hépatoc:ytes ln Sé\en piglets inV'ěStigated. Ali parallěters Investigated exhibited dlfferences among the animals, as illustrated ln (A} fcr thé number..weig'lt ed mean vd'l.l'lle of monooudear hepatOCytes vN(l\Ari-lIEP), (B) for thé nl.l'llber ...,ighted mean volume ol blnuclear hepatocytes 17. (Bri<EP). (C) for the fractla, ol binudear hepatoc:ytes among all hepatOCytes I(Bri<EP} and (D) fcr the numěrlcal den.s:l ty of hepatOCytes N .,.(HEP). Signlficant dlffél'encéS (• .. p < 0 .00 1) Identified using the Kruslca Wallis:N«:NAare presented. Data arě dL'Splayed as median valueswith boxes thatspan thellml1:sd the first and third quartilesa ndw twsk:ers that span thé minimll'll and maxf. mum w luesfcr ead'l animal.

Table 2 Eštimated nl.l'llber-weightedlllěan vdl.l'lleof mooonude.ar hepat ocytes: VN(Mn H EP)and binudear hepatO(ytes V.-.<BnHEP), fr.Ktiood bin-uclear hepatOCytes among all hepatOCytes f(Bri-!EP),and numěrlcal děn.s:lty of hépatoc:ytesN .,.(HEP)ln thrěemac:l'OSCX>picreglol'lSof the pordnellvěr with respect to thěir i:roxlmity to thellvěr WSO.Jlaturě. Data are prěsented as medlansandquartilé ranges(the wlue of the 75th percéntlie minus thé value d thé 25th percenitle, lnduding 50% d théc.ases).

		Paraportal rregi ons	Peripheral regions	Paracaval reg lons
ii. (MnHEP) (μm')	Median	3536	3880	3593
	Quartil e range	841	1277	866
ii. (8nHEP) (,un')	M edian	7015	7397	6739
	Quartile range	3258	2991	2786
f(8nHE" (-)	M edia n	4 %	4 %	4 %
	Quart il e range	3%	3%	3%
Nv(HE" (mɪn ')	M edian	150 776	141 4 13	148 845
	Quart il e range	20 328	20 963	19 693

findings of Gerlyng et al. **(1993)** suggest that polyploid hepatocytes in rat liver have a greater tendency to replicate;

however, they precbminately divide into mononuclear daughter cells. Diploid and polyploid hepatocyte, originate predominantly through failed cytokinesis (Duncan. 2013; Gentric & Desdouets. 2014) and it was suggested that they

promo te liver homeostatic and regenerative turnover (Gen t rie et al. 2012; Pandit et al. 2013; Wang et al. 2015). More-over, aneuploid hepatocytes seem to be different ially resistant to chronic li ver in jury, and regenerate the liver (Duncan, 2013.)

Ir respective of the cause of intr alobar di fferences in the hepatocyte size and density, it should be not ed that the morphometry of hepatocytes *may* **be** easil y biased when samples for qu anútative histology are coll ected from regions wit h different positions bet'\Neen large vessels and t he periphery of li ver lobes.

Size, density and fraction of BnHEP exhibit considerable inll!rindividual differences

As shown in Fig. 5, healthy pigs of the same age and breed with a simliar t ctal liver volum e of 813 ± 85 ml (mean ± SD, see Table SI), and ident ical diet an:I handling may have signif icant interin dividual diffe rences at the l evel of hepat ocytes evaluated within t heir tissu e cont ext. How-ever, Stegemann et al. (2000) demonstrate d with sufficient precision and accur acy that the average size of isolated por· cin e hepat ocytes remained consistent among individuals. The number of animals in our study does not enable a reli· able comparison between males and females; hCYt.Never, '\Ne speculate that sexual dimorphism *may* explain, at least in part, t his variabi lit y. Neverthelesi;, the pig lets inv estigate d were prepubescent. and t he in fluence of sex hormo nes could be expected to remain smalL Detailed studies on t he rat li ver (M arcos et al. 2015b, 2016) show ed t hat t he female liver contained an increased number of hepat ocytes per gram and cont ained more binucleate cell.s, th us suggestin g an increased rege nerative po t enti al in fema le compare d with male rat liver. Moreove,r it shoul d be not ed that the number of nuclei visible in the optical microscope usin g routi n e histological sections is comp lete ly diffe rent from the cell ploidy hepatocyte, assessed us in g a dvanced meth-ods. such as sin gle molecule fl uorescence *in situ* hybridiza-. t io n. H epat ocytes wit h one or two nud ei *may* cont ain tw o, foor, eight or even more haploid chromosome sets (Tanami et al. 2016). In contra st to our findings (4% of 8nHEP), Vinogradov et al. (2001) reported in pig of unknown age t hat 54% of binuclear hepatocyte, had 2 x 2c ploidy. The most probable explanation of this discrepancy is t he grad· ual increase in liver ploi dy durin g agin g, which has been reported by Guidotti et al. (2003) and Gentr ic & Desdouets (2014).

Distribution of larger MnHEP correlale s with a larger volume of BnHEP and a smaller numerical density of all hepatocytes

rather t han ent irely independent. Our stu dy demonstrated that in regions with largeMnHEP, the size of BnHEP was al.so large . Simult aneously, the regions that contained hepa-, tocytes with a large volume had a tendency to contain a small er ratio n of BnHEP. It remains to be determined whether an increased degree of polyploidy, which has been indicated to lead to larger ceU sizes and increased occur· rence of bi- and multinuclear cells (Pand it et al. 2013), *may* provi de an explanation for these correlations.

Regionswith a greater volume of hepatocyte, exhibited a small er density of hepatocyte, per unit of volume. Dne expl anation for this correlation i s t hat if cells grow large,r fewer cells frt in the reference space. How ever, t he volume fractions of t he hepatic vascul ar bed and the int ralobular connectivet issue \W uld have to betaken into account for a correct interpr etation of this finding. Mappin g of t he vol ume fractions of the main ix,rcine liver components remains to be performed. .*As* show n in recent studies (Del> baut et al. 2012, 2014b), this *may* be done economically by combin in g t he histological approach wit h a thr ee--dimen sional analysis and stereo logi cal quantifi cation of hepatic

When i nterpreti n g the bio logical meanin g of the numeria1l results of hepatocyte morp hometry, it should be not ed that the morphological parameters are statisticaUy corr elate d,

microvessels using high resolution X..ray microtomography of vascular corrosion casts (Eberlova et al. 2016; Jiřík et al. 2016). Moreover, quantitative data regarding the size and density of hepatocyte, *may* also be combined with multi - level modeling of hepaticperfusion (Debbaut et al. 2014a; Peeters et al. 2015).

Study implications

When evaluating the histomorphometry of hepatocyte, in experiments with porcine livers, a high variability should be expected in interindividual differences. Another major source of variability as demonstrated in our study was the position of the tissue probes with respect to the distance from the major hepatic blood vessels. Moreover, the samples collected from various hepatic lobes exhibited oo differerces in healthy piglets; hCYt.Never, int ertobar differences have been reported in various cases of liver alterations(e.g. Richardson et al. 1986; Faa et al. 1995; Regev et al. 2002; Irwin et al. 2005; Palladini et al. 2012). Dur findings fully comply with thewelJ•known recommendations of Gundersen & 0sterby (1981), who demonstratedthatin atypical biological experi‐ment. the interindividual biolaj cal variabliity is responsible for up to 70% of the overaU observedvariance.

.As practical suggestions for further studies that involve stereological evaluation of the size. nuclearity and den.sity of hepatocytes in porcine liver, we \Wuld recommend the following: the use of sufficient numbers of pigs per study group; a power analysis for estimating the minimum number of animals per study group *may* be easily calculated from our results. For example. when planning an experi‐ment and expeding the mean numerical density to be decreased by 20% ij.e. from 97 500 to 78 000 cells µm . the minimum number of animals per group \Wuld be

eight, using t he t ypical test power 1 - P• 0.8 (where P is t he type Ii error) an:l a=0. 05 (where « i s the type I error) (Chow et al. **2008).** sr.;tematicall y obtaining histological probes from both the peripheral parts of the hepatic lobes **and the vicniity of the major int rahepatic blood vessels; using the six hepatic lobes of porcin e li ver in dependently** in experiments that address partia l lobectomy or hepatec- **tomy because tissue probes sampled from various lobes** yield comparable results provi ded t hat the paraportal and

peripheral regions are sampled accordingly; when analyz· ing 36 probes per animal, t he **CE was** reasonably low (0.014-0 .018); reducing the number of probes to **18 w** ould **result in an in creased CE of app roximately 0. 15, which can be derived from the Table Sl. Therefore we recommend obt ain in g at least 20 histological probes per individua!. Future studies in the pig w ould provide important addi· tional information with respect to other quantitative**

parameters, such as the distribution of intralobular and int erlobular connective tissue (Marcos et al. 201Sa), as we ll as the lobular zonality of liver pathologies (e.g. Schwe n et al. 2016) and gene expression i n h epat ocyte, (S<h we n et al. 2015).

This study has several limitatio ns. Atthough none of the samples showed **histopat hologi cal alterations, no liver biochemical tests '\Nere done and the liver fun ction was not assessed in this study. Our study was based on one** type of simple PAS staining out lining the glycocalyx **of**

t he ceU membra ne. This approach may al.so re p- esent an advant age when processi g archive samples. H 0'\NeVer, h epat ocyte, laden **with PAS-positive** glycoge n granul e, parti al ly lose contra st betwee n the cytoplasm and cell **membrane. For some application.s, complex stain in g pro-,** t ocols enable the interpretation of the morphometry of **hepatocytes w ithin the conte xt of t he liver tissue** (Hoehme et al. 2010; Schw ier et al. 2013; Hammad et al.

2014.) Moreover, whole-slide im aging (Hoehme et al.

2010; Schw ier et al. 2013; Hammad et al. 2014) is a good **choice to handle the variabliity in the level of more histo-, logical sections.**

Our study generated a number of more detailed ques- tions t hat, to **our** knowledge, have not been addressed to **date forthe porcin e liver.**

rn The size, nu clearity an d density of hepatocyte, will have to be mapped with respect to the position of the hepatocytes w ith in t he classical morph ological hepatic lobules (Marcos et al. 2016), as w ell as wit h in **the zones of the liver acin i. The identi'fication of the lobular and acinar distributions of the stereo logi cal characteristics of hep at ocytes w ould contribute to understa nding their role in pathological processes that preferentiaUy involve zones of a liver lob ule, such as demonstrated durin g cent rilobular necrosisas a result of passive congestion, after poor oxygenati on of** hepatocyte, (Saxena, 2011), in dru g-in duced po r- **cin**

a porcin e model of sma U·for--size synd rome **(Kelly** et al. 2009) or in a p orcine hepatectomy mod el wit h a port ocaval shun t (li da et al. 2007). Simi larly, peri - **porta l n e crosis was fo un d to be pr esent in a porcin e** model of acid aspirati on (Heuer et al. 2012) and in **to xin in duced fuJmi nant h epati c failure (Collins et al.** 1994).

GQ Sexual dimorp hism of t he hepat ocyte size an d den- sity (Marcos et al. 2016) examin ed together wit h enzyme activity (Bode et al. 2010) may elu cidate t he **regenerative potenti al of male vs. female porcin e liv·** ers.

It remains unkn own how t he size, nuclearity and density of hepatocyte, are associated w it h t heir pro- **liferation rate.**

From a stereo logi cal point of view, t he t otal amounts of hepatocytes w ith in the lo bes of the por- cin e liver remain to be estimated using the **fraction** ator sampli ng sch eme (Marcos et al. 2012). This **parameter is not biased by shrink age and is there,. fore useful when comparing the fun ctional capacity** of t he lobes.

Conclusion

The mean number•w eighted volume of porcin e mononu clear hepat ocyte, was 3670 ± 805 µm^3 (mean ± SD, cor• rected for shrinking duri ng histological processin g). T h e **mean number-weight ed volume of binuclear hepat ocytes** was 7050 ± 2550 µm^3 The fraction of SnHEP hepatocyte, was 4 ± 2%. The numerical density of all hepatocytes was 146 997 ± 15 738 cells mm • . Porcine hepatic lobes con- tained hepatocyte, of a comparable size, nucl earity and **den.sity without signi'ficant di fferences betw een the lobes.** Peripheral ROIs of hepatic lobes cont ained the largest

M nHEPw ith the small est numerical density. Thehepatocyte

e models of liver failure **(Newsome et al. 2010), in**

size and density, as'\Nell as the fraction of BnHEP exhibited considerable interindividual differences even in healthy young animals. The distribution of larger M nHEP was ccrre,. lated with a larger volume of BnHEP and a smaller nu meri· cal density of all hepatocytes. The complete primary stereologi cal data in the form of cont inuous variables have been made available as a supp leme nt to t his paper. Practi· cal recommeOOations for designin g studies that involve stereologi cal evaluations of the size. nu clearity and den.sity of h epat ocytes in po rcin e liver are provided. Ho'\Never, a number of biologically relevant parameters remain to be mapped w ithin the porcin e liver.

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Conflict of interest

The aut horsdedare t hat theyhaveno confli ct of interest.

Author contributions

References

Alex.aids N. Galdopo ulou H. Dimitri ou , C et al (200,8) Live r hls,.
t olo gy alterations du.ring c.ar bon dioxide p ne umo pe rltoneu m
in a po rd ne model Surg Enc/ose 2003, 415-420.

Altookaynak BZ. Oz:bek E (2009) Overweight and structural alterations
of the liver in female rats fed a higt't-fat d iet : a ste reo log ical
and hist olog ie.al st udy. Turk J Gastroenterol
2009, 93- 103.

Arb d opo ulos N, Defterevos,G Na.stos C, et aJ. (2011) Develop.

ment of a pord ne model of post.-hepat e<tomy liver failu re. J
Surg R s170. 3 3-e2 4 2.

A.saoka Y, Toga.shi Y, Mu.tsuga ,M et al. (2016) Hist opatho log i• c.al
in age anatjs ls of chem ic.al- indu ced hepatoce llular hyper. t
rophy in mice. EXp Toxicol Pathol 68, 233- 239.

Bahr ,A K.a.ser T, Kemter , E e t aJ. (20 16) Ubiq uitous LEA29Y
expression blocks T cell a:>-stimulation but permitssexual repro-
dutt ion in genetically modified pigs. PloSONE 11, e0155676.

Best,J Manka P, Syn WK. et aJ. (2015) Role of fiver pr og enitors

in live r rege ne rat ion . Hepatobiliary Surg Nutr 48--58.

Bode G. Clau sing P, Gerw i.s F, et aJ. (2010} The ut ility of the

isolated hepatocytes, and the intact liver: studies with in duced
livers involving diazepam. Drug Metab Dispos 2S. 903-911.

OK>w 5, Sihao,J Wang H (200,8) Sample Size Calrulations in CJin-

ical Re search. 2nd edn. Boe.a Raton: Chapman & Hall/CRC Bi:>·
st.at ist ics Series.

Colli ns BH. Chari RS, Magee JC. e t aJ. (1994) Mechanisms of
in jury in pordne llvers perfused w1th bloo d of patients w ith fu
lminant he pat ic fa ilure. Transplantation 15, 1162- 1171.

Cooper OK. Ek.ser B, Tect or AJ (2015) A brief hist ory of d inic.al
xen ot rans p lan tat io n. IntJ Surg 23, 205-2 10.

Court F, Wemyu -Holde n S, Mo rrison C, et aJ. (200.3) Segment al
natu re of the pord ne liver and its potent ial as a model for
experimenta l part ial he pate <tomy. Br J Surg 90, 444.

Darni.s, B Mohkam K. Schmitt Z. e t al. (2015) Subtota l hepatec•
torny in swine for studyin g small.for-size syndrome and port.al
in flow mo du lat ion: is it re liab le? HPB (Oxfor d) 17, 881-888.

Debbaut C. Vie ren dee .s, J Ca.ste leyn C, et aJ. (201 2) Perfusion
cha ratt erist ics of the human hepatic mic:roc irculat ion ba.sed on
t hree-d imensiona l reco nstrutt ions and a:>mputat io na l fluid d
ynamic analysls. J Biomed> Eng 134, 011003.

Debbaut C. Monball u O, Segers P (2014a) Eng ineer in g p oin t of
v iew on live r t rans planta tion st rateg ies: mu lti-level modeling of
hep atic perfusion. Transplant Proc 46. 3143- 3146.

Debbaut C. Seger.s P, ComiUei P, e t aJ. (2014b) Analyzing the
human liver va.scular a rc:hite<ture by combining va.scula r co rro.
sio n cast ing and mic:ro-cr sc.anning: a feas ibili ty st udy. JAnat
224, 509- 517.

Deng ,M Kleinert ,R Huang H, et aJ. (2009) Statistical and eo::,.

nom ic.al effic:iency in a.ssessment of liver rege nerat ion using def
ined sample slze and ielett ion in m mbin ation with a fully
aull'.lmated image ana slssy<tem. J Histochem Cytochem 57,
107>- 1035.

Dorp Pete rsen K,A Nyen gaard Jft Gundersen HJ (2001) Ti.ssue
sh rin kage and unbiased stereologic.al est in at io n of part ic le
number and slze. J Microsc 204 , 23 2- 246 .

Dlw'lcan AW (2013) Aneuploid y, polyploidy and ploidy reversal in
the liver. Semi n Cel/ Dev Biol 24, 347- 356.

Eber'lo w L. U.ska V, Mirka H, et aJ. (2016) Pord ne liver va.scular
bed in Biod ur E20 co rrosio n c.asts. Folia Morphol (Warsz) IS. 154-
-161.

Faa,G N...-chi V, Demelia, L et aJ. (1995) Uneven hepatic m pper
dist ribution in Wil_g)n ' s d i.sease. J Hepatol 22, 303- 30&

Fausto N, Campbe U JS (2003) The role of hepatocytes and oval

cells in liver rege nerat k>n and rep o p u lat io n . Mech Dev 120.
117- 130.

Fingar DC. Sa lama 5, Tsou C, et aJ. (2002) Mammalian cell slze is
cont rolled by mTOR and its do wnstream targets S6K1 and 4
EBP1/e1F4E. GenesDev 16. 1472-1487.

Garde lla O, Hatt on WJ Rind HB, et al. (2003) Dlfferentlal t issue
sh rin kage and m mpression in the z..axis: im plic.atio ns fo r op ti·
c.al d i.sector co unt ing in vib rato me-, plastiC· and c ryosections. J

Neuroscl Methods 124, 45- 59.

min ipig as an an imal model in regulatory toxia:>lo gy. J Phar•
macol Toxicol Methods 62. 19 220.

Garrido Ft Zabka TS, Tao, J et aJ. (201.3) Quan t ltat lve h ist o log ie.al a.ssessment of xenobiotic.ind uced liver enzyme indutt k>n

586 Bruha, J al Ncita LO, Tonar, Z, e t.aL (201S) Mono do nal an tibod y. Analysis al size and density of porcine hepatocytES, Ro L Jun ataset al. against t ranrlo rming growth factor Beta 1 does not influence liver regene ration after resection in large animal experiments. *ln Viw,* 29, 327- 340.

Car'IIle ,OJ Zomorodi K. Houst on JB (1997) Scaling factors to relate drug metabolic d earance in hepatic mia osomes.

and pi tu i ta ry-thyroid axis stin ulat ion in rats using whole--slide automated image analysls. *J Histod,em Cytod,em* 61, 362- 371.

Gentric,G Oesdouets C (2014) Polyploldiza tion in liver t i.ssue. *Am J Pathol* 184, 322- 331.

Gentric G. D&.sdo ue ts ,C Celt on-Mori:tl.W' S (20 1 2) Hepatocytes polyploidization and cell cyde control in liver physiopatho l·ogy. */nt J Hepatol* 2012, 282430.

Ger1yng P, Abyholm A. Grotrnol T, et al (1993) Bin uc:leat io n and po ly p lo id iza t io n patterns in developmental and reg enerat ive rat liver grO'INt.h. *Cel/ Prolif* 26, 557- 565.

Gravante G. Ong SL. West K. et aJ. (2012) Patt erns of hist olog i-e.al changes following he pat ic electrolytic ablat ion in an ex. vivo perfused model. *Pathol Oncol Res* 1 8, 1035--1oa9.

Guidotti JE. Brégerie O, Robert A. e t aJ. (200.3) Live r ce ll poly. ploidlza tion: a pivotal role for binudea r hepatocytes. *J Biol Chem.* 218, 19095-19101.

Goodersen HJ (1988) The nucleato r. *J Microsc* 151, 3- 21.

Goodersen HJ. 0s t erby R (198 1) Opt im lzing sampling effic:lency of stereo log ic.al stud ies in bi o log y: or 'do more less well!'. *J Microsc* 1 21. 65--73.

Goodersen HJ. Bagger P, Bendtsen TF, et aJ. (1988) The new ste reological t oo ls: disect or, fract ionato r, nucleato r and point sampled intercepts and t heir use in pathologic.al rese arch and d iag nosls. *APM/S 96,* 857-881.

Goodersen HJ. Jensen EB, Kieu K. et aJ. (1999) The efficiency of systematic sampling in stereology..reo::,nside red . *J Microsc* 193, 199-211.

Haga S, Ogawa W, lnoue H, et al (2005) Compensatory recovery of liver mass by Akt·mediated hepatocellular hypertro phy in liver..spec:lfic STAT3..deficient mice. *J Hepatol* 43. 799-80 7.

Hammad S, Hoehme S, Friebel A. e t al. (2014) Protoco ls for st.ain ing of bile c.ana licular and sinusoida! net\Norks of humal\ mouse and pig livers, t hree..dimensiona l reconst ruction and q uant lfication of t i.ssue microa rc:hitectu re by im age proc essin g and analysls. *Ard> Toxicol* Ba 1161- 1183.

Ha%ato n JP, O-Oron RL. Dod:son GM. et aJ. (2015) Compari.son of at rioc.aval shuntin g with perihepatic padcing versus perl· hepatic packing alone for retrohepatic vena c.ava in juri es in a swine mode l. *Injury* 46, 1 7 5I764

Hessheimer AJ Esœb• B, Munoz, J et aJ. (2014) Somatost.atin therapy prote<ts porcine livers in small.for-slze liver t rans plan . ta t ion . *Am J Tramp/ant* 14, 1 1816.

Heuer JF, Sauter P,Pelosi P, et aJ. (2012) Effe<ts of pulmonary add aspiration on the lungs and extra. pulmonary organs: a rand omized study in pigs. *Crit Care*16, R35.

Hoehme $, Brulport M. Bauer A. e t aJ. (2010) Prediction and val· idation of cell alignmen t along microvessels as order principie to restore t i.ssue a rchite<ture in liver regene ration. *Proc Natl Aa,d Sci U S A* 107, 10371- 10376.

HoffmaM EK. Oooham PB (1995) Mem brane mechan isms and intracellular signalling in ce ll volume regulatio n. */nt Rev Cyt.ol* **161**, 173- 262.

Howard ev, Reed MG (2005) *Unbiased Stereology. Three-Dimen·sional Measurement in Microscopy,* 2nd edn. New York: Springer.

Hussein KH, Pari(KM. Kim HM. et aJ. (2015) Construction of a biooompatible decellularlzed porcine he pat ic lobe for liver bioeng in eering. */nt J Artif Organs* 38, 9 114.

lid a T, Yagl $, T,, igu d'li K. e t al. (2007) lmpro vement of mor·pholog ical changes after 70% hepatectomy with portocaval shunt: preclinical study in pordne model. *J Surg Res* 143, 238-- 246.

Irwin R. Pat ker,J lobenhofer E. e t al. (2005) Transcriptional pro. filing of the left and median llver lo bes of male F344/N ratsfol. lowing exposure to acetaminophen. *Toxicol* Patho / 33, 111 - 117.

Ji:t'k M. Tonar Z. KrálJt ková A. et aJ. (2016) Stereo logic.al quan. tific.at ion of microvessels using semiautomated evaluation of x. ray mia otomography of hep at ic vaSOJlar co rr osion casts. */nt J Comput Asskt Radio/ Surg* 11, 180.3--1 8 1 9.

K.ar'balay-Doust $, Nooraktian A (2009) Stereolog ic.al study of the effects of nandrolone dec.anoate on the mouse llver. *Micron* 40, 471-475.

Keily DM. ZhuX. Sihiba H. et aJ. (2000) ftd enosine restores t he hep. at ic artery buffe r respo nse and improves survival in a pordne model of small.for.sl.2e syndrome. *UverTranspl* 15, 1448--1457.

KWon ,YJ Lee KG. 0-01 o (2015) (linie.al im plic.at ions of advances in liver regene ration. *Clin Mol Hepatol* 21, 7- 13.

u•a v, Treska v, Mirka H. e t al (2012) lmmed iate ly preo pera. t ive use of biologie.al t he ra py does not influen ce liver rege ner. at ion ah! r large resection - porcine experimental model with mono do nal ant ibody against epidermal growt.h fa<to r. *In Viw,* 26, 6.83-691.

Manda rin'Mfe- Lacerda CA (200.3) Stereo logic.al too ls in bio med i·c.al rese arch . *An A cad Bras Cienc1S.* 469-48 6.

Marcos Ft Mo nte iro RA. Rocha E (2004) Ettimation of the num. ber of stellate cells in a liver with the smooth fractionator. *J Microsc* 215, 174--182

Marcos Ft Mon teiro RA. Rocha E (2007) Oeterm ination of hepa·tocellularlty numbe r in the rat. *Toxicol /n Vitro* 21, 1692 1693.

Marcos Ft Monte iro RA. Rocha E (2012) The use of design.ba.sed ste reo lo gy to evaluate volumes and numbers in the liver: a review with practic.al gu ide lines . *J Anat* 220, 30.3--317 .

Marcos Ft Braga B, Fontes•Sousa AP (2015a) Image analysls or stereology: which to choose for quantifying fibrosls? *J His--tod>em Cyt.ochem* 63, 734--736.

Marcos Ii. CorreiaaGomes, C **Miranda K** et al. (2015b) Livor gen•de r dimo rp hism - inslg hts from quant lta tive mo rp ho logy. *His--tol Histopathol* 30, 1431- 1437.

Marcos R. Lopes C. Malh ao F, et al. (2016) Stereological a.ssess-ment of sexual dimorphism in the rat liver revea ls d ifferences in hepatocyte-s and Ku pffer cells but not he patic stellate ce lls . *J Anat* 228, 99 1005.

Mattf eldt T, Mali,G Ghareh bagh i H. et aJ. (1990) Est imation of surface area and length with the orientator. *J Microsc* 159, 301- 317.

Myaoka Y, B>ato K. Kalo H, et al. (2012) Hypertrophy and unconventional ce ll d iv is ion of hepatocytes undedie liver rege nerat ion . *Cu" Biol* 22, 11 1175.

Mouton PR (2002) *Prindples and Practices of Unbiased Stereo/.ogy. An Introduction for Biosdentists.* Baltimore: Jo hns Hop·kins Unive rsity Press.

Muhlfeld C. Nyengaard JR. MayhEPN TM (2010) A review of st.ate-of.the·art stereology for better quantltatlve 30 morphol·*ogy* in cardlac research. *Cardiovasc Pathol* 19, 6>82 .

Newsome PN, Henderson NC. Nei.son u. e t aJ. (2010) Oevelop. ment of an in vasively monito red porcine model of acetamino·phert-indu ced arute liver failure. *BMCGastroenterol* 10, 34.

Nic ola.s CT, Wang Y, Nyberg SL (2016) Cell t herapy in chronic liver dise ase. *Curr Opin Gastroenterol* 32, 189-194.

Nyengaard Jft Goodersen HJG (2006) Samplin g fo r stereo lo gy in lungs. *Eur Re$) ir Rev 1S.* 107- 114.

Jac k EM. Bentley P, Bieri F, et aJ. (1990) ln a ease in hepatocyte and nuclear volume and decrease in the population of binu. cleated cells in

preneo plastic foci of rat liver: a stereological study using the nucleato r method. *Hepatology* 11, 28 297.

586 Analysis *al* size and density of porcine hepatocytES, K. L Jun ataset al.

Odad E. Bien H. Had muftuoglu A. e t aJ. (2000) Long.te rm t reatments w ith low· and high dose olanzapine change hepa· tocyte numbers in rats. A stereological and hist opatho log ical stu dy. *Arch Med Res* 40, 139- 145.

PaUadi ni G. f.ertlgno A. Ri:zzo v, e t aJ. (201 2) Lo b e--spec:ific hete roge neity and matrix metalloproteinase activation after

ischemia/reperfusion injury in rat livers. *Toxicol Pathol* 40, 722–730.

Pandit SK, Westendorp B, De Bruin A (2013) Physiological significance of polyploidization in mammalian cells. *Trends Cell Biol* 23, 556–566.

Park GC, Song GW, Moon DB, et al. (2016) A review of current status of living donor liver transplantation. *Hepatobiliary Surg Nutr* 5, 107–117.

Peeters G, Debbaut C, Cornillie P, et al. (2015) A multilevel modeling framework to study hepatic perfusion characteristics in case of liver cirrhosis. *J Biomech Eng* 137, 051007.

Peinado A, Vilda P, Quesada A, et al. (1990) Binucleated hepatocyte subpopulations in male and female rats fed with olive and sunflower oils. *Acta Stereol* 9, 3–8.

Regev A, Berho M, Jeffers L, et al. (2002) Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 97, 2614–2618.

Richardson F, Boucheron J, Dyroff M, et al. (1986) Biochemical and morphologic studies of heterogeneous lobe responses in hepatocarcinogenesis. *Carcinogenesis* 7, 247–251.

Rohr HP, Lüthy J, Gudat F, et al. (1976) Stereology of liver biopsies from healthy volunteers. *Virchows Arch A Path Anat Histol* 371, 251–263.

Romeis B (1989) *Mikroskopische Technik*, 16th edn. Munich: Urban und Schwarzenberg.

Saxena R (2011) *Practical Hepatic Pathology: A Diagnostic Approach*. Philadelphia: Elsevier/Saunders.

Schwen LO, Schenk A, Kreutz C, et al. (2015) Representative sinusoids for hepatic four-scale pharmacokinetics simulations. *PLoS ONE* 10, e0133653.

Schwen LO, Homeyer A, Schwier M, et al. (2016) Zonated quantification of steatosis in an entire mouse liver. *Comput Biol Med* 73, 108–118.

Schwier M, Böhler T, Hahn HK, et al. (2013) Registration of histological whole slide images guided by vessel structures. *J Pathol Inform* 30, S10.

Shen MR, Droogmans G, Eggermont J, et al. (2000) Differential expression of volume-regulated anion channels during cell cycle progression of human cervical cancer cells. *J Physiol* 529, 385–394.

Sohlenius-Sternbeck AK (2006) Determination of the hepatocellularity number for human, dog, rabbit, rat and mouse livers from protein concentration measurements. *Toxicol In Vitro* 20, 1582–1586.

Song T (2015) Recent advances in surgical treatment of hepatocellular carcinoma. *Drug Discov Ther* 9, 319–330.

Spear B, Jin L, Ramasamy S, et al. (2006) Transcriptional control in the mammalian liver: liver development, perinatal repression, and zonal gene regulation. *Cell Mol Life Sci* 63, 2922–2938.

Stegemann J, Raina S, Nicholson D, et al. (2000) Comparison of analytical methods for quantitation of isolated porcine hepatocyte yields. *Tissue Eng* 6, 253–264.

Tanami S, Ben-Moshe S, Elkayam A, et al. (2016) Dynamic zonation of liver polyploidy. *Cell Tissue Res*. In Press. DOI 10.1007/s00441-016-2427-5.

Vinogradov AE, Anatskaya OV, Kudryavtsev BN (2001) Relationship of hepatocyte ploidy levels with body size and growth rate in mammals. *Genome* 44, 350–360.

Vrtkova I (2015) Genetic admixture analysis in Prestice Black-Pied pigs. *Arch Anim Breed* 58, 115–121.

Wang H, Yang YG (2012) Innate cellular immunity and xenotransplantation. *Curr Opin Organ Transplant* 17, 162–167.

Wang B, Zhao L, Fish M, et al. (2015) Self-renewing diploid Axin2(+) cells fuel homeostatic renewal of the liver. *Nature* 524, 180–185.

