# 2023

# The XXXIV EAVA-congress at Sundvolden Hotel



**Congress program & book of abstracts** 



Norwegian University of Life Sciences



# Contents

Program	2
Plenary key-note speakers	6
Professor emeritus Harald Kryvi, University of Bergen: THE NOTOCHORD: A FORGOTTEN HERO	6
Professor Finn-Eirik Johansen, University of Oslo: The peculiar immune system of Atlantic cod	6
Professor Øystein Lie, Tempogene AS: Contemporary genetic toolbox to advance aquaculture with epigenetics representing a potential paradigm shift in fish welfare enhancement	h 7
Abstracts – oral presentations	8
Wednesday – July 26 <sup>th</sup>	8
Session 1 – Teaching	8
Session 2 – Gross anatomy1	.3
Thursday – July 27 <sup>th</sup> 1	.7
Session 1 Microanatomy1	.7
Friday – July 28 <sup>th</sup> 2	2
Session 1 – Molecular medicine2	2
Session 2 – Molecular/cellular research2	7
Session 3 Teaching/Gross Anatomy3	2
Abstracts – Poster presentations	7
Teaching and gross anatomy (Wednesday – July 26th)3	7
Microanatomy and Molecular/Cellular (Thursday – July 27th)6	9

## Program

Welcome to the XXXIV EAVA-congress at Sundvolden Hotel outside Oslo with arrival at Tuesday, July 25<sup>th</sup> 2023 and departure on Saturday, July 29<sup>th</sup> after breakfast.

#### Tuesday, July 25<sup>th</sup>

Arrival. Registration and check in (check in open at 15:00).

19:00 Welcome drink and finger food

#### Wednesday, July 26th

07:00 – 08:30 Breakfast

#### 08:45 – 09:05 Opening ceremony

09.15-10:00 Plenary speaker: Professor emeritus, Harald Kryvi University of Bergen. The notochord: a forgotten hero (co-author of the presentation: Professor John Long, Vassar College, NY, USA).

10:00 – 10:15 Break

10:15 – 11:30 Session 1 Teaching

Chairs: Claudia Wolschrijn and Stefan Arnhold

- 1) 10:15 D. Fietz: Formalin exposition in veterinary anatomy dissection courses still needed in times of digital teaching?
- 2) 10:30 M. Soares: Teaching veterinary anatomy: the use of a multimodal system
- 3) 10:45 B. Hierck (presented by C. Wolschrijn): The role of visualspatial ability in learning veterinary anatomy; towards personalized learning
- 4) 11:00 I. Cazimir: The implicit learning of practical histology beyond declarative knowledge
- 5) 11:15 C. Press: Development and use of e-learning work tools for teaching the basic sciences in veterinary education
- 11:30 11:55 Presentation from Congress main sponsor Anatomage

12:00 – 13:00 Lunch

13:00 – 14:00 Session 2 Gross anatomy

#### Chairs: Johann Maierl and Paula Papa

1) 13:00 R. Senos: Comparative study of the digestive system of piscivorous wild avian species

- 2) 13:15 J. Rieger: Intrahepatic venous vascular anatomy of the feline liver as an anatomical basis for surgical intervention
- 3) 13:30 F. Biebl: Measurement of pressure distribution under bovine claws and different blocks
- 4) 13:45 E. Engelke: Anatomy, histology and radiography of larynx, trachea and lungs in inland bearded dragons (*Pogona vitticeps*)

14:00-14:30 Break

- 14:30 16:30 Excursion to Kongens utsikt (The King's View)
- 16:30 17:00 Poster session 1 Teaching and gross anatomy

19:00 – Dinner

#### Thursday, July 27<sup>th</sup>

07:00 – 09:00 Breakfast

09.15 – 10:00: Plenary speaker: Professor Finn-Eirik Johansen, University of Oslo. The peculiar immune system of Atlantic cod.

10:00 – 10:15 Break

10:15 – 11:30 Session 1 Microanatomy

Chairs: Wim van den Broek and Ivan Alic

- 1) 10:15 R. Möller: Histologic evaluation of elastic fibre and smooth muscle cell architecture of blood vessels in uterus, stomach and Kiesselbach plexus in porcine von Willebrand disease
- 2) 10:30 H. Jackowiak: Microvascularization of the thyroid gland of the domestic cat during prenatal development
- 3) 10:45 M. Crisan: Comparative study of the ultrasonographic, anatomopathological and histopathological examination of deep digital flexor injuries of isolated limbs
- 4) 11:00 F. Barac: B cell distribution in the gills of Atlantic salmon and the effects of systemic vaccination on B cell abundance
- 5) 11:15 S. M. Aberoumandi: Aspects of caspase-8 biology in human ovarian cancer studied in anorthotopic mouse model

11:30 – 13:00 Lunch

#### 13:15 – 17:00 Excursion to Kistefoss (art museum and culture park)

#### 17:00 - 18:00 Poster session 2 - Microanatomy and molecular/cellular

19:00 - Dinner

#### Friday, July 28th

07:00 – 09:00 Breakfast

09.00 – 09:45 Plenary speaker: Professor Øystein Lie, TempoGene AS, Oslo. Contemporary genetic tool box to advance aquaculture with epigenetics as 5<sup>th</sup> gear.

09:45 – 10:15 Break

10:15 – 11:30 Session 1 Molecular medicine

Chairs: Christiane Pfarrer and Khan Junatas

- 1) 10:15 K. Klisch: Fuse or not divide: different avenues lead to the formation of multinucleate cells in the placenta of domestic animals
- 2) 10:30 C. Herre: Vim, tpi, mat2a and their role in *in vitro* angiogenesis
- 3) 10:45 K. Junatas: Telocytes of the porcine myocardial sleeves and conduction system
- 4) 11:00 I. Alic: Cellular modelling in neuroscience
- 5) 11:15 M. P. Kowalewski: Canine decidualization: *in vivo* observations and in vitro functional studies of intracellular signalling pathways

#### 11:30 – 13:00 Lunch

#### 13:00 – 14:15 Session 2 Molecular/cellular research

Chairs: Baljit Singh and Hanna Jackowiak

- 1) 13:00 B. Singh: Recruitment of monocytes/macrophages into alveolar septa of lungs of sars-cov2 infected animals
- 2) 13:15 F. Vanden Broucke: Annexin v-fitc apoptosis assay on porcine cardiac telocytes *in vitro*
- 3) 13:30 M. Verstraete: Equine placenta in the lab: development of equine placental organoids
- 4) 13:45 M. Pallaoro: The influence of breed and different seasons on muscle and skeletal development at birth in newborn piglets
- 5) 14:00 H. Bjørgen: Melano-macrophages and their relation to muscle inflammation in Atlantic salmon (*Salmo salar*).

14:15–14:30 Break

#### 14:30 - 15:45 Session 3 Teaching/Gross Anatomy

Charis: Michal Kyllar and Catrin Rutland

- 1) 14:30 R. Schirone: Effects of 3d scans on veterinary students learning process compared to traditional 2d images
- 2) 14:45 R. Tartsch: "Taking the bull by the omentum" using different teaching modalities to explain the ruminant mesogastrium

- 3) 15:00 I. Aktan: Veterinarians' opinions towards dissection during the undergraduate veterinary medicine course and its effectiveness in preparing them for surgery and veterinary life
- 4) 15:15 P. Reyes: Recent developments in developing a novel corrosion casting material
- 5) 15:30 C. Wolschrijn: The development of simove; a virtual reality canine ovariectomy simulator

16:00 – 17:00 EAVA General Assembly

18:00 – Aperitif

18:30: Gala Dinner

#### Saturday, July 29<sup>th</sup>

Departure from Sundvolden. Check-out time is before 11:00

Heads, Congress organizing committee: Professor Erling Olaf Koppang and Associate Professor Håvard Bjørgen, Unit of Anatomy, Veterinary Faculty, Norwegian University of Life Sciences.

Congress organizing committee: Members of Unit of Anatomy, Veterinary Faculty, Norwegian University of Life Sciences and Dr. Hege Hellberg, Pharmaq Analytiq, Bergen, Norway.

Technical organization: Camilla Grefsli and Gro Irene Helmersen, Centre for Continuing Education, Norwegian University of Life Sciences.

# Plenary key-note speakers

# Professor emeritus Harald Kryvi, University of Bergen: *THE NOTOCHORD: A* FORGOTTEN HERO

The notochord is the first axial support system in every vertebrate embryo. As the embryo grows, the notochord does so too, and the Atlantic salmon, with its complex life cycle, is an excellent model organism for studying these changes. The salmon's notochord undergoes dramatic cellular reorganization throughout its lifespan, with differentiation of an epithelium, vacuolated chordocytes, and, later, dense fibre-filled chordocytes. Deeper in the centre of the juvenile's notochord, the chordocytes are arranged into transverse septae that are connected in series along one longitudinal strand, together spanning the entire length of the vertebral column. The strand of chordocytes is surrounded by large extracellular fluid-filled lacunae. In adults, the lacunae shrink, leaving vacuolated chordocytes and lacunae are surrounded by an extracellular sheath, composed of layers of collagen II and elastin fibres. This sheath, built by chordoblasts, is the site of the first ossification of vertebrae, a process that connects the notochord to the formation of the vertebrate column and the evolution of the vertebrate sub-phylum. The notochord starts and ends in the head and the tail, where it differs from that seen in the body.

# Professor Finn-Eirik Johansen, University of Oslo: *The peculiar immune system of* Atlantic cod

In-depth knowledge of physiological systems essential for human and animal health often comes from functional experiments in a few model organisms, neglecting possible divergent evolutionary adaptations. Our understanding of the adaptive immune system, which evolved about 500 million years ago and is common to all jawed vertebrates, largely stems from mouse experiments. Whole genome sequencing of an increasing number of vertebrates has revealed alterations in immune genes, previously thought to be conserved. The Atlantic cod (*Gadus morhua*) was the first vertebrate discovered to have lost Major Histocompatibility (MHC) genes class II and the T helper cell receptor CD4. Given the evolutionary success of codfishes, this was a surprise because mice with targeted disruption of these genes are severely immunocompromised. Similarly, humans with congenital mutations in MHC class II or acquired loss of CD4 T cells do not have a functional adaptive immune system.

To characterize the immune system of Atlantic cod we performed single-cell RNA sequencing of dispersed spleen cells. Unbiased clustering of 57 000 cells gave 15 distinct populations based on transcription profile. Lymphocytes (T and B cells) accounted for 41%, while myeloid immune cells accounted for 19%. A deeper analysis of 14 000 T cells revealed three main populations characterized by expression of unique marker genes. Surprisingly the majority of T cells did not express CD8 and were therefore double negative (CD4-CD8-), unlike what is found in mammals.

Pseudotime trajectory analysis of 9000 B cells suggested two differentiation pathways, where the major pathway culminates in plasma cells. Plasma cells are dedicated to antibody production so we characterized the B cell receptor loci and antibody responses to T-dependent (TD) and T-independent (TI) antigens. We found that Atlantic cod only responded to T-independent type II antigens. Finally, we immunized a cohort of cod with formalin-fixed *Vibrio anguillarum* (a TI type II antigen) and found that the specific antibody response mediated protection against lethal vibriosis.

As more vertebrate genomes become available, new evolutionary adaptations will continue to surprise. New technologies such as single-cell RNA sequencing and CRISPR/Cas9-mediated genome

editing, facilitates research on non-model organisms, previously not possible for the small independent research group.

# Professor Øystein Lie, Tempogene AS: Contemporary genetic toolbox to advance aquaculture with epigenetics representing a potential paradigm shift in fish welfare enhancement

Although fish farming, and particularly the aquaculture of salmonid species, is considered a successful young industry in many temperate countries, with Norway as a leading player, there are still many challenges. Several of these challenges are related to fish health and welfare and to environmental issues, and the challenges have considerable impact on the economics and the sustainability of the sector. Major components of the problem are infectious diseases, parasite infestations like sea lice, developmental malformations, highly variable growth rates in the sea phase, and adverse environmental impacts including fish escapes, wastes, transfer of parasites and infections to wild stocks.

Genetic enhancements technology generations: mass selection, family selection, marker assisted selection and genomic selection have been deployed quite successfully over the years to address the above challenges improving robustness, growth rate, disease resistance to major salmon killers like IPN etc. Moreover, in the wake of the concerted revolutions within next generation sequencing (NGS) and in silico sciences, i.e. bioinformatics and A.I., completely new opportunities are here to take advantage of totally new insight into biology, epigenetics. Whereas DNA (the genome) is the master recipe of life, epigenetics (the epigenome) is, by turning on and off genes, the master of the function of life by governing development, organ differentiation, responding to environmental changes, has memory both within an organism's life span as well as through generations, is reversible and programmable.

The epigenetic markers, since responding to and memorizing any impact, and in part being heritable, they will likely serve dual purpose: become a new additional breeding tool and the optimal mediator to find out about the status of the well beings of our farmed species and thus pave the ground to enhance their welfare.

The present talk will briefly review current status and future opportunities of contemporary genetics employed in aquaculture with particular focus on epigenetics and will also share some of our findings.

Wednesday – July 26<sup>th</sup> Session 1 – Teaching

## FORMALIN EXPOSITION IN VETERINARY ANATOMY DISSECTION COURSES – STILL NEEDED IN TIMES OF DIGITAL TEACHING?

AUTHORS: Fietz D<sup>1</sup>, Thullner I<sup>2</sup>, Hieke H<sup>3</sup>, Ziegenberg K<sup>4</sup>, Kuhlmann MM<sup>4</sup>, Arnhold S<sup>1</sup>

AFFILIATION: <sup>1</sup>Justus Liebig University of Giessen, Institute for Veterinary Anatomy, Histology and Embryology, Giessen, Germany <sup>2</sup>German Social Accident Insurance Institution for the public sector in Hessen, Frankfurt, Germany <sup>3</sup>Justus Liebig University of Giessen, Department of Technology, Giessen, Germany <sup>4</sup>StuKO

ABSTRACT: Formalin has been used as fixative in dissection courses in Anatomy departments for a long time. In 2014, formalin was categorized as a potential carcinogen (category 1b) with the new EU Binding Occupational Exposure Limit (Directive 2019/983) set to 0.37 mg/m<sup>3</sup>. The German Association of Anatomists stated, that the complete substitution of formalin as the only fixative authorized by the European biocide law is not possible. During the pandemic, dissection courses were widely cancelled and the question arose: Are dissection courses still "state of the art" or could digital teaching surrogate them – and lower students' formalin exposition by this? Here, we want to introduce technical and organizational measures taken at the Dept. of Veterinary Anatomy in Giessen to meet the exposure limit and how we included digital teaching in anatomy classes.

In Giessen, fixation of cadavers with 3.5% formalin in aqueous solution, watering and use of InfuTrace<sup>TM</sup> to bind excessive formalin before course is combined with potent technical equipment (low room temperature, table/room ventilation, "shielding" by supply air). With these measures, the formalin concentration was below the Occupational Exposure Limit of 0.37 mg/m<sup>3</sup> in 2019. With the start of the Covid pandemic restrictions, the dissections courses were paused from March 2020 to June 2020 in Giessen and digital teaching was implemented (video material, virtual courses, online lectures). After that, with the start of the winter term 2020/2021, dissection courses resumed under difficult conditions (smaller group sizes and less material).

The use of online resources decreased the exposition of students to formalin significantly, but official surveys carried out by the study coordination team underlined the need for a practical dissection course. We conclude that the combination of technical/organizational measures to decrease formalin exposition and sophisticated digital teaching tools are the new state of the art for teaching veterinary anatomy.

# TEACHING VETERINARY ANATOMY: THE USE OF A MULTIMODAL SYSTEM.

AUTHORS: Soares M, Assunção R, Trindade A, Pequito M, Alves L

AFFILIATION: Egas Moniz Center for Interdisciplinary Research, Egas Moniz School of Health & Science 2829-511 Caparica, Portugal.

ABSTRACT: Anatomy, considered one of the cornerstones of Medicine, and fundamental for students to develop their clinical skills, has seen the number of hours dedicated to its theoretical and practical teaching reduced in recent curricula. This triggered the need to use different teaching and learning techniques to maximize the opportunity for anatomy learning.