Watanabe T, Tanaka Y (1982) Age-related alterations in the size of human hepatocytes. A study of mononuclear and binucleate cells. *Virchows Arch B Cell Pathol Incl Mol Pathol* 39, 9–20. Wu Q, Bao J, Zhou YJ, et al. (2015) Optimizing perfusion-decellularization methods of porcine livers for clinical-scale whole-organ bioengineering. *Biomed Res Int* 2015, 785474.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primary stereological data on porcine hepatocytes in all samples investigated.

Příloha IV:

MALEČKOVÁ, A., Z. TONAR, P. **MIK**, K. MICHALOVÁ, V. LIŠKA, R. PÁLEK, J. ROSENDORF, M. KRÁLÍČKOVÁ a V. TŘEŠKA, 2019. Animal models of liver diseases and their application in experimental surgery. Rozhledy V Chirurgii: Mesicnik Ceskoslovenske Chirurgicke Spolecnosti. 98(3), 100–109. ISSN 0035-9351. Bez IF.

Zvířecí modely jaterních onemocnění a jejich využití v experimentální chirurgii

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Souhrn

Akutní a chronicktl jaterní onemocněnínpl edstavujf Široku skupinu chorob, které t.asto ohrožuj(pacienty na životě. Pochopení mecharismOsto jf- ckh za patogenezi a progresi jaternlho poškození je kUfO'lé pro vyrojnových terapeutických strategi a lékQ. Nejvýznamnějšm faktorem limittJl- cfm studium patogeneza a progreseje jaterních onemocněnije nedostatek vhodných chzvffedchmodE!lO. Do soulas doby byloe tabloWnomnotstvf zvírecfch modelů napodobujících jaterní onemoc: nJu lid f. 1-llodavd, konkrétně po dcani a myši, pl'edstavuj f největ!f skupinu modelO jaternfch onemocněnf Tyto modely orlem dokážou manifestova YSechny klinickÉ aspekty jaterního onemocněnJu lidí jen do omezené míry, předevš(m pro jejich omezenou anatomkkou a fyziologk ou podobnost s lidmi. Velké zvírecf modely reprezentové prasetem významMz ejména v oblasti modeloW!nf akutnfho jaterního hoselM nf, jsoustt Uet astěji využtvttny v rrodelov ní dal ích ak.utnkhi chronických jaterních onemocněnlPl'enos vý- sledkO testovanl novýchl ebných metod do humannl medicinyjez.lvisl) l navývoji dokooalejSlch zvffeclchmodelO refl ek tu jlck h prOběhjaternrch onemocněnf u lidl Toto prehledM sdělení shrnuje dosud publikovanéz vffecf modely chronických i akutnich jaternich onemocněnf se zvtištnfm dOrazem na velk zvffecf modely a jejich využití v experiment Jní chirurg ii

Kllovd slova: onemocnění jater- zvífecf modely - experiment Jnfchi rurgie , prase

Summary
Animal models of liver diseases and their appl ication in experimental surgery
A. Malec:kova, Z. Tonar, P. Mik, K. Michalova, V. Liska, R. Palek, J. Rosendorf, M. Kralickova, V. Treska

Both acute and chronic liver diseasesare frequent and potentia Jy letha'l cond ition s.. Development of new therapeutic strategjesand drugsde- pends on understanding of liverinjury pathogenesisand progression, wnich canbe studied on suitableanimal models. Oue to the complexity of liverinjury, the understanding of underlyi"ng m ec.hanismsof liver diseasesand their treatment has been limited by the lack of satisfactory animal models.. SO far, a wide variety of animalshas been used to mimic human liřver disease, however, none of *the* models indudeall its clinic.al aspects seen in humans. Rodents, namely ratsand mice, represent the largest group of liverdisease mode ls despite their limited resemblance to human. On the other hand, large animal modelslike pigs, previously used mostty in acuteliver failure modeling. are now playinganimportant role in studying variousacute and chronic liver diseases. Alth ough significant progresshas been made the research in hepatology should continue to establishan imal modelsanatomicaly andphysiologic.aJy asclose to human aspossible to allow for translation of the experimenta resultsto human medicine. This review presentsvarious approachesto the study of acute and chronic liverdiseasesin animal models, with special emphasis on largeanimal models andtheir rolein experimental surgery.

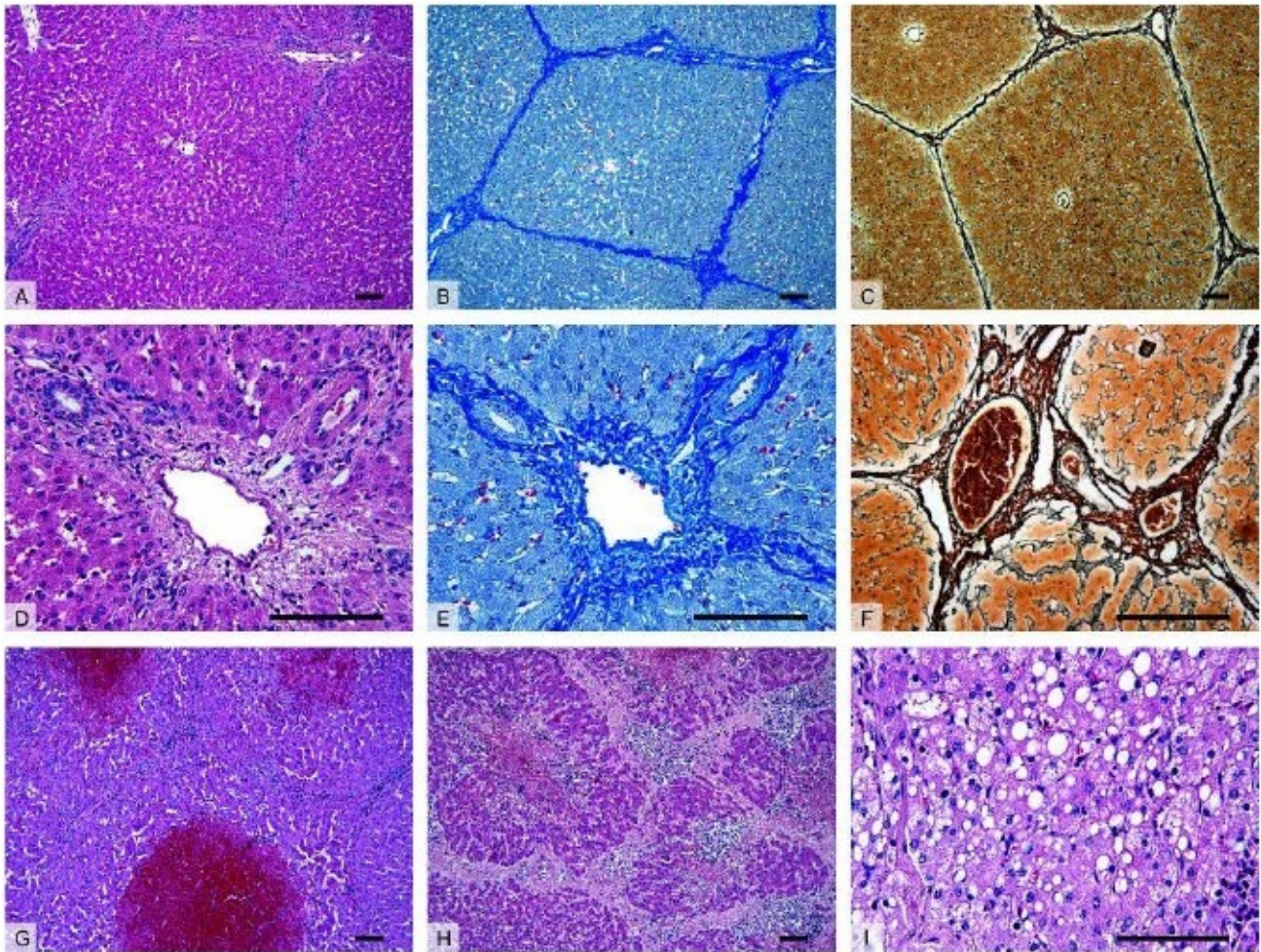*words:* liver disease- animal models - experimental surgery- pig

ÚVOD

Akutní jaterní onemocnění (acute hepaticinjury, acu- te liver disease ALD) a chronická jaterní onemocnění (chronic liverdiseaseCLO) před stavují širokou skupinu chorobjater srCJznou etiolog i í a mechanismem vzniku jaterního poškození. **ALD** mo hou vyú stit v akutn í selhá- ní jater (acuteliverfailure ALF) nebo přejít do chronici- *ty.* CLO typicky vedou k progresivní destrukci jaterního parenchymu a ve svém důsledku vyúsťují v jaterní fib- rózua cirhózu.

Navzdory pokroku v diagnostických a léčebných metodáchv hepatologii stále zČJstává řada nezodpo- vězených otázek týkajících se patogeneze, progrese a případného terapeutického ovlivnění jaterních one- mocnění. Jednou z vhodných cest ke zkoumání těch- to mezer v poznání je experimentální využití zvířecích modelCJjaterních onem ocnění.

Do současné doby byly formoupřehledných sdělení zmapovány oblasti zvířecích modelCJakutních i chro- nických jaterních onemocnění [1- 5]. ALD i CLO jsou nejčastěji modelovány na malých laboratorních sav- cích, kteří se uplatňují zejména *díky* své snadné gene- tické manipulovatelnosti, krátkému životnímu cyklu (dospějí v případě CLO do stadia fibrózy či cirhózy [51] a cenové dostupnosti. Ovšem jejich malá anatomická a fyziologická podobnost s člověkem omezuje apliko- vatelnostvýsledkCJstudií do humánní medicíny. Proto

Obr.1: Histologický obrazzdravých **(A-F) a** patologickyzměněných jater prasete(G-1)

A-C- Přehlednézvětšenízobrazujícíjaterníparenchymčleněnýv jednotlivé jaterníIaIOčky, kteréjsou u prasete ohraničené oproti člověku významně větším množstvímvaziva.

D-F- Detailportob iliám ích prostoro bsahujících hepatickoutrias- interlo bulá mívěteva. hepatica propria, v. portae a žlučovodu.

G- Centrilobulámíhemoragická nekróza u zvířete se sinusoidáJnímobstrukčnímsyndromem vyvolanýmexperimentálnía plikací pyn o lizidinového alkaloidu monocrotalinu.

H - Nodulární transformace jaterní tkáněs porušenou mikrostrukturoujater a tyPickyzmnoženým interlobulárním vazivem u jaternícirhózy.

I - Steatóza jater charakterizována nahromaděním tukových vakuolv cytoplazměhepatocytía v dOsledku toxického poškození jater.

Barven í: hemato xylin a eosin - přeh led né barvení (A„D G-1), a n ilinová modř a jádrová červeň - detekce kolagenního vaziva{B, E), stříbřen í{im preg načn í metoda){C F}- detekce retikulá rních vlá ken. Měřítka: 100 l‚lm.

F i g. 1: Histologyof healthy **(A-F)** and diseased (G- 1) porcineliver

A-C- Theliver parenchymadivided into morphological hepatic lobul‚es in pigs the lobules are demarcated bya significa ntly higher amountofconnective tissue when compared to human.

D-F- Detail ofportaltractscontaining bile ductsand branchesoftl‚e hepaticarteryand portalveinsurrounded byconnectivetissue. G - Centrilo bular hemorrhagic nea osis seen in animal with Sinu‚soidalObstruction Syndrome experiment included the applica- tion of alkaloid monocrotaline.

H - Cirrho tic liver histologicallyrepresented by nodula r transforma tion of liver parenchyma with disruptionsof liver microarchitec- ture and excessive amo unt of intertobular connective tissue.

I - Steatosischaracterizedbylipiddropletsaccumulationwithinhepatocytescausedbytoxicliverinjury.

Staining: hematoxylin and eosin staining{A„D G-1},an iline blue staining, counterstained with nuclearfastred- detection of collagen connect ive tissue{B, E).silver impreg nation technique - detection of reticularfibers.

Scale bars: 100 µm.

jsou zde tendence provádět experimenty pC.vodně s malými savci na modelech relevantnějších pro humánní medicínu, jako např. na praseti [6), jehož játra jsou do velké míry anatomicky ahistologickypodobná játn'.lm lidským (Obr. 1).

Naproti tomu v oblasit modelování ALF převazuJ1 velké zvířecí modely, které umožňují napojení na mimotělní oběh, opakované odběry krve a jsou vhodné pro nácvik chirurgickýchtechnik,kteréjsouna rozdíl od mikrochirurgickýchtechnik používaných nahlodavcích

		onemocnění	Výhody	Nevýhody
Velké zvířecí modely	prase		anatomickya fyziologickypodobné lidem	vysoká cena **nutnostaplikace opakovaných** dávek hepatotoxických láte k dlouhá doba potřebná krozvoji CLD
	pes	**akutníselháníjater**	**možnost opakovaných odběrů krve napojení namimotělníoběh** nácvik chirurgických techn ik	
Malé zvířecí modely	myš	**akutníjaterníonemocnění**	krátká,doba potřebná krozvoji CLD	anatomickya fyziologicky málo podobné lidem
	potkan	**chronická jaterní**	**levné**	**velikostneumožňujícío pako-**
	králík	**onemomění**	možnost genetické modifikace	**vanéodběry krvea napojenína mimotělní oběh**
	Geneticky modifikované myš			

*VywM/ivky:CUJ-chronickéjateml*onemocnhl/*(chronicNverdi sease).*

j ednak méněnáročnéna dovednosti operatéra, ale především bližšíchirurgické praxi(Tab. 1).

V literatuře nacházíme množstvízpůsobů dělenízvířecíchmodelů jaterních onemocněn,íkteré se do jisté míry překrývají a duplikují. Liu et al. [4] navrhl rozdělení modelú CLD dle mechanismu vzniku jaterní fibrózy a cirhózy na (i.) klasické modely: neberou v potaz etiologii jaterního poškození, aplikacihepatotoxických látekdosahují u zvířat jaterní fibrózy, (ii.)modely napodobující specifická jaterní onemocnění: kladou důraz na etiologii CLD, kterávýznamným způsobem ovlivňuje patogenezi, progresi a prognózu CLD Poněkud přehlednějšímjezdenavrženédělení,kterézahrnujevětšinu možností modelování jak akutního, tak chronického jaterního poškození (Tab.2).

Cílem tohoto sdělení je představit zvířecí modely jaterních onemocnění ajejich současné využitiv experimentu. Pro zachování kompat ibility se zahraničními zdroji ponecháváme vedle některých pojmů v češtině i jejich ustálené protějškyv anglickém jazyce.

Akutní selhání jater(ALF}

O,irurgické modely

Farma kolog ické modely

Infekční mode ly

Toxické poškození jater

O,emickyindukované modely

Lékové poškoze ní jater

Alkohol**ové poškozeň**jater

M ode ly imitu jící specifická jaterní onemocnění

Modelyauto imunitní hepatitidy (AIH)

Modely primární biliárnícirhózy (PBO

Modely primárnísklerozujícícholang iticly (PS( )

Modely nealkoholické steatózyjater (NAFLD) a steatohepatitidy(NASHJ

Modelyvirové hepatitidy

Invazivní modeljaterní fibrózy

1. Zvířecí modely akutních a chronických jaterních onemocnění

Následující text shrnuje vybrané modely zvířecích modelů akutních a chronických jaterních onemocně- ní na základě literární rešerše. Rešerše byla provedena v databázi PubMed k datu 30. 8. 2018 s využitím klíčo- vých slov: acute liver failure, chronic liver disease, ani- mal models. Přehledná sdělení (review) byla následně doplněna o konkrétní studie s využitím klíčových slov nalezených v literatuře, např.: acetaminophen, acute li- ver failure, animal model, pig. V předkládaném článku jsou uvedena jak přehledná sdělení (review) na dané téma, tak příklady studií, které využily zvířecí model ke konkrétním experimentům, např. k testování podpúr- ných jaterních systémů (Tab. 4). Zahrnuty byly zejména studie s prasečími modely jaterních onemocnění.

1.1 Modely akutního jaterního selhání

Navzdory vysoké regenerační kapacitě hepatocytú a možnosti spontánního návratu jaterních funkcí zústá- vá pro pacienty s ALF bez uspokojivé regenerace jedi- nou terapeutickou možností ortotopická transplantace jater (orthotopic liver transplantation OLT). Ve většině případú OLT zlepšuje celkové přežití, ovšem počet paci- entú na čekací listině pro transplantaci neustále přibývá [1]. Zlepšení regenerační kapacity jater lze dosáhnou t arterializací portální *vény* (portal vein arterialization PVA), jak bylo testováno v experimentu na potkanech [7] a prasatech [8]. Současné léčebné možnosti použí- vané k překlenutí čekací doby na OLT (tzv.• bridging•) jsou omezené a zahrnují transplantaci hepatocytli [9] a využití podpůrných jaterních systémú, tzv.• umě lých jater (liver suppo rt system s) [10,11].

Pokusy o vysvětlení patofyziologických procesů a propojení metabolických změn s klinickými projevy **ALF** vyú stily ve formulování dvou teorií:. toxin hypo- thesis• a. critical mass theory·•. Toxin hypothesis" před- pokládala, že zaklinické projevy ALF jsou zodpovědné nahromaděné látky jako např. amoniak, fenoly či volné žlučové kyseliny, které jsou ve zdravých játrech odbou- rávány.• Critical mass theory pak za příčinu jaterního selhání považovala ztrátu hepatocytú pod určitou kri- tickou mez, kdy již játra nejsou schopna udržovat svou

Tab. 3: Krité ria pro ideáln ízvířecímodel akutního selhání jate r(Alf)- převzatoa upraveno dle Terblanche a Hickman (131
Tab.3: Criter ia for an idea l animal model of acuteliverfailure,(Alf) postulated byTerblanche and Hickma n(13]

Kritérium	Charakteristik
Vratnost - reversibility	Při zavedení vhodné léčb y by mělo být ALF potencionálně reverzibilnía model by měl přiadekvátníléčbě přežít.
Opakovatelnost- re,producibility	Mode l musíbýt jednoduše reprodukovatelný.
Smrtz příčinjate rního selhání- deathfrom liverfailure	Měly by být přítomny biochem ické histologické a klinické změny od rážejíá průběh jaterního selhániu lidía jaterní poškození by mělovést přímo kesmrti mode lu.
Terapeutické okno- therapeutic window	Mezi inzultem a úmrtím zvířete musí být dostatečnýčas knasazeni a posouzeníefektu lé čby.
Vdký zvířecímod - largeanimalmodel	Velikostzvi řete mus í umož nit pravidelné odběry krve a aplikaci mimotě lního oběhu a model by měl být anatomicky a fyziologicky co nejbližší lidem.
Bezpečnost - minimaIriskfor personnel	Použité techniky a toxiny musí představovat minimální rizikopro personál

metabolickou akti vitu na takové úrovni, aby dokázala zajistit podporu funkce dalších periferních orgánCJ. Hlub ší studium problematiky ALF ovšem ukázalo, že mechanismus nahromaděni toxických látek a ztráty syntetické kapacity jater nejsou jedinými mechanis- my a že vlastní poškozené hepatocyty se pod íleji na

prohlou beni a progresi ALF uvolňováním zánětlivých

cytokinCJ či vazoaktivních látek [12]. Přestododnes zCJstávají některé mechanismy ALF nepop sány. Prostudium patogenezeALFa testovánínovýchterapeutických přístupCJ z h l edi ska jejich bezpečnosti a efektivity je proto nezbytné etablovat zvířecímodel, kterýbudeco nejvícereflektbvat prCJběh ALF u člověka.

Terblanche a Hickman [13) počátkem 90. let navrhli systém šesti kritérií, která by měl ideální zvířecí model ALF splňovat (Tab. 3). V současnosti žádný zvířecí model ALF nesplňuje všechna popsaná kritéria.Pro *vytvoření* modeluALF sevyužívají tři hlavnípřístupy: chirurgický, farmakologický (toxický) a infekčnímodel [1-3].

1.1.1 Chirurgické modely ALF

Chirurgické modely s výhodou využívají velká zvířata. Rozděluj í se do 3 základních skupin: velká resekce (partial hepatectomy), hepatektomie (anhepatic model) aischemickýmodel (ischemie model, devaskularizace)(Tab. 4).

Modely velké jaterní resekce jsou pro svoji náročnost provedení, jež závisí na techni ckém vybavení a zkušenostech operatér CJ, těžkoreprodukovatelné. Alternativouk prostéresekcimCJžebýt kom binace resekce sischemíí zbytkujaterníhoparenchymu[14].

Anhepatický model zahrnuje odstranění celého

orgánu [1,5) je oproti předchozímu snadněji reprodukovatelný. Pro jeho využití v experimentu je mimo jiné dCJležitá i pooperační intenzivní péče: monitorování hemodynamických parametrCJ, zajištění ventilace, monitorování a případná úprava mikce, monitorování množství hemoglobinu, hematokritu a laktátu, elektrolytCJ v séru, acidobazické rovnováhy, krevních plynů a hladinyglukózy které ovlivňují délku přežití zvířete, a tedy délku terapeutickéhookna pro použitía testovánínovýchléčebných strategií[16].

Ischemický mod el představuje dvoustupňový chirurgický výkon, při kterém se *vytváří* po rt okavální shunt s následným uzávěrem hepatické arterie [17]. OkluzemCJžebýt dočas ná, pa k je modelpotenciálněreverzibilní, *ovšem* pn)běh ALFje značněnestálý atěžko reprodukovatelný.Jiným přístupem k vytvoření ischemického modelu je přerušovaná okluze *v*. portae a a. hepatica snáslednou arteriálníligací[18]. Tentomodel splňuje všechna kritéria dle Terblanche a Hickmana svýjimkou reverzibility [13].

Tab. 4: Přehledchirurgických mode lů akutního sel háníjater a jejichsoučasnévyužití v experimentu na praseti
Tab. 4: Surgical modelsofacute live r failure an d t he ircurrent application in experimental research on pigs

Typmodel	Klinická relevanc	Výhody	Omezení	Využití prasečího modelu v experiment
Velká ja e rní resekce	resekční výkon		technickynáročné	
Partial hepatectomy	pronádorové o nemocněn	reverzibilita	nereprod ukovatelné	studium regenerace[48]
	hepa tek tom ie pro rozsáh lé		ireverzibilní	
Hepatektomie Anhepatic model	trauma čiakutní rejekci orgánu po	transplantaci		reprodukovatelno st vyloučení vliv poškozených

hBAL [49] hepatocytů na progresi ALF

Ischemický model	dočasná ischémie	reverzibilita	nereprodukovatelný	BAL [SOJ
Ischemie model	při OLT		krátké přežití	

1/)'SVM/ivl<y:OI.T- onotopickátransplantacejater (orthotopic/ivertransplantation),BAL- bioartmcial Hver.

Název sloučeniny	Mechanismus účinku poškození jater	Výhody	Omezení	Využití prasečího modelu v experimentu
Galaktosamin	narušení metabolismu RNA hepatocytů	zanedbatelné e.xtrahepatální účinky	klinicky nerelevantní nereprodukovatelný riziko pro personál cena	BAL [S1] aplikace MSC a jej i ch efekt na ALF (52)
Aceta mi nofen APAP	metabolizace pomocí cytochromu P450 a vznik toxického NAPQI	klinicky relevantní bezpečný	variabilita v odpovědi na dávku a v přežití extrahepatální toxicita: nefrotoxicita, kardiotoxicita methemoglobinémie	BAL (53)
Tetrachlormetan CCl₄	metabolizace pomocí cytochromu P4SO2E1 se vznikem toxických radikálů	vysoká mortalita	krátké terapeutické okno extrahepatální toxicita	PI/A (8)

Vysvětlivky: ALF - akutní selhání jater (acute liver failure), NAPQI - N-acetyl-j:>-benzoquinoneimine, BAL - bioartificial liver, PVA - portal vein arterializatian, NO - oxid dusnatý, MSC - mezenchymální kmenové buňky (mesenchymal stem cells).

Použitá látka	Mechanismus poškození (54)	Poškození jater		Využití modelu v experimentu
Skutečné hepatotoxiny				
• Tetrachlormethan CCl4	metabolizace pomocí cytochromu P450 2E1 se vznikem toxických	akutní selhání jater fibróza/cirhó za	potkan	studium hepatoprotektivních látek [SS] rolemiRNA ve fibrogenezí (56)
	radikálů		myš	terapie otrav CCl, (57) studium hepatoprotektivních látek (58)
• Thioaceta mid TAA	vzrik intennediární metabolitu thioaceta- mid-S-oxid ua zvýšení oxidativního hostresu	fibróza/cirhóza za HCC, Cholangiokarci- nom	potkan	studium hepatoprotektivních látek (59)
			myš	role mikrobiomu ve fibrogenezi (60)
• Dimethylnitrosamin Diethy lnitr osamin DMN/DEN	Alkylace DNA	fibróza/cirhóza HCC	potkan	role miRNA ve fibrogenezí (61) ovlivnění progrese fibrózy do HCC (62)
			myš	role miRNA ve fibrogenezí [63]
Potenciální hepat- xiny				
• Chemoterapeutika	specifický v závislosti na použitém chemo-	Sinusoidální obstruk-č ní syndrom (SOS)	potkan	terapeutické ovlivnění SOS (65)
	terapeutiku (64) indukce cytochromu P450 HE1	CASH ASH fibróza/cirhóza	myš prase	studium patogeneze SOS [66] regenerace jater s CASH (67)
Ethanol	zvýšená hladina ace- taldehydu oxidativní stres		potkan myš	studium hepatoprotektivních látek (68) studium hepatoprotektivních látek [69)

Vysvětlivky: HCC - hepatocelulární karcinom CASH - steatohepatitida , pciend s chemoterapii /chemotherapy-associated steatohepatitis), ASH - alkoholová steatohepatitida /alcoholic steatohepatitis), A1D - alkoholové po! kození jater /alcoholic liver disease}.

1.1.2 Farmakologické modely

Většinu farmakologických zvířecích modelů ALF představují m alí labo ratorn í savci [19- 21]. Velké zvíře- cí modely jsou zatížené nutno stí opakované aplikace poměrně vysokých dávek toxinu s nestálým klinickým prCJběhem ALF. Přesto velkých zvířecích modelů v sou- časnosti přibývá, a to zejména pro testo vání jaterních

podpCJrných systémÍJ. Nejvýznam nějšími toxíny, jejíchž účinky byly stuďovány na řadě zvířecích druhů a které jsou v současnosti používány k vývoji modelu ALF, patří: galakt osamin [22], acetaminofen [23] a tetrachlor-methan[8,24] (Tab. 5).

1.1.3 Infekční modely

Navzdory tom u, že vírové hepatitidy jsou celosvětově jednou z nejčastějších příčin jaterního selhání, *byly* snahy o vytvoření zvířecího modelu na podkladě infekce dlou- hou dobu neúspěšné. Myší mode ly hepatiti dy B při spěly ke studiu patogeneze ALF, ovšem tento malý zvířecí mo-del neumožňova l opakované krevní rozbory a připojení namimotělní eliminačnímetody. Z tohoto důvodu seir>-fekční modely prozatím ve studiu ALF neup latnily.

1.2 Modely toxického poškození jater

Játra hrají zásadn í rol i v biot ransformaci a elim inaci

Tab.7: Animal models mimicking specific chronic liver diseases

Onemocnění	Kritéria ("wish list") pro ideální model		Princip modelu
Autoimunitní hepatitida AIH[26]	přítomnost auto protilátek (ANA, ASMA) chronický zánět s přechodem do fibrózy přítomnost antigenně-specifických T-lymfocytů přítomnost nekróz na histologickém vyšetření	myš králík	indukované modely geneticky modifikované spontánní modely geneticky modifikované indukované modely přenos antigenů pomocí virů
Primární biliární cirhóza PBC[29]	převaha rozvoje PBC u samic přítomnost autoprotilátek (AMA) chronický zánět malých a středních intra-hepatálních žlučovodů floridní duktulární reakce na histologickém vyšetření	myš	geneticky modifikované spontánní modely modely vyvolané environmentálními faktory geneticky modifikované autoimunitní modely
Primární sklerozující cholangitida PSC[35]	převaha rozvoje PSC u samců progresivní zánět intra- a extrahepatických žlučovodů koncentrická periduktální fibróza (onion skinning) na histologickém vyšetření přítomnost nespecifického zánětu střev (UC) přechod do cholangiokarcinomu	potkan myš	chemicky indukované modely modely využívající infekční agens geneticky modifikované modely model GVHD
Nealkoholická steatóza a steatohepatitida HAFLD, NASH (37)	obezita dyslipidemie inzulinová rezistence steatóza, zánět a balonová degenerace hepatocytů progredující ve fibrózu na histologickém vyšetření přechod do HCC	myš, potkan	dietary models MCD diet CDAA diet atherogenní dieta high-fat dieta a její variace geneticky modifikované modely
Virová hepatitida B a C HBV[46] a HCV (45)	vnímavost k infekci replikace viru v organismu imunitní odpověď srovnatelná s lidmi Progrese do cirhózy přechod do HCC	šimpanz myš potkan kachna sviší	šimpanzí modely myši exprimují virové proteiny myši exprimují receptory pro HCV chimerické myší modely imunodeficientní chimerické modely imunokompetentní

VYSVĚTLIVKY: ANA - antinukleární autoprotilátky, ASMA - autoprotilátky proti hladkým svalovým buňkám, AMA - antimitochondriální protilátky, UC - ulcerózní kolitida, HCC - hepatocelulární karcinom, GVHD - reakce štěpu proti hostiteli, MCD diet - methionine and choline deficency diet, CDAA diet - choline-deficient L-amino acid-defined diet.

exogenních látek, jejichž nadměrnou expozicí často dochází k jaternímu poškození. Opakované aplikace hepatotoxických látek představují klasický způsob indukce jaterní fibrózy u experimentálních zvířat. V závislosti na způsobu podávání ale mohou hepatotoxické látky místo jaterní fibrózy způsobit akutní jaterní poškození, až selhání (viz kapitola 1.1.2 Farmakologické modely ALF) (Tab. 5).

Z preventivního hlediska lze hepatotoxické látky, a tedy i zvířecí modely toxického poškození jater, dělit do dvou kategorii: (i.) skutečné hepatotoxiny - jejich podání vyvolá poškození jater u všech jedinců, jde především o produkty chemického průmyslu jako tetrachlormethan CCl₄, thioacetamid (TAA), dimethylnitrosamin (DMN) a diethylnitrosamin (DEN) - a (ii.) potenciální hepatotoxiny - vyvolají poškození jater jen u části jedinců, jedná se především o léky, např. chemoterapeutika (Tab. 6). Zvláštní kapitolu tvoří alkoholové poškození jater.

1.2.1 Alkoholové poškození jater

Nadměrná konzumace alkoholu, ať už akutní, či chronická, je závažným sociálním, ekonomickým i zdravotním problémem. Alkoholové postižení jater zahrnuje stavy od poměrně nezávažné steatózy až po cirhózu jater s možnosti vzniku hepatocelulárního karcinomu.

Alkoho lové po škození jater se nejčastěji modeluje na myších a potkanech. Nej rozšířenějším modelem je chronické podávání alkoholu (Líeber- DeCarli liquid diet;LD diet), chronická intragastrickáaplikace alkoho- lu nebo kombinace podávání alkoholu se specifickou dietou (high-fat diet), s hepatotoxickýmilátkami nebo u geneticky modifikovanýchmyší [25].

1.3 Modely imitující specifická jaterní onemocnění

Společným znakem většiny chronických jaterních onemocnění je jejich vliv na histologickou stavbu jaterního parenchymu se vznikem jaterní fibrózy až cirhózy. Etio logic ké a patogenetické mechanismy zodpovědné za tyto změny se ovšem u jednotlivých specifických jaterníchonemocnění liší a často je ani neznáme.Vytvoření zvířecích modelú je tak kompliko- váno nejen neznalostí etiopatogenezeonemocnění, ale také tím, že některé choroby se vy skytují pouze u lidí (virové hepatitidy). Prozatím se nedaří modely, které by měly všechny atributy lidského onemocnění, vytvořit (Tab. 7). Navzdory tomu jsou zvířecí modely specifických jaterních onemocnění ideálním nástro-

jem **k** pochopení vzniku a progrese CLO a k nalezení jejichúčinné léčby.

1.3.1 Modely autoimunitní hepatitidy (autoimmune hepatitis AIH)

AIH je chronické zánětlivé onemocnění jater neznámé etiologie charakterizované přítomností autoprotilátek, histopatologickými změnami v játrech a klinickými příznaky vyplývajícími z jaterního poškození - únava, letargie, méně často ikterus. Charakteristickým diagnostickým klinickým znakem AIH je rychlá odpověď na imunosupresivní léčbu, která se vyskytuje asi u 90 % pacientů. Léčba AIH je založena na imunosupresivní terapii.