In this context, the Integrated Master Degree in Veterinary Medicine (MIMV) of the Egas Moniz School of Health & Science implemented a multimodal system, applying different teaching-learning methodologies, in order to expose the students to different species while reducing the use of cadavers. First, the students receive theoretical lectures, where anatomical softwares can be used, allowing a first digital three-dimensional contact with the structures in different species. When appropriate, specific clinical correlates are performed. Furthermore, active learning techniques, like problem-based learning, flipped classroom or jigsaw methodologies are also used, developing important soft and hard skills. Finally, in the practical classes, the students first explore anatomical softwares (e.g. IVALA, easyanatomy, Virtual Veterinary Anatomy), a digital dissection table (Table Vet from Anatomage), 3D anatomy models and plastinated specimens, where they can manipulate the structures, training the visual-spatial ability. These also improve understanding of the anatomical differences between species. Finally, whenever irreplaceable, the students are exposed to dissection classes. In those classes, the students contact with prosected specimens and have some cadavers where they can apply dissection techniques.

This multimodal system has demonstrated several advantages over the more traditional method, including 1) higher student motivation due to the use of methodologies that promote an integrated and comprehensive knowledge of the anatomical structures and how they are related; 2) responds to the Universal Learning Design by exploring different ways of teaching-learning of a specific subject; and, 3) reduction of the number of cadavers used without compromising the quality of learning and demand.

Funding: This work was funding by the awards of the 2nd Seminar of Pedagogic Inovation of Egas Moniz Health of School & Science.

#### THE ROLE OF VISUALSPATIAL ABILITY IN LEARNING VETERINARY ANATOMY; TOWARDS PERSONALIZED LEARNING.

AUTHORS: <u>Hierck B.P.<sup>1</sup></u>, van Leeuwen B.S.<sup>1</sup>, Wolschrijn C.F.<sup>1</sup>, Salvatori D.<sup>1</sup>

AFFILIATION: <sup>1</sup>Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

ABSTRACT: VisualSpatial Ability (VSA) is the competence to make a mental 3-dimensional (3D) representation of a 2D image, and to predict its appearance upon mental rotation. The Mental Rotation Test (MRT) is the golden standard to measure VSA, and MRT scores are normally distributed in the population. That means that ideally spatial learning should be approached in a personalized fashion.

Historically anatomy learning has been supported by 2D learning materials, such as images in books and on computer/tablet/cellphone screens, and by hands-on dissection on cadaveric materials (3D). However, curricular time available for hands-on dissection has been decreased extensively in the last 2 decades, which has led to a relative increase in 2D learning. That poses a risk because there is domain specificity in storing knowledge in our brains, and interdomain transfer of knowledge is not trivial for everybody. With increasing computer power and the approaching "Metaverse" the use of 3D technology to support learning is increasing rapidly. Many examples of rotating 3D anatomy models exist online, and students increasingly use them to support their learning. However, we have shown that for students with below-average VSA learning outcomes significantly drop when they use these rotating models compared to using 3D models in Virtual or Augmented Reality. We have now further finetuned this research by investigating the use of screen-based 3D models in veterinary education, comparing rotation models vs fixed key-views, and show that students with below-average VSA learn equally well with both modalities.

Herewith we focus on the individual learning requirements of students when it comes to spatial learning. We have developed *AvatarZOO*, an application in which veterinary students can learn 3D anatomy in augmented reality. In this contribution we will present *AvatarZOO* as well as our research on anatomy learning.

# THE IMPLICIT LEARNING OF PRACTICAL HISTOLOGY BEYOND DECLARATIVE KNOWLEDGE.

#### AUTHORS: Cazimir I<sup>1</sup>, Bobei T<sup>2</sup>

AFFILIATION: <sup>1</sup>University of Agronomic Sciences and Veterinary Medicine (USAMV), Bucharest, Romania. <sup>2</sup>Babeş-Bolyai University (BBU), Cluj-Napoca, Romania.

ABSTRACT: Histological study methodology has suffered significant changes within the last 10 years, as a result of exceptional technological progress but also due to the COVID-19 pandemic which modified behaviors, especially study-wise. The transition from face-to-face to online and vice-versa has also impacted the medical study field in terms of interpreting microscopy. Electronic data bases have exploded and histology teaching moved from mostly practical, on individual microscopes, to mostly electronical through analyzing images, attempting to create a declarative-procedural balance. The jump in quality was huge, bettering student results, but a certain problem has remained unchanged. Practically, no matter how efficient teaching methods have gotten and how much the feedback and suggestions received from students were taken into account, a series of aspects regarding correct interpretations of images still remain unanswered. In the present study we have analyzed the expertise of students which have used a work technique designed on the principle of logical succession of wellestablished steps for learning histology in the lab. The method applied last year had a clear but complex character, based on textually describing points of interest, accompanied by labeled images, videos, worksheets and quizzes. The explanations and direct usage of the microscope complete this work method. However, it has been noticed that developing declarative knowledge does not transfer as well practically, on a microscope. There is a missing piece in the ability to develop procedural knowledge especially as a result of determined work times. Optimizing these aspects will take into account developing implicit abilities of students who will be able to easily identify under a microscope essential aspects of a structure. In this way, an increase of specificity is desired in identifying formations in the microscopic field by developing the intuitive character and mental flexibility, enabling students to look further than the provided examples.

## DEVELOPMENT AND USE OF E-LEARNING WORK TOOLS FOR TEACHING THE BASIC SCIENCES IN VETERINARY EDUCATION.

AUTHORS: Press, C.<sup>1</sup>, Rasmussen, E.<sup>1</sup>, Sverdvik, H.<sup>1</sup>, Høyheim, B.<sup>1</sup>

AFFILIATION: <sup>1</sup>Norwegian University of Life Sciences (NMBU), Ås, Norway.

ABSTRACT: We have developed an e-learning tool, the e-ZFapp<sup>\*</sup>, for teaching using the zebrafish model. The app collects and organizes e-learning modules consisting of text, pictures, sound, video clips and quizzes. The app facilitates navigating between courses and organizes the content of each specific course. At present the content of the app consists of one 3-day lab course in embryology and genotyping and a VR video on zebrafish care procedures. The app has been used in teaching of veterinary students at NMBU since 2016 and has given good results. The use of e-learning platforms in the basic sciences fosters student active learning and promotes formative testing and increased student feedback. In particular, the app enabled active learning with "flipped classroom/lab course teaching" as a tool for preparation, use during teaching events and in revision before exams. A demonstration of the app will be performed.

\*) e-ZFapp can be accessed at https://www.zebrafishlab.net.

Funding: The e-ZFbook research project was funded by Olav Thon Foundation, Norway 2015-2018.

# COMPARATIVE STUDY OF THE DIGESTIVE SYSTEM OF PISCIVOROUS WILD AVIAN SPECIES.

AUTHORS: <u>Senos R<sup>1-2</sup></u>, Penha JCQ<sup>2</sup>, Quaglio LS<sup>2</sup>, Silva FF<sup>2</sup>, Gartz H<sup>2</sup>, Milhomem R, Azeredo PC, Benedicto HG<sup>2</sup>

AFFILIATION: <sup>1</sup>Cummings School of Veterinary Medicine, Tufts University, North Grafton, USA. <sup>2</sup>Department of Morphology, Universidade Federal Fluminense, Niteroi, Brazil.

ABSTRACT: Although the studies of wild avian species has increased recently, basic morphological information is still scarce. The anatomy of the digestive system plays important role in nutritional and enteric drugs absorption, imaging interpretation, surgery, comprehension of species biology and phylogeny. The aim of this study is to compare the gross anatomy and morphometrics of the digestive system of wild avian species found in the coast of Rio de Janeiro. We studied the digestive system of 6 piscivorous avian species both males and females of the *Thalassarche chlororhynchos* (1), Nycticorax nycticorax (10), Fregata magnificens (5), Ardea alba (5), Sula leucogaster (10) and Spheniscus magellanicus (5). The dissection was made in 10% formalin fixed specimens. The S. leucogaster do not have choana in the oropharynx. The F. magnificens and the A. alba, presented the choana and infundibulum fused is the same ostium. No species presented crop (nor even vestigial). The stomachs were observed in many shapes but the proventriculus and ventriculus were fused in all studied species except for the T. chlororhynchos. The proventriculus bigger than the ventriculus in all species. The cecum size was minimal in all species that presented it. The cecum is paired in T. chlororhynchos, single in the N. nycticorax, F. magnificens, A. alba, and S. magellanicus. The cecum was absent in the S. leucogaster. The common features suggest that piscivorous diet do not require maceration no storage in the crop. In addition, the chemical digestion into proventriculus looks like having more relevance than the mechanical one into ventriculus, especially if compared to the granivorous domestic species. The cecum have little or no function in fermenting and absorbing the products of the digestion in those species. Despite some differences, species which diet is based in fishes and crustaceous present similar anatomy for the digestive system.

### INTRAHEPATIC VENOUS VASCULAR ANATOMY OF THE FELINE LIVER AS AN ANATOMICAL BASIS FOR SURGICAL INTERVENTION.

AUTHORS: Metzger MD<sup>1</sup>, Van der Vekens E<sup>2</sup>, <u>Rieger J<sup>3,4</sup></u>, Forterre F<sup>1</sup>, Vincenti S<sup>1</sup>

AFFILIATION: <sup>1</sup>Division of Small Animal Clinical Surgery, Vetsuisse-Faculty, University of Bern, Bern, Switzerland. <sup>2</sup>Division of Clinical Radiology, Vetsuisse-Faculty, University of Bern, Bern, Switzerland. <sup>3</sup>Division of Veterinary Anatomy, Vetsuisse-Faculty, University of Bern, Bern, Switzerland. <sup>4</sup>Department of Human Medicine, Faculty of Medicine, MSB Medical School Berlin, Berlin, Germany.

ABSTRACT: The anatomy of the feline intrahepatic veins has never been fully described; therefore, veterinary surgeons have based their techniques on prior knowledge of canine liver anatomy for liver surgery. The aim of the study was to identify and characterize the intrahepatic venous vascular anatomy of cat liver using corrosion casting and computed tomography, identify possible physiological variations in the vascular anatomy, and compare these data to canine anatomy. In a cadaveric anatomical study in adult cats (n=7), healthy livers were surgically resected and resin casts of portal and hepatic veins were evaluated using computed tomography (CT). The liver casts were then macerated to visualize the venous vasculature and compare to the CT scans. It was found that the feline livers had consistent intrahepatic portal and venous vascular anatomy with only minor disparities in numbers of secondary and tertiary branches. Several hepatic lobes shared common portal or hepatic veins with adjacent lobes, following a steady pattern in most specimens. The feline portal vein consistently divided into two major branches and not three, as previously described in the literature for cats. The finding of a portal vein originating from the right medial lobe branch leading to the quadrate lobe in 4/7 specimens is a novelty of the feline anatomy that was not previously described in dogs. A partial to complete fusion of the caudate process of the caudate lobe and the right lateral lobe with a lack of clear vessel separation between the lobes was present in two specimens. In conclusion, CT imaging of corrosion casts allowed for a detailed study of the feline intrahepatic portal and hepatic venous vasculature. The anatomy is consistent with previous findings about canine intrahepatic anatomy, though showing some variations between specimens which could have a great impact on surgical treatment of hepatic masses. Further anatomical studies should be encouraged to confirm the present findings and to investigate the utility of this information in a surgical setting or interventional radiological procedures.

Funding: This research was funded by the Vetsuisse Faculty of the University of Bern and the Kommission des Naturhistorischen Museums der Bürgergemeinde Bern (funding number 2021-727).

# MEASUREMENT OF PRESSURE DISTRIBUTION UNDER BOVINE CLAWS AND DIFFERENT BLOCKS.

#### AUTHORS: Biebl F, Mülling CKW

AFFILIATION: Leipzig University, Germany.

ABSTRACT: Many dairy cattle experience compromised claw health during their productive lifetime and show lameness due to pain associated with claw lesions. Complicated cases of sole ulcers require therapy with a block attached to the healthy ipsilateral claw which results in remarkable pain relief and promotes healing of the injured side. Despite the well-known sensitivity of cows' feet to especially hard floors there are no studies about claw blocks and their effects on the pressure distribution under bovine claws.

Aim of the project was to measure the possible impact of different blocks and application techniques on pressure distribution. Wooden blocks with polyurethane and methyl methacrylate glue, soft synthetic blocks with superglue and innovative orthoses with polyurethane glue were tested on 50 cadaver distal hind limbs. The feet were loaded in a hydraulic press with 150kg for 10s. Maximum pressure, average pressure and loaded area were measured with a piezoresistive sensor between claw and block.

First results show that softer blocks reduce the average pressure by 38% (23,46N/cm<sup>2</sup>) and increase the loaded area by 83% (40,81cm<sup>2</sup>) compared to wooden blocks (37,91N/cm<sup>2</sup> and 22,28cm<sup>2</sup> respectively). There are also clear differences when the application technique is modified. Including the claw wall in the block adherence with polyurethane and methyl methacrylate resulted in 26% lower maximum pressure compared to block attachment only to the sole.

Measurements and their analysis confirm that softer blocks are less stressful for the claw. There is also evidence that the application technique has a significant impact on the extent of pressure load. The results of our ex-vivo-study clearly show how important the evaluation of claw blocking is. There should be further in-vivo-studies to investigate the differences between possible block-glue-combinations in order to collect more conclusive information.

Funding: Friedrich-Naumann-Stiftung für die Freiheit, scholarship number 9482/P622.

## ANATOMY, HISTOLOGY AND RADIOGRAPHY OF LARYNX, TRACHEA AND LUNGS IN INLAND BEARDED DRAGONS (*POGONA VITTICEPS*).

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ABSTRACT: Since specific knowledge concerning the anatomy of the respiratory tract in bearded dragons is sparse, this study examined its larynx, trachea and lungs. Fifteen Inland Bearded Dragons, euthanized because of severe illness or injury, were used for macroscopic and histologic examination. Twelve cadavers (seven females, three males) were dissected for macroscopic documentation. In two of them, the pulmonary blood vessels were injected with coloured latex prior to dissection. For histology, samples of larynx, trachea, and lungs were fixed with formalin, and paraffin sections were stained with hematoxylin-eosin, Masson-Goldner or PAS. Thirty-nine healthy bearded dragons (13 males; 26 females, thereof 12 with follicles) were examined radiographically. The larynx contains one cricoid and two arytenoid cartilages. Its respiratory mucosa has a multi-layered epithelium with squamous to cuboid cells interspersed with goblet cells. The trachea consists of about 50 hyaline cartilaginous rings. Its respiratory mucosa has mostly a two-layered epithelium with small goblet cells. Dorsally to the heart, the trachea divides into two main bronchi, entering the respective lung in its cranial quarter and ending at the hilum without ramification. Both lungs are shaped sac-like and situated dorsally in the pleuroperitoneal cavity, extending caudally to the level of the gonads. In female bearded dragons, the pulmonary length depends on the presence of follicles. Two pulmonary arteries and a common pulmonary vein are the main blood vessels for the lungs. The pulmonary inner structure is formed by septa protruding from the outer wall into the lumen, surrounding irregularly shaped to round hollow spaces (faveoli).

The study was approved by the Institutional Animal Care and Use Committee at the University of Veterinary Medicine Hannover, Germany and the Lower Saxony State Office for Consumer Protection and Food Safety (Protocol number: 33.9-42502-05-18A281).

# HISTOLOGIC EVALUATION OF ELASTIC FIBRE AND SMOOTH MUSCLE CELL ARCHITECTURE OF BLOOD VESSELS IN UTERUS, STOMACH AND KIESSELBACK PLEXUS IN PORCINE VON WILLEBRAND DISEASE.

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ABSTRACT: Von Willebrand Factor (VWF) is the main protein mediating blood coagulation. Patients with von Willebrand Disease (VWD) type 3 (T3) suffer mucosal bleeding in several organs, caused by a quantitative defect of VWF. The influence of VWF on angiogenesis is known and angiodysplasia was reported in individuals with VWD. The pig model of this project matches clinically with human VWD patients. To analyse how VWF deficiency affects the blood vessel structure, we investigated the elastic fibre (EF) and smooth muscle cell (SMC) architecture of blood vessels in uterus, stomach and Kiesselbach plexus in porcine VWD type 3 in comparison to healthy wildtype pigs.

Three pigs homozygous for the causal VWF gene mutation, phenotypically matching T3, and three healthy pigs without mutation were chosen. Organs were harvested directly after sacrifice, processed for histology and stained with resorcin fuchsin and nuclear fast red. The presence and distribution of EF and organization of SMC in phenotypically unremarkable blood vessels was evaluated semi-quantitatively by scoring from 0 (none) to 3 (very good).

In every organ EF of unremarkable blood vessels were significantly more organized in T3 animals (e.g. stomach veins 1,41 vs 0,89, p=0,0006) and significantly more stained (stomach veins 1,76 vs 1,15, p<0,0001). In contrast, SMC were significantly more organized in WT animals (e.g. stomach veins 1,91 vs 1,64, p<0,0001). Angiodysplastic vessels were not uniform, specifically the SMC layer appeared disrupted, vessel walls seemed thin and dilated, and EF showed varying staining intensity.

To conclude, angiogenesis in VWD affected animals seemed to be altered. The enhanced staining intensity and organization of EF in unremarkable blood vessels of T3 animals indicates a compensation for compromised organization of SMC. Thus, not only obviously angiodysplastic vessels are affected. The observed alterations could lead to destabilization which may facilitate bleeding events in VWD affected individuals.

Animal experiments were approved by Lower Saxony state veterinary office (AZ33.19-42502-04-182940)

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# MICROVASCULARIZATION OF THE THYROID GLAND OF THE DOMESTIC CAT DURING PRENATAL DEVELOPMENT.

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ABSTRACT: As the first endocrine gland, the thyroid gland is essential for regulating the basal metabolic rate and somatic growth. The development of the thyroid gland in cats starts at day 30 p.c. when both undifferentiated primordia lay near the ventral surface of the trachea by the common carotid arteries. Between days 38-40 p.c. begins formation of the first glandular follicles, whose number increases until birth. This process causes an extensive and rapid development of nutritional and functional vascular networks. Therefore, we aimed to show the sequence of histological changes of glandular parenchyma together with a formation-specific vascular system. For the study, the formalinfixed cat fetuses aged from 28 to 63 days p.c. were dissected to describe the thyroid gland position. Some samples were processed routinely for histological LM observation. Additionally, vascular corrosion casts (VCC) for scanning electron microscopy after injection of Mercox resin were prepared. According to Polish law and the EU directive (no. 2010/63/EU), the research did not require the approval of the Local Ethical Committee. The macroscopical observations of the thyroid between days 28 - 38 p.c. have revealed that both primordia of the thyroid have a typical pattern of the main blood vessels, the same as in adult animals. Between days 28 - 25 p.c. in the undifferentiated parenchyma of the primordial thyroid, the loose, irregular network of voluminous capillaries between epithelial bands of cells, with signs of intussusceptive angiogenesis, was recognized. Around day 40 p.c., the glandular follicles appear, surrounded by 3 - 4 own capillaries. VCC of thyroid from day 45 p.c revealed the presence of a superficial vascular network and interlobular arterioles. In the follicular capillary network occur signs of sprouting angiogenesis. After day 48 p.c., when glandular follicles start to produce a colloid, the basketlike and mesh-like capillary networks appear around them, resembling the thyroid's micro vascularization pattern in adult cats.

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## COMPARATIVE STUDY OF THE ULTRASONOGRAPHIC, ANATOMOPATHOLOGICAL AND HISTOPATHOLOGICAL EXAMINATION OF DEEP DIGITAL FLEXOR INJURIES ON ISOLATED LIMBS.

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ABSTRACT: This study was performed on 36 isolated thoracic limbs collected from 36 skeletally mature horses from Alba County (Romania) and Alençon (France). The horses were humanely destroyed for causes unrelated to orthopedic problems but for public consumption (slaughter). Each forefoot was sectioned at the level of the carpo-metacarpal joint and identified. No history of the horses was known, but all were adult light draft or sport horses. Inspection and palpation of the feet were realized before the ultrasound exam. No attempt was made to evaluate limb conformation or hoof shape. The deep digital flexor tendon (DDFT) was examined using ultrasound and gross postmortem examinations followed by histopathology. To describe DDFT lesion localization in a proximodistal direction, three levels were established related to the distal sesamoid bone: suprasesamoidean, sesamoidean and infrasesamoidean. Our work hypothesis was confirmed, the ultrasonography had a very high overall sensitivity (96%, p < 0.0001 for the anatomopathological exam and 83.87%, p=0.0094 for the histopathological exam). The method was less specific (respectively 88.89% for the anatomopathological exam and 80.00% for the histopathological exam). In the sesamoidean region the ultrasound exam had the lowest specificity (38.10% for gross postmortem exam and 35.71 for histopathological exam) with an increased number of false negatives, but the results were not statistically significant (p=0.0514 and p=0.3884). In the infrasesamoidean region of DDFT the sensitivity of ultrasound (78.57% and 58.33%) was lower than specificity (86.36% and 91.67%) and these results were statistically very significant (p=0.0002 and p=0.005).

## B CELL DISTRIBUTION IN THE GILLS OF ATLANTIC SALMON AND THE EFFECTS OF SYSTEMIC VACCINATION ON B CELL ABUNDANCE.

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ABSTRACT: This study aimed to enhance our understanding of mucosal immunology and B cell responses by examining the gills of Atlantic salmon (Salmo salar L), focusing specifically on the interbranchial lymphoid tissue (ILT). The B-cell repertoire in the gills was investigated at various time points following vaccination. Two sampling periods were conducted, the first four months after intraperitoneal injection vaccination (average weight 150g), and the second over a year later (average weight 1700g). Both vaccinated and naïve fish were included in the analysis for the two size groups. The RNA Scope system, an in-situ hybridization method targeting specific RNA sequences in tissues, was utilized to identify distinct B cell populations. A comprehensive analysis of immune responses in the ILT revealed no statistically significant disparities between the unvaccinated and vaccinated animals. Nevertheless, a notable disparity emerged between the younger and older animals in the ILT in terms of IgM levels, with a significant increase observed in the older population. Through quantitative analysis, we determined that IgD levels were significantly higher compared to other immunoglobulin classes within the gill tissue. This observation suggests a potential role for IgD in the immune response and defense mechanisms specific to the gill environment. The predominance of IgD expression in the gills, especially in younger animals, underscores its potential importance in early immune development and adaptation to environmental challenges in this tissue. Further investigation into the functional significance of IgD in gill immunity could provide valuable insights into the mechanisms governing immune responses in aquatic organisms. These results help elucidate the complexity of gill immune system by measuring different B cells both qualitatively and quantitatively, and by putting them in an anatomical context. In addition, the presence of activationinduced deaminase (AID) was assessed to determine if secondary maturation of lymphocytes occurs within the ILT.

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### ASPECTS OF CASPASE-8 BIOLOGY IN HUMAN OVARIAN CANCER STUDIED IN AN ORTHOTOPIC MOUSE MODEL.

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ABSTRACT: Ovarian cancer (OC) is the deadliest gynaecological malignancy in women due to latestage diagnosis in an already metastatic phase. Therapy regimens relying on surgery and chemotherapy show good first response but frequent relapses. Novel therapies are eagerly needed addressing cancer cell biology with focus on programmed cell death, esp. Caspase-8 as a key protein in the apoptotic cascade. We aimed at analysing the impact of Caspase-8 depletion in an orthotopic mouse model of human OC. We used human OC cells (OVCAR8) derived from ovarian adenocarcinoma modelling high grade serous OC. Wildtype (WT) and Caspase-8 knockout (KO) OVCAR8 cells transfected with luciferase reporter were injected in the bursa ovarica of n=4 NMRI nu/nu mice (n=2 WT, n=2 KO). Starting one week after surgery and until the mice were sacrificed, luciferin signalling reflecting tumour cell proliferation and spreading was measured every week using an in vivo imaging system (IVIS). Ovaries and organs showing signs of tumour infiltration were harvested and fixed for IHC-P or stored in liquid nitrogen for protein/mRNA analyses. There was a significant signalling increase in the Caspase-8 KO group in comparison with the WT group demonstrating increased tumour growth after depletion of Caspase-8. Staining of the cells with annexin V as a marker of apoptosis showed slightly more apoptotic cells in the Caspase-8 KO group in comparison with WT, but there were no significant differences in cell cycle distribution and cell proliferation as tested by cell cycle and CellTiter-Blue test. RNAseq datasets will be used for in-depth analysis of tumour cell transcriptome linked to Caspase-8 to reveal differentially expressed genes. Focus will be on Connexin 43 in view of its importance for OC growth and metastasis. These experiments will provide insight into OC cell biology and tumour cell behaviour linked to Caspase-8.

# FUSE OR NOT DIVIDE: DIFFERENT AVENUES LEAD TO THE FORMATION OF MULTINUCLEATE CELLS IN THE PLACENTA OF DOMESTIC MAMMALS.

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ABSTRACT: In mammals the placenta is probably the organ with the greatest structural variability. One aspect of this variability is the presence of large multinucleate cells in many species. Generally, multinucleate cells develop by two completely different mechanisms: either cell fusion or different types of incomplete (e.g. acytokinetic) mitosis. Because these two pathways might be easily confused, especially in the placenta, we aim to give some morphological tools for their differentiation.

Glutalaldehyde fixed, epon embedded placentas from mid- to late pregnant animals were used. These were from domestic dog (*Canis familiaris*), cow (*Bos taurus*), sheep (*Ovis aries*) and alpaca (*Vicugna pacos*) (each n=3). Sections were studied by light microscopy and transmission electron microscopy.

In the dog placenta cytotrophoblast and syncytiotrophoblast are present and could be distinguished. Mitoses were only observed in the former, but never in the latter.

In ruminants (cow and sheep) mitotic activity was found in uninucleate and binucleate trophoblast cells. The nuclei in binucleate trophoblast cells were frequently large, with abundant chromatin. Stages of cell fusion between binucleate trophoblast cells and cells in the caruncular (maternal) epithelium were observed. Mitoses in the caruncular epithelium occurred frequently in the uninucleate cells of the cow placenta, but were not observed in the ovine caruncular epithelium.

In the alpaca, mitoses were seen in the uninucleate trophoblast and in large trophoblast giant cells and in the uterine epithelium. In the trophoblast giant cells mitoses were large and multipolar and the cell in interphase had multiple large lobulated nuclei.

Four structural criteria for the differentiation emerged: 1. multinucleate cells, which originate from cell fusion show no mitotic activity; 2. cell fusion can be observed in development of these cells; 3. mitotic figures can be seen in cells which undergo acytokinetic mitosis; 4. polyploidisation of nuclei can result from subsequent acytokineteic mitoses.

### VIM, TPI, MAT2A AND THEIR ROLE IN IN VITRO ANGIOGENESIS.

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ABSTRACT: As a requirement for versatile pathological events, e.g. tumorigenesis, endothelial cells (ECs) are compelled to perform angiogenesis. The research field of angiogenesis is currently focusing on tissue engineering and wound healing. Putatively based on the heterogenous character of ECs, in vitro assays of angiogenesis still face difficulties considering their reproducibility. Morphologically classified into angiogenic and non-angiogenic, ECs revealed alterations in their expression of eight specific proteins (1). Among them, vimentin (VIM), triosephosphate isomerase (TPI) and adenosylmethionine synthetase isoform type-2 (MAT2A), are selected for this study in order to get analyzed considering their association to *in vitro* angiogenesis. Therefore, tip and stalk cell distribution within cell populations of human dermal microvascular endothelial cells were analyzed via VEGFR-1 and -2 expression. A shRNA mediated knockdown of VIM and TPI was initiated separately. Cells were long-term cultivated using proangiogenic media and morphologically staged into their respective angiogenic stage, according to the all-in-one assay (2). During cultivation, protein and mRNA expression profiles of VIM, TPI and MAT2A were determined. Native cells displayed a high expression of VIM and MAT2A in early angiogenic stages and TPI throughout angiogenesis in vitro. VIM and TPI knockdown, respectively, resulted in cells not being able to enter late stages of in vitro angiogenesis, opposed to native ECs. By knocking down VIM, only cells with higher VEGFR-1 expression survived showing an increase in MAT2A and TPI expression. Concluding that VIM and MAT2A are indicated to be relevant in beginning stages and TPI during the course of angiogenesis in vitro. VIM and TPI knockdown led to a deceleration of in vitro angiogenesis, whereby knocking down VIM resulted in cell death of populations with less stalk cells and alterations of TPI and MAT2A expression.

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# TELOCYTES OF THE PORCINE MYOCARDIAL SLEEVES AND CONDUCTION SYSTEM.

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ABSTRACT: A novel type of cell known as telocyte (TC) was discovered over a decade ago. Described primarily in the interstitial spaces of various organs of humans and non-human species, TCs are defined by unique morphological features and diverse functions such as cellular communication, tissue organization, immune surveillance, and regeneration. Vandecasteele et al. (2017) have identified cardiac TCs for the first time in the wall of porcine pulmonary veins near the myocardial sleeves (MS) of the atriopulmonary junction. The study aimed to provide additional evidence indicating the presence of TCs in the MS and the porcine heart regions comprising the conduction system. Hearts were collected from 4 to 10 weeks old piglets and processed for light and electron microscopic investigations. Masson's trichrome staining confirmed the correct isolation of MS from the cranial and caudal vena cavae and pulmonary veins, the sinoatrial node, atrioventricular node, trabeculae septomarginalis, and Bundle of His. Immunohistochemistry and immunofluorescence revealed the presence of CD34+ cells with TC phenotypes. Double immunolabeling also detected CD34 and PDGFR $\alpha$  coexpression in these cells. Finally, transmission electron microscopy clearly showed the existence of TCs characterized by the presence of small, spindle-shaped cell bodies and cytoplasmic extensions known as telopodes (Tp). Tps have a mean thickness of  $<2 \mu m$ , are hundreds of microns long, and exhibit the typical moniliform appearance. The basal lamina in these prolongations was absent vis-à-vis with cellular units that possess this layer. In all regions, TCs were generally found in between muscle bundles. Specifically, Tps were observed proximate to or interacting with fellow TCs, blood/lymphatic endothelial cells, collagen fibers, pale and transitional cells, working myocardium, Purkinje cells, and ganglia. In conclusion, the combined findings unequivocally demonstrate the existence of TCs in the specialized regions of the porcine heart, suggesting their possible participation in impulse transmission and associated cardiac pathologies.

### CELLULAR MODELING IN NEUROSCIENCE.

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ABSTRACT: Mammalian Brain is the most complex organ in the human as well as animal body. To understand neuronal morphological differences and layerisation in different parts of the brain, in vitro models are crucial. In our previous work we published expression of different NSC, Neuronal and Glia specific markers during mouse embryonic development as well as during in vitro differentiation of human cerebral organoids. Primary mouse NSCs were isolated from E14.5 telencephalons while the human mature neurons and organoids were iPSCs derived. Both mouse and human in vitro differentiated neurons were stained with specific neuronal markers, imaged by confocal microscope and analysed by IMARIS software. Cells were stained with (Nestin, SOX2, PAX6, Reelin, FOXG1, SATB2, TBR1, Ctip2, Brn2 and GFAP) to analyse cell population and differentiation to mature neurons. Dendrites were labelled with MAP2 antibody while the axons were labeled with SMI312. Our data showed significant differences between mouse and human neurons in the number of dendrites, branching points, terminal points, initial axonal segment, thickness of dendrites and shill diagram. In our group we were focused on cell morphology and branching of dendrites as well as axons. With filament tracer we showed significant differences between genotypes and species. The same cell phenotype we showed in human as well as in mouse brain. Based on our data, there is no prefect model for any research, but for complete picture we need everything in vitro and in vivo data generated on gyri-cephalic and lissencephalic brain.

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# CANINE DECIDUALIZATION: IN VIVO OBSERVATIONS AND IN VITRO FUNCTIONAL STUDIES OF INTRACELLULAR SIGNALLING PATHWAYS.

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ABSTRACT: Among the characteristic features of the canine endotheliochorial placenta is the speciesspecific, embryo-driven decidualization, i.e. differentiation of endometrial stromal cells into decidual cells, associated with mesenchymal-epithelial transition.

Following placentation, decidual cells, together with maternal endothelial cells, evade the trophoblast invasion and become the only placental cells expressing the nuclear progesterone receptor (PGR). This directly links the placenta to the maintenance/termination of canine pregnancy, as interfering with PGR signaling, e.g. by applying blockers of PGR function (antigestagens), terminates pregnancy. The underlying signaling cascades evoked by antigestagens resemble the natural prepartum withdrawal of PGR function, providing unique clinically relevant opportunities for investigating canine placental function. *In vitro*, cAMP can be used to induce decidualization in immortalized dog uterine stromal (DUS) cells. The downstream cascades involve the activity of PKA, a serine/threonine kinase (STK), but the involvement of other intercellular signaling pathways is obscure.