Zvířecí modely AIH lze vytvořit několika způsoby. V období před možností genetické manipulace modelových organismů se AIH indukovala aplikaci jaterních antigenů neboro rostlinného lektinu concavalinu A, který nespecificky aktivuje *T-lymfocyty* a vede k poškození jater. Velkou skupinu modelů **AIH** ovšem představují geneticky modifikované myší modely [26] (Tab. 7).

1.3.2 Primární biliární cirhóza (primary biliary cirrhosis PBC)

PBC je chronické cholestatické onemocnění postihující malé a střední intrahepatální žlučovody a objevující se nejčastěji u žen středního věku (v pátéa šestédekádě života). Klasická forma PBC je spojena s přítomností antimitochondriálních protilátek v séru [27]. Aspekty ovlivňující etiologii ovšem zatím zůstávají neznámé a studie se zaměřují je nejen na vnitřní, ale i environmentální *faktory* v rozvoji onemocnění [28]. Do současné doby bylo ke studiu etiopatogeneze a interakce možných genetických a environmentálních faktorů vytvořeno množství zvířecích modelů PBC (myši) [29]. PBC u těchto modelů vzniká buď spontánně u geneticky modifikovaných myší [30- 3,2] nebo je indukována působením environmentálních faktorů na zvíře, jako je expozice xenobiotikům (např. 2-octynoic acid (2-0A) [32]) nebo působení infekční agens [33].

13.3 Primární sklerozující cholangitida (primary sclerosing cholangitis PSC)

Primární sklerozující cholangitida (primary sclerosing cholangitis PSC) patří mezi další chronické zánětlivé onemocnění jater postihující intra- a extrahepatické žlučové cesty a pomalu může progredovat k biliární cirhóze a jaternímu selhání. PSC se často vyskytuje u pacientů) s nespecifickým střevním zánětem, zejména s ulcerózní kolitidou [34]. Klinické a laboratorní nálezy jako T-lymfocytární infiltrace v okolí cílových žlučovodů, přítomnost autoprotilátek pANCA (perinukleární autoprotilátky proti cytoplasmě neutrofilů, perinuclear antineutrophil cytoplasmatic antibodies) a spojitost s určitými HLA haplotypy a dalšími autoimunitními chorobami (UC) svědčí o autoimunitně zprostředkovaném mechanismu vzniku a progrese onemocnění. Ovšem v porovnání s jinými autoimunitními chorobami PSC špatně reaguje na imunosupresivní léčbu, vyskytuje se častěji u mužů) (2- 3:1) a je zde jen slabá korelace mezi hladinou sérových autoprotilátek s klinickými i a laboratorními nálezy.

Pollheimer a Fickert [35] rozdělují zvířecí modely PSC do několika skupin dle mechanismu indukce onemocně-

ní. Žádný z modelů ovšem ještě nezodpověděl všechny otázky týkající se epatogeneze a progrese onemocnění.

1.3.4 Nealkoholická steatóza jater (non-ak oholic fatty liver disease NAFLD) a nealkoholická steatohepatitida (non-ako holic steatohepatitis NASH)

NAFLD je v současné době nejčastějším chronickým progresivním jaterním onemocněním v rozvinutém světě jehož prevalence se odhaduje až na 30% populace. Ve většině případů) je NAFLD úzce spojena s nadváhou a obezitou a s nimi spojenými metabolickými projevy, a to především inzulinovou rezistencí. Pokud jsou v játrech současně přítomny steatóza, zánět a poškození hepatocyt) (balonovádegenerace, ballooning), hovoříme o NASH, která může být doprovázena progresivní fibrózou a je spojena s vyšším rizikem vzniku hepatocelulámího karcinomu.

Nejlépe popsanými a nejčastěji používanými modely NAFLD jsou tzv. dietary models kde je navozeno jaterní poškození změnami ve stravě modelového organismu. Rózné typy diet jsou pro zvířecí modely NASH klíčové. Představují buď vlastní příčinu NASH, nebo slouží jako spouštěče jaterního poškození u geneticky modifikovaných modelů. Diety jsou založeny buď na kaloricky bohaté stravě- fruktózová dieta, high-fat diet (HFD)- nebo na deficitu určitých živin podílejících se na správném metabolismu tuků - methionine and choline deficient diet (MCD diet), choline-deficient L-amino acid-defined diet (CDAA diet). Z geneticky modifiko vaných model) se nejčastěji uplatňují modely myší s diabetem [36,37].

1.3.5 Modely virové hepatitidy

Studium virové hepatitidy B a C bylo dlouho omezeno na *in vitro* podmínky nebo na studie probíhající na jediném zvířeti, které je k infekci těmito hepatotropními viry vnímavé - na šimpanzovi [38,39]. Šimpanzí model hrál klíčovou roli při studiu interakce hostitel-vir a bylo na něm možno testovat první antivirové strategie v léčbě infekce. Přelomovým objevem bylo objevení buněčných receptorů pro HBV a HCV, díky nimž bylo možné vytvořit buněčné linie pro studium životního cyklu a jednotlivých virových komponent *in vitro*. Základních strategií tvorby *in vivo* myších modelů virové infekce je několik. Jednou z nich je exprese virových protein) nebo receptorů pro lidské viry samotnými hepatocyty u transgenních myší [40]. Další možností je vytvoření chimerických myší, a to transplantací lidských hepatocytli imunodeficientním myším [41.42]. Imunodeficientní organismy ovšem nedovolují studium imunitních mechanismó. Z tohoto dóvodu byly vyvinuty imunokompetentní modely (potkan), kterých bylo dosaženo současnou transplantací lidských *kr*vetvo rných kme nových buněk [43,44].

V současnosti máme *tedy* k dispozici množství modelů jak pro preklinické testování antivirové terapie (imunodeficitní), tak pro studium imunitní odpovědi na léčbu (imunokompetentní). Žádný z model) ovšem nesplňuje všechna kritéria pro ideální model, a to zejména přechod do chronicity se vznikem jaterní cirhózy a eventuálně HCC [45,46].

1.4 Invaz ivní mode l jatern í fibrózy

Podvaz *ductushepaticus communis* (bíleduetlig ation BOL) je invazivní metodou vyvoláni sekundární biliární cirhózy. Obstrukce toku žluá způsobuje intrahepa tální cholestázu , proliferaci žlučovodů, zánět v portobiliárních prosto rech a fibrózu a vede ke vzniku sekundární biliární cirhózy a jaternímu selhá ní. BOL se provádí u potkanů, kteří nemají žlučník[47]

1.5 Geneticky modifikované myši jako zvířecí

modely jaterních onemocnění

Geneticky modifikované myši se používají **k** modelová ní jaterní fibrózy kdy vyřazení specifických genů nebo naopak jejich over -exprese umožňuje studium

jejich fibrogenn iho nebo antifibrogenního vlivu exprimovaných faktorů[5].Transgenn í myši se uplatňují i při mode lová ní specifických jaterních onemocnění, jak je uvedeno v předchozích kapitolách.

ZAVER

Snahy o vytvoření zvířecích modelů jaterních ooemocnění probíhají již několik desetiletí s většími a menšími úspěchy o piibližení se průběhu jaterních chorob u lidí. Největší skupinu tvoří malé zvířecí modely reprezentované myšmi a potkany, které se využívají nejčastěji ke studiu etiopatogeneie a progrese onemocnění. Testování nCN)dl terapeutickjdl

přístupů,a to jak lékiJ, tak invazivních metod,**je** ovšem z těchto modelů jenomezeně přeoositelné do humánní mediány.
Na druhou stranu velké zvířecí modely, zektel)dl je často používaným modelem prase, představuij spojuijá ďánek mezi experimentální medicínou a klinickou praxí.Uplatňuij íse zejména při studiu akutního selhání jater (Tab.1),kde jel.<el-

kézvíře je jedním ze zásadních kritérií ideálního modelu,a stále častěji při vývoji nových chirurgick)dl přístupů a invazivních terapeutických metod, jako jsou podpůrné jaterní *systémy.*

Využití anatomické a fyziologické podobnosti jater prase tes játry člověka může být s výhodou využito při přenosu výsled ků experimentá lních výzkumů do humánní klinické medicíny. Vývoj nových velkých zvířecích modelů jate rních one mocnění např. v ob lasti autoimunit ních jaterních onemocnění, jeovšem zatížen řadou nevýhod spojených s prací s velkými zvířaty. Menší počet jedinců

v porovnávaných skupinách ztěžuje statistické vyhodnocení experimentů s možným ovlivněním výsledků značnou interindividuální variab litou. Oproti laborato rním hlodavc ům by ale prasečí modely chronických jaterních onemocnění mohly přinést nové poznatky o mechanismech způsobujících jaterní poškození a umožnit tak přesnější diag nózu i účinnější léčb u našich pacientů.

Seznam zkratek
AIH - a utoimunitní hepatitida, autoimmune

APAP	- acetaminophen
ASH	- alkoholová steato hepa titida(alcoholic steatohepatitis)
ASMA	- autoprotilát ky proti hladkým svalo vým buňkám, a nti-smooth muscle a ntibod ies
BAL	- bioa rtificial liver
BOL	- podvaz *ductus hepaticuscommuni,s* bíle duetligation
CASH	- steatohepatitida spojená s chemoterap ií, chemotherapy-associatedsteatohepatitis
(DAA diet	- choline-defícíentL-a minoacicklefined diet
CCI,	- tetrach lo rmethan
CLO	- chronická jaterní onemocnění,chronicliver disease
DEN	- d iethylnitrosamin
DNM	- dimethylnitrosamin
GVHD	- reakce štěpu proti hostiteli,g raft versus host disease
HBV	- virus hepatitidy B, hepatitis **B** virus
HCC	- he patocelulární karcinom,hepatocellular **carcinoma**
HCV	- virus hepatitidy C, hepatitisCvirus
HFD	- d ieta boha tá na tuky, high-fat diet
MCDdiet	- methionineand cholin deficiencyd iet
MSC	- mezenchymální kmenové buňky, mese nchymal stemcells
NAFLD	- nealkoholická steatóza jater, non-alcoh ol ic fatty liver disease
NAPQI	- N-acety l parabenzoquinoneimine
NASH	- nealkoholická steatohepatitida, non-alcoholic steatohe patitis
NO	- oxid d usnatý, nitríc oxide
OLT	- ortotop ická transp lantacejater, ortho topic live rtransplantation
pANCA	- perinukleá rní autoprotilá tky proti cytoplasmě neutrofilů, perinuclea r a ntineutrophilcytoplasmaticantibodies
PBC	- primární biliární cirhóza, primary biliary cirrhosis
PSC	- primární sklerozující cho langitida, primary sclerosing cholang itis
PI/A	- a rterializaceportálnívé ny,porta!vein a rterialization
TAA	- thioacetamid

hepatitis
ALD - a kutní jaterní onemocně ní, acuteliverd isease ALF - akutní selhá ní jater, acuteliver failure
AMA - antmitochondriální protilátky, anti- mitoch ondrial a ntibod ies
ANA - antinukleární protilátky, antinuclear antibodies

UC   - ulcerózní kolitida , ulcerativecolitis

Konflikt zájmů
Autoři článku prohlašují, že nejsou v souvislosti se vzni- kemtohotočlánku vestřetuzájmůaže tentočlánek nebyl publikován v žádném jinémčasopise

LITERATURA

1. Van Oe Kerkhove MP, Hoek.stra R, Van Gulik TM, et al. Large animal models of fulminant hepatic failure in artificial and bioartificial liver sup port research . Biomaterials 2004;25:1613- 25.

2. Rahman TM, Hodg son HI. Animal models of acute hepatic failure. Iot J Exp Pathol 2000;81:145-57.

3. Newsome PN, Plevris JN, Nelson u. et al Animal models of fulminant hepatic failure: acritical evaluation. Li ver Transpl 2000;6:21-31.

4. Uu Y, Meyer C, Xu C, el al Animal models of chronic liver diseases. AJP Gastrointest. Liver Physio l 2013;304:G449-68.

S. Starkel P, Leclercq 1A Animal models for the study of hepatic fibrosis. Best Pract Res ClinGastroenterol 2011;25:319-33.

6. P lek R, Li!l<a V, Tie!l<a V, et al.Sinusoida! obstructionsyndromeinducedbymonocrotaline in a large animal experiment - a pilot study. Rozhl Chir 2018;97:214-21.

7. Fan YD, Praet M, van Huysse J, et al Effectsofportalveinarterializationonliver regeneration after partial hepatectomy in therat. Uver Transplant 2002;8 1 52.

8. ZuUo A, Cannist@M, (a vallari G, et al liver regenerationinducedby extracorporeal portal veinarterialization a swi"ne model of carbon tetrachloride intoxicatioo. TransplantProc 201S;47:21HS.

9. Hughes RO, Mitry RR. Ohawan A. Current status of hepatocyte transplantation. Transplantation 2012;93:342-7.

10. Laleman W, Mime, A, Evenepoel P, et al Review article: No biologic.al l iver support in liver failure. Aliment Pharmacol Ther 2006;23:3S1-6 3.

11. Ryska O, Pantoftllek T, Usz ikova E, et al. Soufasný význam bio logkkých a nebiologických eliminalnJch metod v lé&ě akutníhoselhttnJjater. Rozhl Chir 2008·87:291-6

12. LosM-R,Pa}'en O. Mechanisms of liver damage. SeminUver Dis 1996;16:357-67.

13. Terblanche J, Hickman R Animal models of fulminant hepatic failure. Oig Ois Sci 1991;36:770-4

14. LadurnerR.Hochleitner B,Schneeberger S, et al. Extended liver resection and hepaticischemia in pigs: A new, potentially reversible model to induce acute liver failure and study artificial liver support systems. EurSurgRes2005;37:365-9.

1S. Sose! MN, Van Gulik Total hepatec- tomy modelin pigs:Revised method for vascular reconstruction using a rigidvascular prosthesis.Eur SurgRes 2004;36:8-12.

16. Thiel C, Thiel K. Elspue ler A, el al Standard ized intensive care unit management in an anhepatic pig model: new standardsfor analyzing liver supportsystems.Crit Care 2010;14:RI 38.

17. NieuwoudtM, Kunnike R.SmutsM,etal Standardization criteria for an ischemie surgic.al model ofacutehepaticfailurein pigs.Bio materials 2006;27:3836-4 S.

18. Lee KU, Zheng L. Cho YB, et al An experimental animal model of fulminant hepatic failure in pigs. J Korean Med Sci 2005;20:427-32.

19. Saracyn M.Hepatoprotective effect of ni. trieoxidein experimental model ofacute hepatic failure. Workt J Gastroenterol 2014;20:17407.

20. Carvalho NR,. Tassi CC, Oobraschinski F, et al. Rewrsal of lbioenergeticsdysfunction by diphenyt diselenide is critical to protection ag;ainst the acetamin phenindocedacute liver failure. Ufe Sci 2017;180:42-50

21. Zhang S, Zhu Z, Wang Y, et al Therapeutic potential of Barna miniature pig ad pose stem cells illduced hepatocytes in a mouse model with acute liver failure. Cytotechnology 2018;701:131-4 1.

22. Kalpana K, Ong H5, Soo KC, et a1. An improve<in ode l of ga lactosamine-induceo fulminanthepaticifailurein the pig.JSur g Res1999;82:121-30.

23. Thiel C, Thiel K, Ets pueler A, et al. A reproducible porcine model of acute liver failure induced by intrajE!juna I acetaminophen administration. Eur SurgRes 2011;46:118-26.

24. NayakNC, Chopra P, Ohar A, et al. O verse mechanism s of hepatoceUular in juries due to cíhemicals: evidence in rats administered carbon tetrachloride or dimethytnitrosanine. Br J Exp Pathol 1975;56:103-12 .

25. Ghosh Oastidar S, Wamer J, Warner O, et al. Rodent models of alcoholk liver disease: Role of binge ethanol administratioo . Biomolecules 2018;8&3.

26. Christen U, Holdener M, Hintermann E. Animalmodelsof autoimmune hepatitis. AutoimmunRev 2007;6:306-11.

27. Oertelt S, Rieger R,Selmi C, et al A sensitive bead assay for ant imitochondrial antibóáaes:Chipp ing awayat AMA-negativeprimary biliary cirrhosis.Hepatology 2007;4S:6S9-6.S

28. Tanaka A, Leung PSC, Young HA, et a1. Towardsolving the etiologicalmysteryof primarybiliarycholangiti s..Hepatol Co mun 2017;1:275-87.

29. Katsumi T.Tomita K, Leung PSC, et al An imal models of primary biliary cirrhosis. Oin Rev AllergyImmunol 2015;4:8142-53.

30. Koarada S, Wu Y, Fertig N, et al. Genetic control of autoimmunity: Protection from diabetes, blit spontaneous aut im muliary d'isease in a oononobese diabetic congenic strain. J ImmunoL 2004;173:231S-23.

31. Oertelt S, lian Z X. Cheng C-M et al. Anti-mitochondrial antibodies and pr mary biliary cirrhosis in TGF- receptor U dominant-negat ive mice. J Immunol 2006;177:16SS-6.0

32. wakabayashiK,LianZ-X.Mor okiY,et al. I1-2 receptora–/– miceandthedevelop. ment of primary biliary drrhosis. Hepatology 2006;44;1240-9,

33. wangJJ,Y ng GX,Z hangWC,eta l. Escherichia coli infection inducesautoimmune cho&angitis and anti-mitochondrial ant bodies in oon-0bese diabetic (NOO).B6 (lddl0/ldd18) mice. Clin Exp lmmunol 2014;175:192-201.

34. Fausa O, Schrumpf E, Elgjo K. Relatiooship of inflammatory bowel disease and primary sc:lerosi119 cho langit is. Semin U.,,, Dis 1991;11:31-9.

35. Pollheimer MJ, Fickert P. Animal models in primary biliary cirrhosis and primary scterosing cholang itis. Oin Rev AUergy lmmund 2015;48:207-17.

36. Santhelcadur PK, Kumar DP, Sanyal AJ. Preclinical models: of non-alcoholic fatty liverdisease.J Hepatol 2018;68:230-7.

37. van Herek MA, Vooghia L. FrancqueSM. Animal modelsof nonalcoholic fatty liver disease-a starter's guide. Nutrients 2017;9:1-13.

38. BukhJ. A critic.al role for the chimpanzee model in the study of hepatitis C Hepatology 2004;391:469-7S.

39. 'Meland SF. The ch im panzee model for hepatitis B virus infection. Cold Spring Hart>.P<!rspect Med 2015;5:1-19.

40. Moriya K. Fujie H, Shintani Y,et al The core protein of hepat is C virus induces hepatocelllar carciooma in transgenk mice. Nat Med 1998;41065-7.

41. Dandri M, Burda MR, TOrok E, et al Repopulation of mouseliver withhuman hepatocytesandinv ivoinfectionwithhepot isBvirus.Hepato logy2001;33:981-8.

42. Bissig K-0, Wieland SF, TranP, et al. Humanlĩver chimeric miceprovidea model for hepatitis e and C virus infection and treatment.JClinlnvest2010120:92430.

43. Washbum ML. Bilily MT, Zhang L, et a1. A humanized mouse model to study hepatitis C virus infection, immune response,and liver disease. Gastroenterology2011;140:134-44.

44. Billerbeck E, Mommersteeg MC, Shlomai A, et al Humarized mice efficiently engrafted with fetal hepatoblasts and syngeneic immune cells develop human monocyt.es and NK ceUs.J Hepatol 2016;6S:334-4

45. Vercauteren K, De Jong VP, Meu lemanP. HCV animal models and liver disease.J Hepatol 2014;61:S26-33.

46. Dandri M.. Petersen J. Animal models of HBVinfection.BestPractResClin Gastroenterol 2017;31:273-9.

47. Abd EI Motteleb DM, Ibrahim IAAEH, Elshazly SM. Sildenafil protects against bile duet ligation induced hepatic fibro- sis in rats: Potential role for silent information regulat0< 1 (SlRTI). Toxicol Appl Pharmacol 2017;3364-71.

48. Michalopolos GK. Liver regeneration after partial hepatectomy. Am J Pathol 2010;176:2-13.

49. Abrahamse SL. Van De Ker1chove MP, Sosef MN,et al. Treatment of acute liver failure in pigs rEduces hepatocyte function in a bioartfficial liversupportsystem. lntJArtifOrgans2002;25:966-74.

S0. LeeJ.H,Lee D-H, Lee S, et al Functional evaluation of a bioartificial liver support system using immobi lized hepatocyte spheroids in a porcine model of acute liver failure. Sci Rep 2017;73804. doi: 10,1038/s41598-017-03424-2.

S1. Lv G, Zhao L. Zhang A et al Bioartificial liversystem based onchoanoid ffuidized bed bioreactor improvethe survival time of fulminanthepatic failurepigs.. Bi o t echnol Bioeng 2011;108:2229-36.

S2. Sang J-F, Shi X-l, Han B, et a l. lntraportal mesenchyma l stem cell transplantation prewntsacuteliverfailurethroughpromoting cell proliferation and inhibiting apoptosis.HepatobiliaryPancreatOis Int 2016;1S:602-11.

S3. He G-L, Feng L. Cai L, et al Artificial liver support in pigs Mlh acetaminopheni-nducedacuteliver failure.World JGastroenterol 2017;23:3262.

54. Saxena R. Practical hepatic pathology:

A diagnostic approach. Elsevier/Saunde", Philadelphia 2011.

SS. Ham id M, Abdulrahim Y, liu O, et aL The hepatoprot ective effect of selenium-enriched yeast and gum Arabic combination on c.arbo n t etrachloride-induced chronic liver injury in rats. J. Food Sci 2018;83:525-34.

56. **Ma** L. Yang X, Wei R, et al. MicroRNA-214 promotes hepatic stellate ceU activation and liver fibrosis by suppressing Sufu expression. Cen Death Dis 2018;9:1-B.

57. Alam **Mf,** Safhi **MM, Arrwe<** T, *et* al Therapeutc potential of Vanillyacetone against CC4 indoced hepatotoxicity by suppressing thE! serum marker, oxidative str&, inflammatory cytokin **M andapop.** tosis in Swiss albino mice Exp Mol Pathol 2018;105:81-8.

58. **Song** YC, Liu YC, Chao PH, et al Combined defivery of sorafenib and a MEK inhibitor using CXC targe,ted nanopartid es reduces hepatic fibrosis and prevents tumor development Theranositcs 2018·8:894-905

59. Czechowsl<a G, éelinski K, Korolczuk A, et al Protective effects of melatonin against

thioacetamide-ind uced liver fibrosis in rats. J Physio l Pharmacol 2015;66:56779.

60. Mazagova M, Wang L. Anfora AT, et a l. Commensal microbiota is hepatoprotective and prevents: liver fibrosis in mice. FASEBJ 2015;29: IOH - 55.

61. . Chandel R, Saxena R, oas A, et a l. Association of rno-miR-183- 182 cluster with diethyinitrosamine indoced liver fibrosis in Wistar rats. J Cell Biochem 2018;119:4072-84.

62. Jilkova ZM, Kuyucu Al., Kurma K, et al. Combination of AKTinhibitor ARQ 092 and sorafenib potentiates inhibition of tumor progression in cirrhotic rat model of hE!patcxe Uular card noma. Oncotarget 2018;911 145-SS.

63. Marrone **AK,** Shp yre vaS, Chappell G, et a l. Oiffaential ty expr;essed microRNAs pr vide mechanistic i nsight into fibrosis-associated liver carei nogenesis in mice. Mol Carcinog 2016;55:808-17.

64. King PO, Perry **MC..** Hepatotoxicity of chemotherapy. Oncologist 2001;6:162- 76'

65. Nakamura K, 1-latano E.. Narita M, et aL Sorafenib attenuates moncxrotaUne-induced sinusoida) obstruction syndrome

in rats through suppression of JNK and **MMP-9.1** He patol 2012;57:1037-4 3.

66. Robinson SM, Mann DA, Manas DM, *et* al The potential contribution of tumour-related factors to the development of FOLFOX-induced sinusoida! obstruction syndrome. Br J Cancer 2013;1@.2396-403.

67. Bruha J, Yycital O, Tonar Z, et al. Monodonal antibody against transforming growth factor beta 1 does not influence liver regeneration after resection. Large Animal Experiments 2015;340:3240.

68. Chen X. Ying X. Sun W, et al. The therapeutic effect of fraxetin on ethand-induced hepatic fibrosis by enhandng ethanol lM!aboíosm, inhibiting oxidatiV<! stress and modulating inflammabry mediatorsin rats. Int Immunopharmacol 2018;56:931-04.

69. Choi Y, Abdelmegeed MA, Song BJ. Preventive effects of indole-3-carbinol against ak ohol-induced liver injury in mice via antioxidant, anti-inftammatory, and anti-apoptotic mechanisms: Role of gu t-liver-adipose tissue axis. J Nutr Bio. chem 2018;5512-25.

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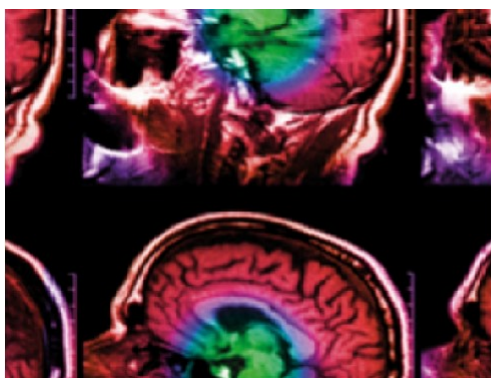*ann a.m* æckov *a@lfp cunia*

Příloha V:

MALEČKOVÁ, Anna, Petra KOCHOVA, Richard PÁLEK, Václav LIŠKA, Patrik **MIK**, Tomasz BOŃKOWSKI, Miroslav HORÁK a Zbyněk TONAR, 2021. Blunt injury of liver – mechanical response of porcine liver in experimental impact test. Physiological Measurement [online]. [vid. 2021-02-24]. ISSN 0967-3334. Dostupné z: doi:10.1088/1361-6579/abdf3c. **IF$_{2020}$=2,833, Q2**(Biomedical, Engineering)**.**

PAPER

Blunt injury of liver: mechanical response of porcine liver in experimental impact test

Physiological Measurement

IPEM Institute of Physics and Engineering in Medicine

CORRIGENDUM

Corrigendum: Blunt injury of liver: mechanical response of porcine liver in experimental impact test (2021 *Physiol. Meas.* 42 025008)

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This corrigendum corrects the wrong description of hepatic lobes in 'Blunt injury of liver—mechanical response of porcine liver in experimental impact test' (Malečková *et al* 2021). Right hepatic lobes were wrongly described as left hepatic lobes and vice versa, namely the right lateral lobe was wrongly described as the left lateral lobe; the

Figure 4. Typical external injury patterns of the porcine liver after the impact test. (A) Laceration of the visceral surface of the liver affecting more than a half of the right lateral lobe, injury grade V. (B) Rupture of the visceral surface of the left medial lobe, that propagates from the hilum along the big vessels and the stromal connective tissue. (C) Peripheral rupture starting at the border of the left lateral lobe. (D) Occasional shallow rupture on the diaphragmatic surface of the liver, most likely caused by the direct impact of the wood plate used in the impact test.

left medial lobe was wrongly described as the right medial lobe. Therefore, the figure caption in figure 4 should be as follows:

Additionally, this error also occurred in Results. The formulation in the section 3.3.1 'Ruptures propagated from the hilum along big vessels and were mostly located in the left lateral (figure 4(A)) and right medial lobes (figure 4(B))' should be changed to 'Ruptures propagated from the hilum along big vessels and were mostly located in the right lateral (figure 4(A)) and left medial lobes (figure 4(B))'.

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Reference

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Physiological Measurement

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PAPER

Blunt injury of liver: mechanical response of porcine liver in experimental impact test

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Abstract

Objective. The liver is frequently injured in blunt abdominal trauma caused by road traffic accidents. The testing of safety performance of vehicles, e.g. belt usage, head support, seat shape, or air bag shape, material, pressure and reaction, could lead to reduction of the injury seriousness. Current trends in safety testing include development of accurate computational human body models (HBMs) based on the anatomical, morphological, and mechanical behavior of tissues under high strain. *Approach.* The aim of this study was to describe the internal pressure changes within porcine liver, the severity of liver injury and the relation between the porcine liver microstructure and rupture propagation in an experimental impact test. Porcine liver specimens ($n = 24$) were uniformly compressed using a drop tower technique and four impact heights (200, 300, 400 and 500 mm; corresponding velocities: 1.72, 2.17, 2.54 and 2.88 m s^{-1}). The changes in intravascular pressure were measured via catheters placed in portal vein and caudate vena cava. The induced injuries were analyzed on the macroscopic level according to AAST grade and AIS severity. Rupture propagation with respect to liver microstructure was analyzed using stereological methods. *Main results.* Macroscopic ruptures affected mostly the interface between connective tissue surrounding big vessels and liver parenchyma. Histological analysis revealed that the ruptures avoided reticular fibers and interlobular septa made of connective tissue on the microscopic level. *Significance.* The present findings can be used for evaluation of HBMs of liver behavior in impact situations.

1. Introduction

Road traffic accidents (RTAs) are one of the main causes of blunt abdominal trauma, which is often accompanied by internal organ injuries, most frequently to the spleen, the liver and the kidney (Elhagediab and Rouhana 1998, Augenstein *et al* 1999, Monchal *et al* 2018). Although soft tissue injuries and bone fractures are the most common types of road traffic injuries, liver trauma is responsible for significant morbidity and mortality (Doklestić *et al* 2015, Vyčítal *et al* 2019). According to the Global Status Report On Road Safety issued by the World Health Organization (2018), vehicle safety is one of the key factors reducing the likelihood of serious injury in RTAs. The importance of both air bag deployment and safety belt restraint in preventing the liver injuries was demonstrated by Holbrook *et al* (2007) in a study that analyzed 311 patients with injuries

suffered in motor vehicle crashes. Therefore, improving safety systems for car occupants became an important tool for the reduction of traffic accident injuries and casualties (Xu *et al* 2018).

Testing the safety performance of newly developed vehicles is usually carried out in two distinct ways: (i) standardized car crash tests using anthropometric testing devices (ATDs) commonly called dummies or (ii) car crash simulations using computational models of ATDs or human body models (HBMs) (Xu *et al* 2018). The current trend in predicting trauma impact on human tissues and organs relies on the development of computational modeling, as only a few large corporations use mechanical dummies for car crash tests (Xu *et al* 2018) and the ATD outputs are only injury criteria based on physically measurable parameters (acceleration, force, compression of chest). The models are based on morphometric data collected from human cadavers (Tropiano *et al* 2004) or imaging modalities such as CT and MRI (Gayzik *et al* 2011), and could be morphed for the vide variety of anthropometries.

The HBMs used for abdominal injury prediction include e.g. the HUman MOdel of Safety, HUMOS (Behr *et al* 2003, Arnoux *et al* 2008), Total Human Model for Safety, THUMS (Golman *et al* 2014), Global Human Body Models Consortium, GHBMC (Schoell *et al* 2015, Schwartz *et al* 2015), Wayne State Human Body Models, WSUHAM (Lee and Yang 2001). Arnoux *et al* (2008) used in their experiment a hybrid approach coupling numerical simulation (HUMOS) with experimental data from free fall tests using human cadaver trunks. They provided a complex analysis of the liver behavior within the abdomen in frontal crash situations and proved the importance of experimental data for computational modeling of inner organ behavior. The study has been followed by several experimental studies on human (Cheynel *et al* 2009, Conte *et al* 2012, Untaroiu *et al* 2015) and porcine liver (Chen *et al* 2018, Chen *et al* 2019) biomechanical behavior since then. The studies were mostly focused on the effect of sudden deceleration and impact, two phenomena that were identified as the main causes of liver injury (King and Yang 1995, Cheynel *et al* 2009). The results of those tests were influenced by parameters such as velocity, mass, size, surface area of the impactors, compression and duration of the applied force (Untaroiu *et al* 2015). However, only a few studies were focused on the relationship between liver injury and internal pressure changes (Sparks *et al* 2007, 2008). That is despite the extensive vascular supply of the liver and the previously described dependence of the liver injury severity on rapid increase in internal fluid pressure (Sparks *et al* 2007, 2008, Sparks and Dupaix 2008).