Our *in vivo* observation included formalin and glutaraldehyde fixed, paraffin- or epon-embedded placentas from animals in late pregnancy. *In vitro*, a functional kinomics approach was used, involving the PamChip assay (PamGene) with decidualized DUS cells, for the prediction of STKs activity.

Besides confirming their close localization to maternal vessels, as a new finding from serial sectioning, decidual cells had cytoplasmic projections that spread around maternal vessels and between the fetal trophoblast compartments. Among STKs, 85 showed increased activity in decidualized DUS cells (e.g., PKA, PKC, ERK1/2, ATR and Akt1/2). Antigestagen (aglepristone and mifepristone) treatment for 6h reversed the increased activity of all kinases. The involvement of selected kinases was further dissected in functional studies by applying specific blockers and assessing the expression of decidualization markers in DUS cells (e.g. *PRLR*, *IGF1*, *PTGES*, *PTGS2*). PKA and MAPKs evoked stronger effects than PKC, indicating that signaling downstream of PKC differed from that of PKA and MAPKs.

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# RECRUITMENT OF MONOCYTES/MACROPHAGES INTO ALVEOLAR SEPTA OF LUNGS OF SARS-COV2 INFECTED ANIMALS.

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ABSTRACT: Upon a viral infection, on of the first cells to interact with pathogens are macrophages/monocytes. The infected macrophages/monocytes help in spread of virus by direct cellto-cell contact and interaction with other cell types. During their active migration to the sites of injury, monocytes and macrophages produce chemokines and cytokines that are crucial for immune and inflammatory processes. Due to the significant morbidity and mortality caused by severe clinical symptoms of SARS-CoV-2 infection including cytokine storm and multiple organ failure and the long COVID disease, we still need better understanding of immune responses in the lungs. In this study, we divided Syrian golden hamsters into 6 groups. The uninfected group served as control. Three groups of SARS-CoV-2 only infected (unvaccinated) animals were studied at 2-, 5-, and 14-days post infection (p.i.). Two infected+vaccinated groups were studied at 5- and 14-days p.i., (n=4 per group). We quantified both the percentage of lung parenchyma occupied by fluorescent stained cells and the normalized fluorescent intensity ratio (FIR) of each immune marker (myeloperoxidase for neutrophils, CCR2 and CX3CR1 for classical and non-classical monocytes/macrophages, respectively, IBA-1 for macrophages and CD3 for T cells). The levels of the immune cell types were elevated in unvaccinated animals, 2 days p.i. A higher presence of macrophages, predominantly septal, was detected on days 2 (72.7%, p<0.01), 5 (63.6%, p<0.05) and 14 (65.2%, p<0.05) p.i. in unvaccinated animals compared to noninfected control (34.4%). The FIR of IBA-1<sup>+</sup> and CX3CR1<sup>+</sup> cells, was higher 2 days p.i. for unvaccinated animals, when compared to vaccinated ones and to 5and 14-days p.i (p<0.05). Our data are the first to suggest recruitment of septal macrophages into the lungs of SARS-CoV-2 infected animals and a reduced recruitment of these cells in vaccinatedinfected animals. These findings are important because septal macrophages are established as proinflammatory cells.

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# ANNEXIN V-FITC APOPTOSIS ASSAY ON PORCINE CARDIAC TELOCYTES *IN VITRO*.

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#### ABSTRACT:

Telocytes (TCs) are a relatively unknown cell type characterized by their moniliform, thin and long processes known as telopodes. However, the existence of TCs remains a subject of debate. Transmission Electron Microscopy is currently regarded as the gold standard for the identification of TCs, primarily relying on their localization and two-dimensional morphology. This approach can be prone to misinterpretation. Nevertheless, discovering their role and function may provide valuable insights into the etiopathogenesis of cardiovascular and other diseases. In this regard, *in vitro* studies are needed to further investigate TC functionality. Obtaining TC cell cultures remains a challenge, and cultured TCs can be confused with cells undergoing apoptosis. In this study, we aimed to compare TC morphology with apoptotic cells *in vitro*.

Porcine myocardial tissue was processed using a TC isolation protocol. Passage 2 cells with TC phenotype were stained with Annexin V-FITC (Abcam) and DAPI (Sigma) to detect early-stage apoptosis. To induce apoptosis in the positive control cells, TCs were incubated with staurosporin (Abcam) at a final concentration of 0.5  $\mu$ M for 1 hour. At this timepoint, obvious apoptotic morphological traits were visible using inverted phase microscopy. Imaging was performed using an epifluorescence microscope (ZEISS Palm microbeam).

Preliminary findings suggest that telopodes and the prolongations of apoptotic cells share some morphological similarities. On the staurosporin treated cells, various stages of apoptosis were observed. The apoptopodia, a type of apoptotic membrane protrusions, can resemble the beaded appearance of telopodes. Nevertheless, the annexin V staining of our cultured TCs was negative, indicating that these cells are different from apoptotic cells. This adds to the evidence that TCs are an individual cell type. Obtaining a consensus on the existence of TCs is a vital step in order to spark more research interest and potentially use TCs in cell therapy of cardiovascular diseases.

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# EQUINE PLACENTA IN THE LAB: DEVELOPMENT OF EQUINE PLACENTAL ORGANOIDS.

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ABSTRACT: A fundamental understanding of placental development and its associated pathologies is crucial to mitigate the losses associated with placental disorders. Organoids are widely used to investigate the developmental biology of organs and diseases. In this study, we aimed to (1) develop placental organoids using first trimester and term equine placentae, and (2) evaluate the organoid structures. Chorioallantois (CA) was collected from healthy placentae following elective abortion (80-100d of gestation) and spontaneous term parturition. CA samples were processed for culture, fixed for histology, and stored in RNAlater for transcriptomic analysis. For culture, each CA sample was enzymatically digested to a single cell suspension and seeded into Matrigel droplets surrounded by medium. Passage-3 organoids (P3) were used for histology (H&E), transmission electron microscopy (TEM) and transcriptomic analysis. After optimization of culture conditions, we were able to produce 3D-organoids (Pre-Term, n=6 and Term, n=3). Organoids were passaged up to 12 times over  $\pm 3$ months and were successfully frozen and re-cultured post-thaw. Total extracted RNA from initial CA samples and organoids was sequenced (150PE; ~43 million reads/sample). The genes expressed in the organoids closely resemble those of the tissue of origin, 17.6% of the expressed genes were differentially expressed between the initial CA and P3 (n=3,587 genes; FDR<0.01 and  $|\log_2F_0|dChange|\geq 2$ ). The expression of trophoblast-enriched genes PLAC8B, GATA3, KRT7, EGFR, TFAP2A, TFAP2C, and GATA2 in the organoids confirm their trophoblast identity. H&E staining of the organoids confirmed their multicellularity and indicated the epithelial origin of the cells. Our preliminary TEM analysis suggests the presence of microvilli on the luminal side of the cells. To conclude, this is the first report of equine placental organoids. The organoids show structural and transcriptomic similarities to the equine placenta in vivo and consequently show great promise as an in vitro model to study placental development and associated pathologies.

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#### THE INFLUENCE OF BREED AND DIFFERENT SEASONS ON MUSCLE AND SKELETAL DEVELOPMENT AT BIRTH IN NEWBORN PIGLETS.

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ABSTRACT: The development of the musculoskeletal system is a complex mechanism regulated by genotype and environmental factors. Together, they play a role in defining the outcome and the timing of the process. This study aimed to assess musculoskeletal maturity at birth in Nero di Lomellina (NL), an Italian local pig well known and appreciated for its high-quality meat. Analyses were performed in winter and in summer to evaluate the effects of different temperatures during gestation. Results were compared with those obtained for Commercial Hybrid (CH) newborns. We considered a farm where NL and CH were equally bred: this provided us with a robust and reliable developmental model.

No animal was sacrificed for research purposes: piglets, born alive and crushed under the sow at birth, were enrolled for the study. Muscle development was assessed in *Longissimus dorsi* (LD) by morphological and molecular analyses. Skeletal maturity was scored by a radiological evaluation of limb Secondary Ossification Centers (SOCs). In summer, NL's LD showed significantly wider fibers than NL's winter and CH's summer ones (p<0,05), while cell count resulted significantly higher in winter only inside each breed (p<0,05). Both breeds revealed an apparent higher, though non-significantly, expression of Myogenic Regulatory Factors (MRF) in winter, except for NL's summer expression of Myf6, which resulted significantly higher than the winter one (p<0,01) and CH's summer one (p<0,05). The analyses of SOCs revealed a higher skeletal maturity in NL in both seasons.

These preliminary results suggest a different musculoskeletal development at birth according to the season and the breed, which could influence the resulting productive performances of adult animals. Not only does this study provide a characterization of skeletal muscle tissue at birth, but it also represents a starting point to study the mechanisms involved in muscle development. Further studies are required to better clarify this aspect.

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# MELANO-MACROPHAGES AND THEIR RELATION TO MUSCLE INFLAMMATION I ATLANTIC SALMON (SALMO SALAR).

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#### ABSTRACT:

Melano-macrophages are typically found in the kidney and spleen of healthy fish, where they can form melano-macrophage centers that resemble germinal centers. While they may occasionally appear in other organs, they are commonly associated with chronic inflammatory processes. Notably, in Atlantic salmon, their presence in the abdominal muscle leads to the formation of melanized spots, necessitating the trimming and down-classification of fillets, which poses significant challenges for the industry. These melanized spots are observed in salmon-producing countries worldwide. In our research, we have investigated the development of these changes, which initiate with muscle bleeding and progress into chronic granulomatous inflammation characterized by the presence of melano-macrophages. Furthermore, we have explored the impact of Piscine orthoreovirus 1 (PRV-1) infection on this condition. In this study, we provide evidence for the establishment of melano-macrophages within the lesions. By examining sequential samples throughout the development of these lesions, we trace the emergence of this cell population and observe their responses over time. Our findings suggest that the synthesis of melanin occurs within the cells in situ and is not solely a result of the engulfment of extracellular material.

Funding: FHF project # 901501

# EFFECTS OF 3D SCANS ON VETERINARY STUDENTS' LEARNING PROCESS COMPARED TO TRADITIONAL 2D IMAGES.

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ABSTRACT: Students often experience difficulty in interpreting conventional textbook images [1,2]. One of the most challenging topics in veterinary anatomy is to identify the correct structures of skulls in different species. The aim of the study was to examine the impact of 3D scans on students' learning process compared to traditional 2D images. For this purpose, annotated 3D scans of horse and pig skulls were generated and made available on Sketchfab. During practical classes a total of 118 second-year students were divided into three groups, which received different learning materials (3D scans, 2D images annotated with arrows or with numbers). The anatomical knowledge of students was tested using an objective structured practical examination before and after the learning session. In addition, spatial ability was tested using the mental rotation test. After the study, the students could provide feedback on quality and usability of the different learning tools. All groups showed significantly improved results in the post-test compared to the pre-test. The 3D scans group scored the best, followed by the 2D arrows group and then by the 2D numbers group. However, results between the groups did not differ significantly from each other. The results of the mental rotation test were positively correlated with the results of the 3D scans and the 2D arrows group, but negatively correlated with the results of the 2D numbers group. The survey of the students revealed that they perceived 3D scans as beneficial learning material. They also indicated a great interest in this alternative learning material. These data suggest that 3D scans could be useful as an alternative learning material to ease the learning process and improve motivation of students, even though they do not necessarily contribute to a better learning outcome than 2D images.

- [1] Preece, D., Williams, S. B., Lam, R., & Weller, R. (2013). "Let's get physical": Advantages of a physical model over 3D computer models and textbooks in learning imaging anatomy. Anatomical Sciences Education, 6(4), 216–224.
- [2] Wainman, B., Wolak, L., Pukas, G., Zheng, E., & Norman, G. R. (2018). The superiority of three-dimensional physical models to two-dimensional computer presentations in anatomy learning. Medical Education, 52(11), 1138–1146.

# "TAKING THE BULL BY THE OMENTUM" – USING DIFFERENT TEACHING MODALITIES TO EXPLAIN THE RUMINANT MESOGASTRIUM.

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AFFILIATION: Leipzig University, Leipzig, Germany.

ABSTRACT: The omental arrangement associated with the ruminant stomach is very complex. Understanding three-dimensional anatomical morphology such as the mesogastrium makes cadaver dissection an essential component in the students' curriculum. Dissecting a ruminant, however, is not always possible. Especially in lectures or for students at home information on this topic will be conveyed through two-dimensional imagery or textbook learning.

The presented project's goal is to help students grasp this challenging topic and develop a solid threedimensional appreciation of the mesogastrium by using different teaching modalities. Studying with a cost effective, low-fidelity model can facilitate a learning success similar to that of the dissection experience. For this purpose, galvanised chicken wire was used to mould the ruminant oesophagus, stomach, duodenum, and adjacent diaphragm. It will be covered by papier mâché, coloured, and will serve as framework for lengths of fabric representing the omentum. Different colours of the material will help identify the deep and superficial layers of the greater omentum. In addition to covering the kinaesthetic learning style by building a model, a small ruminant will be embalmed and dissected. Short instructive videoclips will be prepared in order to include an audiovisual approach. They will then be revised using a video editing software and uploaded to the students' online learning platform.

As an ongoing study the evaluation by the students will be conducted during the summer term.

Past experience integrating different teaching methods has shown that the learning effect for theoretical understanding was enhanced. Furthermore, the acceptance and satisfaction of learning with a hands-on experience by the students has proven to be very positive. As a conclusion implementing these methods could be a chance for lecturers to make their topic more graspable upgrading their overall teaching.

# VETERINARIANS' OPINIONS TOWARDS DISSECTION DURING THE UNDERGRADUATE VETERINARY MEDICINE COURSE AND ITS EFFECTIVENESS IN PREPARING THEM FOR SURGERY AND VETERINARY LIFE.

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ABSTRACT: Studies are aplenty when it comes to understanding the opinions of veterinary students towards dissection and maximising their experience in the dissection lab, however very little has been done exploring the opinions of practising veterinarians on the usefulness of dissection as part of the undergraduate veterinary medicine degree.

Methods: An online survey was distributed via two veterinary social media pages on Facebook in order to ascertain the views of veterinarians. Responses were organised into subgroups to allow for data analysis.

Results: 221 people responded to the request for participants. There was little difference in opinions between the subgroups, with the only significant difference identified being in the anatomical and clinical confidence between different numbers of years in practice (p = 0.045). Veterinarians identified the importance of dissection in anatomical instruction and the development of practical skills, however there was variability in the perception of dissections role in developing certain attitudes such as professionalism and emotional coping strategies.

Conclusions: The results strongly support the continued use of dissection in undergraduate veterinary medicine, whilst also recognizing the need to improve its relevance to clinical knowledge and increase its utilisation in later clinical years. It also recognizes the need to use modern methods, such as virtual software, alongside traditional dissection rather than as a replacement.

Funding: NA.

### **RECENT DEVELOPMENTS IN DEVELOPING A NOVEL CORROSION CASTING MATERIAL.**

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ABSTRACT: Corrosion casting is the three-dimensional physical replication of hollow anatomical structures, to further analyze them either macroscopically, or indirectly by microscopy, or other imaging techniques. It is characterized by the injection of a fluid with solidifying capabilities, commonly a polymeric resin. Most of the commercially available casting materials have limitations regarding pot life, viscosity of the fluid, structural integrity after solidification, or high cost. Our work has explored the potential of novel acrylic resins with superior performance as the corrosion casting material. Here we focused on the characterization of different formulations based on acrylic monomers. Firstly, we studied binary formulations. Kinetic, mechanical and rheological analysis of those formed the basis to develop more complex mixtures. Then, 15 second-generation four-component formulations have proven to have a potential for different applications. Testing this potential, several practices have been identified as challenging for successfully executing a corrosion casting procedure. (1) For a study on chicken embryos (16~17 days old), we used an ultra-low viscosity ( $\sim 3 \text{ mPa} \cdot \text{s}$ ) resin. After euthanizing the embryos, injection was done via the external iliac veins with an outflow point created at the level of the jugular veins. The injection went remarkably easy due to the low viscosity. The perfusion was high, replicating lungs, kidneys, heart, and most of the digestive tract and the sacs. The cast was noticeably sturdy, especially compared to available acrylic resins in the market. (2) Mice casting studies showed that the compatibility of the resin with a newly created contrast agent (hafnium nanocrystals) is excellent as the dispersion was homogeneous with neglectable change in the size distribution. With the contrast agent, we acquired outstanding images via micro-CT scans.