Experiments conducted on human cadavers or organs are valuable sources of biomechanical data (Kemper *et al* 2010, Conte *et al* 2012). Many previous experiments and most of the current liver models, however, include the use of animal tissue to simulate human organs (Melvin *et al* 1973, Wang *et al* 1992, Tamura *et al* 2002, Santago *et al* 2009, Marchesseau *et al* 2017). Using animal cadavers and livers is therefore a plausible and more feasible approach to obtaining biomechanical data. Pig is particularly convenient as it is widely used in different fields of biomedicine for its anatomical and physiological similarities with humans (Eberlova *et al* 2016, Malečková *et al* 2019, Eberlova *et al* 2020). Nevertheless, the anatomical and microscopic differences between human and porcine liver need to be taken into consideration when interpreting data from animal experiments (Huelke *et al* 1986, Kruepunga *et al* 2019). The porcine liver microstructure has already been described in detail using well-established stereological methods (Junatas *et al* 2017, Mik *et al* 2018). The microstructure is organized into well-defined roughly hexagonal hepatic lobules demarcated by fibrous interlobular septa containing portal triads. The interlobular connective tissue originates from the fibrous capsule and enters the liver via the hilum along big branches of hepatic vessels—the portal vein and hepatic artery. The hepatocytes are arranged within lobules into trabeculae surrounded by a delicate network of reticular fibers that separates the hepatocytes from hepatic sinusoids (see supplement S1 (available online at stacks.iop.org/PMEA/42/025008/mmedia)).

The aim of our study was to describe the biomechanical behavior of the whole porcine liver in an experimental impact test, with special emphasis on the internal pressure changes depending on the impact energy. The experiment was designed to enable us to study the effect of impact alone with no influence of deceleration. The severity of liver damage on the macroscopic level was evaluated using the American Association for the Surgery of Trauma (AAST) organ injury scaling and Abbreviated Injury Scale (AIS severity), 2018 update (Kozar *et al* 2018). As the mechanical properties of the soft tissue organs depend on their microscopic structure, we wanted to specifically focus on the relationship between rupture propagation and tissue microstructure, namely on the main fibrillary components of the hepatic interstitial connective tissue matrix. Hence, our biomechanical data were accompanied by histological quantitative analysis. Our aim was also to provide complete primary biomechanical data and make them publicly available for biomechanical modeling of liver crash tests.

2. Methods

2.1. Liver sample preparation

Liver samples were obtained from Prestice Black-Pied pigs (Vrtkova 2015) ($n = 24$). The animals received standard care in compliance with the European Convention on Animal Care, and the project no. MSMT-4428/2018-2 was

approved by the Faculty Committee for the Prevention of Cruelty to Animals. The piglets were pre-medicated by intramuscular administration of 10 mg kg^{-1} ketamine (Narkamon; Spofa, A.S., Prague, Czech Republic), 5 mg kg^{-1} azaperon (Stresnil; Jannssen Pharmaceutica NV, Beerse, Belgium) and 1 mg atropine (Atropin Biotika; Hoechst Biotika, Martin, Slovak Republic). For general anaesthesia propofol (1% mixture 5–10 mg^{-1} kg^{-1} h^{-1} propofol; Fresenius Kabi Norges AS, Halden, Norway) was administered intravenously. Continuous analgesia was provided by fentanyl (1–2 μg^{-1} kg^{-1} h^{-1} Fentanyl Torre; Chiesi CZ s.r.o, Prague, Czech Republic). Airways were secured by endotracheal intubation and pigs were mechanically ventilated. The piglets received infusion and volume substitution when necessary (Hartmann's Solution; B.Braun Melsungen AG, Melsungen, Germany and Plasmalyte; Baxter Healthcare Ltd, Compton, UK).

Midline laparotomy was performed to enter the abdominal cavity. All the ligaments attaching the liver to the surrounding structures were transected, the common bile duct was ligated and divided, and the portal vein, hepatic artery and the infrahepatic part of the caudate vena cava (equivalent to the inferior vena cava in human anatomy) were dissected. The thoracic cavity was opened to gain access to the thoracic part of the caudate vena cava. The diaphragm was transected in the ventrodorsal direction leaving a small rim around the caudate vena cava. The animal was then heparinized (10 000 IU of heparin; Zentiva, k.s., Prague, Czech Republic) and the portal vein and the thoracic part of the caudate vena cava were cannulated by infusion tubes. These tubes were later used for measuring intravascular pressure in the liver. Finally, all the vessels supplying and draining the liver were ligated and divided and the whole organ, filled with blood, was removed from the body. The animals were sacrificed under general anesthesia via an intravenous administration of a cardioplegic solution (potassium chloride).

2.2. Impact test

Prior to the mechanical experiment, all the specimens were checked for the following conditions that might have been caused by specimen handling: contusions, ruptures and bleeding. Damaged specimens were excluded from further analysis. In total, 24 livers were used for the mechanical experiment. The livers were divided into four groups (six livers per group) based on the impact height (200, 300, 400 and 500 mm and corresponding impact velocity 1.72, 2.17, 2.54 and 2.88 m s^{-1}, respectively). The specimens were kept cool on ice until the mechanical impact test. The maximum time between surgery and impact test was three hours. Firstly, each liver was pressurized through the infusion tubes inserted in the portal vein and caudate vena cava at 7 cm H$_2$O by 0.9% NaCl solution. Maintaining the turgor of the liver at a level comparable to normal hemodynamic pressures was necessary for simulation of impact injuries (Melvin *et al* 1973). Then the size and weight of the pressurized liver was measured. After that, the livers were put into the measurement device—a drop tower (Sparks *et al* 2008). The livers were uniformly compressed using an impact 6.22 kg plate made of wood with impact area of 29.5 cm × 29.5 cm. The plate was firmly connected to an aluminum beam, which could freely move along the aluminum frame. We neglected the beam friction to simulate the plate free fall by the use of Teflon sliders between the beam and the frame. The plate was covered by a plastic foil and spread with tempera color to visualize the contact area between the plate and the liver.

The pressure was recorded at the portal vein and the caudate vena cava during the impact test using a Silicon Pressure Sensor MPXV5100DP (GND +5V) (NXP Semiconductors N.V., Eindhoven, Netherlands). The sensor was connected to the external opening of the infusion tube, while the intravascular opening was placed in the blood vessels, in a way similar to the setting previously proposed by Sparks *et al* (2008).

Maximal pressure values were used for further quantitative analysis. The position of the impact plate before the impact was measured by two lasers optoNCDT 2300-50 (Micro-Epsilon GmbH, Frankfurt am Main, Germany) on each side of the impacting beam. The velocity of the impact was derived from the position measurement and then averaged from both lasers. Measured signals were acquired by the NI CompactDAQ (National Instruments, Austin, TX, USA) system with SignalExpress software (National Instruments, Austin, TX, USA). The system energy was determined by two formulas:

$$E_K = \frac{1}{2}mv^2$$

where m was the weight of the impact plate and v was the velocity of the impact plate before impact, and

$$E_P = mgh$$

where m was the weight of the impact plate, g was acceleration of gravity, and h was the height of the impact given as impact height minus the liver thickness. The loading area was calculated from the colored part of the liver using Ellipse software (ViDiTo, Košice, Slovak Republic). The impact energy was then calculated as the energy of the plate divided by the loaded area. The loaded area in percentage was calculated as the colored part of liver divided by the total liver area. Both parameters were estimated with Ellipse software using the 'POLYGON' tool in calibrated photographs.

2.3. Macroscopic injury analysis

Immediately after the impact test, ruptures on the surface of the liver were captured by camera and the projection length of the ruptures was measured. The position of the ruptures with respect to the liver lobes was registered to determine the most frequently damaged part of the liver. After that, each liver was cut completely into 1 cm thick slices and the depth of the ruptures and the depth of the contused parenchyma were measured. The depth was defined as the distance between the liver surface and the deepest point of the damaged parenchyma perpendicular to the liver surface. Both the projection length and the depth of the ruptures were measured in calibrated photographs using the Ellipse software 'LINE' tool (ViDiTo, Kosice, Slovak Republic). The injury grade and AIS severity were then determined according to liver injury scaling (Moore *et al* 1989, Kozar *et al* 2018).

2.4. Microscopic injury analysis

For the histological analysis, every fourth tissue slice was selected using systematic uniform random sampling. The first slice was selected randomly using the RANDBETWEEN function in Microsoft Office Excel. The selected slices were trimmed into tissue blocks with dimensions $1 \times 1 \times 1$ cm. Every eighth tissue block was then processed histologically. Each sample was carefully examined for liver abnormalities or signs of liver damage, especially inflammation and liver fibrosis. For the quantitative analysis of the ruptures we selected tissue samples with a macroscopically visible crack, 27 tissue blocks in total. The tissue samples were fixed in 4% buffered formalin, dehydrated and embedded in paraffin blocks. Each selected tissue block was cut into 5 μm thick sections. The section plane was perpendicular to the liver capsule. Sections were stained with hematoxylin–eosin (HE) and with Reticulin kit (BioGnost Ltd, Zagreb, Croatia) in order to visualize reticular fibers (type III collagen). Reticular fibers are located within the interlobular septa. They also represent the essential part of a delicate network, that separates the hepatic sinusoids and hepatocytes within the hepatic lobules (supplement S1). The results were based on 189 sections.

To determine the spatial relationship of ruptures and reticular fibers, we used a method previously published by Tonar *et al* (2009) and Kubíková *et al* (2017). Briefly, an isotropic stereological grid with known geometrical parameters was superimposed over the microphotographs. The number of intersections between this grid and reticular fibers was then counted. We compared the estimated value of the intersection intensity of reticular fibers along the rupture $P'_{L(\text{ret.})}$ with the theoretical value $P_{L(\text{ret.})}$ that should be expected in the case of random propagation of the microcracks throughout the liver parenchyma. The values were calculated with the assumption of independence of the ruptures on the reticular fibers.

The theoretical intersection intensity $P_{L(\text{ret.})}$ was calculated as the intersection intensity of a randomly oriented line (i.e. the rupture) with the reticular fibers (Stoyan *et al* 1995). It was defined as follows:

$$P_{L\,(\text{ret.})} = \frac{2}{p} L_{A\,(\text{ret.})}$$

where $L_{A(\text{ret.})}$ was the 2D length density of reticular fibers within liver. For each analyzed sample the length density L_A was assessed individually as follows:

$$L_A = \frac{L}{A}$$

where L is the length of the reticular fibers and A is the area of the reference space. L was estimated by counting the intersections of circular arcs randomly positioned on the microphotographs with reticular fibers. A was estimated using the point grid.

The intersection intensity of reticular fibers $P'_{L(\text{ret.})}$ was defined as follows:
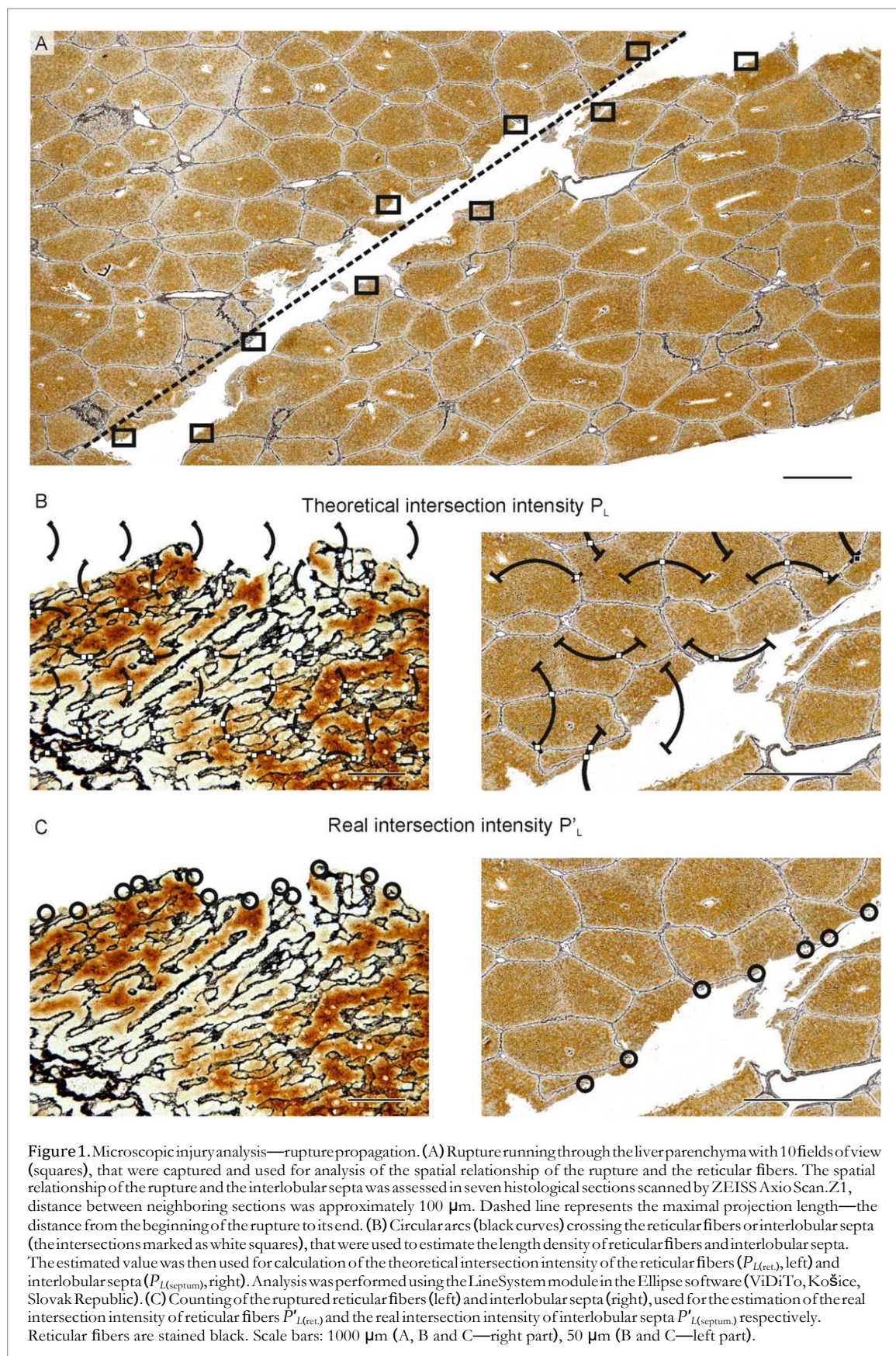
$$P\prime_{L(\text{ret.})} = \frac{p(\text{ret.})}{l(\text{ret.})}$$

where $p_{(\text{ret.})}$ is the real number of the intersections counted in the histological sections along the rupture of the length l. The length $l_{(\text{ret.})}$ was defined as the shortest distance between the beginning and the end of the crack, the so-called maximal projection length. To estimate all the parameters, we used Ellipse software (ViDiTo, Košice, Slovak Republic) (figure 1).

The same analysis was performed to determine the spatial relationship of ruptures and the interlobular septa. We compared the estimated value of the intersection intensity of interlobular septa along the rupture $P'_{L(\text{septum})}$ with its theoretical value $P_{L(\text{septum})}$.

If the actual propagation of the ruptures throughout the liver parenchyma were at random, there would be no significant difference between the real intersection intensity and the theoretical intersection intensity ($P'_L = P_L$). Therefore, a significant difference between the theoretical value of intersection intensity P_L and the observed intersection intensity P'_L would indicate that the rupture ran predominantly across the reticular fibers

or interlobular septa $(P'_L > P_L)$ or along the reticular fibers or interlobular septa $(P'_L < P_L)$.

Figure 1. Microscopic injury analysis—rupture propagation. (A) Rupture running through the liver parenchyma with 10 fields of view (squares), that were captured and used for analysis of the spatial relationship of the rupture and the reticular fibers. The spatial relationship of the rupture and the interlobular septa was assessed in seven histological sections scanned by ZEISS Axio Scan.Z1, distance between neighboring sections was approximately 100 μm. Dashed line represents the maximal projection length—the distance from the beginning of the rupture to its end. (B) Circular arcs (black curves) crossing the reticular fibers or interlobular septa (the intersections marked as white squares), that were used to estimate the length density of reticular fibers and interlobular septa. The estimated value was then used for calculation of the theoretical intersection intensity of the reticular fibers ($P_{L(\text{ret.})}$, left) and interlobular septa ($P_{L(\text{septum})}$, right). Analysis was performed using the LineSystem module in the Ellipse software (ViDiTo, Košice, Slovak Republic). (C) Counting of the ruptured reticular fibers (left) and interlobular septa (right), used for the estimation of the real intersection intensity of reticular fibers $P'_{L(\text{ret.})}$ and the real intersection intensity of interlobular septa $P'_{L(\text{septum.})}$ respectively. Reticular fibers are stained black. Scale bars: 1000 μm (A, B and C—right part), 50 μm (B and C—left part).

2.5. Statistical analysis

The data were processed using Statistica Base 10 (StatSoft, Inc., Tulsa, OK, USA). The normality of the data was tested using the Shapiro–Wilk W-test. All the statistical tests were non-parametric methods, because some data sets did not pass the normality test. To assess the differences among the impact groups, we used the Kruskal–Wallis

Table 1. Morphometric data on pigs and livers.

Impact height (mm)/ impact velocity (m s^{-1})	Sex	Age (weeks)	Pig weight (kg)	Liver weight (kg)	Liver width (mm)	Liver height (mm)	Liver depth (mm)
200/1.74	Female	14	30	1.570	300	216	33
200/1.70	Female	16	25	1.328	278	208	50
200/1.72	Female	16	35	1.636	299	224	47
200/1.74	Male	14	27	1.472	274	241	35
200/1.70	Male	14	24	1.192	267	222	40
200/1.71	Male	14	24.5	1.232	296	216	38
300/2.15	Male	17	28.5	1.336	264	219	47
300/2.16	Male	15	27.2	1.278	298	285	45
300/2.20	Female	15	29.6	1.348	288	240	33
300/2.17	Male	15	36	1.394	280	250	45
300/2.20	Male	16	34.2	1.112	283	208	33
300/2.14	Female	16	37.8	1.400	283	220	37
400/2.52	Female	17	42.8	1.446	273	224	43
400/2.56	Male	15	28.5	1.054	247	226	41
400/2.50	Male	16	33	1.252	257	232	39
400/2.62	Male	16	37	1.650	315	245	40
400/2.51	Male	16	41.5	2.044	287	265	55
400/2.50	Female	16	39.4	1.492	265	275	38
500/2.89	Male	15	36.5	1.030	240	194	37
500/2.92	Male	15	28	1.070	256	184	35
500/2.85	Male	15	25.5	1.108	246	198	40
500/2.84	Male	15	25	1.060	244	191	43
500/2.95	Male	16	37.7	1.572	307	248	40
500/2.86	Male	16	35	1.700	283	235	55

ANOVA test. To compare the paired values of real intersection intensity (P'_L) and theoretical intersection intensity (P_L), we used the Wilcoxon signed-rank test. The level of significance was 0.05.

3. Results

3.1. Liver samples

The livers were collected from 24 Prestice Black-Pied pigs aged 14–17 weeks and weighing 24–42.8 kg (31.9 ± 5.6 kg, mean ± standard deviation [SD]). Seventeen piglets were neutered male and seven female. The fresh liver weight ranged from 1.030 to 2.044 kg (1.364 ± 0.24 kg, mean ± standard deviation [SD]) (table 1). None of the specimens had signs of microscopic injury such as inflammatory infiltration or fibrosis.

3.2. Impact test

3.2.1. Greater impact velocity (heights) resulted in increased maximal intravascular pressure accompanied by more severe injury.
The impact plate hit less than a half or about a half of the liver in all cases, figure 2(A). There was an increasing trend of maximal pressure related to the impact velocity, but it did not reach the level of significance, figure 2(B). The impact energies (given by liver height and by plate position measured by laser, figure 2(C)) increased with impact velocity. The higher impact velocity led to more severe injury, figure 2(D, E). The linear regression analysis of the AIS severity and injury grade is shown in figure 2(F). These two parameters are interchangeable. The regression analysis of AIS severity and maximal pressure, and impact energy is shown in figure 3(A), and 3(B), respectively. The results are summarized in table 2 and shown in figure 2. Complete primary biomechanical data are available as supplement S2.

3.3. Macroscopic injury analysis

3.3.1. Ruptures affected mostly the interface between connective tissue surrounding big vessels and liver parenchyma
The major injuries affected the visceral surface of the liver as shown in figure 4. Ruptures propagated from the hilum along big vessels and were mostly located in the left lateral (figure 4(A)) and right medial lobes (figure 4(B)). The depth of the lacerations and contused parenchyma differed among experimental groups as well as among livers within the groups. The injury grade was based both on the length and on the depth of the ruptures. Most severe injuries were found in the group with impact velocity 2.88 m s^{-1} (impact height 500 mm)
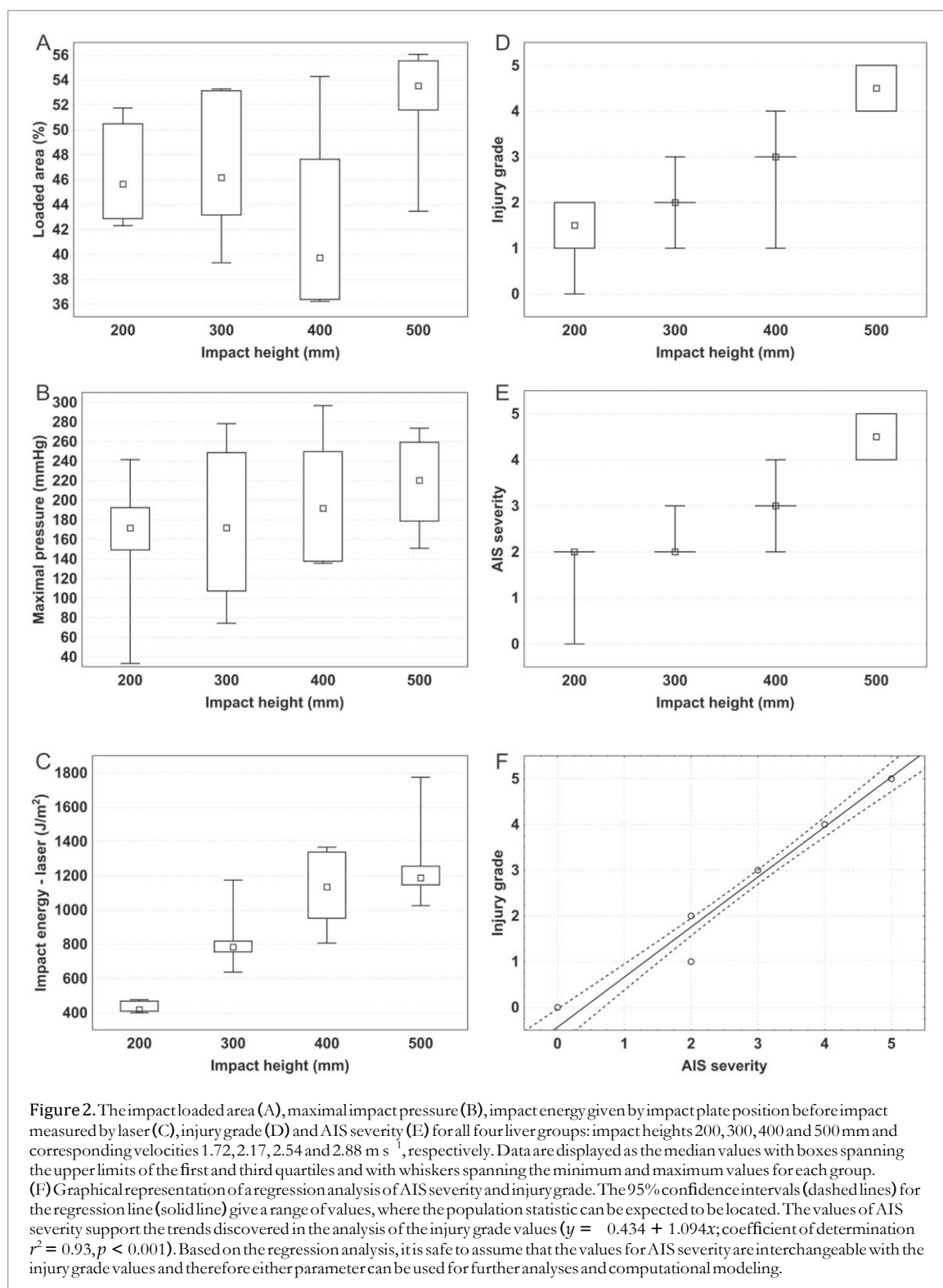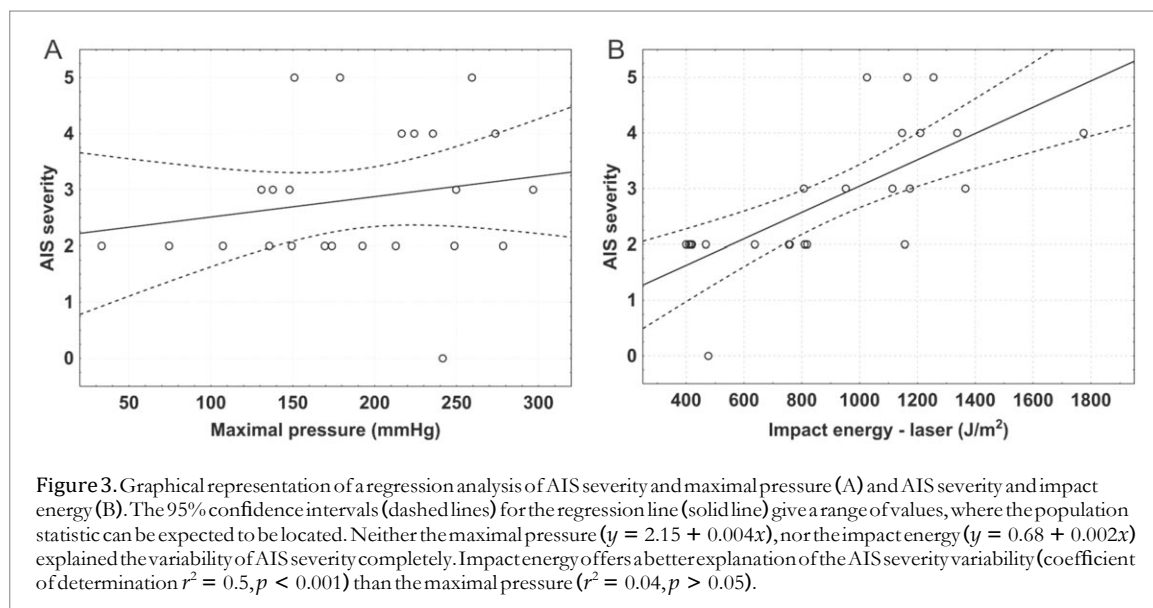
Figure 2. The impact loaded area (A), maximal impact pressure (B), impact energy given by impact plate position before impact measured by laser (C), injury grade (D) and AIS severity (E) for all four liver groups: impact heights 200, 300, 400 and 500 mm and corresponding velocities 1.72, 2.17, 2.54 and 2.88 m s^{-1}, respectively. Data are displayed as the median values with boxes spanning the upper limits of the first and third quartiles and with whiskers spanning the minimum and maximum values for each group. (F) Graphical representation of a regression analysis of AIS severity and injury grade. The 95% confidence intervals (dashed lines) for the regression line (solid line) give a range of values, where the population statistic can be expected to be located. The values of AIS severity support the trends discovered in the analysis of the injury grade values ($y = 0.434 + 1.094x$; coefficient of determination $r^2 = 0.93$, $p < 0.001$). Based on the regression analysis, it is safe to assume that the values for AIS severity are interchangeable with the injury grade values and therefore either parameter can be used for further analyses and computational modeling.

(table 2). In some cases, the ruptures were located on the periphery of the lobe (figure 4(C)) and, occasionally, we found small lacerations on the diaphragmatic surface of the liver (figure 4(D)).

3.4. Microscopic injury analysis

3.4.1. *The ruptures avoided reticular fibers and interlobular septa on the microscopic level*
The real intersection intensity P'_L was smaller than the theoretical intersection intensity P_L for both the reticular fibers and for the interlobular septa (Wilcoxon signed-rank test, $p < 0.001$) (figure 5). The ruptures thus did not

Figure 3. Graphical representation of a regression analysis of AIS severity and maximal pressure (A) and AIS severity and impact energy (B). The 95% confidence intervals (dashed lines) for the regression line (solid line) give a range of values, where the population statistic can be expected to be located. Neither the maximal pressure ($y = 2.15 + 0.004x$), nor the impact energy ($y = 0.68 + 0.002x$) explained the variability of AIS severity completely. Impact energy offers a better explanation of the AIS severity variability (coefficient of determination $r^2 = 0.5, p < 0.001$) than the maximal pressure ($r^2 = 0.04, p > 0.05$).

propagate randomly through the liver parenchyma but predominantly ran along the reticular fibers and interlobular septa avoiding intersections with reticular fibers and interlobular septa.

4. Discussion

This study on porcine liver injury focused on description of the tissue damage and the biomechanical behavior of liver tissue. We combined an impact approach using a drop tower technique, while registering the changes of intravascular pressure in portal vein and caudate vena cava. Our mechanical data focused on the internal fluid pressure changes in impact situations. Even though the liver tissue damage in car crashes is the consequence of both deceleration and impact (Cheynel *et al* 2009), the sole impact approach allowed us to exclude the influence of the deceleration on the biomechanical behavior and to study the effect of impact alone. A similar approach, albeit, by simulating the deceleration injury alone has already been used in studies by Cheynel *et al* (2006, 2009) and by Arnoux *et al* (2008). Such experiments provide data that can be used for validation of the computational models of liver injury.

The finite element method (FEM)-based models of liver are mostly based on the detailed geometry of the real liver. Our study showed that the ruptures were spreading around the vascular branches, thus the vascular tree should be included in the FEM models because of its important contribution to the injury mechanism.

Another factor that should be taken into account in the models is the increased inner liver pressure caused by impact loading. High pressure levels can cause ruptures adjacent to the vascular trees. These ruptures propagate along the reticular fibers and interlobular connective tissue septa as shown in our study.

Our results are further suitable for validation of impact test results in terms of the impact energy and velocity threshold. The impact velocities and the impact energies used in the impact tests in our study should lead to the same injury levels also when used in the simulated impact tests with HBMs. An impact velocity of 1.72 m s^{-1} with impact energy 418 J m^{-2} leads to low injury grade (0–II) and AIS severity (0–2), while the higher impact velocity (energy) caused the injury connected with large blood outflow. An impact velocity higher than 2.88 m s^{-1}, related to an impact energy higher than 1187 J m^{-2}, leads to total disruption of the liver. Also the internal liver pressure in the HBMs should be the same as during our impact test. Note that the high levels of pressure could also lead to the artery or internal structures ruptures.

A recent study by Chen *et al* (2019) provided detailed data on material behavior of porcine liver during high strain rate compression. However, their measurements were performed on tissue samples separated from the whole liver, thus excluding the effect of the fibrous capsule on the biomechanical behavior. It was proven that the content of connective tissue influenced the stiffness of the liver tissue (Mazza *et al* 2007). Neglecting the influence of the capsule could result in significant differences when assessing the biomechanical behavior of the liver parenchyma (Hollenstein *et al* 2006). The test on tissue samples provided us only with information on local material properties. Moreover, the proposed study design using samples of liver tissue cannot be used for studying the relation of internal fluid pressure changes on the impact injury. As previously described, rapid increase in internal fluid pressure is related to the severity of liver injury on the whole organ level (Sparks *et al* 2008). Therefore

Table 2. The impact loaded area, maximal impact pressure, impact velocity, impact energy (given by position of the plate before the impact) measured by laser and given by liver height respectively, AAST injury grade and AIS severity for all four liver groups: impact height 200, 300, 400 and 500 mm. N is the number of specimens. The values of impact velocity are presented as mean values. The values of loaded area, maximal vascular pressure and impact energy are presented as medians (lower quartile–upper quartile).