Funding: This research was funded by the Special Research Fund (Bijzonder Onderzoeksfonds-BOF) of Ghent University, under a Concerted Research Actions Project (Geconcerteerde Onderzoeksacties-GOA) under the number BOF.GOA.2018.0002.03.
## THE DEVELOPMENT OF SimOVE; A Virtual Reality CANINE Ovariectomy SIMULATOR.

AUTHORS: Wolschrijn C.F<sup>1</sup>., Hierck B.P.<sup>1</sup>, Leeuwen van B.S.<sup>1</sup>, Sanders A<sup>2</sup>., Salvatori D<sup>1</sup>.

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ABSTRACT: Ovariohysterectomy (OVE) in the dog is in most veterinary curricula considered a socalled Day-1 competence, which should be mastered at graduation before entering clinical practice. Since the implementation of EU directive 2010/63/EU, animal use in education is only allowed if there are no alternatives to reach the learning objectives which has major implications for veterinary education. The learning objective of spaying can be 'dissected' into smaller learning goals like basic anatomy of the female urogenital tract and abdominal wall, anesthesia, aseptic procedures, the procedural steps and, the actual performing of the procedure on a patient. Except for the last one, all other learning outcomes can be covered without live dogs such as with books, e-modules including films and overlays, plastinated specimens and simulators. What is lacking is a hands-on experience that allows practice of the procedure time- and space-independent, allowing a better preparation before performing the surgery on a live client-owned dog, and also boosting the self-confidence of the student.

With a to-be-developed simulation model it should be possible to reach the following learning goals; proper opening of the abdomen, performing an OVE of the bitch with the focus on locating the ovaries in the abdomen by means of the descending duodenum and the colon, clipping of the relevant structures and checking on complete removal of the ovaries and bleeding. In order to achieve this, a commercially available 3D virtual reality dog model was brought into the 3D surgical theatre of IntoSurgery.com (holodeckOR) in which patients can be operated in VR. The desired (student) learning goals were guidelines for the technicians that adapted the model accordingly.

With the presentation of the prototype the added value of VR simulation in teaching the anatomy of a surgical procedure will be demonstrated, which can be expanded to other procedures as well.

Funding: none.

### Abstracts – Poster presentations

Teaching and gross anatomy (Wednesday - July 26th)

## 1) CYCLIC TENSILE TEST ON CANINE CRANIAL AND CAUDAL CRUCIATE LIGAMENTS.

AUTHORS: Schulda N, Reese S

INSTITUTION: University of Munich (LMU), Institute of Anatomy, Munich, Germany.

ABSTRACT: Knowledge of the biomechanical properties of the canine cruciate ligaments has always been of high clinical relevance in veterinary medicine. Several publications concentrate on tensile tests on ultimately extended stifle joints to primarily determine the failure load of the cranial cruciate ligament (CrCL). This study is designed as a cyclic tensile test in which the viscoelastic properties of the cranial and caudal cruciate ligament (CaCL), get assessed and compared. This abstract presents the initial results of 15 CrCL and CaCL tests.

The stifle joints were prepared as bone-ligament-bone complexes. The cruciate ligaments' crosssectional areas were determined, and the samples were inserted into a material testing machine at a 90° flexing angle. A cyclic tensile test (CrCL: mean of 921 cycles; CaCL: mean of 629 cycles) with increasing levels of strain (from 2% over 20%) was run for either CrCL or CaCL. Hysteresis loops were plotted continually and biomechanical parameters like standard load, tensile strength, and deformation were protocolled until a 50% decrease in maximum tensile strength was registered. The cause of material fatigue was assessed by using a stereo microscope.

The comparison of the area of the hysteresis loops at the range of 2%-18% strain shows a nearly identical decrease of approximately 30% for both cruciate ligaments. Material fatigue occurs at the range of 18%-26% strain and a substantial decrease in the area of the hysteresis loops can be seen in both ligaments. CrCL experiences intraligamentous damage before rupture occurs at further levels of strain (> 20%). CaCL failure is seen at earlier levels of strain (mainly 12%-20%).

Cruciate ligaments show clear viscoelastic properties which are dependent on the level of strain. CrCL and CaCL tolerate a higher level of strain until failure occurs than former studies prove for damage in tendons (12%-15% strain) (Martin et al., 2015).

Martin RB, Burr DB, Sharkey NA, Fyhrie DP. Skeletal Tissue Mechanics: Mechanical Properties of Ligament and Tendon. 2 ed. New York: Springer; 2015: p. 175-225.

Funding: University of Munich (LMU)

## 2) THE CANINE DORSAL PRIMARY BRANCHES OF THORACOLUMBAR SPINAL NERVES: COURSE AND BRANCHING PATTERN.

#### AUTHORS: Bernigau D, Röhrmann N

AFFILIATION: Leipzig University, Leipzig, Germany

ABSTRACT: Exact knowledge about spinal nerves and their corresponding cutaneous areas as well as referred pain syndromes are fundamental for numerous manual and complementary therapies in veterinary medicine. Detailed findings in cats show a remarkable interindividual regularity concerning the covered distances of nerve branches through all body layers until their entry in the skin. There is need for research whether examinations of dogs due to their numerous different breeds reveal comparable results.

Therefore, the aim of this ongoing morphological study is the detailed description of the lateral cutaneous dorsal branches of the thoracolumbar dorsal region in dogs.

Dissection of ten dogs of different breeds, sex and age was carried out in four layers to reveal the course of dorsal primary branches between the spine and their point of entry into the skin. These animals were euthanized in field practices for reasons not related to this study and donated to the Institute of Veterinary Anatomy. After perfusion with 4% formalin, the specimens were placed in a straight prone position. In each layer, the courses and covered distances from the dorsal branches were documented, measured and evaluated on both body sides. The documented findings were compared with those obtained in cats.

Preliminary findings indicate a mean caudal shifting of three back regions (defined as the distance between the cranial tips of two consecutive spinous processes) for the dorsal branches Th9 to L7 in the most superficial layer (skin). These outcomes are in consent with the published results in cats and show remarkable similarities for the course and distances of the dorsal primary branches through all body layers between the spine and skin.

The presented data provide solid anatomical basis for therapies used in complementary veterinary medicine.

### 3) SCIENTIFIC ILLUSTRATION OF PRE-SURGICAL RADIOGRAPHIC POSITIONING, ACCESS AND TECHNIQUES FOR AVIAN ULNA AND RADIUS OSTEOSYNTHESIS IN WILDLIFE REHABILITATION.

AUTHORS: Martins B1\*, Mendes S2\*, Correia M3, Carreira L-M4,6\*\*, Alexandre-Pires G5,6\*\*

AFFILIATION: <sup>1</sup>Faculty of Veterinary Medicine of Lisbon, Lisbon, Portugal. <sup>2</sup>Exoticvets, Lisbon, Portugal. <sup>3</sup>LIDA, School of Arts and Design, Polytechnic of Leiria, Leiria, Portugal. <sup>4</sup>Clinical Department, <sup>5</sup>Department of Morphology and Function, Faculty of Veterinary Medicine of Lisbon, <sup>6</sup>CIISA, AL4AnimalS, Portugal. \* Authors contribute equally and should be considered 1<sup>st</sup> authors, \*\*Authors contribute equally and should be considered last authors.

ABSTRACT: Wild birds medicine requires endeavour from expertizes in order to share information for others planning to work in this field. Scientific illustration is a striking tool, which ensures the possibility of detailing at "a step at a time" manoeuvres, steps, planes and processes involved in medical practices.

As many of the procedures performed in the rehabilitation of wild birds, surgical ones or otherwise, were not previously described, a huge amount of adaptability, creativity and innovation is often required from those involved in their care. Although many may believe that good digital photography can successfully explain delicate surgery, for example of the avian wing in which some structures are no larger than 1 centimetre, we believe that they are mistaken. In fact, when learning highly demanding subjects for the first time, visual references are an invaluable resource and photographs alone, of delicate structures, can be very hard to decipher even for expert professionals. No doubt, when talking about emergent avian wildlife medicine in rehabilitation centres, an endless panoply of new situations is assumed, not described jointly in books and other scientific literature. Therefore, medical illustrations serve as a bridge between what is observable and what can be shown. Experience from professionals of the field is worthy to be share. We have chosen to demonstrate through a practical example how scientific illustration techniques can convey the appropriate techniques for radiographic pre-surgical evaluation of radius and ulna fractures in Eurasian Eagle Owl (Bubo bubo). Accurate illustrations depict the appropriate surgical access for each fracture type, detailing anatomical structures involved, and the techniques available for surgical osteosynthesis of simple fractures in different locations of the aforementioned bones. It is our objective to convey these concepts in a mixed format, combining and comparing illustration, photography and written description, to maximize the potential for information transmission.

### 4) SELF-REGULATED LEARNING IN VETERINARY ANATOMY: COMPARING STRATEGY USAGE AND STRATEGY KNOWLEDGE.

AUTHORS: Dörrenbächer-Ulrich L1, Gerics B2, Steinberg E2

AFFILIATION: 1Saarland University, Saarbrücken, Germany. 2University of Veterinary Medicine, Vienna, Austria.

ABSTRACT: College students face rather autonomous learning settings that are not as structured as in high school. Medical and veterinary college students have to cope with high amounts of learning material and additionally have to acquire new skills within practical-clinical settings. Self-regulated learning (SRL) has been found helpful to overcome learning obstacles in this population. The present study investigates how SRL strategy knowledge and SRL strategy usage are related. We analyzed if first and third semester students differ regarding these variables in their anatomy studies. A sample of 181 veterinary students (first semester n = 108, third semester n = 73) filled out a strategy knowledge test on self-regulated learning as well as a self-report questionnaire. Sixty students filled out both instruments one semester later as well. While there were no differences in neither strategy knowledge or usage between first and third semester students, correlations between instruments were higher for first semester students. While motivational strategy usage increased during one semester, there were no changes in SRL strategy knowledge. Trainings to foster self-regulated learning that helps veterinary anatomy students to cope with challenging learning situations seem to be necessary.

## 5) FUNCTIONAL ANATOMY OF THE TARSAL JOINT OF THE ALPACA (*VICUGNA PACOS*).

#### AUTHORS: Riebow D, Maierl J

AFFILIATION: Chair of Anatomy, Histology and Embryology, Department of Veterinary Sciences, Ludwig-Maximilians-Universität (LMU) Munich, Germany

#### ABSTRACT:

Introduction: The rising number of South American Camelids in Europe is increasingly confronting veterinarians with their clinical problems. This study's objective was to present the anatomical knowledge of the tarsal joint of the alpaca.

Materials and Methods: Pelvic limbs of eight alpacas, euthanized for medical reasons not affecting the locomotor apparatus, were examined. The tarsal joint was dissected and latex milk preparation of the blood vessels and the joint capsules was performed.

Results: The alpaca's hock is a compound joint, made up of four levels and six distinct bones. The alpaca has also developed an independent os malleolare. The proximal row is formed by the talus und calcaneus. The os tarsi centrale articulates proximally with the talus, laterally with the os tarsale quartum and distally with the os tarsale primum and the fused os tarsale secundum et tertium.

The lateral and medial ligaments consist of the ligg. collateralia tarsi longum and breve on each side. On the dorsal aspect is the fan shaped lig. tarsi dorsale, which rises medial at the talus and runs obliquely to the metatarsus. The lig. plantare longum is split in a lateral and a medial limb. The latter of which partially encloses the superficial digital flexor tendon.

The joint capsules are formed separately for each joint level, with the articulatio tarsocruralis being the most spacious including a dorsal and a plantar recess.

The complex structure of the tarsal joint limits its mobility. The m. tibialis cranialis, m. fibularis tertius and M. fibularis longus are responsible for flexion. Due to the absence of the m. soleus, the m. gastrocnemius is the main extensor.

Conclusion: For diagnosis and treatment of hock disorders, e.g. varus/valgus deformities due to rachitis or trauma as well as fractures and luxations, it is important to know the anatomical features of the tarsus.

Funding: Ludwig-Maximilians-Universität Munich

### 6) SKELETAL MUSCLE AND SUBCUTANEUS FAT TRAITS IN INDIGENOUS KRŠKOPOLJE PIG BREED IN RELATION TO DIFFERENT REARING ENVIRONMENT AND DIET.

AUTHORS: Štrbenc M<sup>1</sup>, Farazinc G<sup>1</sup>, Brankovič J<sup>1</sup>, Vrecl Fazarinc M<sup>1</sup>, Čandek-Potokar M<sup>2</sup>, Poklukar K<sup>2</sup>, Batorek Lukač N<sup>2</sup>, Škrlep M<sup>2</sup>

AFFILIATION: <sup>1</sup>University of Ljubljana, Veterinary Faculty, Institute of Preclinical Sciences, Ljubljana, Slovenia. <sup>2</sup>Agricultural Institute of Slovenia, Ljubljana, Slovenia.

ABSTRACT: The Krškopolje pig is a local breed of Slovenia, characterized by slow growth and high fat deposition. The breed is kept in different rearing systems with various levels of intensity, effect of which are still largely unknown. Thus, the aim of the study was to characterize the effects of environment and diet on histo-morphological traits of fat and muscle tissue.

In total 52 Krškopolje pigs were kept either entirely indoors or in outdoor roaming area and fed diets with low (LP) or high protein content (HP). After slaughter, back fat samples with adherent skin at the last rib were collected, fixed in formalin, sectioned, and stained with hematoxylin and eosin. The longissimus dorsi (LD) and semispinalis capitis (SSC) muscles were also sampled, snap frozen in liquid nitrogen and used for enzyme- and immuno-histochemistry, oil-red-O staining, RNA isolation for subsequent RNA sequencing (RNAseq) and Nanostring nCounter analysis.

Diet had no effect on tested adipose tissue parameters, whereas HP diet increased the percentage of IIX and decreased the % of IIB muscle fibers in LD. Rearing environment did not affect interfascicular fat content, but adipocytes in the outer backfat layer were smaller and dermis thicker in indoor compared to outdoor pigs. In skeletal muscles a shift toward faster fiber types was observed in indoor groups in both muscles. Accordingly, RNASeq suggested higher expression of myosin heavy chains IIx and IIb in SSC of indoor groups. In addition, differences in expression of other genes related to contractile (MYO15A, MYL6B, MYOZ2) and metabolic (HK2, SCD, ARG2, FASN) properties were also detected. Validation of muscle transcriptome profiles by nCounter will be presented.

To summarize, in local pig breed the tested diet had little effect compared to the rearing, with indoor housing associated with lower adiposity and increase in faster-twitch muscle fiber types.

Funding: Slovenian Agency of Research (P4-0133, P4-0053, J4-3094, V4-2201), GEroNIMO (EU H2020 GA no. 101000236).

## 7) HOW IMPORTANT IS THE DISSECTION TO LEARN VETERINARY ANATOMY?

### AUTHORS: <u>Senos R<sup>1</sup></u>, Mann P.

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ABSTRACT: Dissection is probably the oldest approach used to teach both human and veterinary anatomy throughout the globe. In the modern vocabulary, dissection is also a way of active-learning, which is related to better knowledge retention and deeper learning. Dissection is also the most common method used to awake kinetics-learning in the students. However, in the contemporaneous educational world, how important is the dissection to learn veterinary anatomy? The aim of this study was test if dissection is essential to learn anatomy. We surveyed 73 DVM students that studied small animals anatomy using dissection as main method and large animals using prosected specimens. We also compared the grades on the horse distal limbs between 15 students that dissected and had access to prosected specimens and students that just had access to prosected specimens. 85% of the students considered dissection essential to learn anatomy. Students attributed that more than 60% of their knowledge were obtained by reviewing the specimens they dissected weeks before against 40% that obtained the knowledge during the dissection. Regarding the performance on the horse distal limb questions of the practical exam, students that dissected performed 11% (p<0.05) better than the students that just had access to prosected specimens. However, the only two students that scored all the questions correct on the exam did not perform any dissection. Although the dissection seems to result in better performance of the students on the exams and students stated that dissection is essential, the results indicate some controversy. Some students that did not dissect performed better than those who dissected. In addition, students stated that the majority of their knowledge was obtained from reviewing the dissected specimens, as studying from prosected specimens. Our results highlighted controversy of the topic and that there is more than one way to learn anatomy effectively.