Impact height (mm)	Impact velocity (m s^{-1})	Loaded area (%)	Maximal vascular pressure (mmHg)	Impact energy—laser (J m^{-2})	Impact energy—height (J m^{-2})	AAST injury grade/AIS severity	N
200	1.72	45.65 (42.88–50.48)	171.69 (149.35–192.52)	418.00 (409.55–467.87)	450.00 (446.57–485.07)	0–II/0–2	6
300	2.17	46.17 (43.17–53.14)	174.85 (107.34–248.69)	783.97 (755.30–818.35)	850.31 (813.06–876.81)	I–III/2–3	6
400	2.54	39.73 (36.40–47.64)	191.77 (137.85–249.69)	1134.79 (952.00–1337.62)	1239.53 (976.22–1435.52)	I–IV/2–4	6
500	2.88	53.53 (51.89–55.54)	220.35 (178.85–259.36)	1187.47 (1146.72–1255.55)	1305.18 (1224.52–1347.71)	IV–V/4–5	6

Figure 4. Typical external injury patterns of the porcine liver after the impact test. (A) Laceration of the visceral surface of the liver affecting more than half of the left lateral lobe, injury grade V. (B) Rupture of the visceral surface of the right medial lobe that propagates from the hilum along the big vessels and the stromal connective tissue. (C) Peripheral rupture starting at the border of the right hepatic lobe. (D) Occasional shallow rupture on the diaphragmatic surface of the liver, most likely caused by the direct impact of the wood plate used in the impact test.

Figure 5. Results of microscopic injury analysis. The Wilcoxon signed-rank test showed statistically significant differences between the real intersection intensity of the reticular fibers $P'_{L(ret.)} = 42$ (38–51) mm^{-1}; median (lower quartile–upper quartile) and the theoretical intersection intensity of the reticular fibers $P_{L(ret.)} = 57$ (54–60) mm^{-1}; median (lower quartile–upper quartile), ($p < 0.001$). Significant differences were also found between the real intersection intensity of the interlobular septa ($P'_{L(septum)} = 1.2$ (1–1.3) mm^{-1}; median (lower quartile–upper quartile) and the theoretical intersection intensity of the interlobular septa $P_{L(septum)} = 1.5$ (1.4–1.7) mm^{-1}, median (lower quartile–upper quartile), ($p < 0.001$). Data are displayed as the median values with boxes spanning the upper limits of the first and third quartiles and with whiskers spanning the minimum and maximum values for each group.

experiments on whole porcine liver are a necessary source of data on liver behavior from the pressure changes point of view.

The whole liver was also used in experiments with human organs. Conte *et al* (2012) investigated macroscopic damage of human liver in a uniaxial compression test. The macroscopic injury consisted mostly of

lacerations of the right lobe. In some cases the lacerations originated in close proximity to the big vessels and propagated along the connective tissue, which was in concordance with the macroscopic injury pattern in our study. Occasional lacerations of the liver diaphragmatic surface found in our study were mostly caused by the setting of the experiment. The diaphragmatic surface was exposed to the direct impact of the wooden plate, which did not correspond to the injury mechanism *in vivo*.

The multiscale analysis by Conte *et al* (2012) also included histological analysis of the damaged tissue. The microscopic injury patterns presented in human liver tissue were described as 'cracking' and 'cavitation' that appeared to propagate within the hepatic lobules. According to this analysis the cracks' origin appeared to be located at the interface between the vessel wall and the parenchyma. Their data, however, did not clearly answer how the tissue damage spread in the context of liver microstructure, namely in the context of the fibrous components of the connective tissue matrix.

Our histological quantitative analysis based on stereological methods revealed that the ruptures did not propagate randomly through the liver parenchyma, but rather followed a pattern that was closely related to the tissue microstructure. The weak link of the tissue appeared to be the interface between the reticular fibers and the hepatocytes arranged into trabeculae, as ruptures crossing the reticular fibers were statistically proven to be less likely to occur than ruptures propagating along the above-mentioned interface. Moreover, the interlobular septa that are composed of connective tissue also seemed to represent a strong border that ruptures did not cross.

The components of the extracellular connective tissue matrix, such as collagen III in reticular fibers, influence the response of the organ to mechanical stimuli. Collagen is a rather stiff and hard protein whereas cells on the other hand tend to respond to mechanical stimuli in various ways (Meyers *et al* 2008). The cell rigidity is dependent on its environment, including cell–extracellular matrix interactions. The difference in microscopic structures of porcine and human liver therefore needs to be taken into consideration when extrapolating data from experiments on porcine organs to the human model.

Based on our study and works focused on the macro- and micro-structure of liver, there are three main differences between human and porcine liver that are important for the mathematical modeling. The microstructure of porcine liver is organized into only roughly hexagonal hepatic lobules, while the microstructure of human liver is organized into regular hexagonal hepatic lobules. This difference is important for the mathematical models based on the microstructure. The second main difference is the shape of the whole liver (Ntonas *et al* 2020). The shape of porcine liver is lobular and thick in the centre, becoming thinner at the perimeter of the organ, while human liver has a triangular prism shape, and the size decreases from right to left. Both human and porcine livers consist of sinister, dexter, quadrate, and caudate lobes. Because of the more lobular shape of the porcine liver, the sinister and dexter lobes are each divided into lateral and medial lobes. The arterial vascular system in both humans and pigs have the same structure. The portal veins of the species have minor differences. In human, the division of the portal vein occurs outside the liver parenchyma, while in pigs, it occurs at the hilum. The third difference is the amount of connective tissue, which is significantly higher in pigs compared to humans (Mik *et al* 2018). One of the main aims of our study was to find connection between the impact energy and the range of injury. The results are based on the porcine liver, but the level of injury, the rupture around veins and the rupture spreading should be similar for both species due to similarities in the tissue structure (however, this claim is yet to be verified).

Velocity is recognized to be a critical factor in evaluating the possibility of abdominal organ injuries. According to Brasel and Nirula (2005) the probability of abdominal injury significantly increases at velocity >20 km h^{-1}. However, in our study, relatively low velocities of the impact plates at the moment of impact still led to severe liver injury. This was probably caused by the absence of the protective effect of the rib cage and of the abdominal wall. Despite this limitation, the analysis showed an increasing trend of maximal pressure related to the impact height and impact velocities, which supported the view that the severity of injury was dependent on the impact energy. The highest loaded velocity (impact height) caused the most severe lacerations.

5. Conclusion

Using an experimental impact approach, this study provides a multiscale analysis of the mechanism of porcine liver injury that gives an accurate description of damage evolution which can also be applied to human liver tissue. Biomechanical behavior of porcine tissue, namely changes in intravascular pressure, was coupled with analysis of rupture propagation on both the macroscopic and microscopic level. The microscopic analysis proved that connective tissue containing reticular fibers influenced the rupture propagation through liver. The pattern of rupture propagation, with regards to differences in the amount of connective tissue in porcine and human liver, should be especially considered when extrapolating data from animal experiments to the human model. The present findings can be used to improve (by incorporating the vascular tree) and validate computational models of liver behavior in impact situations.

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Authorship

Study design, literature search, data analysis, data interpretation, and writing—AM, PK, ZT, animal care—RP, VL, histology—AM, PM, ZT, biomechanical measurements—PK, TB, MH, critical revision—ZT, VL, PM.

Disclosure

The authors declare no conflicts of interest.

References

Arnoux P J, Serre T, Cheynel N, Thollon L, Behr M, Baque P and Brunet C 2008 Liver injuries in frontal crash situations a coupled numerical —experimental approach *Comput. Methods Biomech. Biomed. Engin.* **11** 189–203

Augenstein J, Bowen J, Perdeck E, Singer M, Stratton J, Horton T, Rao A, Digges K H, Malliaris A C and Steps J 1999 Injury patterns in near-side collisions *Annu. Proc. Assoc. Adv. Automot. Med.* **43** 139–58 (PMCID: PMC3400216)

Behr M, Arnoux P J, Serre T, Bidal S, Kang H S, Thollon L, Cavallero C, Kayvantash K and Brunet C 2003 A human model for road safety: from geometrical acquisition to model validation with radioss *Comput. Methods Biomech. Biomed. Engin.* **6** 263–73

Brasel K J and Nirula R 2005 What mechanism justifies abdominal evaluation in motor vehicle crashes? *J. Trauma* **59** 1057–61   Chen J, Brazile B, Prabhu R, Patnaik S S, Bertucci R, Rhee H, Horstemeyer M F, Hong Y, Williams L N and Liao J 2018 Quantitative analysis of tissue damage evolution in porcine liver with interrupted mechanical testing under tension, compression, and shear *J. Biomech. Eng.* **140** 0710101–07101010

Chen J, Patnaik S S, Prabhu R K, Priddy L B, Bouvard J L, Marin E, Horstemeyer M F, Liao J and Williams L N 2019 Mechanical response of porcine liver tissue under high strain rate compression *Bioengineering (Basel)* **6** 49

Cheynel N, Serre T, Arnoux P J, Baque P, Benoit L, Berdah S V and Brunet C 2006 Biomechanic study of the human liver during a frontal deceleration *J. Trauma* **61** 855–61

Cheynel N, Serre T, Arnoux P J, Ortega-Deballon P, Benoit L and Brunet C 2009 Comparison of the biomechanical behavior of the liver during frontal and lateral deceleration *J. Trauma* **67** 40–4

Conte C, Garcia S, Arnoux P J and Masson C 2012 Experimental multiscale analysis of liver damage and failure process under compression *J. Trauma Acute Care Surg.* **72** 727–32

Doklestić K *et al* 2015 Surgical management of AAST grades III–V hepatic trauma by damage control surgery with perihepatic packing and definitive hepatic repair-single centre experience *World J. Emerg. Surg.* **10** 34   Eberlova L *et al* 2016 Porcine liver vascular bed in Biodur E20 corrosion casts *Folia Morphol. (Warsz)* **75** 154–61   Eberlova L, Maleckova A, Mik P, Tonar Z, Jirik M, Mirka H, Palek R, Leupen S and Liska V 2020 Porcine liver anatomy applied to biomedicine *J. Surg. Res.* **250** 70–9

Elhagediab A and Rouhana S 1998 Patterns of abdominal injury in frontal automotive crashes *The 16 Int. Technical Conf. on the Enhanced Safety of Vehicles*

Gayzik F S, Moreno D P, Geer C P, Wuertzer S D, Martin R S and Stitzel J D 2011 Development of a full body CAD dataset for computational modeling: a multi-modality approach *Ann. Biomed. Eng.* **39** 2568–83

Golman A J, Danelson K A, Miller L E and Stitzel J D 2014 Injury prediction in a side impact crash using human body model simulation *Accid. Anal. Prev.* **3** 1–8

Holbrook T L, Hoyt D B, Eastman A B, Sise M J, Kennedy F, Velky T, Conroy C, Pacyna S and Erwin S 2007 The impact of safety belt use on liver injuries in motor vehicle crashes: the importance of motor vehicle safety systems *J. Trauma* **63** 300–6

Hollenstein M, Nava A, Valtorta D, Snedeker J G and Mazza E 2006 Mechanical characterization of the liver capsule and parenchyma *ISBMS 2006: Biomedical Simulation (Lecture Notes in Computer Science vol 4072)* ed M Harders and G Székely (Berlin: Springer) (https://doi.org/10.1007/11790273_17)

Huelke D F, Nusholtz G S and Kaiker P S 1986 Use of quadruped models in thoraco-abdominal biomechanics research *J. Biomech.* **19** 969–77

Junatas K L, Tonar Z, Kubíková T, Liška V, Pálek R, Mik P, Králíčková M and Witter K 2017 Stereological analysis of size and density of hepatocytes in the porcine liver *J. Anat.* **230** 575–88

Kemper A R, Santago A C, Stitzel J D, Sparks J L and Duma S M 2010 Biomechanical response of human liver in tensile loading *Ann. Adv. Automot. Med.* **54** 15–26 (PMID: 21050588)

King A I and Yang K H 1995 Research in biomechanics of occupant protection *J. Trauma* **38** 570–6

Kozar R A, Crandall M, Shanmuganathan K, Zarzaur B L, Coburn M, Cribari C, Kaups K, Schuster K, Tominaga G T and AAST Patient Assessment Committee 2018 Organ injury scaling 2018 update: spleen, liver, and kidney [published correction appears in J. Trauma Acute Care Surg. 2019 Aug;87(2):512] *J. Trauma Acute Care Surg.* **85** 1119–22

Kruepunga N, Hakvoort T B M, Hikspoors J P J M, Köhler S E and Lamers W H 2019 Anatomy of rodent and human livers: what are the differences? *Biochim. Biophys. Acta, Mol. Basis Dis.* **1865** 869–78

Kubíková T, Kochová P, Brázdil J, Špatenka J, Burkert J, Králíčková M and Tonar Z 2017 The composition and biomechanical properties of human cryopreserved aortas, pulmonary trunks, and aortic and pulmonary cusps *Ann. Anat.* **212** 17–26

Lee J B and Yang K H 2001 Development of a finite element model of the human abdomen *Stapp Car Crash J.* **11** 79–100

Malečková A *et al* 2019 Animal models of liver diseases and their application in experimental surgery *Rozhl Chir.* **98** 100–9

Marchesseau S, Chatelin S and Delingette H 2017 Non linear biomechanical model of the liver: hyperelastic constitutive laws for finite element modeling *Biomechanics of Living Organs* ed Y Payan and J Ohayon (Cambridge: Academic)

Mazza E, Nava A, Hahnloser D, Jochum W and Bajka M 2007 The mechanical response of human liver and its relation to histology: an *in vivo* study *Med. Image Anal.* **11** 663–72

Melvin J, Stalnaker R, Roberts V and Trollope M 1973 Impact injury mechanisms in abdominal organs *Proc. 17th Stapp Car Crash Conf.* (Warrendale, PA: Society of Automotive Engineers) pp 115–26

Meyers M A, Chen P Y, Lin A Y M and Seki Y 2008 Biological materials: structure and mechanical properties *Prog. Mater. Sci.* **53** 1–206

Mik P *et al* 2018 Distribution of connective tissue in the male and female porcine liver: histological mapping and recommendations for sampling *J. Comp. Pathol.* **162** 1–13

Monchal T, Ndiaye A, Gadegbeku B, Javouhey E and Monneuse O 2018 Abdominopelvic injuries due to road traffic accidents: characteristics in a registry of 162 695 victims *Traffic Inj. Prev.* **19** 529–34

Moore E E *et al* 1989 Organ injury scaling: spleen, liver, and kidney *J. Trauma* **29** 1664–6

Ntonas A, Katsourakis A, Galanis N, Filo E and Noussios G 2020 Comparative anatomical study between the human and swine liver and its importance in xenotransplantation *Cureus* **12** e9411

Santiago A C, Kemper A R, McNally C, Sparks J L and Duma S M 2009 The effect of temperature on the mechanical properties of bovine liver—biomed 2009 *Biomed. Sci. Instrum.* **45** 376–81 (PMID: 19369792)

Sparks J L, Bolte J H 4th, Dupaix R B, Jones K H, Steinberg S M, Herriott R G, Stammen J A and Donnelly B R 2007 Using pressure to predict liver injury risk from blunt impact *Stapp Car Crash J.* **51** 401–32 (PMID: 18278606)

Sparks J L and Dupaix R B 2008 Constitutive modeling of rate-dependent stress-strain behavior of human liver in blunt impact loading *Ann. Biomed. Eng.* **36** 1883–92

Sparks J L, Stammen J, Herriott R and Jones K 2008 Development of a fluid-filled catheter system for dynamic pressure measurement in soft-tissue trauma *Int. J. Crashworthiness* **13** 255–64

Stoyan D, Kendall W S and Mecke J 1995 *Stochastic Geometry and its Applications* 2nd edn (New York: Wiley)

Schoell S L, Weaver A A, Urban J E, Jones D A, Stitzel J D, Hwang E, Reed M P, Rupp J D and Hu J 2015 Development and validation of an older occupant finite element model of a mid-sized male for investigation of age-related injury risk *Stapp Car Crash J.* **11** 359–83 (PMID: 26660751)

Schwartz D, Guleyupoglu B, Koya B, Stitzel J D and Gayzik F S 2015 Development of a computationally efficient full human body finite element model *Traffic Inj. Prev.* **16** S49–56

Tamura A, Omori K, Miki K, Lee J B, Yang K H and King A I 2002 Mechanical characterization of porcine abdominal organs *Stapp Car Crash J.* **11** 55–69 (PMID: 17096218)

Tonar Z, Janácek J, Nedorost L, Grill R, Báca V and Zátura F 2009 Analysis of microcracks caused by drop shatter testing of porcine kidneys *Ann. Anat.* **191** 294–308

Tropiano P, Thollon L, Arnoux P J, Huang R C, Kayvantash K, Poitout D G and Brunet C 2004 Using a finite element model to evaluate human injuries application to the HUMOS model in whiplash situation *Spine (Phila Pa 1976)* **29** 1709–16

Untaroiu C D, Lu Y C, Siripurapu S K and Kemper A R 2015 Modeling the biomechanical and injury response of human liver parenchyma under tensile loading *J. Mech. Behav. Biomed. Mater.* **41** 280–91

Vrtkova I 2015 Genetic admixture analysis in Prestice Black-Pied pigs *Arch. Anim. Breed.* **58** 115–21

Vyčítal O, Horský O, Rosendorf J, Liška V, Skalický T and Třeška V 2019 Treatment of liver injuries at the Trauma Centre of the University Hospital in Pilsen *Rozhl Chir.* **98** 488–91

Wang B C, Wang G R, Yan D H and Liu Y P 1992 An experimental study on biomechanical properties of hepatic tissue using a new measuring method *Biomed. Mater. Eng.* **2** 133–8

World Health Organization 2018 Road traffic injuries *World Road Safety Global Status Report on Road Safety* (https://who.int/news-room/fact-sheets/detail/road-traffic-injuries) (accessed 8 June 2020)

Xu T, Sheng X, Zhang T, Liu H, Liang X and Ding A 2018 Development and validation of dummies and human models used in crash test *Appl. Bionics Biomech.* 3832850

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Anatomie jater prasete domácího jako modelového orgánu v biomedicíně – přílohy

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MIK, P., Z. TONAR, A. MALEČKOVÁ, L. EBERLOVÁ, V. LIŠKA, R. PÁLEK, J. ROSENDORF, M. JIŘÍK, H. MÍRKA, M. KRÁLÍČKOVÁ a K. WITTER, 2018. Distribution of Connective Tissue in the Male and Female Porcine Liver: Histological Mapping and Recommendations for Sampling. Journal of Comparative Pathology [online]. 162, 1–13. ISSN 0021-9975. Dostupné z: doi:10.1016/j.jcpa.2018.05.004 **IF$_{2017}$=1,364, Q2$_{\text{(Veterinary sciences)}}$.**

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SPONTANEOUSLY ARISING DISEASE

Distribution of Connective Tissue in the Male and Female Porcine Liver; Histological Mapping and Recolllllendations for Sampling

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Sununary

TI1e pig is a largea1ůmal model that is often used in experimental medidne. TI1e aimoftl study was toassess, in norma! pig livers, sexual dimorplůsmin tbe norma(fraction of hepatic imerlobular aud imralobu lar conneC* rive ů,sue (Cf) insix hepatic lobesand in three macroscopical regions of intere,t (RO!s) with different positions relative to tbe liver vasculature. Usingstereological point grids, the fractions of Cf were quantified in lůstological sectionsstained widi ruůline blue ruid nud e ar fast red. Samples (415 rissue blolcs) werecollected &om he aldiy piglets, represenring paracaval, paraportal and peripheral ROIs. There was oonsiderable variability in tbe Cf fraction at aH sampling levels. In mates tbe mean fraction of imerlobular Cf was 4.7 ± 2.4% (me rui ± SD) ruid rruiged from 0% to 11.4% . In females the mean fraction of die interlobular Cf was 3.6±2.2% and ranged from 0% to 12.3%. The me:an &acrion ofintralobular (perisinusoidal summed widi pericentral) Cf was <0.2% in bothsexes. TI1e interlobular Cf repre,ented >99.8% ofthetotal hepatic Cf ruid die fractions were highly correlated (Spearmru, , ＝0.998, P <0.05). TI1e smallest Cf &acrion was observed indieleft media!lobeand in die paracaval ROJ a11d d i e large,t CT &acrion was detected in die quad-ratelobeand in tbe peripberal ROJ.Forplanni.ngexperimemsinvolvingtbe lůstolog-icaJ (Juant ification ofliver fibrossi and requiring compar on between tbe liver lob , tb e data fadHtate tbe power ana tysis forsample siz-e needed to detect tbe expected relative increase or decr.e ase in tbe fraction of Cf.

Keywords: connective tissue; histology; liver; pig

ln troduction

Both small and large animals are used to study the mechanisms ofthe origin and spread ofliver 6brosis, often together with the regenerative capacity and healing of the liver. Fíbroses of diffi:rent aetiologies have been studied predominantly in mice (Gcorge et al., 2003; Anstee and Goldin, 2006; Machado et al., 2015) and r,1ts (Yi et al., 2012; Nowatzky et al., 2013; Fakhoury-Sayegh et al., 2015). However, small animal models of liver 6brosis have several limitations due to the small organ size (Lossi et al., 2016). Therefore, it is usually not possible to study phenomena such as portocentr,1) and portoportal bridging 6brosis, modelling of the biomechanics of trauma, lobar resection and regener...1tion or surgical

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techniques. Large animal models are needed for the improved translation of experimental work into human medicino. Apart from shcep liver (Ghodsizad *et al.,* 2012), the pon:ine liver is the most widel y used large animal model (Avritscher *et al.,* 201 I; Kawamu ra *et al.,* 2014; Bruha *et al.,* 2015; Nygrad *et al.,* 2015; Wang *et al.,* 2015) to study improvements in invasive (Croome *et al.,* 2015) and **non-invasive methOOs of liver disease management** (Gnutzmann *etat.,* 2015), to interpret animal experi· ments and to translate the results of animal models to human medicino (Arkadopoulos *et al.,* 2011; Watson *et al.,* 2016; Budai *et al.,* 2017). Chen *et al.* (2013) showed that histological assessment of liver 6brosis in the pig correlates with non-invasive splenic mag• netic resonance imaging. Combining the data ob- tained for connective tissue (CT) with data on tbe microvascular boo of the porcine liver (Eberlova *et al.,* 2016, 2017) would improve existing models of **human liver petfusion. Moreover, fibrosis is an important part of porcine liver diseases, such as in** pigs suffering from biliary and peribiliary cysts (Komíne *et al.,* 2008) or swine hepatitis E (Lee *et al.,* 2010).

Summarizing the present literature, porcine liver 6brosis and cirrhosis of different aetiologies can be used as a model for human liver 6brosis and cirrhosis (Avritscher *etal.,* 2011). ln the porcine liver, 6brosis is usually induced by CCL, (Zha ng *et al.,* 2009), by alcohol (Lee *et al.,* 2017), by a high-fut diet or by a Westem-style diet (Panasevich *et al.,* 2018) orby u:s• ing pentoxifylline (Peterson and Neumeister, 1996). lt has been proposed that porcine liver 6brosis ma y be staged according to human standards using th e Metavir scoring system (Ishak *et al.,* 1995; Zhou *et al.,* 2014; Yin *et al.,* 2017); however, this required several modi6cations (Huang *et al.,* 2014, 2017).

The major 6brogenic cellsinthe liverare the hepat• ic stellate cells, porta) 6broblasts, 6brocytes, bone marrow-derived cellsthat areactivated and transdif- ferentiated into hepatic myo6broblasts and possibly hepatocytesand cholangiocytesthattransition to my• o6broblasts (Forbes and Parola, 2011; Zhao *et al.,* 2016; Kisseleva, 2017). Histological assessment of the location of 6brosis and identi6cation of the source of 6brogenic cells is necessary when assessing the severity of the liver disease and the patient's prognosis (Ta kahashiand Fukusato, 2014; Stasi an d Mila n 2016). Biopsy samples are usually scored in terms of their grade (Scheuer, 1991) and stage (Saxena, 2011). Six speci6c loci of liver 6brogenesis have been proposed for scoring, namely porta!, peri- cellular (i.e. perisinusoidal), pericentral (i.e. perive- nular), centrilobular, dueta) (i.e. periductal) and ductular (Batts and Ludwig, 1995; Gohlke *et al.,*

1996; Brunt *et al.,* 1999; Sakhuja, 2014; Takahashi and Fukusato, 2014).

During chronic hepatitis, 6brosisstartsand spreads from porta) regions, fornúng stellate periportal scars or enlarging the porta) tracts (Lelkowitch, 2007). Steato6brosis in alcoholic liver disease begins in the pericentral region and extends in a perisinusoidal **pattern, where itis more pronounced than in hepatitis** C infection (Zaitoun *et al.,* 200 I). This phenomenon kads to centroportal and portoportal bridging and, together with the regenerating nodular parenchyma, resultsin cirrhosis (Theise, 2013). Similar histological 6ndings have been reported in non-alcoholic futty liver disease (NAFLD) or non-alcoholic steatohepati- tis (NASH), but Jack the pericentral origin of6brosis (Brunt *etal.,* 1999; Klei ner *et al.,*2005;Takahashi and Fukusato, 2014). Primary biliary cirrbosis involves 6brosis of small intrahepatic bíle ducts (Lindor *et al.,* 2009; Working Subgroup for Clinical Practice Guidelines for Primary Biliary Ci rr hosis, 2014). Centra) hepatic veins are often retained in their centrilobular location, even in cirrhosis. Sclerosing cholangitis shows bíle duet scarring biliary 6brosis, leading eventually to cirrhosis (Hirschfield *et al.,* 2013; de Vries *et al.,* 2015).

The amount of CT in the human liver is usually estimated during routine analysisof liver biopsy sam• ples, according to widely used scoring systems (Scheue r, 1991; Ishak *et al.,* 1995; Bedossa a nd Poynard, 1996). However, Standisheta/. (2006) high• lighted several limitationsofsubjective or semiquanti- **tative scoring as both the interobserver and intra•** observer variability might disqualify the datagener• ated from comparative studies or from evaluations of non-invasive methods of liver 6brosis. Therefore, the need foran objective, reproducible measure, pref- **erably generating continuous data, has been articu•** lated (Saxena, 2011).

To the bestofour knowledge, no published dataare available for continuous quantitative histological pa• **rameters that demonstrate the norma) intersexual** and interindividual variability in the fraction of CT in various macroscopical regions of pon:ine liver lobes. Therefore, the aim of the present study was to assess the content and distribution of norma) hepatic CT in the domestic pig and to provide sampling rec· ommendations for further histopathological studies. The following null hypotheses were tested: (I) thevol- umefraction ofCT in the liveris the samein maleand in female pigs, (2) the volume fraction of CT in the liver is the same in all hepatic lobes and (3) the vol- ume fraction of CT in the liver is the same in thrce macroscopical regions with different positions related to the livervasculature (regionsofinterest; ROIs): the peripheral regionsof the liver lobes, the regions near

the porta bepatis and the regions adjacent to tbe **caudal vena cava .**

Material and Method

Animals

As a part of tbisstudy we used liver samples collected previously forstudiesof tbe sizeand den.síty ofbepa - cytes in tbe porcine liver (Junatas *et al.,* 2017). F1ve more animals were added. Bridly, liversamples were obtained from 12 bealtby Prestice black-pied pigs (Vrtková, 2015) aged 9- 15 wecks and wei h!ng 25- 45 kg (34.3± 5.3 kg, mean ± standard dev,atJon [SOJ). Six pigletsweremale andsixpigletswe':female (sec Supplementary Table I for ample d ta ils). -r:be **animak received humane care m comphance w1th** the European Conventionon Animal Careand project number 27374/2011-30 was approved by tbe Faculty Committec for tbe PreventionofCruelty to Animals. Tbe animals were premedicated (with atropine, keta• mine and azaperone), anaestbetized (with propofol andfentanyl), relaxed (witb pancu ronium), intubated and mecbanically ventilated. Fluid infusion and vol- ume restorationwere provided (Plasmalyte™solution and Gelofusine™ solution; B-Br,1un AG, Melsungen, Cermany). Animals were killed under anaestbesia by administr,1tion of cardioplegic solution **(KCI).** I mme• diately after sacrifice, the wbole liver was removed. Tbe fresb liver volumes ranged from 0.640 to 1.200Iand tbe mean ± SD valueswere 0.871 ± 0.1461.

Ti s$11t Sample Colk ction

Eacb liver was scctioned into 1.5 cm tbick slices and immersed in 10% neutra) buffered formalin. From eacb liver, 36 tissue samples (eacb approximately 25 x 25 x 15 mm) were collected to represent six be- patic lobes (i.e. left lateral, left media), rigbt media rigbt later,1), caudate and quadr,1te) and tbrec diffi:rent positions (ROls) relative to tbe liver vascu• lature (Fig. IA). In eacb lobe, two samples repre· scnted tbe peripberal ROi s, two were from tbe paracaval ROls and two were from the par,1portal ROls. Tbe peripber,1) ROls were de6ned as located no more tban I cm from the surface and peripbery of eacb bepatic lobe. Tbe par,1caval location was the region of eacb lobe immediately adjacent to tbe openings of tbe bepatic veins into tbe caudal vena cava. Tbe par,1portal (bilar) location "'.as adja nt to tbe main br,1ncbes of tbe porta) vem followmg from tbe bilum witbin eacb anatomical lobe.

I n total, 415 tissue samples were collec!"d (in tbrec animals, tbe size of the quadrate lobe did not allow **collection of separ....tte tis.sue sample.s; moreover, three** otber samples were lost during processing). Before

cutting, tbe orientation of eacb tissue block was r,1n- domized using tbe orientator scbeme (Ma ttfeldt *et al.,* 1990; Miihlfeld *et al.,* 2010).

Volumdric Analys-is oj &gionsoj fnll!rest

Volumetrie analysis of tbe ROls was based on computed tomograpby. Tbe borders betwecn be ROls were mapped to guar,1ntee tbat eacb region represented approximately one tbird of the volume of eacblobe.Tberefore, the sampling described above was not biased by different relative volumes of tbe ROls. Prior tosampling, tbe liver was scanned using a Somatom De6nition Flasb Dual Source Computed Tomogr,1pby Scanner (Siemens, Erlagen, Germany) and tbe Syngo software kernel. Tbe section tbicknes; was 0.65 mm and tbe voxel size was 0.65 x 0.65 x 0.40 mm^3 A typical tomograpby stack consisted of 700- 800sections. Assignment of tbe liver parencbyma to tbe paracaval, paraportal and peripb· er.11 ROI was done using tbe USA (Liver Surgery Analyzer) software (Jiřík *et al.,* 2018, a frec and open-source application intended for computer• aided liversurgery and measurement. Liver segmen• ta tion was performed using a 30Gr,1pb-Cut algo- ritbm (Boykov and Jolly, 2000). For tbe measurement of ROI respective volumes, tbe porta) vein vascular bed and the bepatic vein vascular bed wo:re segmented separately. Tbe liver paren byma was assigned to tbe respective vascular bed by 1ts po- sition witbin tbe balí-distance betwecn two vascular beds using tbe Euclidean Distance Transform. In t-he second step, we arbitr,1rily cut-off the peripberal th:ird of the liver parencbyma and as;igned it to tbe peripber,1) ROL Tbe mean volumes of eacb ROI wo:re set to one-tbird of tbe total liver parencbyma volume (sec an example in Fig. IB).

Histologil;al Process-ing

T he tissue blocks were proces;ed routinely and embedded in paraffin wax. Tbree sections (3 µm) wo:re cut from eacb block and one was selected randomly and used for furtber analysis. After d -wax• ing and rebydration, nuclei were stained witb nuclear fast red (Waldeck GmbH, Munster, Cermany). Af\er wasbing in distilled water, tbe ections werediffer n i- ated in 5% pbospbotungstic ac1d and wasbed agam m distilled water. Collagen6breswerestained witb 0.5% aniline blue (Merck KGaA, Darmstadt, Cermany).

Microscopic Sampling aná (luantifiration oj Vo/unie Fraction oj Connective Ti ssue

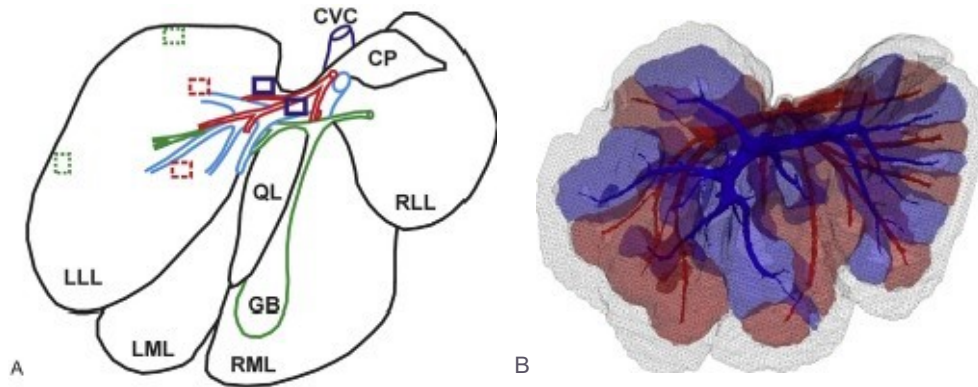Ali quanti6ed par,1meters are listed in Table I. Tbe 6rst step comprised a systematic uniform random

Fig. I. Collcction of tissucsamplcsofthc porcinc Livcr for q uanti t.ative   histo l ogy and thc rdativcvolumcsof the rcgionsofintcn::st (R O ls).
{A) As shownon ad rawingof dle facicsviscccralisof dle pcrcinclivcr, thcsamplingstratcgy wasthcsamcasinJ unatasttal.(2017).
Thcschcme outlincsthe len la1eral lobe (LLL), len med ia ! lobe (LM L), rig ht mcdia l lohe (R M L), rig h t latcra l lobc (R LL),q uad -
ratc lobeQ L) and caudatc lobe (mmdy hidclen bch ind thc vessels, the schcme shows iu caudatc proœs.ş C P, on ly). The ga ll-
blad cler (G B) and thc ca uda l vcna cava are also$hown (CVC). Branching of hepacic art crics is$hown in rcd, branching of thc
porta.I vcin in bluc and branch ing of thc bite ducts ingru:n. ThcLLLisU$CCl as an ex.amplc of collection of histo logica l samp lcs
from thl'C'C R Ols: paraca val region (dark bluc rcctang les drawn \.\ith a continuous Linc) , paraportaJ rcgicn (rccl rcctang les \.\ith
adashedLinc)and thc pcriphcra   lregion(grccnrcctanglcswithadoncdLinc). Sam p les ofthc othc rl obcs were co llected accord   ingl y.
(B) Visua li za ti.on of the R O ls rcpn::scnti.ngapproximatdy thc thrcc third:i of thc total volume of thc porcine Li\.: r. Thc li vcr was
scanned using computed tomography and   thc   reconstruction was rotated $o   that thc visceraJ surface is sho"""fl from a caudaJ
\'lew. I n this individual, the paracava l regionwith a$$0Clated hcpati.cvcinsissho"""fl in rcd(volumc = 0. 3881), thc pcriphcraJ region
is shCM'n in black (volume = 0.427 I)and thc perip,on.al region with dle branching of the pon.al vcin is sho"""fl in bluc (volumc
= 0.400 I). The volumesof thc bloodvesscls wc:rcsubtracted from dle ROIs.

sampling of microscopical image 6elds within each section according to Fíg. 2A.To quantifythe interlobular CT V_{17} (ICT) (i.e. CT surrounding classical lobules), three uniformr ,rndom 6eldsofview (FOVs) at x 1O magni6cation were captu red, while to quanti fy the perisinusoidal CT V'' (PSCT) (i.e. intralobular CT adjacent to the liver sinusoids) and pericentral CT V'' (PCCT) (i.e. Cf surrounding the centra) veins), four uniform random FOVs at x 20 magni6ca• tion were photographed (Fig. 2A) at a resolution of 1,280 x 960 pixels. ln the second step, a stereological

point grid was applied over the FOVs in the Ellipse software (ViDiTo, Kosice, Slovak Republic; T onar *et al.,* 2015; Kubíková *et al.,* 2017) to quantify the volume fraction of each Cf type (Figs. 2B and C). The areas occupied by the Cf were equal to the corresponding volume fractions according to the Deles;e principle (Mouton, 2011). T he 6brous capsule of the liver, which was present in the samples from the pe· ripher,1) region, was omitted from the quanti6 cation. Any tisme processing artifacts, such as microcracks or folds, were not evaluated and were subtracted from

Tablo I

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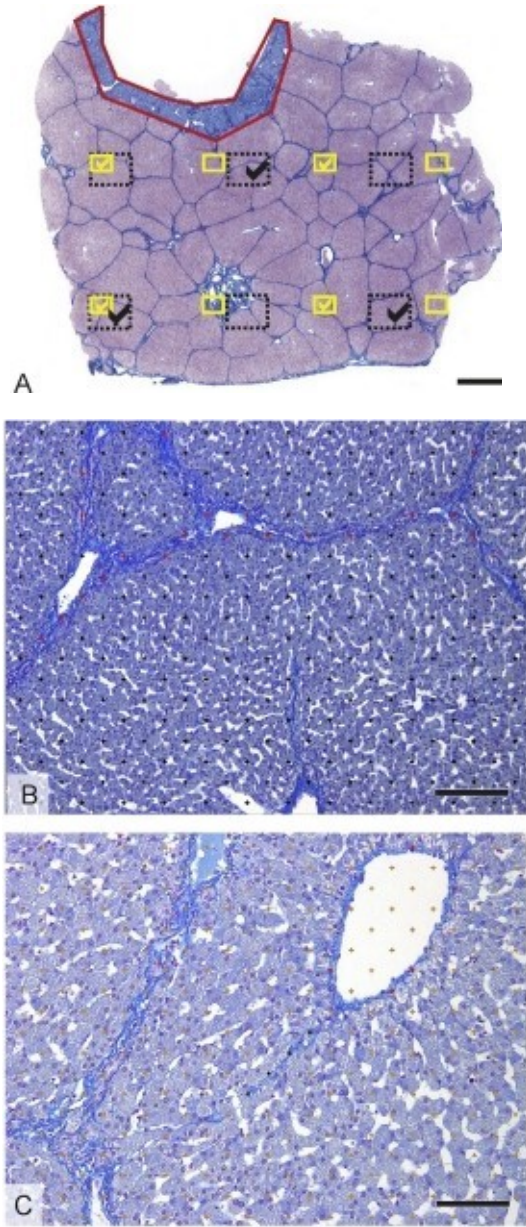|	Abbreviation and unit	Definition	$ura,/ogŭal udtmfu ,u1d fM f"4JlllJl4JíO/t
Volumc fraction of imcrlobular (l()l\llCCci\e ti$$ue	$l'v$ (ICT)	Flrac tion of co mlCCti,•c 0$$uC$Urro und ing c la.ssica l lobu lc w ith in th e li ,,er	Point grid
Volume fraction of pcrisinu 90id al (l()l\llCCci\e ti$$ue	Vv (PSC1)	Fractionofcoa.ulCÇi ,e tis.uc locatcd ator in thc lobularsinu90ids with in th c, li •cr lo b ulcs	Point grid
Volume fraction of pcriccntral coa.mcct ive ti$$ue	$l'v$ (PCCl)	Flra c tion of co mlCCtive tissuc$Urro utld ing th c ccn u a,l •cin with.in th c livcr lobu les	Pointgrid
Vol.um e fra ct ion o f rotal (l()l\llCCci\e ti$$ue	Vv (CT)	Sumofthe $l'v$(ICT) + l' v(PSCT) + Vv (POCT)	None (calculatcd from previous thrce parametcrs)

Fig. 2. Histological sampling and quantification of connective tissue. (A) Fields of view (FOVs) sampled, captured and used for quantification of connective tissue. For interlobular connective tissue (ICT), three FOVs observed with X10 objectives were sampled (blad: dotted rectangles, one FOV = 870 X 653 μm). For perisinusoidal connective tissue (PSCT) and pericentral connective tissue (PCCT), four FOVs with x20 objectives were used (>.:DCM' rectangle C'$, óze o fF O V = 43S x 326μm). The first FOV was poorioned randomly, starting from the top left corner of the section and further FOVs were placed equidistantly using a micr0900pe stage micrometer. The FOVs photographed from this systematic uniform sampling pattern are marked on the scheme with check mark. The hepatic capsule (outlined red) was omitted from the quantification. (B) Stereological point grid used for quantification of CT. Points hitting the ICT are highlighted in red. (C) Stereological point grid used for quantification of PSCT (highlighted in blad:) and PCCT (highlighted in red). Detection of collagen using aniline blue staining counterstaining with nuclear fast red. Bars, 1 mm (A), 200μm (B), 100μm (C).

the reference space. We did not observe any differential shrinkage between the Cf and the liver parenchyma.

Statistical Analys-i,

The study was based on the quantification of 2,905 micrographs sampled from 415 histological tissue blocks. The Statistica Base 11 software package (Statt• Soft, Inc., Tulsa, Oklahoma, USA) was used for the statistical analysis. The Shapiro- Wilk's W-test for normality showed that some of the data were not distributed normally. Therefore, we used non• parametric tests for further analysis. To assess differences between the male and female animals, among the six liver lobes, the differences between the different RO ls, the differences between V_{17} (P SC T) and V_{17} (P CC T) , the Kruskal-Wallis ANOVA and the Mann-Whitney U-test were used. As an example of the use of our data, the possible sample size calculation for the porcine liver is shown: a power analysis (C how *et al.,* 2008) was performed to illustrate the sample size needed to detect the expected relative change in the fraction of C T in various hepatic lobes and ROls .

Coe.ffitimt oj Error

Estimation of the volume fraction of C T and the size of hepatic lobules was based on two tissue sections per tissue block. However, the values of these parameters might differ between individual histological sections. Therefore, we assessed the volume fraction of CT and the size of hepatic lobules in series of 12 equidistant sections in four randomly selected tissue samples. To provide recommendations for sampling the sections, the variation quantified in the serial sections was estimated using the moving average of the CT fraction and the coefficient of error (CE) (Gunderson and Jensen, 1987).

Results

There was considerable variability in the Cf fraction at all sampling levels of the study: between sexes, among individual animals, liver lobes and ROls. The mean fraction of interlobular CT was greater in males than in females *(P* <0.001) (Fig. 3A). In males, the mean fraction of interlobular Cf was 4.7 ± 2 .4% (mean ± SD; Fig. 3B) and ranged from 0% to 11.4%. In females, the mean fraction of interlobular Cf was 3.6 ± 2.2% (Fig. 3C) and ranged from 0% to 12.3%. The mean fraction of intralobular (i.e. perisinusoidal summed with pericentral) C T was <0.2% both in males and in females; the V'' (PSCT) was 0.003 ± 0.02% in males and 0.002 ± 0.02% in
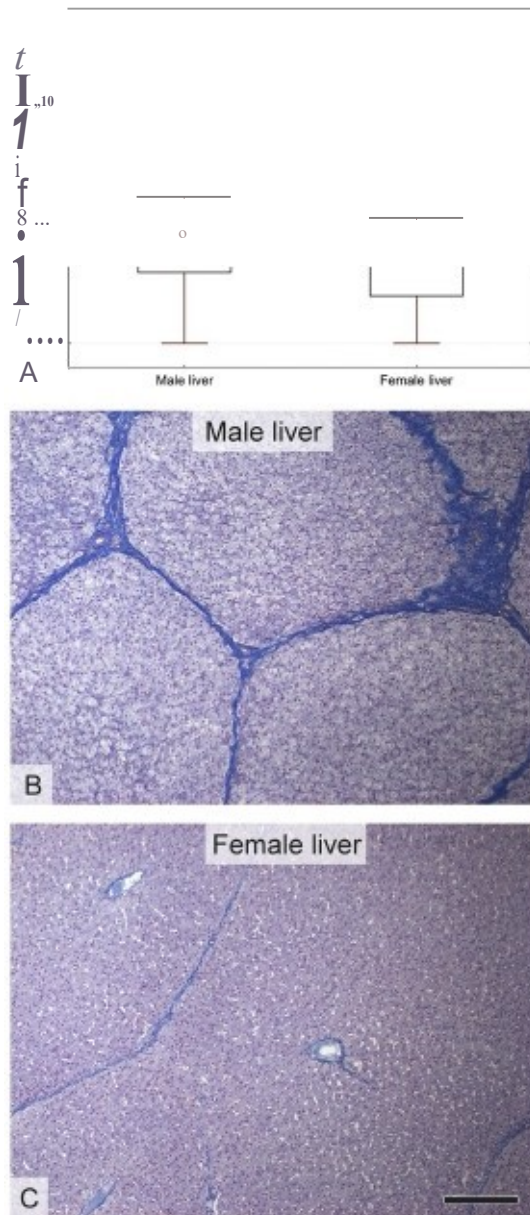
Fig. 3. Fraction of interlobular connective tissue in male versus female animals. **(A)** In the male porcine liver the volume fraction of connective tissue (mean = 4.7%) was significantly higher (• • • *p* <0.00 1, Mann-Whitney U-test) when compared with female liver (mean= 3.6%). The statistical findings are illustrated with histological pictures taken randomly from the paracaval region at interest of the lateral lobe in: **(B)** a male pig and **(C)** a female pig. Aniline blue and nuclear fast red, Bar, 200 μm (B).

females and the V_r (PCCT) was 0.03 ± 0.1% in males and 0.04 ± 0.1 in females. Unlike in females, the fraction of pericentral CT in males was greater than the fraction of the perisinusoidal CT (P <0.0 1). T he interlobular CT represented > 99.9% of the total hepatic CT and their fractions were mutually highly correlated (Spearman r = 0.998, P <0.05). The complete stereological results for all samples are provided in Supplementary Table 1.

Volume Fractions of Connective Tissue

In male pigs, the only significant differences in the fraction of interlobular CT among hepatic lobes were observed between the quadrate and left media) lobe *(P* <0.05) and between the quadrate and right media) lobe *(P* <0.05) (Fig. 4A). The paracaval ROI was the region with the smallest volume fraction of interlobular CT (3.9 ± 2.3%, *P* <0.01). The paraportal ROI (4.9 ± 2.0) and the periphery) ROI (5.2 ± 2.5) had a greater fraction of CT (Fig. 4B). Other differences in interlobular CT among the ROIs were not significant . The same differences were detected when the total CT fraction was analysed. The fractions of both the perisinusoidal and the pericentral CT were negligible (<0.2%).

In female pigs, the volume fractions of interlobular CT differed between the left media) lobe and the caudate lobe *(P* <0.05) and the quadrate lobe *(P* <0.05), and between the right media) lobe and the caudate lobe *(P* <0.05; **Fig.** 4A). The volume fraction of interlobular CT was greater in the peripheral ROI (4.1 ± 2.4) than in the paracaval ROI (3.1 ± 2.0) *(P* <0.05; Fig. 4B). The fractions of both the perisinusoidal and the pericentral) CT were negligible (<0.2%).

Qualitative Findings

Histological images illustrating the typical findings in liver lobes are shown in Supplementary Fig. 1 and Fig. 5. Apart from the typical histological findings of regularly arranged lobules (Fíg. 5A), several less common histological characteristics were found. In the paracaval region of the caudate lobe, the hepatocytes within the lobules below the fibrous capsule showed shape elongation (Fig. 5B), which currendy cannot be distinguished from an artifact. In the periphery) region of the left media) lobe, more (or branching) centr,1) veins were found (Fig. 5C). In tbe peripberal region of tbe left mediaI lobe , incompletely separated lobules (Fig. 5) were found.

Coefftcients of Errors in Serial Sections

Analysis of four series of 12 serial sections revealed the relationship between the CEs and the number of histological sections selected for the analysis (Table 2). The data suggested that using four sections for quantification of both the fraction of CT in liver instead of one section would have reduced the sampling error to an acceptable value of approximately 0.1l.
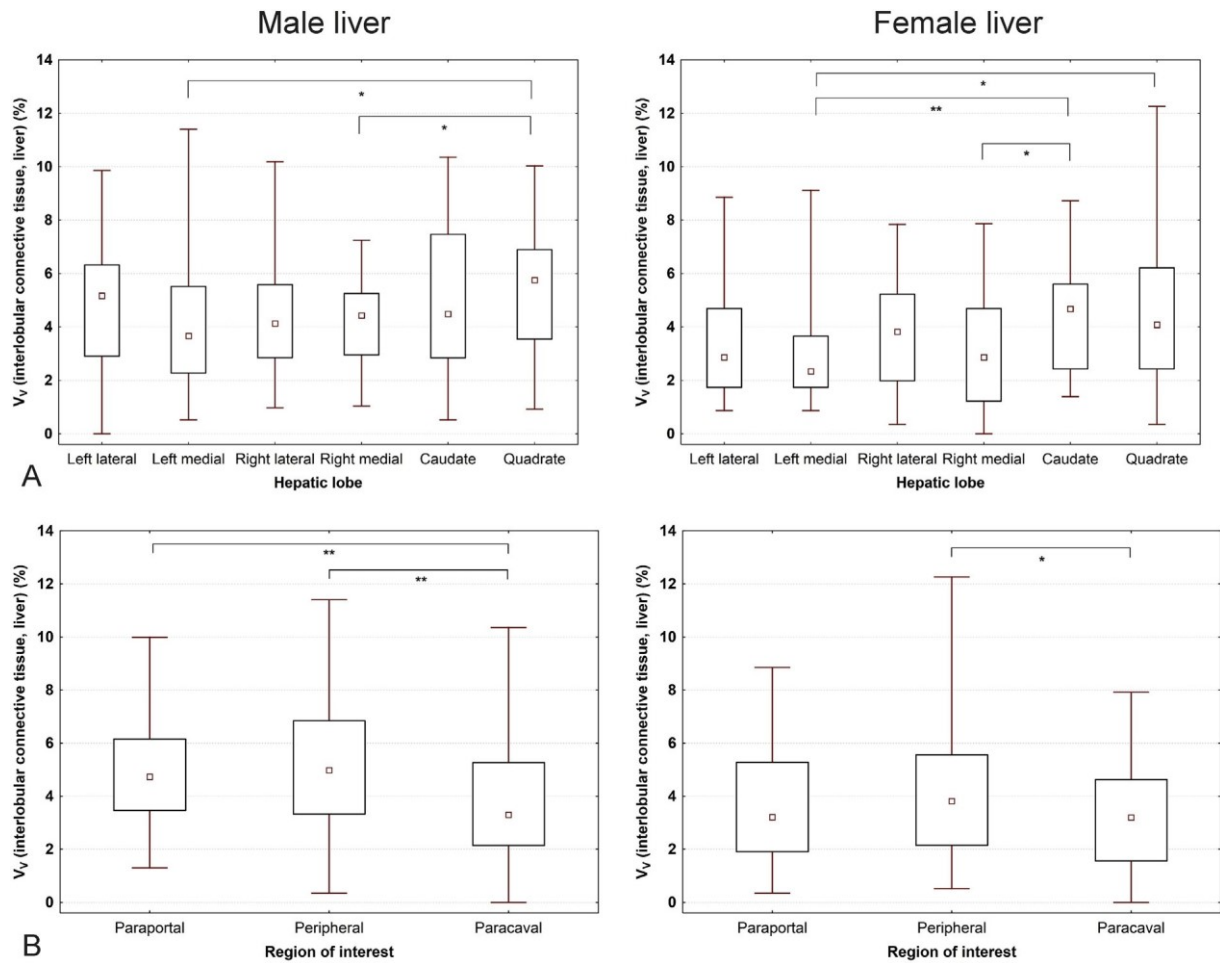
Fig. 4. Fraction of interlobular connective tissue (ICT) in male (on the left) and female (on the right) pig livers. The data are presented in six porcine hepatic lobes (A) and in regions of interest (ROIs) with respect to their proximity to the liver vasculature (B). (A) In males (left), the fraction of ICT was greater in the quadrate lobe than in the left medial and right medial lobes. The left medial lobe contained the smallest fraction of ICT (median 3.67%), the quadrate lobe contained the largest fraction of ICT (median 5.75%). In females (right), the fraction of ICT was greater in the caudate lobe than in the left medial and right medial lobes and was smaller in the left medial lobe than in the quadrate lobe. The left medial lobe contained the smallest fraction of ICT (median 2.34%), the caudate lobe contained the largest fraction of ICT (median 4.67%). (B) In males (left), the fraction of ICT was the smallest in the paracaval ROI (median 3.30%). In females (right), the fraction of ICT was smaller in the paracaval ROI than in the peripheral ROI. Data are displayed as median values with boxes spanning the limits of the rst and third quartiles and whiskers spanning the minimum and maximum values for each group. Signi cant differences (*$P<0.05$, **$P<0.01$, ***$P<0.001$) identi ed by the Manne Whitney U-test are presented. See Supplementary Table 1 for the source data.

Calculating the Sample Size for Histological Evaluation of Hepatic Fibrosis in Pigs

The number of histological samples needed to detect an expected relative increase in the CT fraction in various hepatic lobes and ROIs is shown in Fig. 6. The number of tissue samples needed for comparison rapidly decreased with an increasing expected change. This phenomenon applied especially to lobes with a relatively high (e.g. quadrate lobe, Fig. 6B) or intermediate (e.g. left lateral lobe, Fig. 6A) intralobar variation among the ROIs. We also simulated an experiment with pooled lobes (e.g. for cases where the anatomical origin of the histological samples from the lobes would not be known, but the ROIs would be known) (Fig. 6C). The case with unknown ROIs, but known lobes, is shown in Fig. 6D. Source data for all lobes and ROIs are provided in Supplementary Tables 2 and 3.

Discussion

The detected variability in the volume fraction of CT between sexes can be attributed to the effect of sex hormones, especially oestrogen that has a protective effect against brogenesis (Yang *et al.,* 2014).
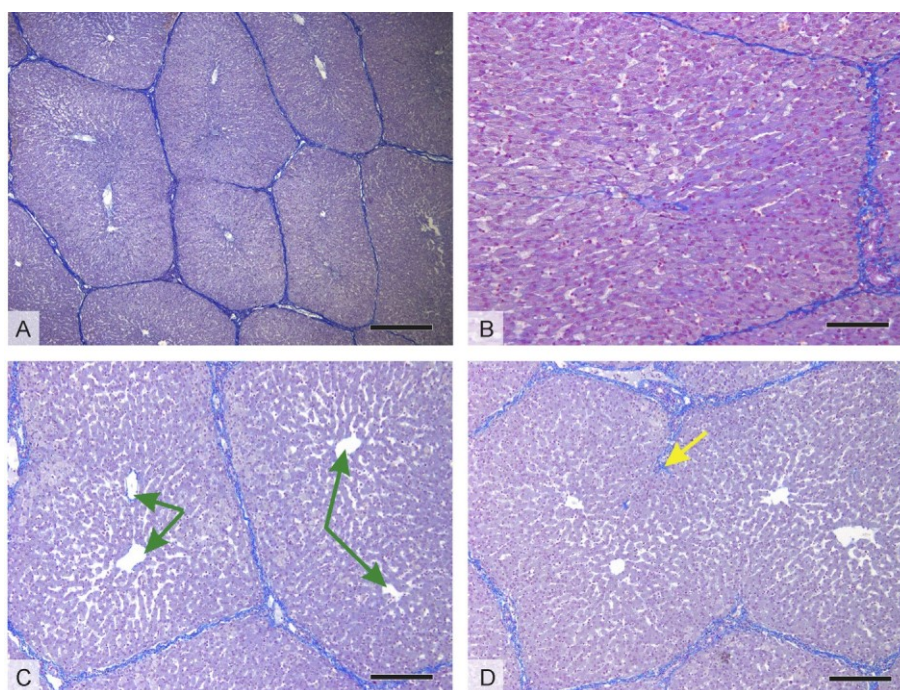
Fig. 5. Illustration of qualitative histological ndings in the porcine liver. (A) Typical histological ndings of regularly arranged lobules. (B) In the paracaval region of the caudate lobe, the hepatocytes within the lobules adjacent to the brous capsule are attened and elongated. The possibility that this could be an artifact cannot be excluded. (C) In the peripheral region of the left medial lobe, more (or branching) central veins are found (green arrows). (D) In the peripheral region of the left medial lobe, incompletely separated pseudolobules (yellow arrow) are found. Aniline blue and nuclear fast red staining. Bars, 500 mm (A), 100 mm (B), 200 mm (C, D).

Table 2   Analysis
of the coefficient of error (CE) estimating the amount of sampling error between the serial histological sections in four
randomly selected samples of the porcine liver

Number of serial sections sampled	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
CE (%)	21.3	14.6	11.3	8.70	8.70	7.49	6.66	5.85	5.47

For each of the four samples the volume fraction of interlobular CT was analysed. The mean CE (Gundersen and Jensen, 1987) decreases with increasing numbers of analysed sections.

Therefore, in pre-menopausal women (Yang *et al.*, 2014), as well as in female Wistar rats (Marcos and Correia-Gomes, 2015), the amounts of CT in the liver tend to reach lower values relative to male counterparts. The sex-related differences in rats included mainly increased perisinusoidal brous tissue deposition in males with ageing (Marcos and Correia-Gomes, 2015). Our results adds to the evidence for a greater volume fraction of CT in male porcine livers when compared with their female counterparts.

To our best of our knowledge, no data explaining the differences in variable fractions of CT in various ROIs and lobes have been published. The only known data show preferential distribution of types of collagen, namely: type I and type III collagen co-localize in the same bre bundles both outside the hepatic lobule (i.e. in the portal tracts) and within the hepatic lobule (Mak *et al.*, 2012), while collagen IV is found only within the hepatic lobule (Mak *et al.*, 2013). Our present ndings on the greater fraction of CT in peripheral ROIs could be answered by further studies: (1) mapping the distribution of brogenic cells, and (2) mapping quantitatively the local differences in the hepatic microvascular bed (Zhang *et al.*, 2015). Although brogenic cells within the liver have been studied (Zhao *et al.*, 2016; Kisseleva, 2017), their differential distribution within liver compartments has not yet been mapped. The greater content of CT in peripheral ROIs found in our study could be explained by the proximity to mesothelial cells that count as liver brogenic cells (Lua *et al.*, 2015).

The fraction of CT in the normal porcine liver in our study was considerably higher than that in most other animals or in man. CT is hardly visible in the dog, goat, horse, cat, rat and man; however, CT is clearly visible in the guinea pig, hamster, sheep and
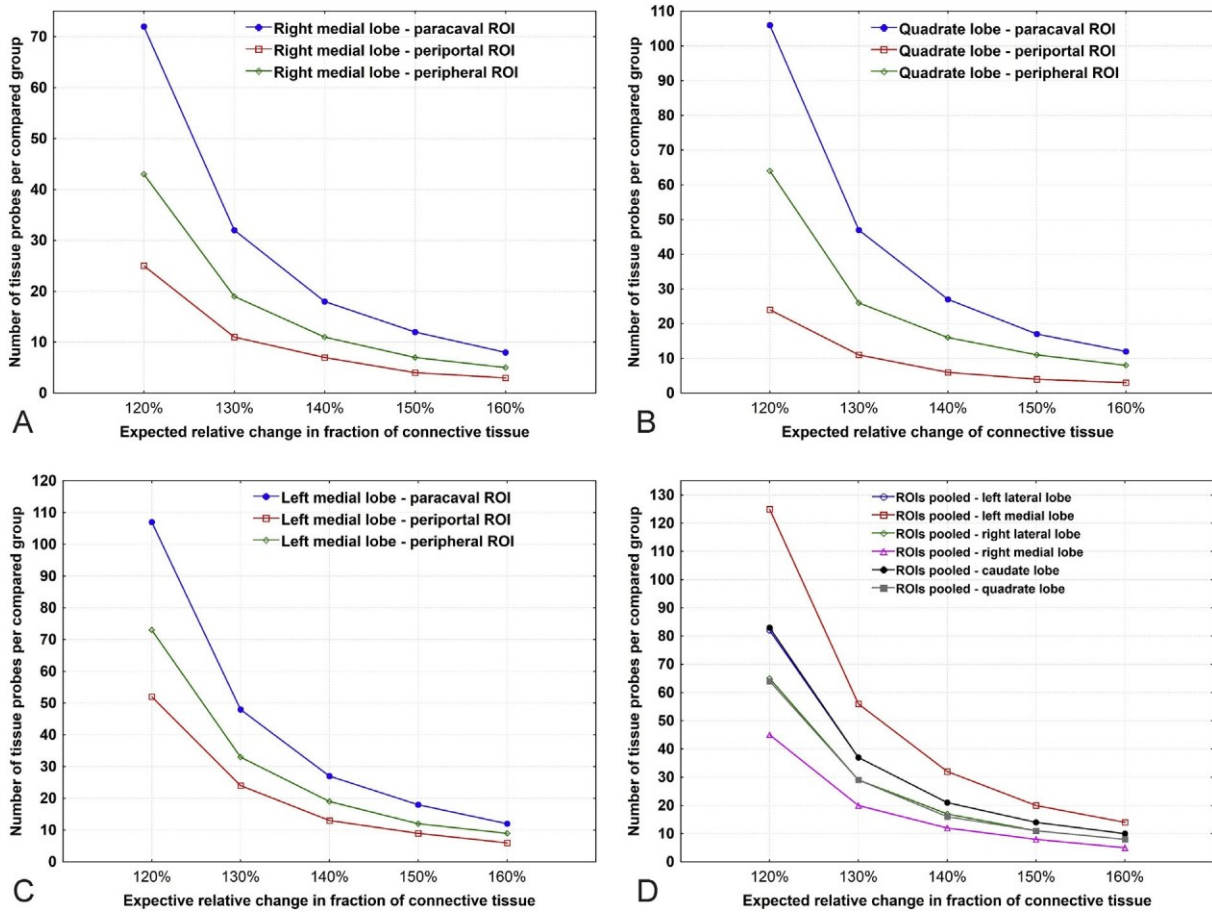
Fig. 6. Examples for calculating the sample size needed to detect a certain expected relative increase in the connective (CT) fraction in various hepatic lobes and ROIs in male pig liver. In each of the graphs, the x-axis shows the expected change in the CT fraction (i.e. an expected 30% increase in the fraction of CT corresponds to 130% on the x-axis). The y-axis shows the number of tissue blocks needed from a group under study to detect the expected change calculated according to Chow *et al.* (2008). The number of tissue blocks needed for comparison rapidly decreases with an increasing expected change. (A) In male porcine liver, in the right medial lobe, the greatest coefficients of intralobar variation were found (Supplementary Table 2). Detecting a 30% increase in CT would require, for example, 40 tissue blocks in the paraportal regions and 61 blocks in the paracaval regions. (B) The quadrate lobe had an intermediate intralobar variability of CT (see the coefficients of variation in Supplementary Table 2), for example, detecting a 30% increase in CT would require 11 tissue blocks in the paraportal regions and 47 tissue samples in the paracaval regions. (C) When all of the lobes are pooled, detection of a 30% increase in the fraction of CT requires at least 24 tissue blocks (paraportal ROI) in each group under study. (D) When comparing the variability inside the lobes by pooling their paracaval, paraportal and peripheral regions, detecting the same increase in the CT fraction inside the right medial lobe (pink line) would require fewer samples than detecting the same changes in the left medial lobe (red line). This observation shows the importance of respecting the anatomical lobes and ROIs when sampling the porcine liver for histological analyses. Source data are provided for male and female animals separately in Supplementary Tables 2 and 3.

pig. Studies using transmission electron microscopy (Hosoyamada *et al.*, 2000) revealed detailed patterns of length distribution of collagen fibrils. The mean amount of CT in the porcine liver is approximately double that found in the rat liver (Marcos and Correia-Gomes, 2015). Other numerical data are sparse, both for small and large animals. Moreover, few studies in animals have used an unbiased stereological design (Zaitoun *et al.*, 1998; Marcos *et al.*, 2012 and Supplementary Table 4). The importance of a quantitative approach proved to be useful: in

our study, quantification revealed several differences, which were scarcely visible using routine qualitative histology (Supplementary Fig. 1).

For studies of human liver diseases, such as chronic hepatitis C infection (Besusparis *et al.*, 2014), alcoholic liver disease and non-alcoholic liver disease (Sakhuja, 2014), primary biliary cirrhosis (Working Subgroup for Clinical Practice Guidelines for Primary Biliary Cirrhosis, 2014) or sclerosing cholangitis (de Vries *et al.*, 2015), using porcine liver as an experimental model, it should be noted that the

norma) amounts of CT in porcine liver already resemble fibrosis in buman livers (Saxena, 2011). Moreover, tbe distribution ofinterlobular and intralobular CT in pigs and in man bas diffi:rent patterns .