## 8) GROSS MORPHOMETRY OF THE HEART OF THE COMMON SQUIRREL MONKEY.

AUTHORS: Senos R<sup>1-2</sup>, Muniz HKR<sup>2</sup>, Souza DAO<sup>2</sup>, Souza IV<sup>3</sup>, Perea AS<sup>3</sup>, Andrade MCR<sup>3</sup>

AFFILIATION: <sup>1</sup>Cummings School of Veterinary Medicine, Tufts University, North Grafton, USA. <sup>2</sup>Faculdade de Veterinária, Universidade Estácio de Sá, Rio de Janeiro, Brazil. <sup>3</sup>Instituto de Ciência e Tecnologia em Biomodelos, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

ABSTRACT: The Common squirrel monkey (Saimiri scireus) is a South American endemic primate species that has been widely used as an experimental model in biomedical research. Although there are hundreds of published studies using the species, morphological data are scarce. We aimed to describe the morphometric parameters of the heart of the S. scireus kept in captivity for biomedical studies. Twenty hearts (10 males and 10 females) of S. sciureus from the Fundacao Oswaldo Cruz (FIOCRUZ) were collected and fixed in 10% formalin to characterize the gross morphometry. Biometric data were also obtained. Data were grouped in males (M) and females (F). Biometrics averages indicated that M weighted 1012g, while F 640g; body length was 67.10cm for M and 61.30cm for F; and thoracic perimeter was 18.40cm for M and 16.60cm for F. Heart morphometry presented average weight 4.7g(M) and 3.4g(F); relative weight 0.54% (M) and 0.50% (F); perimeter 55.8mm(M) and 47.4mm(F); ventricle height 19.9mm(M) and 16.8mm(F); lateral width 14.2mm(M) and 13.0mm(F); dorsal-ventral width 20.5mm(M) and 17.5mm(F); right ventricle thickness 1.7mm(M) and 1.9mm(F); left ventricle thickness 5.4mm(M) and 4.9mm(F); and interventricular septum 5.7mm(M) and 4.7mm(F). Significant differences were noticed between males and females on biometric aspects and on heart perimeter, heart weight, ventricle height and dorsal-ventral width. Gender dimorphism were characterized in biometrics, including the thoracic perimeter. The dimorphism also reflected in heart morphometry when males and females presented significant differences. Nevertheless, regarding the charge of work, males and females probably have similar conditions since the relative weight of the heart is not significantly different. In comparison to humans, S. sciureus have similar charge of work and gross anatomical aspects. Due to proportional similarity with human morphometry, especially, gender related, the S. sciureus could be used as nonhuman primate experimental model for biomedical studies on heart.

Funding: None

## 9) ANATOMICAL STUDY OF THE ORIGIN AND COURSE OF THE VERTEBRAL ARTERIES IN DOGS.

AUTHORS: Korim F<sup>1</sup>, Kuricová M<sup>2</sup>, Lipták T<sup>2</sup>, Koľvek F<sup>3</sup>

AFFILIATION: <sup>1</sup>Department of Morphological Disciplines, <sup>2</sup>Small Animal Clinic, <sup>3</sup>Equine Clinic, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic

ABSTRACT: Observation of the vertebral arteries was performed using the corrosion casting method and the contrast radiology. We used cadavers of 14 mix-breed dogs and the average age of the dogs was 7 years. The approach to the ascending aorta was made by the left lateral thoracotomy. Cadavers were perfused with saline through the ascending aorta and the contrast agent sodium amidotrizoate (Urografin 76%) was administered through the ascending aorta in 4 dogs. The radiographs were taken using the Gierth HF 200 immediately after application of the contrast agent in ventrodorsal (VD) and laterolateral projections. Duracryl Dental, a self-curing acrylic resin, was applied through the ascending aorta in 10 dogs. After resin polymerization, the cadavers were macerated in automatic device at 60°C temperature, 4 days and 2% sodium hydroxide was used as maceration medium. Based on both methods, we observed that the both vertebral arteries are branches from the subclavian arteries. The course of the both vertebral arteries was standard according to general characteristics in the available literature in 13 dogs. In one case, we observed a non - standard course of the left vertebral artery. The left vertebral artery entered into the transverse foramen of the fifth cervical vertebra. On the other hand, the right vertebral artery expressed higher variability. In 2 dogs, the common trunk with the costocervical trunk was present and very close contact with the costocervical trunk was present in 3 dogs. The relationship with the deep cervical artery was observed in 2 dogs. The obtained results confirmed higher variability of the right vertebral artery. Non – standard course of the left vertebral artery is according to our knowledge the first time described in the dog. The results of this work can be used in clinical practice during imaging examinations and surgeries.

Funding: Work was supported by the grant of CEF and Slovnaft a.s. foundation.

# 10) THE ORBIT OF THE ALPACA – A TOPOGRAPHICAL ANATOMICAL DESCRIPTION.

#### AUTHORS: Weis S, Zandt E, Maierl J

AFFILIATION: University of Munich (LMU), Institute of Veterinary Anatomy, Munich, Germany

#### ABSTRACT:

Introduction:

The alpaca becomes increasingly relevant as domestic and farm animal which makes it necessary to study their anatomical peculiarities in order to provide well-founded medical care. The objective was to describe the clinically applied topographical anatomy of the orbit.

#### Material and Methods:

The animals used in this study were donated after either euthanasia or natural death due to medical reasons not affecting the region of interest.

Specimens were preserved in a solution of sodium chloride with sodium nitrate (0.4%) and formalin (0.05%). Frozen heads were bisected to examine the cranial nerves related to the orbit from lateral and medial.

#### Results:

The optic nerve (II) leaves the cranial cavity via the optic canal and enters the muscular pyramid of m. retractor bulbi on its way to the ocular bulb. The cranial nerves III, IV,  $V_1$  and VI pass through the foramen orbitorotundum. The oculomotor nerve (III) divides into two branches that innervate the m. rectus dorsalis, medialis, ventralis, m. levator palpebrae superioris and m. obliquus ventralis. The trochlear nerve (IV) innervates the m. obliquus dorsalis, which is deflected around the trochlea. The ophthalmic nerve (V<sub>1</sub>) leads to the medial orbital rim and sends a probably proprioceptive branch to the m. rectus dorsalis. Furthermore the ophthalmic nerve crosses under the m. obliquus dorsalis and travels dorsally into the fat tissue of the medial angle of the eye. The abducens nerve (VI) enters both the m. rectus lateralis and the m. retractor bulbi by dividing into two branches.

### Conclusion:

At the current preliminary state of research, the cranial nerves entering the orbit and the eye muscles were examined. Their course and innervation seem identical with other domestic artiodactyls. Further studies regarding innervation and vascularisation of the orbital structures of the alpaca will follow.

## 11) RUMEN CHROMA PHENOTYPE IN DIFFERENT BIOHYDROGENATION PATHWAYS OF LAMBS.

AUTHORS: <u>Luísa Mendes-Jorge<sup>1,2</sup></u>, Joana Catita<sup>1,2,3</sup>, Andreia Valença<sup>1,2,3</sup>, Saeedeh Moradi<sup>1,2</sup>, Ana Cristina Vítor<sup>1,2</sup>, Eliana Jerónimo<sup>4,5</sup>, Chica Manuel<sup>1,2</sup>, Cristina Xavier<sup>1,2</sup>, Susana P. Alves<sup>1,2</sup>, Francisco Sena<sup>1,2</sup>, Andrea Cruz<sup>6</sup>, Graça Alexandre-Pires<sup>1,2</sup>, Rui J. B. Bessa<sup>1,2,4</sup>

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<sup>6</sup> Instituto de Medicina Veterinária, Universidade Federal do Pará, Brasil

ABSTRACT: Reticulo-rumen are gastric compartments of ruminants that harbour a complex microbiota that digest fiber efficiently but also isomerizes and hydrogenates extensively the dietary unsaturated fatty acids through biohydrogenation metabolic pathways (RBH). Generally, RBH yields t11-18:1 as the main intermediate, but when ruminants are fed high-starch diets tend to develop subacute ruminal acidosis and to present altered RBH (i.e. t10-shift) where the t10-18:1 isomer becomes main intermediate with potential deleterious effects on meat nutritional value. Lambs susceptibility to express the t10-shift widely varies, but individual variability remains unexplained. The project Gene2rumen proposes to sample up to 700 intensively finished lambs and use rumen morphological data in a GWAS study. Lamb's reticulo-rumen are being collected from a slaughterhouse for morphometric data, including maximal length and width, and rumen volume, registered. After dissection, the colour of rumen mucosa was determined by visual determination and by using a Minolta Chroma Meter for lightness, redness and yellowness in 5 different areas. Preliminary data (19 animals) indicates that 68% of rumen mucosa were dark-brownish, while 32% were yellower. In accordance, a significant difference in yellow/blue coordinate values was confirmed for the same samples using a chromameter ( $13.5\pm2.4$  vs  $11.7\pm3.0$ ; p=0.006), but no differences were found for its lightness and redness. Averages for maximal rumen length and width were 28.5cm and 27.43cm, respectively, and the average estimated rumen volume was 6.75L. In summary, a morphological and colorimetric assay was successfully developed and two distinct chroma phenotype were established as far as rumen mucosa yellowness variation.

FUNDING: PTDC/CAL-ZOO/4515/2021 - Gene2Rumen, UI/BD/152817/2022 (CIISA), UIDB/00276/2020 (CIISA) and LA/P/0059/2020 (AL4AnimalS).

### 12) INTRAHEPATIC VENOUS VASCULAR ANATOMY OF THE FELINE LIVER AS AN ANATOMICAL BASIS FOR SURGICAL INTERVENTION.

AUTHORS: Metzger MD<sup>1</sup>, Van der Vekens E<sup>2</sup>, <u>Rieger J<sup>3,4</sup></u>, Forterre F<sup>1</sup>, Vincenti S<sup>1</sup>

AFFILIATION: <sup>1</sup>Division of Small Animal Clinical Surgery, Vetsuisse-Faculty, University of Bern, Bern, Switzerland. <sup>2</sup>Division of Clinical Radiology, Vetsuisse-Faculty, University of Bern, Bern, Switzerland. <sup>3</sup>Division of Veterinary Anatomy, Vetsuisse-Faculty, University of Bern, Bern, Switzerland. <sup>4</sup>Department of Human Medicine, Faculty of Medicine, MSB Medical School Berlin, Berlin, Germany.

ABSTRACT: The anatomy of the feline intrahepatic veins has never been fully described; therefore, veterinary surgeons have based their techniques on prior knowledge of canine liver anatomy for liver surgery. The aim of the study was to identify and characterize the intrahepatic venous vascular anatomy of cat liver using corrosion casting and computed tomography, identify possible physiological variations in the vascular anatomy, and compare these data to canine anatomy. In a cadaveric anatomical study in adult cats (n=7), healthy livers were surgically resected and resin casts of portal and hepatic veins were evaluated using computed tomography (CT). The liver casts were then macerated to visualize the venous vasculature and compare to the CT scans. It was found that the feline livers had consistent intrahepatic portal and venous vascular anatomy with only minor disparities in numbers of secondary and tertiary branches. Several hepatic lobes shared common portal or hepatic veins with adjacent lobes, following a steady pattern in most specimens. The feline portal vein consistently divided into two major branches and not three, as previously described in the literature for cats. The finding of a portal vein originating from the right medial lobe branch leading to the quadrate lobe in 4/7 specimens is a novelty of the feline anatomy that was not previously described in dogs. A partial to complete fusion of the caudate process of the caudate lobe and the right lateral lobe with a lack of clear vessel separation between the lobes was present in two specimens. In conclusion, CT imaging of corrosion casts allowed for a detailed study of the feline intrahepatic portal and hepatic venous vasculature. The anatomy is consistent with previous findings about canine intrahepatic anatomy, though showing some variations between specimens which could have a great impact on surgical treatment of hepatic masses. Further anatomical studies should be encouraged to confirm the present findings and to investigate the utility of this information in a surgical setting or interventional radiological procedures.

Funding: This research was funded by the Vetsuisse Faculty of the University of Bern and the Kommission des Naturhistorischen Museums der Bürgergemeinde Bern (funding number 2021-727).

# 13) PLAYFUL LEARNING TRIAL IN LEARNING COMPARATIVE ANATOMY

### AUTHORS: Aktan Í

AFFILIATION: School of Veterinary Medicine, University of Surrey, Department of Comparative Biomedical Sciences, Surrey, United Kingdom.

ABSTRACT: The aim of the study was to evaluate the usefulness of playful learning with use of crossword puzzles, colouring and labelling as a learning tool in the subject of anatomy at the level of undergraduate veterinary curriculum.

The survey was presented to all first-year students from the School of Veterinary Medicine, in the 2019–2020 academic period. After a Topographical Anatomy Practical, practical evaluation form was handed out to students. Out of 144 students, 125 completed half a page survey. %98 of the students that completed the survey said that they think having think having playful learning tools is beneficial to their understanding of anatomy. Breakdown of common responses are presented in the below table.

Most common student responses	n
Enjotable way to learn	34
Consolidating knowledge whilst having fun	34
Great for visual learners	21
Promeotes interaction with peers and tuto	rs 18
Allows to think differently	6
any other	10
lt wasn't useful	2

This preliminary study showed that studying challenging subjects like comparative veterinary anatomy with the use of crossword puzzles, colourings and labelling's has proved to be an effective way of learning.

This session helped to create an environment for active learning, a process that motivates the students and increase their interest in the topic, which contributed towards the positive learning experience as perceived by the students in this study. Most of our students also agreed that crossword puzzles promoted peer discussion and that they would like to have more puzzles to be used in future teaching. Most students also commented positively to the background music. Currently author is trialing use of Instagram in teaching Comparative Anatomy. Anecdotal responses show that students love seeing daily stories related to their Anatomy Learning.

Funding: N/A.

## 14) DOG OWNERS' AWARENESS OF ANIMAL CADAVERS AND THEIR MOTIVATIONS TOWARDS PET CADAVER DONATION

### AUTHORS: Tracey K<sup>1</sup>, Aktan Í<sup>1</sup>

AFFILIATION: <sup>1</sup>School of Veterinary Medicine, University of Surrey, Department of Comparative Biomedical Sciences, Surrey, United Kingdom.

ABSTRACT: Animal cadavers play an essential role in education, as the number of schools increase, the demand for cadavers rises proportionately. Many schools would like to participate in pet cadaver donation schemes but finding owners willing to donate their pet is challenging. The aim of this study was to investigate dog owners' awareness of pet cadaver donation.

The study was conducted using Jisc online surveys with the survey being distributed via social media. In total 708 people answered a ten-question online questionnaire.

The most common reason given for why a dog owner might donate was to assist in the education of future veterinarians (n=396, 56%). When asked to rank the options given to them upon the passing of their pet single animal cremation was overwhelmingly the participant's favoured option. (n=483, 68.2%).

The only incentive shown to increase the likelihood of pet owners to donate to a pet cadaver scheme was to return their pet's ashes to them after the cadaver had served its purpose. This study revealed that dog owners currently have very little knowledge of pet cadaver donation.

Funding: NA.

### 15) THE CURIOUS CASE OF THE SUPRATROCHLEAR FORAMEN IN DOGS AND IBERIAN WOLVES HUMERI

AUTHORS: Joana Catita<sup>1,2,3</sup>, Mariana Batista<sup>1,2,3</sup>, Eduardo Marcelino<sup>1,2,3</sup>, Giovana Braga<sup>1</sup>, Brunna Ciobanu<sup>1</sup>, Luísa Mendes-Jorge<sup>2,3,4</sup>, Graça Alexandre-Pires<sup>2,3,4</sup>, Maria Soares<sup>2,3,5</sup>, Cleia Detry<sup>6</sup>, Sónia Gabriel<sup>7,8</sup>, Dulce Ferreira<sup>7</sup>, Ana Elisabete Pires<sup>1,7,8</sup>

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ABSTRACT: The supratrochlear foramen (STF) is an anatomical feature of canine humeri, that connects the radial and olecranon fossae, but contrary to most foramina, no anatomical structure passes through it. To our knowledge, there is still a lack of detailed studies on STF in dogs and its ancestor, the grey wolf. We aimed to describe STF prevalence and morphometry in different sized humeri from dogs and iberian wolves. Unpaired humeri from 52 dogs and 70 Iberian wolves were measured twice by two independent observers, using a digital caliper. Dog humeri were categorized into small (≤10cm) and medium/large (10,3-22,3cm) according to maximum length; wolf humeri length ranged between 18,0-22,8cm. Analysis revealed that 62,5% (15 of 24) of small dog humeri and 14,3% (4 of 28) of medium/large dog humeri were STF deficient, and only 1,4% of wolf humeri (1 of 70) had absent STF. When present, STF longitudinal and transverse length was lower in small dog humeri (3,23±1,61mm and 3,63±1,58mm) compared to medium/large (5,24±2,37mm and 6,20±2,18mm), although the ratio between both lengths was similar in small (1,17±0,18mm) and medium/large (1,25±0,23mm) humeri. In wolves, STF longitudinal and transverse mean length was 9,90±1,94mm and 10,73±1,55mm, respectively. Oval-shape STF, with a longer transversal axis, was more common in dogs (76%; 25 of 33) and wolves (65%; 45 of 69) than round-shape. STF margin distance to the lateral epicondyle was inferior to the distance to the medial epicondyle in small dog (7,93±1,59mm vs 11,62±1,66mm), medium/large dog (13,88±3,76mm vs 21,02±6,29mm) and wolf humeri (13,96±1,46mm vs 23,63±2,14mm). The study provides new data about STF variation in dogs and wolves. Small dog humeri showed decreased size or absent STF more frequently than medium/large dogs. STF absence was rare in the Iberian wolf. STF was commonly oval-shaped and closer to the lateral epicondyle, where canine humeral condylar fractures are predominant.