Tbe use of our data asa basisfor tbe design of the following studies is explained in Fig. 6 as fo)Jo,..-,s. In tbe male porcine liver, tbe mean fr,1ction ofinterlobular CT in rigbt media) lobes is $4.27 \pm 1.62\%$; tberefore, ifwe expect, lor example, a 30% increase in CT in tbe porcine liver (to 5,99%), tben we sbould compare at least 11 tissue blocks in eacb of the compared groups (power $=$ 0.8; significance level $=$ 0.05); bowever, tbis is only true wben we know the exact position of tbe tissue blocks witb the respect to tbe liver lobe and to tbe ROL If we know only tbe lobe of origin of tbe tissue blocks, tben we sbould compare at least 20 tissue blocks in eacb of tbe compared groups. lf tbe positions of the tissue blocks forcomparison are unknown (i.e. all tbe lobes would be pooled, e.g. wben assessing arcbival sam• ples), the variability between the lobes, the ROls and between tbe individuals would affect the study design in such a way thatat least 36tis;ue blocks foreacb oftbe compared groupssbould besampled to detect tbe samedegreeoffibrosis. Wben tbe sampling scbeme of experiments using the porcine liver re8ects tbe differences between the lobes and tbe ROls, the **same quantitative information may result from a** smaller number of samples and therefore provide a **more efficient comparison to the situation in which** tbe tissue blocks are barvested in a purelyr ,1ndom **manner.**

Tbe population of animals used in tbisstudy wasof **the minimum size allowed for use of non-parametr.ic** statistical tests *(n* > 6). l n large animal models, such as tbe pig, experiments are typically done using relatively small numbersofanimals according to tbe priaciples of the '3Rs' (replacement, reduction and refinement; Emerson, 201O) . Tbis strategy suffe.-s from an increased variability of results caused by interindividual differences, but tbe researcbers are simulated to follow large biological effects that are observable even in small study groups. Since our study was performed on piglets aged 9- 15 week:s, an ontogenetic perspective sbould also be considered in future studies. Age-related differences are to be ex• pected. Altbougb mecbanisms of fibrogenesis duri ng liver development bave been studied (Lepreux and Desmouliere, 2015), to tbe best of our knowledge, no study guantif)'Íng tbe amount of CT during liver development bas been conducted to date.

ln conclusion, for planning experiments involving tbe bistological quantilication of liver fibrosis and requiring comparison between tbe liver lobes, these data facilitate tbe power analysis for the sample size needed to detect tbe expected relative increase or decrease in tbe fraction of CT .

Sup plen1entar y Da ta

Supplementary data related to this article can be found at https:{{doi.o rg/10.1016/j.jcpa.2018.05.004.

Re ferences

Anstee QM, Goldin RD (2006) Mouse models in nonalcoholic fuuyliver diseaseandsteatobepatitis research. *!nUrntJtúmal Joumal oj Experimmtal Pat!toÚ!gy,* **8 7,** 1- 16.

Arkadopoulos N, Dellerevos G, Nastos C, Papalois A, Kalimeris K *et al.* (20 li) Development of a porcine modelofpost-hepatecton,yliverlailure.*JoumalojSu,gi, fAlRtstardi,* 170, e233- e242.

Avritscher R, Wright KC, Javadi S, Udiamandůl R, Gupta S *et al.* (2011) Development of a large animal model ofcirrhosis and porta! hypertension usinghepatic **transarterial emboliz.ation: a study in swine.** *Journal oj VaJ>Cula, and lnu,utntiímal Radwlogy,* 22, 1329- 1334.

Baus KPMD, Lud,,ig JMD (1995) Chronic hepatitis: an updateon terminologyand reporting.*Joumaloj SurgifAl Pat!tOÚ!f!.J,* **1 9 ,** 14()9- 1417.

Bedossa **P,** Poynard T (1996) Analgoridim for diegrading **of act.ivity in chronic hepatitis C.** *Htpatt>logy,* **24,** 289- 293.

Besusparis J, Jokubauskiene S, Plancoulaine B, HerlinP, Laurinaviciene A *et al.* (2014) Quantification accuracy of liver fibrosis by in-vivoelastography and digital image analysis of liver biopsy histodien,istry. *AnalytifAl Cdlula, Patňology,* 2014, e3 17635.

Boykov Y, JoUy M-P (2000) lnteractive organ segmentation usinggraph cuts. ln: *Mt.difAl Image Computing and Compu1,t,-assi.,1,td fn/,tfutntwn - M ICCAI 2000,* SL Delp, **L Scou, AM DiGoia, BJarama:z, F.ds., Springer, Bertin,** pp. 276- 286.

BruhaJ, VyciLal O, Tonar Z, Mirka H, Haidingerova L *et a/,.* (20 15) Monoclonal anŭbody againsLLransforming **g rowŭ1 factor beta I does not influence liver regenera- tion aft.er resection in large animal experiments.** *ln Vivo,* 29, 327- 34-0.

Brum EM, J anney CG, D i Bisceglie AM, Ne usdiwander-Te<ri BA, Bacon BR (1999) NonalooholicsLea LOhepaŭ -Lis:a proposal lorgradingands<agingdie lŭstological le-sions. *Ammllm Joumaloj Gastromurology,* 94, 2467 - 2474.

Budai A, Fulop A, Hahn O, Onody P, Kovacs T *tt a/,.* (20 17) A1ŭmal models for associaŭng tiver parŭŭ on and porLal vein ligaŭon for sLaged hepaLecLomy (ALPPS): aclŭevements a nd fuwre perspectives *Euro- fJMn ,Su gil;al &s,ardi,* **58,** 140--157.

Chen XL, Chen T -W, Zhang X-M, Li Z-L, Li H *et a/,.* (20 13) Spleen magneŭc resonance diffusion-weiglned **imagingforquantitativestaginghepaticfibrosisin** milŭ-awre pigs: an iniLia l st u d y . *Ht(Jaw/ogy &SUJtth,* **43 ,** 1231- 124-0.

Chow S-C, Wang H, Shao J (2008) *SampltSiztCaltulatwns in Clinital &s,ardi,* 2"" EdiL. Chapman and HaU/C R C **B iostatistics Series, Boe.a Ra ton, pp . 49-82.**

Croome KP, Mao SA, Glorioso J M, Krishna M, Nyberg SL *tt al.* (2015) CharacLerizaLion of a porcine **model forassociatingliverpartition and portaI vein liga-** Lionfor a s<aged hepa Lect on,y. *Jouma/,ojt!tt!nUrntJtwnal Ht fJtlto PanaMwBiliaryAJ:totiatwn,* 17, 1130- 1136.

de Vries EMG, Verheij J, Hubscher SG, Leeftang MMG, Boonstra K *tt al.* (2015) Applica biliLy and prognosŭc **value oflŭstologicscoringsystems in primary sclerosing** cholangiLis. *Jouma/, oj Ht(Jawlogy,* 63, 1212- 1219.

Eberlova L, Liska V, Mirka H, Gregor T, Tonar Z *tt al.* (2016) Porcineliver vascular beŭl in Biodur E20 corro-sioncasts. *FoliaMo,pho/agita,* 75, 15'1- 161.

Eberlova L, Liska V, Mirka H, Tonar Z, Haviar S *tt al.* (2017) The use of porcine oorrosion casts for Leaclŭng hum ru, a na, on,y. *Amalsoj Anawmy,* 213, 69- 77.

Emerson M (2010) Refinemem, reducLion a nd replace-**ment approad1es to in-vivo cardiovascular rese.a rch .** *BritiJh Jouma/, ojP/ramu, eo/ogy,* **161,** 749- 754.

Fakhoury-Sayegh N, T rak-Smayra V, Khazzaka A, Esseily F, Obeid *Ott al.* (2015) CharacLer isŭcs of non-**alcoholic fauy liver d ease induced in w tar rats** followingfour diflerem diets. *}{utriti,m RtsMfdt and P,actIl:t,* 9, 350- 357.

Forbes SJ, Parola M (20li) Liver fibroge,ŭcceUs. *B <rtPt ac-tIl:t & &$U1,di Clinital GO$b'omurology,* 25, 207 - 217.

George] , Per a N, P hung N, L ed er cq I, Yun Hou J *tt a/,.* (2003) Lipid peroxidaŭon, sLeDaLe ceU activ aŭon a nd **hepatic fibrogenes in a rat model of**chronic steatohe-paŭŭs. *Journal oj Ht(latology,* 39 , 756- 764.

Ghodsizad A, Faby BN, Wad awczyk S, LiedLke S, Berjon J MG *tt al.* (2012) PorLal applicaLion of hum,ru **unrestrictedsomaticstem cells tosupporthepatic regen-eration aft.er portal embolization and tumor surgery.** *ASA/0 J oumal,* **58,** 255- 261.

Gnurzmann DM, MedielJ , SchmiLz A, Kohler K, KroneD *tt a/,.* (2015) Evaluaŭon of Lhe plasmaLic a nd paren - **chym a l elution kinetics of two differem irinotecan-**

loaded drug-eluŭng embolie, in a pig model. *J oumal oj Va,tula,and lntn'vtntwnal Radú,/agy,* 26, 746- 754.

Gohlke F, Lohse AW, Dienes H P, Lohr H, Marker-Hermann E *tt al.* (1996) Evidence for an overlap syn-**drome ofautoinmrnne hepatitis and primarysclerosing** cholangiLis. *Journal oj Ht(Jawlogy,* **24,** 699- 705.

Gun dersen HJ G, J ensen EB (1987) Tiie efficiency **of**sys-**tematicsampling instereology and its prediction.** *Jout-nal oj Mitrom(Jy,* 147, 229- 263.

Hirschfield GM, Ka rlsen TH , Lindor KD , Adams OH (2013) Primary sclerosing diolru1giŭs . *Lanttt,* 38 2, 1587- 1599.

Hosoyamada V, Kurihara H, Sakai T (2000) Ultraslruc -wral localisaŭon ruid sizedisLribuŭon **of**coUagen fibrils **in Gl son's sheaŭ1 of**rat liver: **implicationsfor mechan-**icalenvironmemand possibleproducingceUs. *Jouma/,oj Anato"!)',* **196,** 327- 340.

Hu ruig SV, Abdelsalam ME, Harmoush S, Ensor J E, CheLLaJ A (2014) Evaluaŭon ofliverfibrosisand hepaŭc **venous pressure gradient wiŭ1 MR elastography in a** novel swine model ofcirrhosis. *J oumal oj Mogntti,; Rtso-nanr:t1maging,* 39, 590- 597.

Hu ruig V, Adams LA, J oseph J , Bulsara MK, J ef&ey GP (20 17) The a bility of Hepascore Lo predicL liver fibrosis **in chro1ŭc liver d ease: a meta-analys .** *Liver lnU:rna-twnal,* 37, 121- 131.

lshak K, BapLisLa A, Biruid i iL, CaUea F, De Groo Le J *tt al.* (1995) HisLological grading ands<aging ofchro,ŭc hep-aŭŭs. *J oumal oj Ht fJtlto/agy,* 22, 696- 699.

J iří k M, Barl oš M, Tomášek P, Malečková A, Kural T *tt al.* (2018) GeneraLing sLrui dar dized image dala lor LesLing **a nd ca librating quantification of volumes, surfaces,** lengllis, and objecL counLS in fibrous and porous maLe-rials using X-ray microLomography. *Mi,;,oseof!Y RtsM,di T tdmiquts,* **8 1,** 551- 568.

J una Las KL, T onar Z, Kubíková T, Liška V, Pálek R *ttal.* (2017) SLere ological analysis ofsize and de,isiLy of hep a -**tocytes in the porcine liver.** *Jouma/. oj Anato"!Y,* **2 3 0 ,** 575 - 588.

Ka wamura V, Ikeda K, Fukuslŭna T, Hara T, Hosaka T *ttal.* (2014) PotenLial ofa no-Loud i pincer ablaLion pro-**cedure for small hepatocellular carcinoma ŭ1at uses a mullipolar radio&equency ablation system: an experi-**memal animalswdy. *Ht(Jaw/ogy &SUJfdt,* **44 ,** I23'1- 124-0.

Kisseleva T (2017) Tiie origin offibroge,ŭc myofibroblasts in fibroŭc liver. *Ht(Jawlogy,* **65,** 1039- 1043.

Kleiner DE, Brum EM, V,ru NaLLa M, Behling C, Comos MJ *tt al.* (2005) Design a nd valida ŭonofa lŭsto-logical scoringsySLen, lor non-alcoholic faLty liver dis-ease. *Ht(Jawlogy,* **41,** 13 13- 1321.

Ko mine M, Kawasako K, Okamoto M, Matsuda K, Hirayama K *tt al.* (2008) HisLOpad i ological disŭncŭon and evaluaLion **of** biliary ruid peribilia ry cysts in pig liver. *J ouma/, ojCompa,ativt Patňolgy,* 139, 202- 207.

Ku biková T, Kochová P, Brázdil] , Špa Len ka J , BurkerLJ *tt al.* (2017) Tiie con,posiŭon and bion,echrui ical prop-**erties of human cryopreserved aortas, pulmonary trunks, and aortic and pulmonary cusps.** *Annalsof Ana-t omy,* 212 , 17- 26.

L<Oe YH, Ha V, Chae C (20 IO) Expression of interfer,oc - alpha and Mx protein in d,e tiversofpigsexperimentally infected wiǔ1swine hepatitis E virus. *Joumal o/Compat.ative Patltoú,gy,* **142 ,** 187- 192.

Lee KS, Santagostino SF, Li D, Ramjit A, Serrano K *tt al.* (2017) Cadieter-directed intraponal detivery of endo- d,elial ceU therapy **for** tiver regeneration: a feasibity s111dy in a large ruǔmal model of cirrhosis. *Raáú>Ú>!{J,* 28 5, 114- 123 .

Leflcowitd> JH (2007) Liver biopsy assessment in dironic hepatitis. *Ardtiw oj Mtdi(JJ[lusMrch,* 38 , 634-643.

Lepreux S, Desmoutiere A (2015) Human tiver myofibro- **blasts during development and <lise.a.ses wiǔ1 a focus on** portaI (myo)fibroblasts.*Frontitrs in Pl!}'wlogy,* 6, 1- 8.

Lindor KD, Gershwin ME, Poupon R, Kaplan f, Bergasa NV *et al.* (2009) Primary bitiary cirrhosis. *He atology,* 5 0 , 29 1- 308 .

Lossi L, D' Angelo L, De Girolamo P, Meriglǔ A (2016) **Anatomie.al feawres for an adequate choi.ce of** exped- mentalaiǔmal model in biomedicine: **li.** Smalllaboratory rodents, rabbit, and pig. *Anna/s oj Anato"!)',* **204 ,** 11- 28 .

Lua I, Li V, PappoeLS, Asalǔna **K** (2015) M yofibrob lastic **conversion and regeneration ofmesoǔ1elial cells in peri-** toneal and tiver fibrosis. *Anurimn Joumal oj Pat!toÚ>!{J,* **18 5,** 3258- 3273 .

Mad,ado MV,Mid,elotri GA, Xie G, de Almeida **TP,** Boursier *Jet al.* (20 15) Mouse models of diet-induced **non-alcoholic steatohepatitis reproduce ǔ1e het.erogene-** ity **of** d,e human disease. *PLoS One,* IO , eOI2799 1.

Mak KM, Chen LL, Lee TF (2013) Codistribution **of** collagen type IV and laminin in liver fibrosis ofelderly **cadavers: immunolǔstochemical marker of perisinusoi- dal basen1ent membrane formation.** *Anaf.ǔmi(.,(Jl &tǔrd,* 296, 953- 964.

Mak KM, Chu E, Lau KHV, Kwong AJ(2012) Liver fibrosis in elderlycadavers: localization ofcoUagen types **I,111, and IV, cx-s mooǔtmuscleacǔn, andelasLic fiber.s.** *Anaum,i,..l&txird,*295, 1159- 1167.

Marcos R, Correia-Gomes C (2015) Liver,ru d gender:are **ǔtere differences in fibrous Lissue before Lhe onseL of** fibrosis? *Htpavlogy,* **61 ,** 1093 - 1094 .

Marcos R, Monteiro RAF, Rod,a E (2012) The use **of** design-based stereology to evaluate volumes ,ru d **numbers in ǔteliver: a review wiǔt pract.icalguidelines.** *Journal oj Anawmy,* 220 , 303 - 3 17 .

Mattfeklt T, MaU G, Gharehbaghi H, Miiller P (1990) E.1timatiQn **of** $urface area and, I:ngt h wid, the o,ri:n ta - tor. *Joumal oj Miéroseopy,* **159 ,** 30 1- 3 17 .

Mouton PR (2011) *Unbiasd Sureo/ogy: A Contise Guide.* Johns HopkinsUniversity Press, Baltimore, pp. 55- 57.

Miihlfeld C, Nyengaard J R, MayhewT M (2010) A review of state-of-the-art stereology lor beuer quantitative 3D **morphology in cardiac research.** *Ca,di tnJa$1.Ula, Patǔology,* 19 , 6 5- 82 .

Nowarzky J , Knorr A, Hirdi-Dietrich C, Siegting A, Volk H-D *et al.* (2013) Inactivated Orfvirus (Parapo"- **virus ovis) elicits anǔfibroǔc act.iviLy in modeb ofliver** fibrosis.*Hepatoú,gy Research,* 43 , 535- 546.

Nygárd JE, Mortensen KE, Hedegaard J , Conley LN, Bendixen C *et al.* (2015) 1issue remodeUing following **resecLion of porcine liver.** *Bi.oMM & suuth lnU:rnati.onal,* 201 5, 1- 10 .

P ru, asevid, MR, Meers GM, Linden MA, Bood, FW, Perfield J W *et al.* (2018) High-fut, lǔgh -fructose, lǔgh - d, olesterolfeeding ca usessevere NASH and cecal micro- **biota dysbiosis injuve1ǔle Ossabawswine.** *Ametitan] ou,- nal oj Physiǔ,gy. &dot:rino/ogy and Metabolism,* **314 ,** e78- e92 .

Peterson TC, Neumeister M (1996) Effect Qfpentoxifyltine in rat and swine models ofhepaticfibrosis: roleoffibro- **proliferaLion in its mechanism.** *l m.mu nophar!]llt0logy,* **31,** 183 - 193.

Sakhuja P (2014) Pathologyofalcoholic tiver disease, can it **be ditrerentiated from nonalcoholic steatohepatiLis?** *World Joumal oj Gastroenurology,* 20 , 16474 - 16479 .

Saxena R (20 li) *Prattil:al Htpati, Patlto/ogy: A Diagnostil: A(>proach.* Saunders, Plǔladelplǔa, pp. 203- 213.

Scheuer PJ (1991) Classificationofdironicviral hepatitis: a need lor reassessment. *Joumal oj Hepatd>gy,* 13 , 372- 374 .

St ruid ish RA, Cholongitas E, Dlǔllon A, Burroughs AK, Dlǔllon AP (2006) An appraisal ofthehistopat hological assessment ofliver fibrosis. *Gut,* **55,** 569- 578.

Stasi C, Milani S (2016) Non-invasive assessment **of** liver **fibrosis: between prediction/prevenǔon of outcomes** ,rud cost-elfectiveness.*Worlá Journal oj Gastroenuroú,gy,* 22 , 17 11 - 1720 .

Takal,ashi V, Fukusato T (2014) Histopat hology of non- alcohoticfatty liverdisease/nonalcohoticsteatol>epatitis. *World Joumal oj Gastroenurology,* 20 , 155 39 - 155 48.

TI,eise ND (2013) Histopad, ologyofalcoliolic tiver disease. *Clini,..l Liver Disease,* 2 , 64 - 6 7.

Tonar Z, Kubíková T, Prior C, Demjén E, Liška V *tt al.* (2015) Segmental and age differences in d,e elastin **network, collagen, and smoot.h muscle phenotype in ǔte wnica media ofǔteporcine aorta.** *Amrals of Anawmy,* 201 , 79- 90.

Vrtková I (2015) Genetic admixture analysis in Prestice black- piedpigs.*ArchivesojAnimalBrt.tding,*58, 115- 121.

Wang L, He F-L, Liu F-Q, Yue Z0 , Zhao H-W (2015) Establishment ofa hepatic cirrhosis and porta! hyper- ten-.sion model by hepatic arterial perfusion wiǔt 80% alcohol. *World]oumal ojGastroenuroú,gy,*21 , 95#- 955 3.

Watson AL, Carlson DF, Largaespada DA, Hackett PB, Fahrenkrug SC (2016) Engineered $winemodel$ of can- cer.,*F ontiers inGmttia,* **7, 78.**

Working Subgroup (Englishversi,oc) for Cli,ǔcal Practice Guidelines for Primary Biliary Cirrhosis (2014) Guide- **lines for the management of primary biliary cirrhosis.** *Htpato/ogy Research,* **44 ,** 7 1- 90 .

YangJO, Abdelmalek MF, Pang H, Guy CD, Smith AD *et al.* (2014) Gender and menopause impactseverity of **fibrosis antongpatients wiǔt non-alcoholic steatohepati-** ris.*Hepatoú,gy,* 59 , 1406- 1414.

Yi X, SongM, Yuan V, ZhangX, Chen W *eta/.* (2012) He- **paticstimulator subsLance alleviates toxin-induced and**

immune-mediated liver injury and fibrosis in rats. *Digestive Diseases and Sciences,* 57, 2079-2087.

Yin M, Glaser KJ, Manduca A, Mounajjed T, Mailů H (2017) Disringuishing between hepatic inflammation and fibrosis with magnetic resonance elastography. *Radiology, 284,* 694-705.

Zaitoun AAM, Awad S, Ukabam S, Makadisi S, Record C (2001) Quantitarive assessment of fibrosis and steatosis in liver biopsies from patients with chronic hepatit C. *Journal of Clinical Pathology,* **54,** 461-465.

Zaitoun AAM, Mardini H A, Record CO (1998) Stereology and morphonetry of steatos in human alcoholic (ALD) and non-alcoholic liver disease (NALD). *Acta Stereologica,* 17, 49-58.

Zhang JJ, Meng XK, Dong C, Qjao JL, Zhang RF *et al.* (2009) Development of a new ruminal model of liver cirrhos in swine *F.umptan SurgitAl Rr.stt1tdt.,* **42,** 35-39.

ZbangZ, Zhru1gF, Lu Y, ZhengS (2015) Updateon implication. sru1dmecbruůsm.s ofangiogenesisinliverfibros. *HepatoŮ!gy Resumh,* **45,** 162-178.

Zbao YL, Zhu R-T, Sun Y-L (2016) Epid1elial-mesenchymal transiůon in liver fibrosis. *BWm.t.ditAl &port.s,* **4,** 269-27+.

Zbou L, Chen T, Zhang X, Yang Z, Tang H *eta/.* (201+) **Liverdynamitcontrast-enhanced MR.I forstaging liver** fibrosis in a piglet model. *Joumal oj Magnetit &sonante lmaging,* 39, 872-878.

Příloha VII:

PÁLEK, R., V. LIŠKA, L. EBERLOVÁ, H. MÍRKA, M. SVOBODA, S. HAVIAR, M. EMINGR, O. BRZOŇ, P. **MIK** a V. TŘEŠKA, 2018. Experimental processing of corrosion casts of large animal organs. Rozhledy V Chirurgii: Mesicnik Ceskoslovenske Chirurgicke Spolecnosti. 97(5), 222–228. ISSN 0035-9351. Bez IF.

Experimentální příprava korozivních preparátů orgánů velkého zvířete

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So uhrn

úvod: Koro zivnI preparáty (KP) slouž k viru alizaá a hodnocení morfologie dutých struktur. **KP** s napl ným kapilárním retiJtěm umožňuj za po-
moá zobrazovacích metod Dskánl da t použitelných pro matematické modelování orgánovéperfuze Nárofnost ptfpravy KP stoupá s objemem
cévního rem tě. protoby touto metodou doposud zkoumány zejména malé živocišné druhy. cnem této studie bylo optimalizovatprotokoly
prípravykorozivnlchpreparátOrOznýchorgánOprasetedomádho, ato vzhledemkdostupnosti těchto orgánOavýznamuprasetedomácího v ex-
perimentálnímedicíně.

Metoda: Byly použity orgány Oátra,slezina, ledviny a tenké strevo) zdravého prasete domáclho. Celkem1O p rasat(6 samic),plemenopre!tické
ternostrakaté, hmotnost 35-45 kg.Orgánybyty vypreparoványd o systémového rem tě bytaplikovánheparin anásed byl cévní systémorgánu
propláchnut buď in sil u Oátra), nebo po vyjmuť z těla zvrrete Oedviny . slezina, tenké strevo) heparinizovaným fyziologickým roztokem.Veškerá
manipulaces vyjmutými orgánypak probIhala podvodnl hladinouzďOvodu prevence embolizacevzduchu do cévnlho retiJtě. Dalším krokem
byla intraarteriální (u jater i intraportálnQ aplikacepryskyfice Biodur E20·(Heidelberg.Německo).Po ztuhnuti pryskyrice byly odstraněny okolní
tká pomocí 15%roztoku KOH apotévymytyvodou. Objeméprepar.!ty bytyuchovávány v 70%denaturovaném alkohdu men!Ibytyvysu!Qny
mrazem. Hotové KP byly zkoumány pomocí stereonikroskopub ěžné vý"pot etn f tomografie (CT) mikro-cr. skenovací elektronové mikroskopie
(SEM)nebovysokorozli!ovacfho digitálnlho mikroskopu.

Výsledky: Dfky odběru orgánO za specifických podmínek,použití vhodnépryskyrice i metodiky nástřiku sepodarilo zlskat kvalitní **KP** jater,ledvin.
slezinya tenkého streva prasete domácıho. Možnost barvenípryskyřice zlepšıla makroskopic<ou pt ehlednostpreparáO. Bylo provedenoskeno-
vání pomocí běžného CT,které seukázalojako vhodná metodapro zkoumání preparátujater.Mikr T.SEM a vysokorozli!ovacl digitální mikro-
skopiepakpfinesly obrazynejdrobnějších struktur reti!t ě zkoumaných orgánO. I když seSEMpro kontrolu kvalityodlitkOještě stálejeví jakone-
zastupitelná, zdá se..že mOže být tásteálě nahrazena vysokorozfi.šovacím digitálním mikroskopem Mikro-CTumožnilo získándat o prostorovém
uspořádáníreti!t ě a datprobudoucısoftwarové modelováni orgánovéperfuzezkoumanýcorgánO.Vysoká kva ita zlskanýd preparáOumo žrila
jejich využití i vevýuce anatomie t lov ka.

Zóvr: Podafiloseoptimalizovatprotokol ptfpravyKPjater,led\nn,slezinya tenkéhosttevaprase.tedomácího. S pou.žitfm rOznýchzobrazovacích
modalitmohou být tytoKP využıty pro získánídatoprostorové architektonicecévníhore6ště.Tato datamohou být využıtapropttpravumatema
tickýchmodelO orgánové perfuze využitelnýchnapříkladpro optimalizaд orgánovýchresekcí.

Kllčov áslova:korozivní preparáty- kapilární reti!t ě- Biodur E20"- prasedomácl- animální model

Summary

Experlmentalprocesslng of corrosloncast s o:f la rgeanimal organs

R. Palek, V. Liska, L. Eberlova, H. Mirka, M. Svo boda, S. Hav ia r,M. Emingr,O. Brzon, P. Mik, V.Treska

Introduction: Corrosion casts(CCs) are used forthe visualization and assessment of hollow structures.*CCs* with filled capillariesenable (with
the help of imaging methods) to obta in data for mathematical organ perfusion modelling. As the process ing is more difficult in case of
organswith greater volume of the vasculature, mainly organs from small animals have been cast upto now. The aim of this study was to
opt imize the protocol of corrosion casting of different organs of pigi. Porcine organsare relatively easily accessible and frequently used in
experimenta l medicine.

Method: Organsfrom 1Ohe althy Prestice Black-Pied pigs(6 females, body weight 35-45 kg),*were* used in this study (liver,spleen,kidneys and
small intestine).The organswere dissecteJd heparin wasadministered into the systemic circulat ion and then the vascular bed of the
organswasflushed with heparinized saline either insltu (liver) oraftertheir removal (spleenJ kidney,small intestine).Ali handling wasdone unde
r the watersurface to prevent air embolization.The nextstepwas an intraarterial (in caseof the liveralso intraporta administration of Biodur
E20-(Heidelberg, Germany) resin. After hardening of the res in the organ tissue was dissolvedby 15%KOH and the specimen was rinsed with
tap water.Voluminous casts were stored in 70% denatu r,ed alcohol, the smaller oneswere lyophilized. Thecastswere assessed with
astereomicroscope,.computed and microcomputed tomography (CTand microCT),a scanning electron microscope (SEM)and high-re-
solution digital microscope (HROM).

Results: High-quality CCs of the po rcine liver, kidneys.spleen and small intestine werecreated owing to the sophisticatedorgan harvesting,.
the suitable resinandcastingprocedure. Macroscopicclaritywasimproved thanksto the possibilityofresindying.ScanningbyCTwasper-
forrned and showed to be a suitable method forthe livercast e.xamination. MicroCT,SEMand HROM produced images of the most detailed
structures of vascula r bed. Despite the fact thatSEM seems to be an irreplaceable method for CCsq uality control,itseemsthat this modality
could be partly replaced by HROM. MicroCTenabled to obta in data aboutthree-dimensional layoutof the vascularbed and data formathe-

matical modelling of organ perfusion. With regard to the quality of the CCs, they could also be used to teach human anatomy.

Conclusions: The protocol of the corrosion casting of the porcine liver, kidneys, spleen and small intestine CCs was optimized. Thanks to different imaging methods, the CCs can be used as a source of data on three-dimensional architecture of the vascular bed. These data can be used for mathematical modeling of organ perfusion which can be helpful for example for optimization of organ resections.

Key words: corrosion casts – microvasculature – Biodur E20® – domestic pig – animal model

ÚVOD

Korozivní preparáty (KP) slouží k vizualizaci a hodnocení morfologie dutých struktur. Jejich příprava spočívá ve vyplnění prostor polymerizující látkou a v následném odleptání okolních tkání. Vzniklý preparát je tedy odlitkem dutiny (lumina). Historie KP sahá až do středověku. Obvykle je za objevitele a průkopníka této metodiky považován Leonardo da Vinci, který na začátku 16. století vyplňoval hovězí mozkové komory a srdeční dutiny voskem, po jeho ztuhnutí však okolní tkáně odstraňoval mechanicky [1,2]. Nejednalo se tedy přímo o korozi (proces definovaný jako samovolné, postupné rozrušení hmoty vlivem chemické reakce s okolím), z tohoto hlediska je historie KP mnohem starší. V tzv. prekorozivním období např. již Alessandra Giliani (1307–1326), italská vědkyně a anatomka, jako první naplnila velké cévy lidského těla barevnou tvrdnoucí látkou, aby usnadnila jejich sledování. První skutečný korozivní preparát připravil nejspíš až holandský anatom Govert Bidloo (1649–1713), jenž naplnil dýchací cesty člověka roztaveným kovem a po jeho ztuhnutí odstranil okolní tkáň varem [3]. V následujících stoletích se anatomové snažili vylepšit jak aplikovaná média, tak techniku samotné aplikace nebo odstranění okolních tkání tak, aby získali co nejpřesnější odlitky zkoumaných struktur [4].

KP nacházejí své využití zejména při studiu cévního řečiště jednak normálních a jednak patologicky změněných orgánů [5]. Pro hodnocení cévního stromu je nutná dobrá náplň kapilárního řečiště [6].