FUNDING: 2022/23 Exploratory project grant (SupT-Dog) by Faculty of Veterinary Medicine of Lusófona University (FMV-ULusófona).

# 16) PRENATAL DEVELOPMENT OF THE URETERS IN THE DOMESTIC CAT (FELIS SILVESTRIS CATUS)

AUTHORS:, Prozorowska - Basińska E<sup>1</sup>, Nowikowska K<sup>1</sup>, Jackowiak H<sup>1</sup>,

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ABSTRACT: The ureters develop from the ureteral buds, which separate from the lower part of the mesonephric ducts and elongate towards the blastema of the metanephroi. The early ureters access the wall of the urogenital sinus, which differentiates into urinary bladder. The study aimed to analyze the cat ureters' development regarding changes in their topography, microstructure, and connection to the urinary bladder. The research materials were embryos and fetuses aged 27 - 63 days p.c. (postconception). The research methods included macroscopic and microscopic observations, SEM observations, and 3D reconstructions. According to Polish law and the EU directive (no. 2010/63/EU) the research did not require approval of the Local Ethical Committee. Macroscopic observations showed that between days 27 - 36 p.c., the straight ureters lie in the dorsal part of the abdominal cavity and stay partially covered by mesonephroi. After day 44 p.c., the mesonephroi disappear, exposing the ureters characterized by a wavy course. The 3D models revealed that the ureters reach the urinary bladder at an oblique angle and then bend at a right angle and penetrate across the urinary bladder wall. SEM observations showed that inside the wall of the urinary bladder form V-shaped ureteral folds containing openings of the ureters, which become unobstructed after day 36 p.c. Until day 36 p.c., the ureters have an embryonic appearance, as their wall consists of simple cuboidal epithelium surrounded by mesenchyme. About day 44 p.c., a layered arrangement appears in the wall of the ureters, with a distinct mucosa, muscularis, and adventitia. The transitional epithelium with umbrella cells establishes around day 56 p.c. At birth, the wall of the ureters consists of a flat mucosa lined with urothelium and a two-layered muscularis covered by adventitia. The differentiation of the ureters continues postnatally and involves folding of the mucosa and expansion of the outer muscular layer.

Funding: Research conducted by the statutory funding No. 506.539.04.00 of the Department of Histology and Embryology in the Faculty of Veterinary Medicine and Animal Science, Poznan University of Life Sciences, Poland.

# 17) PRENATAL DEVELOPMENT OF THE URETERS IN THE DOMESTIC CAT (FELIS SILVESTRIS CATUS)

AUTHORS:, Prozorowska - Basińska E<sup>1</sup>, Nowikowska K<sup>1</sup>, Jackowiak H<sup>1</sup>,

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Funding: Research conducted by the statutory funding No. 506.539.04.00 of the Department of Histology and Embryology in the Faculty of Veterinary Medicine and Animal Science, Poznan University of Life Sciences, Poland.

### 18) THE SCIENTIFIC COLLECTION OF THE VETERINARY ANATOMY MUSEUM OF THE UNIVERSITY OF MURCIA. HIGHLIGHTS AFTER SIX MONTHS OF EXPERIENCE

AUTHORS: Gil F<sup>1</sup>, Latorre R<sup>1</sup>, Orenes M<sup>1</sup>, Albarracín J<sup>1</sup>, Pellicer M T<sup>2</sup>, Ramirez G<sup>1</sup>, Ayala MD<sup>1</sup>, Autón JM<sup>1</sup>, Lopez Albors O<sup>1</sup>

AFFILIATION/INSTITUTION: <sup>1</sup> Dept Anatomy & Comparative Pahtology, Veterinary Faculty, University of Murcia 30100 Murcia, Spain. <sup>2</sup> Dept. Audiovisual Communication & Advertising, Faculty of Communication & Documentation. University of Murcia 30100 Murcia, Spain.

ABSTRACT: The Scientific Collection the Veterinary Museum (SCVM) is an new exhibition area of the University of Murcia aimed at promoting the study, research and dissemination of anatomy from a comparative perspective. The SCVM consists of nine exhibition halls dedicated to primates, equines, ruminants, suids, carnivores, marine mammals, fish, reptiles and birds. In addition to a broad number of skeletons (n=30), the museum is praised by the amount of silicone plastinated specimens (n=90). Apart from some human specimens, donated Medical Schools (University of Valladolid, Spain) and a public exhibition (Human Bodies®), all exhibits were prepared in the Veterinary Faculty (dissection room and plastination laboratory). The museum was officially opened in October 2022. Visits are always tutored by the academic staff of the Veterinary Anatomy Area according to a their agenda. Students of Secondary and High Schools are the main visitors of the museum, although visits are also commonly requested by students of the Veterinary Degree, Arts Degree, Vets in practice and general public. Additionally, a virtual visit to the museum is also possible in the following web site (https://www.um.es/web/museo-veterinario/visitas/virtual). All specimens on display are identified by an ID card with QR code linked to an explanatory podcast with relevant information. For Secondary School students tuition is complemented by an assessment on-line form Google®. Most relevant figures after a six months experience are, total number of visits 882, number of Secondary and High Schools 20 (with 56 teachers), number of podcasts visits 1510, average score obtained by Secondary students 8.3/10. It can be concluded that the SCVM is a valuable and effective exhibition site to disseminate comparative anatomy knowledge, both formally and informally, not only to Secondary students but to a broad profile of visitors further than the Veterinary scope.

Funding: OTRI University of Murcia, Musealia Entertainement® (donation)

# **19) IS SERIOUS GAMING A GOOD EDUCATIONAL TOOL FOR TEACHING VETERINARY ANATOMY?**

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AFFILIATION: <sup>1</sup>ToNIC, Toulouse NeuroImaging Center, Université de Toulouse, Inserm, UPS, ENVT, Toulouse, France.

ABSTRACT: Serious games are computer applications designed to provide users with an engaging and interactive learning experience. They can be used to support teaching and learning in a variety of knowledge areas, including veterinary medicine.

Serious games have several advantages as educational tools. They can be particularly useful in veterinary medicine. Veterinary students are often confronted with complex concepts that require a deep understanding to apply them correctly in clinical practice. Serious games can help students better understand these concepts by providing them with realistic simulation scenarios that mimic real clinical situations.

In this study, 39 students participated in a one-hour course on heart anatomy, which they had never dealt with before. Then, three groups of 13 students were formed to deepen their knowledge of this topic for 45 minutes using a different pedagogical method. The first group participated in a traditional method that involved dissecting a sheep's heart to realistically study its anatomy. The second group participated in a serious game that simulated a clinical case requiring knowledge of heart anatomy. The third group was tasked with repeating the course on their own with the support provided. All students then completed the same written exam, which included a theoretical section and a clinical case.

The first group achieved the best overall results on the exam. However, the second group scored better on the clinical portion. In conclusion, classical methods, including hands-on work, seem to be the most effective for mastering the theoretical principles of veterinary anatomy. However, serious games seem to provide a better understanding of clinical situations. They are a good addition in this area to help students with the practical side of learning.

## 20) VIRTUAL SERIOUS GAME: AN INNOVATIVE AND DIDACTIC REVISION TOOL FOR STUDENTS

AUTHORS: Cartiaux B<sup>1</sup>, Montel C<sup>1</sup> and Mogicato G<sup>1</sup>

AFFILIATION: <sup>1</sup>ToNIC, Toulouse NeuroImaging Center, Université de Toulouse, Inserm, UPS, ENVT, Toulouse, France.

ABSTRACT: The last Covid-19 pandemic has led to unprecedented repercussions on our approach to teaching and on the learning methods of our students. Indeed, successive confinements have forced us to familiarize ourselves with a large number of digital tools (such as Genially, Zoom, Socrative, H5P, etc.) in order to ensure our courses, tutorials and to organize exam sessions.

Based on this experience, we wanted to provide students with a new way to review through a virtual serious game using the Genially software, an online platform dedicated to creating interactive content. For the past three years, we have built several virtual serious games that took place in the Anatomy Museum and clinics of the National Veterinary School of Toulouse where players can move around in different rooms and interact with a large number of elements present. This allowed us to highlight the heritage of the museum and to discover clinics while augmenting knowledge acquisition in a fun, team-based learning experience using the codes of an escape game.

These serious games were tested by second-year students, divided into groups of 3, during a two-hour slot in the amphitheater. Progressing through the rooms requires completion of many puzzles, each dealing with different notions of the anatomy program through the integration of different games, videos, and sound passages. We will present to you the many possibilities of revisions that Genially can offer based on examples taken from our different serious games.

In conclusion, these virtual serious games have had huge success with our students: it seems like a good way for them to test their knowledge of anatomy. Thus, we have recruited three thesis students to create other virtual escape games that will be more specialized in specific areas of anatomy such as arthrology, splanchnology, imagery, or cardiology.

Funding: None.

### 21) COMPUTED TOMOGRAPHY AND CROSS-SECTIONAL ANATOMY OF THE NASAL CAVITIES AND PARANASAL SINUSES OF CATTLE AT DIFFERENT AGES.

AUTHORS: Isler F<sup>1</sup>, Cartiaux B<sup>2</sup>, Montel C<sup>3</sup>, Maillard R<sup>1</sup>, Mogicato G<sup>2</sup>

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ABSTRACT: Computed Tomography (CT) is more and more described as a routine imaging technique in small animals. In bovine medicine, this technique is less common due to a more difficult set up in large animals. Yet, CT can be useful in genetically selected animals with high economic value. Moreover, this imaging technique allows a better exploration of nasal cavities and paranasal sinuses which are often difficult to explore in cattle, although often affected. The aim of this study was to compare head anatomical cross-sections with corresponding CT scans of cattle at different ages in order to provide a valuable tool to clinicians.

CT bone window scans of five bovine heads aged 5 days to 48 months and belonging to two races, Prim'Holstein and Blonde d'Aquitaine, were obtained in ventral recumbency. After the imaging procedures, the heads were frozen at -20°C. Head cross-sections were obtained in the same plane as the one used for CT scans, i.e. transverse. Relevant anatomical structures were identified and labelled on each cross-section and corresponding CT images. Our results show a good correlation of most of the anatomical features between both techniques.

CT scans produced an excellent definition of the bony and air-filled structures and allowed us to successfully identify most of the bovine head bone structures. Overall, our study provides a detailed anatomical description of the head structures in cattle at different ages and can be used as a guide for the interpretation of imaging studies.

All husbandry and experimental procedures were approved by the institutional ethics committee and the Ministry of Higher Education, Research and Innovation

Funding: no funding

## 22) EQUINE PLACENTA IN THE LAB: DEVELOPMENT OF EQUINE PLACENTAL ORGANOIDS

AUTHORS: Verstraete M1,2,, van Heule M1,2, Couck L1, Martin-Pelaez S2, Ortega A2, Loux S3,Van Den Broeck W1, De Spiegelaere W1, Daels P1, Dini P2

AFFILIATION: 1Faculty of Veterinary Medicine (UGent), Merelbeke, Belgium. 2School of Veterinary Medicine (UCDavis), California, USA. 3Gluck Research Center (UKY), Kentucky, USA.

ABSTRACT: A fundamental understanding of placental development and its associated pathologies is crucial to mitigate the losses associated with placental disorders. Organoids are widely used to investigate the developmental biology of organs and diseases. In this study, we aimed to (1) develop placental organoids using first trimester and term equine placentae, and (2) evaluate the organoid structures. Chorioallantois (CA) was collected from healthy placentae following elective abortion (80-100d of gestation) and spontaneous term parturition. CA samples were processed for culture, fixed for histology, and stored in RNAlater for transcriptomic analysis. For culture, each CA sample was enzymatically digested to a single cell suspension and seeded into Matrigel droplets surrounded by medium. Passage-3 organoids (P3) were used for histology (H&E), transmission electron microscopy (TEM) and transcriptomic analysis. After optimization of culture conditions, we were able to produce 3D-organoids (Pre- erm, n=6 and Term, n=3). Organoids were passaged up to 12 times over • }3 months and were successfully frozen and re-cultured post-thaw. Total extracted RNA from initial CA samples and organoids was sequenced (150PE; ~43 million reads/sample). The genes expressed in the organoids closely resemble those of the tissue of origin, 17.6% of the expressed genes were differentially expressed between the initial CA and P3 (n=3,587 genes; FDR<0.01 and llog2FoldChange >2). The expression of trophoblast-enriched genes PLAC8B, GATA3, KRT7, EGFR, TFAP2A, TFAP2C, and GATA2 in the organoids confirm their trophoblast identity. H&E staining of the organoids confirmed their multicellularity and indicated the epithelial origin of the cells. Our preliminary TEM analysis suggests the presence of microvilli on the luminal side of the cells. To conclude, this is the first report of equine placental organoids. The organoids show structural and transcriptomic similarities to the equine placenta *in vivo* and consequently show great promise as an *in* vitro model to study placental development and associated pathologies.

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## 23) CASE REPORT OF AN ABSENT RIGHT VENA CAVA AND PERSISTENT LEFT VENA CAVA IN A CANINE CADAVER

### AUTHORS: Irimescu I1

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ABSTRACT: An uncommon occurrence of an atypical vena cava was discovered during student dissection in LIU CVM's Anatomy lab in the Fall semester of 2022. The sample comes from a medium size male adult American Pitbull mix. Exploration of the cranial mediastinum, after removing the lungs revealed the absence of the right cranial vena cava (RCVC). A persistent left cranial vena cava (LCVC) was found draining the brachiocephalic veins and crossing the left aspect of both the aortic arch and of the pulmonary trunk, before emptying into the right atrium, which was slightly expanded at the caudal aspect of the base of the heart. The right azygos vein emptied into the LCVC, after crossing from right to left the dorsal aspect of the thoracic descending aorta, at the level of the fifth intercostal space. The caudal vena cava presented no atypical variations. Cases of both LCVC and RCVC present have been occasionally described in literature for man, dog, horse and ruminants, but the absence of a RCVC is a rare occurrence. As the LCVC emptied into the right atrium, it is likely that this defect did not have a clinical impact on the individual. A limitation in determining this is the lack of medical history (as the cadaver was purchased embalmed, without medical records). To further expand the details of this unique presentation, we intend follow up with imaging of this heart sample with its large vessels.

Funding: not funded

## 24) MORPHOLOGY, WELFARE AND SECURITY ASPECT OF IMPORTANCE IN TRAFFICKING OF EXOTIC AND WILDLIFE ANIMALS

### AUTHORS: Kubale V1, Rutland CS2, Dvojmoc M3

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ABSTRACT: Currently, the illegal wildlife trade is one of the most profitable illegal industries in the world. Countries are interconnected and some of them are important transit countries. The aim of our study based on literature review and interviews was to determine the current situation in Slovenia in terms of wildlife trade, wildlife transit and its impact on animal welfare, focusing on the morphological characteristics of the animals that make them interesting. Comparison with other countries was made, especially UK.

According to our study, Slovenia is currently mainly a transit country, but it is difficult to estimate the actual volume of trade, which, although not extensive, is not insignificant. The most common species involved in illegal trade of endangered wildlife in Slovenia are brown bear, peregrine falcon, date mussel, lady's slipper orchid, common snowdrop, cyclamen, sea turtle, otter and various reptile species. The smuggling of shells (date shells), ivory (ivory products), certain plants and trophies of various animals (bears, big cats) has decreased in recent years. Some of the animals are interesting as delicacies, but some animals are also involved in trafficking due to their morphological characteristics.