Ke zkoumání KP jsou využívány různé zobrazovací techniky. V 70. letech minulého století byly publikovány první práce využívající skenovací elektronovou mikroskopii (SEM) pro diagnostiku různých struktur kapilár- ního řečiště [7]. Jeho dobrá náplň byla umožněna nově syntetizovanými akrylovými a epoxidovými pryskyřicemi. Pro zkoumání KP je poměrně nově využívána metoda mikroCT. Zdá se, že se zavedením vysokorozlišovací tomografie (mikroCT) prožívají KP opět svoji renesanci [8,9,10]. Ačkoli je SEM pro kontrolu kvality odlitků stále nezastupitelná, při použití RTG kontrastních pryskyřic nabízí mikroCT v kombinaci s využitím v klinické praxi běžně používaných softwarů zcela nové možnosti 3D zobrazení s rozlišením struktur na úrovni 2D histologických řezů [11]. CT skeny je však možné použít i pro potřeby modelování perfuze nebo kvantifikaci jednotlivých parametrů mikrocirkulačního řečiště (míra větvení, úhel odstupu cév atd.) [10,12]. Jako velmi nadějná zobrazovací technika se pro studium cévní morfologie založené na KP jeví i vysokorozlišovací digitální mikroskopy, které díky mobilnímu objektivu a pohybu v Z ose umožňují 3D rekonstrukce s možností zvětšení až 2000x, a to v rozli-

šení odpovídajícím možnostem SEM. Avšak na rozdíl od SEM můžeme s digitálním mikroskopem odečítat povrchové struktury do hloubky až 1 cm bez nutnosti řezání preparátu nebo následné fixace či pokovování vzorku. Digitální fotografie umožňují reálný barevný obraz, instalovaný software pak možnost 2D i 3D měření.
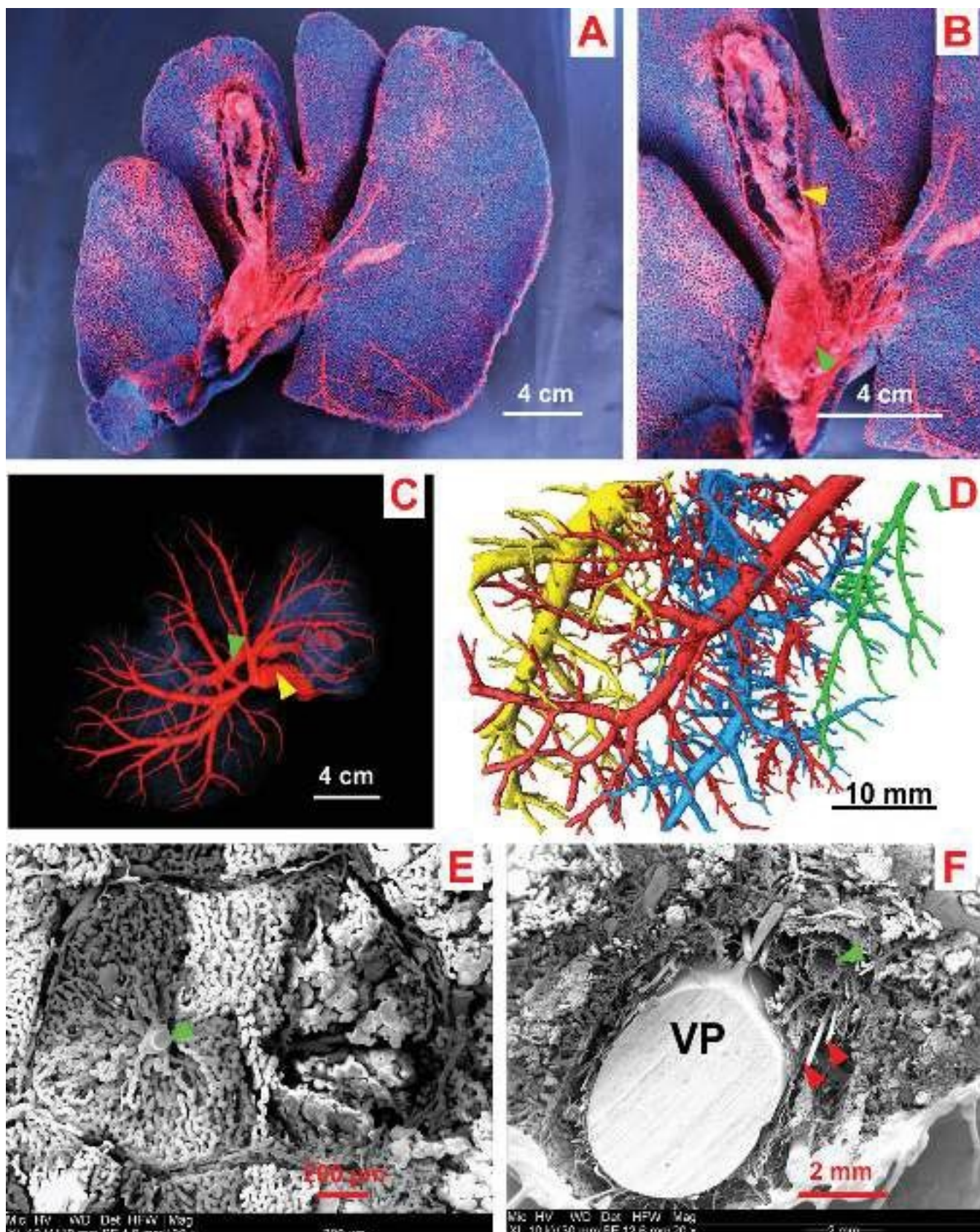
Pro dobrou náplň kapilárního řečiště je nutné odebírat orgán za specifických podmínek (systémová heparinizace, průplach řečiště izotonickým roztokem). Z toho důvodu jsou lidské orgány pro tento účel prakticky nedostupné. Výjimku tvoří jen orgány vyřazené z transplantace [8,13]. Cílem této studie bylo optimalizovat protokoly přípravy korozivních preparátů různých orgánů prasete domácího, a to vzhledem k dostupnosti těchto orgánů a významu prasete domácího v experimentální medicíně.

METODA

Studie byla provedena dle platné legislativy a s povolením Ministerstva zemědělství ČR. Byly dodrženy stávající předpisy a směrnice pro chov a experimentální využívání zvířat v souladu se zákonem č. 246/1992 upraveným vyhláškou č. 207/2004 s následným výkladem k vyhlášce č. 39/2009. Orgány byly získány z prasete domácího (plemeno přeštické černostrakaté prase), které je na Lékařské fakultě UK v Plzni využíváno jako animální model pro výzkumné účely [14,15,16,17]. Pracovalo se s 10 zdravými jedinci z jatečního chovu (6 samic, váha 35–45 kg).

Zvířata byla operována v celkové anestezii za použití mechanické ventilace. Orgány byly vypreparovány a ihned poté zvířeti aplikován heparin (100 IU/kg váhy). Následovalo propláchnutí cévního systému orgánu heparinizovaným fyziologickým roztokem (5000 IU/1 l fyziologického roztoku). V případě jater byl proveden proplach *in situ* pomocí katétrů zavedených suprahepaticky do *vena cava caudalis* a do *arteria hepatica*. *Vena portae* zůstala volná pro odtok krve a fyziologického roztoku, infrahepatická část *vena cava caudalis* byla podvázána. V případě ostatních orgánů byl proplach proveden intraarteriálně po vynětí orgánu z těla zvířete. Žíly byly ponechány volné pro odtok. Obě ledviny byly ponechány jako jeden preparát a proplachování bylo provedeno intraaortálně s odtokem cestou *vena cava caudalis*. Po propláchnutí byly cévy vyjmutých orgánů zasvorkovány a orgány přeneseny a proplněny pryskyřicí pod vodní hladinou z důvodu prevence embolizace vzduchu do cévního řečiště.

Plnění cévního stromu pryskyřicí Biodur E20® (Heidelberg, Německo) bylo provedeno tepennou cestou (ledviny, slezina, střevo), u jater i cestou *vena portae*.

Obr. 1: Korozivní preparát cévního řečiště jater prasete domácího

A, B – Biodur E20® aplikován cestou *v. portae* (modrá) a cestou a*. hepatica* (červená); cévy žlučníku (žlutá šipka), *vasa vasorum v. portae* (zelená šipka). C – makroCT, VRT, syntopické zobrazení *v. portae* (zelená šipka) a *v. cava caudalis* (žlutá šipka). D – mikroCT, 3D rekonstrukce, preparát plněný pouze cestou *v. portae*. E – SEM, *lobulus venae centralis* (zelená šipka). F – SEM, preparát plněný cestou *vena portae* (VP) a *a. hepatica* (červené šipky); peribiliární arteriální pleteň (zelená šipka).

Fig. 1: Vascular corrosion cast of the domestic pig liver

A, B – Biodur E20® injected via the portal vein (blue arrow) and via the hepatic artery (red arrow); vessels of the gallbladder (yellow arrow), vasa vasorum of the portal vein (green arrow). C – macroCT, VRT, syntopic view of the portal (green arrow) and hepatic ve- nous systems (yellow arrow). D – microCT, 3-D reconstruction, cast filled via the portal vein. E – SEM, liver lobule. Central vein (green arrow). F – SEM, cast fill via the portal vein (VP) and the hepatic artery (red arrows). Peribiliary plexus (green arrow).
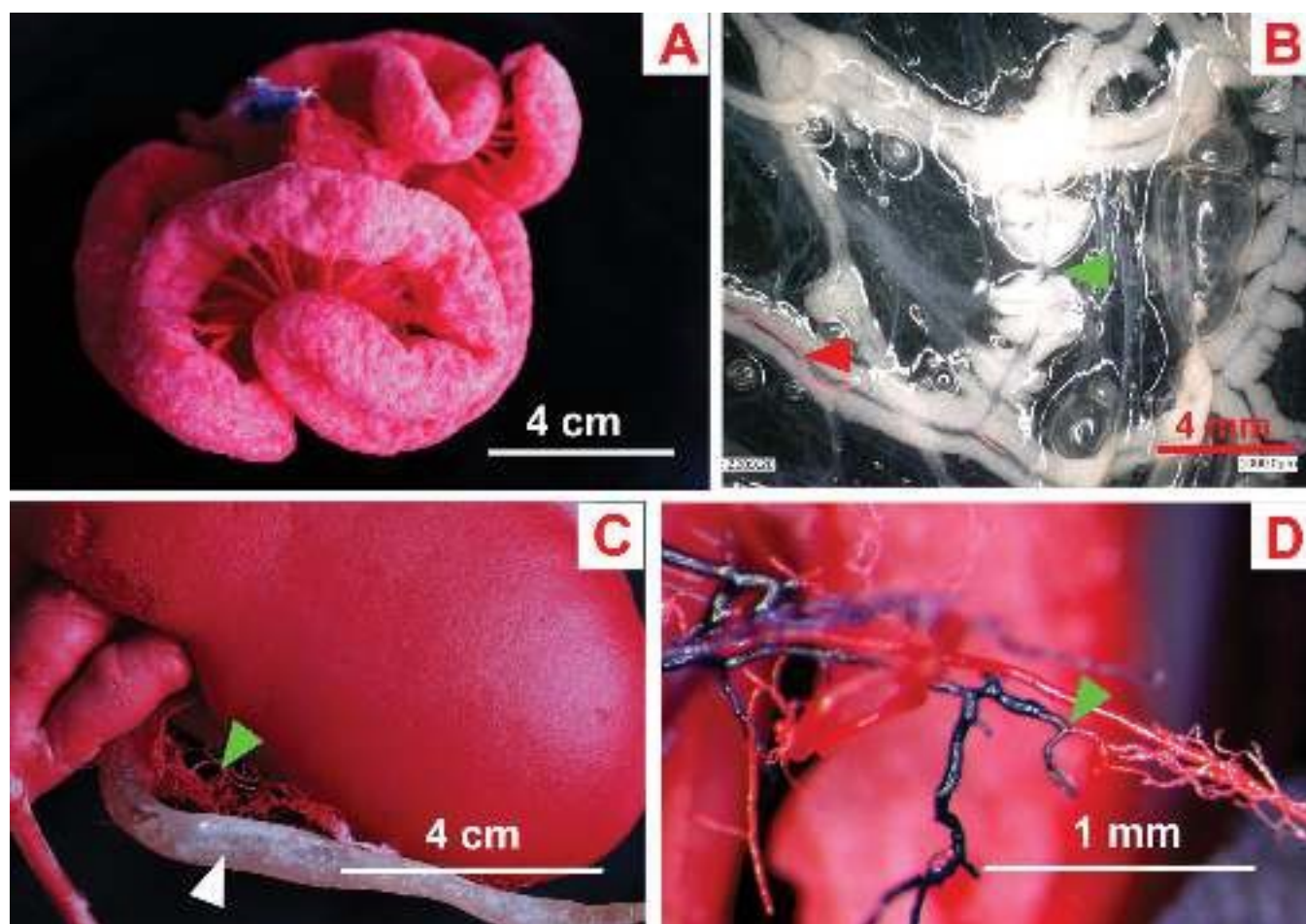
U některých preparátů jater byly navíc naplněny i žlučové cesty včetně žlučníku cestou *ductus choledochus*, v případě ledvin pak retrográdně močovod a ledvinová pánvička. Některé preparáty ledvin byly plněny jak cestou intraarteriální, tak intravenózní. Při aplikaci pryskyřice více cestami bylo vždy provedeno barvení pryskyřice k následnému usnadnění makroskopického rozlišení jednotlivých řečišť a struktur. Po aplikaci pryskyřice orgány zůstaly ve vodní lázni o pokojové teplotě. Po ukončení polymerizace pryskyřice (asi 24 hod.) byl orgán přemístěn do 15% roztoku KOH, jenž rozpustil organické tkáně. Hotové KP byly uchovány v 70% denaturovaném alkoholu, menší byly vysušeny mrazem.

Pro studium a ověření kvality hotových KP byly použity následující zobrazovací metody: pro přehledové vyšetření větších struktur běžné CT (Somatom Sensation 64, Siemens, Forchheim, Německo), pro detailnější zobrazení stereomikroskop (Olympus SZX7; Tokio, Japonsko), dále mikroCT (Xradia μXCT 400, Pleasanton, CA, USA), SEM SU-70 (Hitachi, Japonsko) a digitální mikroskop Keyence VHX-6000 (Osaka, Japonsko). Při použití technik mikroCT, SEM a stereomikroskopu nelze vyšetřit preparáty velkých orgánů jako jeden celek. Preparáty tedy byly zamraženy v destilované vodě a nařezány na pásové pile na menší díly o velikosti přibližně 1 cm³. Pro zkoumání

pomocí SEM bylo dále potřeba tyto nařezané kostky na 5 minut ponořit do 5% kyseliny mravenčí, opláchnout v destilované vodě, usušit na vzduchu a pokrýt zlatem. Až po této přípravě mohlo být provedeno vyšetření SEM.

VÝSLEDKY

Byla vypracována metodika přípravy KP různých orgánů prasete domácího (Obr. 1–3). Díky proplachu cévního řečiště orgánů při jejich odběru, prevenci vzduchové embolizace a díky použití pryskyřice s požadovanými vlastnostmi (přiměřená viskozita, dostatečný manipulační čas před ztuhnutím, nízká lámavost po ztuhnutí) se podařilo naplnit celé cévní řečiště včetně kapilár. Při aplikaci pryskyřice arteriální cestou došlo v případě ledvin, sleziny a střeva k naplnění celého řečiště skrz kapiláry až do žil. U jater došlo při aplikaci intraarteriální a intraportální k naplnění sinusoid a skrz *venae centrales* postupně *venae hepaticae* a *vena cava caudalis* (Obr. 1 A, B). Použitá pryskyřice Biodur E20 umožňuje zároveň zobrazení velkých struktur (vrátnicová žíla, jaterní tepny a žíly, ledvinové a slezinové tepny a žíly, žlučové cesty se žlučníkem) i mikrocirkulace (glomeruly, jaterní sinusoidy). Možnost barvení této
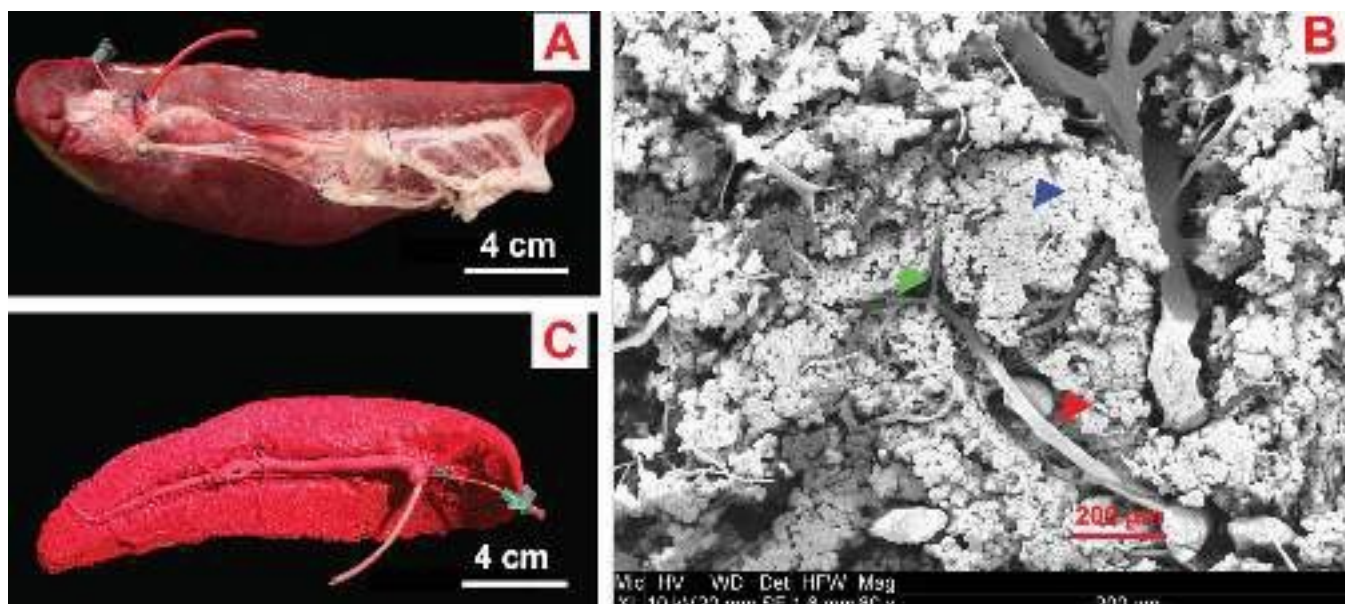

Obr. 2: Korozivní preparáty tenkého střeva (A, B) a ledviny (C, D) prasete domácího
Biodur E20® aplikován tepnou – A, B, C, tepnou a žilou –D. B –tepna mezenteria (červená šipka), mízní cévy (zelená šipka). C – přední stěna dolního pólu levé ledviny, *rami ureterici* (zelená šipka), močovod (bílá šipka). D – ledvina, arteriovenózní anastomóza.
Fig. 2: Vascular corrosion casts of the small intestine (A, B) and kidney (C, D), domestic pig
Biodur E20® injected only via artery – A, B, C, via artery and vein – D. B – artery of the mesentery (red arrow), lymphatic vessels (green arrow). C– anterior aspect of the lower pole, left kidney. Ureteric branches (green arrow), ureter (white arrow). D – kidney, arteriovenous anastomosis.

Obr. 3: Korozivní preparáty sleziny prasete domácího, použit Biodur E20®, plněno tepnou
A – preparát před korozí, C – po korozi. B – SEM; *a. centralis* (červená šipka), *a. penicillata* (zelená šipka), pryskyřice mimo cévní řečiště červené pulpy (modrá šipka).

Fig. 3: Vascular corrosion casts of the porcine spleen. Biodur E20®, artery filling
A – spleen before, C – after corrosion. B – SEM; central artery (red arrow), penicillate artery (green arrow), resin extravasation in the red pulp (blue arrow).

pryskyřice pak zlepšuje makroskopickou přehlednost připravovaných preparátů.

Díky dostatečné radioopacitě lze takto získané preparáty vyšetřovat pomocí CT. Běžné CT je však z důvodu velikosti orgánu a vyšetřovaných struktur použitelné pouze u jater (Obr. 1 C). Softwarové programy umožňují prostorové rekonstrukce větších cév a odlišení jednotlivých cévních řečišť. Autoři prezentují rekonstrukci *pomocí volume rendering technique (VRT) s patrným řečištěm vena portae a vena* cava caudalis (Obr. 1 C). Díky běžnému CT mohou být vytipovány dobře naplněné části orgánů vhodné k detailnějšímu zobrazení. Podobně jako běžné CT, umožňuje i mikroCT provedení prostorových rekonstrukcí a měření včetně kvantifikací (Obr. 1 D) [10]. I přes technický pokrok se jeví SEM pro kontrolu kvality odlitku včetně diagnostiky různých oddílů cévního stromu jako nezastupitelná. Autoři představují obraz jaterního lalůčku včetně *vena centralis* a obraz peribiliární arteriální pleteně (Obr. 1 E, F). Na KP jater prasete je také demonstrována přítomnost pěti laloků na rozdíl od lidských jater (Obr. 1 A).

V případě tenkého střeva byly zobrazeny cévy mezenteria, došlo k naplnění cestou *a. mesenterica superi- or* a *arteriae rectae* až do mikrocirkulace vlastního stře- va a dále pak k naplnění přes drobné žíly mezenteria do přítoků *vena mesenterica superior* (Obr. 2 A). Na preparátu střeva před samotnou korozí jsou patrné mízní cévy mezenteria (Obr. 2 B).

Dále autoři prezentují makroskopický obraz preparátu ledvin plněný jednak arteriální cestou a jednak skrz močovod. Patrné jsou cévy vyživující močovod (Obr. 2 C). Při plnění jak arteriálním, tak venózním řečištěm se podařilo zobrazit arteriovenózní anastomózu (Obr. 2 D).

V případě sleziny došlo také k naplnění mikrocirkulace, ovšem aplikovaná pryskyřice přestoupila do intersticia,

v němž vytvořila kvůli svým hydrofobním vlastnostem typické kulovité útvary, patrné na snímcích ze SEM (Obr. 3 B). Dále autoři představují makroskopický obraz celého preparátu sleziny před korozí a po ní (Obr. 3 A, C).

MikroCT umožnilo získání dat o prostorovém uspořádání mikrocirkulace a dat pro budoucí softwarové modelování orgánové perfuze zkoumaných orgánů. Vysoká kvalita získaných preparátů umožnila jejich využití i ve výuce anatomie člověka.

DISKUZE

Zhotovené preparáty umožnily demonstraci rozdílů mezi anatomií prasečích a lidských orgánů. Vzhledem k tomu, že byly použity orgány prasete pro studii s předpokládaným přesahem do lidské medicíny, je potřeba zmínit tyto odlišnosti mezi lidskými a prasečími orgány. Například játra prasete domácího mají makroskopicky obvykle pět laloků (*lobus lateralis dexter et sinister, lobus medialis dexter et sinister, lobus caudatus*). *Lobus quadratus* je variabilní. Cévní a žlučový strom je však lidským játrům bližší, utváří také osm segmentů, které jsou zhruba shodné s lidskými [18]. Játra prasete domácího jsou tak vhodným modelem pro nácvik chirurgických technik [19,20], studium regenerace [21,22] nebo pro matematické modelování [23]. Histologicky je u zdravých jater prasete popisováno vazivo ohraničující jednotlivé jaterní lalůčky, zatímco u člověka je toto vazivo patrné pouze za patologických stavů, jako jsou jaterní fibróza či cirhóza [24].

Ledviny prasete jsou podobné lidským svou velikos- tí, hmotností, počtem a stavbou nefronů včetně architektury mikrocirkulace [25,26]. Liší se však počtem segmentů. Zatímco lidské ledviny mají obvykle pět

segmentů, ledviny prasete mají pouze čtyři segmenty. *Arteria renalis* se u prasat větví před ledvinovou brankou na horní a dolní pólovou tepnu a poté na přední a zadní segmentové tepny [27].

Slezina prasete se liší svým tvarem od sleziny lidské. Je významně delší a užší, její mikrostruktura doposud nebyla detailně zkoumána.

Lidské orgány jsou také využívány pro přípravu cévních korozivních preparátů, v průběhu odběru je však řečiště již zpravidla vyplněno koaguly, a naplnění mikrocirkulace je tudíž obtížné [28,29], avšak ne nemož- né [30], což může činit problémy zejména při pokusu   o dokonalou náplň kapilár. Výjimku tvoří lidské orgány vyřazené z transplantace, jejichž řečiště je propláchnuto speciálními roztoky, a riziko trombotizace je tak eliminováno [8]. Získání takového orgánu je však možné jen ve výjimečných situacích. Je až zarážející, že u tak často používaného animálního modelu ještě chybějí základní morfologické údaje – např. množství vaziva v játrech zdravého prasete dosud nebylo kvantifikováno, ačkoli stupeň fibrotizace je klíčový pro hodnocení výsledku experimentu včetně jeho převodu do lidské medicíny [31].

U KP sleziny sice byla naplněna mikrocirkulace, ovšem došlo k extravazaci pryskyřice. Získané zobrazení mikrocirkulačního řečiště sleziny odpovídá otevřené cirkulaci, jež umožňuje přestup pryskyřice do interstícia. Tento nález je v souladu s prací Lametschwandtnera a kol. a ukazuje, že pro studium morfologie cévního řečiště sleziny prasete je nutné použití imunohistochemie nebo SEM z nativních vzorků [32].

Do budoucna je možné využití dat o cévním řečiš-   ti získaných pomocí KP a mikroCT k matematickému modelování perfuze jednotlivých orgánů. Tyto modely mohou mít význam například pro zkoumání vlivu izolované orgánové perfuze, ale i pro plánování a optimalizaci některých chirurgických výkonů, např. jaterních resekcí [33]. Pokud by bylo možné pomocí těchto perfuzních modelů predikovat jaterní regeneraci, mohla by se zvýšit operabilita pacientů s jaterními malignitami. Nová zjištění mohou přinést i KP patologicky změněných orgánů. Již dnes je známo například využití KP jater pro výpočet smykového napětí na stěně vrátnicové žíly u myšího modelu portální hypertenze [34].

ZÁVĚR

Byla vypracována metodika přípravy KP různých orgánů prasete domácího. Za mimořádný úspěch lze označit optimalizaci protokolu pro přípravu KP ja- ter prasete s ohledem na komplikované cévní řečiště a náročnost operačního protokolu. Předpokládáme možnost použití této metodiky i pro jiná velká zvířata včetně lidských jater. V této studii zhotovené preparáty mohou sloužit pro výuku anatomie a jejich výhoda spočívá v možnosti zkoumání postupně od makroskopických až po mikroskopické struktury v rámci jednoho preparátu. Je možné vytvořit trojrozměrné modely mikrocirkulace z dat získaných pomocí mikroCT. Získaná data o prostorovém uspořádání mikrocirkulačního řečiště pak mohou být přínosná pro matematické modely orgánové perfuze s významem pro chirurgické resekce a transplantační chirurgii.

Konflikt zájmů
Autoři článku prohlašují, že nejsou v souvislosti se vznikemtohotočlánkuve střetuzájmůaže tentočláneknebyl publikován v žádném jiném časopise.

LITERATURA

1. Hermiz DJ, O´Sullivan DJ, Lujan HL, et al. Constructivist learning of anatomy: gaining knowledge by creating anatomical casts. Anat Sci Educ 2011;4:98–104.
2. De Sordi N, Bombardi C, Chiocchetti R, et al. A new method of producing cas- ts for anatomical studies. Anat Sci Int 2014;89:255–65.
3. Davies A. The evolution of bronchial casts. Med Hist 1973;17:386–91.
4. Haenssgen K, Makanya AN, Djonov V. Casting materials and their application in research and teaching. Microsc Microanal 2014;20:493–513.
5. Lametschwandtner A, Lametschwandtner U, Weiger T. Scanning electron microscopy of vascular corrosion casts – technique and applications: updated review. Scanning Microsc 1990;4:889–941.
6. Aharinejad SH, Lametschwandtner A. Microvascular corrosion casting in scanning electron microscopy, first edition. Springer, Berlin-Heidelberg-Wien 1992.
7. Murakami T. Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. Arch Histol Jpn 1971;32:445–54.
8. Debbaut C, Segers P, Cornillie P, et al. Analyzing the human liver vascular architecture by combining vascular corrosion casting and micro-CT scanning: a feasibility study. J Anat 2014;224:509–17.
9. Pabst AM, Ackermann M, Wagner W, et al. Imaging angiogenesis: perspectives and opportunities in tumour research – a method display. J Craniomaxillofac Surg 2014;42:915–23.
10. Jirik M, Tonar Z, Kralickova A, et al. Stereological quantification of microvessels using semiautomated evaluation of X-ray microtomography of hepatic vascular corrosion casts. Int J Comput Assist Radiol Surg 2016;11:1803–19.
11. Eberlova L, Liska V, Mirka H, et al. Porcine liver vascular bed in Biodur E20 corrosion casts. Folia Morphol 2016;75:154–61.
12. Debbaut C, Monbaliu DR, Segers P. Validation and calibration of an electrical analog model of human liver perfusion based on hypothermic machine perfusion experiments. Int J Artif Organs 2014;37:486–98.
13. Peloso A, Petrosyan A, Da Sacco S, et al. Renal extracellular matrix scaffolds from discarded kidneys maintain glomerular morphometry and vascular resilience and retains critical growth factors. Transplantation 2015;99:1807–16.
14. Vrtkova I. Genetic admixture analysis in Prestice Black-Pied pigs. Arch Anim Breed 2015;58,115–21.
15. Bruha J, Vycital O, Tonar Z, et al. Monoclonal antibody against transforming growth factor Beta 1 does not influence liver regeneration after resection in large animal experiments. In Vivo 2015;29:327–40.
16. Junatas KL, Tonar Z, Kubikova T, et al. Stereological analysis of size and density of hepatocytes in the porcine liver. J Anat 2017;230:575–88.
17. Eberlova L, Liska V, Mirka H, et al. The use of porcine corrosion casts for teaching human anatomy. Ann Anat 2017;213:69–77.
18. Couinaud C. Liver lobes and segments: notes on the anatomical architectu-

re and surgery of the liver. Presse Med 1954;62:709–12.

19. Bedoya M, del Rio AM, Chiang J, et al. Microwave ablation energy delivery: influence of power pulsing on ablation results in an ex vivo and in vivo liver model. Med Phys 2014;41:123301.

20. Okada N, Mizuta K, Oshima M, et al. A novel split liver protocol using the subnormothermic oxygenated circuit system in a porcine model of a marginal donor procedure. Transplant Proc 2015;47:419–26.

21. Mortensen KE, Revhaug A, et al. Liver regeneration in surgical animal models - a historical perspective and clinical implications. Eur Surg Res 2011;46:1–18.

22. Avritscher R, Abdelsalam ME, Javadi S, et al. Percutaneous intraportal application of adipose tissue-derived mesenchymal stem cells using a balloon occlusion catheter in a porcine model of liver fibrosis. J Vasc Interv Radiol 2013;24:1871–8.

23. Fu YB, Chui CK. Modelling and simulation of porcine liver tissue indentation using finite element method and uniaxial stress-strain data. J Biomech 2014;47:2430–5.

24. Besusparis J, Jokubauskiene S, Plancoulaine B, et al. Quantification accuracy of liver fibrosis by in vivo elastography and digital image analysis of liver biopsy histochemistry. Anal Cell Pathol (Amst) 2014. Available from: 10.1155/2014/317635.

25. Rytand DA. The number and size of mammalian glomeruli as related to kidney and to body weight, with methods for their enumeration and measurement. Am J Anat 1938;62:507–20.

26. Friis C. Postnatal development of the pig kidney: ultrastucure of the glome- rulus and the proximal tubule. J Anat 1980;130(Pt 3):513–26.

27. Evan AP, Connors BA, Lingeman JE, et al. Branching patterns of the renal artery of the pig. Anat Rec 1996;246:217–23.

28. Rani N, Singh S, Dhar P, et al. Surgical importance of arterial segments of human kidneys: an angiography and corrosion cast study. J Clin Diagn Res 2014;8:1–3.

29. Mazur M, Walocha K, Kuniewicz M, et al. Application of Duracryl plus for preparation of corrosion casts of venous coronary tree of human heart. Folia Med Cracov 2015;55:69–75.

30. Bereza T, Tomaszewski KA, Lis GJ, et al. ´Venous lakes´– a corrosion cast scanning electron microscopy study of regular and myomatous human uterine blood vessels. Folia Morphol (Warsz) 2014;73:164–8.

31. Avritscher R, Wright KC, Javadi S, et al. Development of a large animal model of cirrhosis and portal hypertension using hepatic transarterial embolizati- on: a study in swine. J Vasc Interv Radiol 2011;22:1329–34.

32. Lametschwandtner A, Radner C, Minnich B. Microvascularization of the spleen in larval and adult Xenopus laevis: Histomorphology and scanning electron microscopy of vascular corrosion casts. J Morphol 2016;277:1559–69.

33. Svobodova M, Jirik M, Vcelak P, et al. Software LISA – virtual liver resection to accelerate and facilitate preoperative planning. Rozhl Chir 2016;94:485–90.

34. Van Steenkiste C, Trachet B, Casteleyn C, et al. Vascular corrosion casting: analyzing wall shear stress in the portal vein and vascular abnormalities in portal hypertensive and cirrhotic rodents. Lab Invest 2010;90:1558–72.

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