Animal trafficking can have severe consequences on the health and well-being of individual animals and entire populations. It can lead to the extinction of species, disrupt ecosystems, and spread diseases. Additionally, it is often associated with other illegal activities, such as money laundering, corruption, and human trafficking. Efforts to combat animal trafficking involve enforcing laws and regulations, educating the public, and reducing demand for animal products. Many countries have implemented measures to protect endangered species and control the trade of animals and their parts. It is important to address animal trafficking to ensure the long-term survival and well-being of animal populations and their ecosystems.

Funding: The authors gratefully acknowledge financial support from the Slovenian Research Agency P4-0053.

## **25) BOVINE EXPLANTS AND ORGANOTYPIC 3D-MODELLING IN VITRO.**

AUTHORS: Michler J<sup>1</sup>, Baumbach C<sup>1</sup>, Anantama N<sup>1</sup>, Mülling, C<sup>1</sup> and Schinköthe J<sup>2</sup>.

AFFILIATION/INSTITUTION: University of Leipzig, Institute of Veterinary Anatomy, An den Tierkliniken 43, 04103 Leipzig. University of Leipzig, Institute of Veterinary Pathology, An den Tierkliniken 33, 04103 Leipzig.

ABSTRACT: In dermatological research, two 3D-approaches, explant cultures and reconstructed organotypic skin models, are mainly used. Advantages and disadvantages of these techniques have been extensively reviewed. However, for bovine medicine, there is a gap in basic research articles although the modelling of skin seems an obvious approach due to skin diseases with only partially clarified pathogenesis (e.g. digital dermatitis, claw horn disruption, laminitis). In total, 19 distal limbs were obtained from local abattoirs as waste material. We cultured explants of interdigital skin (both aerob and anaerob) up to a week for the aerobic cultures and sampled at day 0, 2, 5 and 7. Primary cultures of keratinocytes and fibroblasts for the organotypic skin equivalents were established. To seed the fibroblasts in collagen gels, proliferation-inhibiting using mitomycin C was performed. On top of these equivalents, we seeded keratinocytes and cultured with an air-liquid interface with sampling on day 7, 14 and 21. Standard H&E-evaluation, immunohisto-/immunocytochemical analysis for lineage markers of epidermal and dermal cells and a histopathological analysis for the explants followed. Antibodies used were Keratin 14, 10, Desmoglein-1, Vimentin, Ki67 and Caspase-3. Both explant and organotypic cultures were successfully established. The viability of the skin explants was assessed by analysis of H&E-stained slides and by detection of basal cells in active cell division by Ki67 assay. Cell division of basal cells was evident at day 0 and then declined respectively was absent at day 7 with Caspase-3-positive cells appearing in the cut edges. For the dermal collagen pads, interspersed postmitotic fibroblasts labeled Vimentin-positive. The keratinocytes showed Desmoglein-1-staining and Keratin14 in basal and suprabasal layers. Challenges of maintaining long-term viability (> 7 days) due to restricted nutrient supply (at least inner cells) and metabolite removal stay neuralgic points for explants. For the reconstructed skin, to achieve correct stratification is demanding and requires critical monitoring.

Funding: The study was self-funded

## 26) Development of innervation of the gastric groove in domestic cattle (*Bos taurus*).

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AFFILIATIONS: Department of Animal Anatomy, Faculty of Veterinary Medicine, University of

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ABSTRACT: Digestion in young ruminants is similar to that in monogastric animals. A very specific part of the fore-stomach is responsible for this fact - the gastric groove. It is a structure made of two lips and the floor. It extends from the orifice of cardia until the pylorus, and it is divided into three segments: reticular, omasal and abomasal groove. When sucking milk, the contraction of the gastric groove is activated forming a hollow structure - a duct along the wall of the reticulum, which connects the oesophagus to the reticulo-omasal orifice, as a result, food goes directly to the abomasum. With the introduction of larger amounts of roughage and the simultaneous growth of the forestomachs, the reflex of contraction of the gastric groove weakens. Innervation of this structure plays essential role in regulation of its activity, thus taking under consideration changes in its activity, we decided to study development of its innervation.

The studies were carried out on animals from six age groups (n=5). All material was collected at the slaughterhouse immediately after slaughter. The three groups of foetuses covered the first, second and third trimesters of pregnancy. Next groups included 6-week-old calves, 2-year-old and 4-year-old cows. Tissues were cut using freeze microtome, and obtained sections were stained using anti-acetyl tubulin, PGP-9.5 and Hu antisera. Best results were obtained with antibodies against acetylated tubulin.

The obtained sections were analysed using a confocal microscope. It has been shown that during foetal life, the number of nerve cells increases, reaching the highest number in the oldest foetuses. Subsequently, the number of nerve cells present in gastric groove gradually decreases, and it is the lowest in the oldest animals studied.

The observed decrease in the number of neurons may be the direct cause of the motor activity reduction of the gastric groove.

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## 27) DISTRIBUTION AND CHEMICAL CODING OF NEURONS IN THE PORCINE MALE INTRAMURAL GANGLIA OF THE URINARY BLADDER TRIGONE AFTER ONE-SIDED AXOTOMY

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AFFILIATION: University of Warmia and Mazury in Olsztyn, Department of Animal Anatomy, Faculty of Veterinary Medicine, Poland.

ABSTRACT: Function of the lower urinary tract to store and periodically release urine is dependent on neural circuits located in the brain, spinal cord and peripheral ganglia. Intramural ganglia of the urinary bladder trigone (IG-UBT) are important sources of nerve fibres supplying the lower urinary tract in mammals including the pig. Since our knowledge on the distribution and chemical coding of IG-UBT neurons in the male pig is very limited, we have used retrograde tracing combined with onesided axotomy and double-labelling immunohistochemistry to elucidate these questions. The study was performed on 10 juvenile male pigs of the Large White Polish breed. All the pigs were deeply anaesthetized with sodium pentobarbital. A midline laparotomy was performed to administer the fluorescent retrograde tracer Fast Blue into the wall of the UBT. After three weeks, in the same animals right-sided axotomy of nerve fibres projecting from the anterior pelvic ganglion (APG) to UBT was performed. Then, IG-UBT's were collected and prepared for morphological and immunohistochemical research. Double-labelling immunofluorescence with antibodies against tyrosine hydroxylase (TH), dopamine  $\beta$ -hydroxylase (DBH), neuropeptide Y (NPY), somatostatin (SOM), galanin (GAL), vasoactive intestinal polypeptide (VIP), nitric oxide synthase (NOS), calcitonin gene-related peptide (CGRP), substance P (SP) and vesicular acetylcholine transporter (VAChT) were performed.

The IG-UBT neurons formed characteristic clusters (consisting from a few to tens neuronal cells) found under visceral peritoneum or in the outer muscular layer. After axotomy, a dramatic reduction in the number of IG-UBT neurons, amounting up to 75 % as compared to the values found in the control pigs, was determined. Immunohistochemistry revealed several subpopulations in UBT-IG neurons, namely noradrenergic, cholinergic, non-adrenergic/non-cholinergic nerve cells, NPY-, SOM-, VIP- and NOS- immunoreactive (IR). This study has revealed a relatively large population of intramural UBT neurons, which probably contribute to the complexity of the urinary bladder neural pathways.

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## 28) SECTIONAL ANATOMY, MICRO-COMPUTED TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING OF SYRIAN HAMSTER (MESOCRICETUS AURATUS) HEAD

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ABSTRACT: Although hamsters are very popular as pets, and are widely used as experimental animals, no reports have been made on sectional imaging anatomy of this species. The current study was aimed at investigating the sectional anatomy and imaging of the head in Syrian hamsters due to the complex anatomy of this region and the small size of these animals. Eight healthy adult Syrian hamsters were used in the present study. Sectioned heads were first reviewed, marked and then were compared with their corresponding micro-computed tomography (micro-CT) and magnetic resonance imaging (MRI) images. All recognizable anatomic structures were then labeled on the images. Micro-CT images provided precise visualization of different bony structures, dentition and airways of the Syrian hamster head, whereas soft tissue structures were identified on MRI images. Our findings provide for the first time a basic atlas of normal sectional anatomy and imaging of the Syrian hamsters head for veterinary anatomists, radiologists, clinicians, surgeons and researchers.

Funding: The Research Council of Shahid Chamran University of Ahvaz, Iran. Grant number: SCU.VB1401.770.

## 29) ANATOMICAL AND PATHOLOGICAL CHARACTERISTICS OF RIBS IN THE ATLANTIC SALMON (*SALMO SALAR* L.) AND ITS RELEVANCE TO SOFT TISSUE CHANGES

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### ABSTRACT:

Studies on the anatomical and pathological characteristics of ribs in farmed Atlantic salmon (*Salmo salar* L.) are warranted due to their possible association with red and melanized focal changes (RFC and MFC) in the fillet, a major quality and animal welfare concern. In this work, we provide an anatomical description of ribs based on radiographical and histological analyses. We also address various pathological rib changes and their association to RFC and MFC. In total, 129 fish were investigated; captured wild (n = 10) and hatchery reared (n = 119) Atlantic salmon (3.5–6.1 kg). The fish were selected based on the macroscopic presence of RFC, MFC or no changes (controls). Radiographic results revealed costal abnormalities in all fish groups. By histological investigations of the variations herein, our results provide new insight into the anatomical characteristics including vascularization within the ribs; a potential site for haemorrhage following costal fractures. Costal fractures were detected by radiology in 40 of 129 samples (RFC: 38.4%, MFC: 47.2%, controls: 9.5%). A statistically significant association was found between costal fractures and red (p = 0.007) and melanized changes (p = 0.000). However, red and melanized changes were also observed in samples with no costal fractures (n = 45), indicating that also other factors influence the development of RFC/MFC.

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## **30) HOW USEFUL ARE PLASTINATED ORGANS AS SUPPLEMENTARY RESOURCES IN DISSECTION COURSES FOR VETERINARY STUDENTS? – An ongoing project at the Norwegian University of Life Sciences**

AUTHORS: Sudmann S, Godager L, Westad E, Rørtveit R

AFFILIATION: Norwegian University of Life Sciences (NMBU), Ås, Norway.

#### ABSTRACT:

<u>Background</u>: Organized dissection courses have a high learning value in veterinary anatomy courses. However, assistance and guidance from teachers are necessary to maximize the learning outcome and this is challenged by limitations in staff resources and an increasing number of students. Plastinated anatomical specimens available for the students during the dissection courses could have the potential to solve this issue.

In the new buildings of the Veterinary Faculty at NMBU a brand new plastination laboratory is available. In 2022 we received funding from the program for innovative teaching at NMBU to make plastinated specimens for teaching veterinary anatomy.

<u>Aim:</u> The aim of the present study is to evaluate whether plastinated specimens, as supplementary learning resources, could increase the veterinary students' learning outcome and have an effect on the student's self-evaluation of learning outcome during organized dissection courses.

<u>Materials and methods</u>: Anatomical specimens from dog cadavers showing relevant structures including muscles, vessels, nerves and organs have been prepared. The specimens are currently undergoing S10 plastination. Flash cards naming structures belonging to each specimen will be produced.

During four sessions of the dissection of the dog course at NMBU in September 2023, the dissection groups will be randomized into a control group and an experimental group. The experimental group will have access to the plastinated specimens and flash cards during the session, whereas the control group will not. During the four teaching sessions, all students will switch between being control and experimental group.

### End points:

- Real learning outcome. Pre- and post- session quizzes testing knowledge before and after each teaching session. Results from experimental group and control group will be compared.
- Self-evaluation of learning outcome. Questionnaires mapping self-evaluated learning outcome in the experimental group after each teaching session.

Funding: Program for innovative teaching, Learning Center, NMBU

### 31) IMAGING, MORPHOLOGICAL AND HISTOPATHOLOGICAL CORRELATION IN METASTATIC SENTINEL LYMPH NODES THAT DRAIN MAMMARY CARCINOMAS IN FEMALE DOGS

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ABSTRACT: Introduction: Mammary tumors occupy the first place in the pathology of mammary glands in dogs. Half of these tumors are malignant. Most carcinomas metastasize through the lymphatic system. In the presence of mammary carcinomas, the evaluation of sentinel lymph nodes (SLNs) is of major importance in staging, prognosis and therapeutic attitude. Non-invasive imaging techniques have a main place in the initial evaluation of SLNs. The purpose of this study is to establish the correlation between the histological and imaging patterns of metastatic sentinel lymph nodes.

Material and methods: A retrospective observational study was performed in which 36 sentinel lymph nodes that drained mammary gland carcinomas were evaluated by ultrasound. The histopathological examination confirmed the presence of metastases in these SLNs. Using two-dimensional ultrasound, Doppler technique, contrast enhanced ultrasound (CEUS) and real time elastography, we described the shape, size, echostructure, echogenicity, appearance of the hilum and capsule, type of vascularization, enhanced pattern and degree of stiffness.

Results: There is a direct correlation between the rounded shape with Solbiati index >0.5, perilymphonodal fat infiltration, inhomogeneous echostructure, mixed or peripheral vascularization, inhomogeneous pattern after peritumoral administration of contrast agent, with the presence of areas without contrast agent, stiffness score above 3 and the presence of metastases (p<0.001). The size, echogenicity, appearance of the hilum and the capsule are not directly correlated with the presence of metastases in the sentinel lymph nodes (p<0.057).

Conclusion: The specific imaging characteristics that are correlated with the presence of metastases in the lymph nodes provide a pertinent preliminary analysis and may justify the need for SLNs biopsy in the presence of mammary gland carcinomas in female dogs.

All procedures were approved by the Bioethics Committee of the Faculty of Veterinary Medicine, Cluj Napoca, nr of approval 68/21.11.2019

## 32) CEUS AND INDIRECT LYMPHOGRAPHY ACCURATELY IDENTIFY SENTINEL LYMPH NODES THAT DRAIN MAMMARY CARCINOMAS IN FEMALE DOGS

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AFFILIATION: <sup>1</sup>University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj Napoca, Romania. <sup>2</sup>Ross University School of Veterinary Medicine, Basseterre, St. Kitts and Nevis.

ABSTRACT: In veterinary medicine, the techniques that identify sentinel lymph nodes of mammary carcinomas are not fully standardized. The aim of this study was to achieve a real identification of SLNs that drain mammary carcinomas in female dogs.

36 bitches diagnosed with carcinomas of the abdominal and inguinal mammary glands by histopathological examination were included in the study. The contrast agent (SonoVue, Barraco, Italy) was injected peritumorally in two points. The lymphatic pathways were visualized on contrast pulse sequencing and followed to the draining sentinel lymph node that accumulates the contrast agent, corresponding to the tumor drainage. For indirect lymphography, the dye was injected in the same points, followed 24 hours later by the regional dissection during surgery.

On CEUS, 35 SLNs were identified and the dye identified 34. On CEUS, Lymphatic vessels were identified as linear structures, well defined in their route to the SLNs. In two subjects, lymphatic vessels bypassing the superficial inguinal lymph nodes were visualized. The dye impregnated the lymphatic vessels which showed an increased peritumoral density. In two subjects, the dye did not identify any SLN. Initially, the lymphatic vessels had a superficial peritumoral route, to then penetrate the subcutaneous cellular tissue, orienting in the perilymphonodal adipose tissue as deep lymphatic vessels. In their route, the lymphatic vessels presented numerous anastomoses, organizing themselves into one or two lymphatic vessels that approached the lymph node at the level of the capsule. Compared to SonoVue, which was phagocytosed by macrophages, the dye impregnated the efferent lymphatic vessels too.

In conclusion, CEUS is one of the simple and effective methods that can identify the SLN topography and guide its biopsy if necessary. In addition, CEUS has the advantage of being non-invasive, low-cost and easy to perform due to the fact that it identifies, evaluates and characterizes SLNs pertinently.

All procedures were approved by the Bioethics Committee of the Faculty of Veterinary Medicine, Cluj Napoca, nr of approval 68/21.11.2019

### 1) HISTOGENESIS OF DUODENUM IN PRENATAL INDIAN GOATS

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ABSTRACT: The present research was conducted on the developing duodenum of 36 goat embryos/foeti irrespective of breed and sex. The tissues were fixed in 10% neutral buffered formalin and in cold acetone. Thin paraffin sections (5µ) were cut and stained with Haematoxylin and Eosin for routine fibrocellular architecture, Masson's Trichrome stain for collagen fibres, Gorden and sweet's stain for reticular fibers and Verhoeff's stain for elastic fibres. Different histochemical techniques were applied for detection and localization of neutral mucopolysaccharides, acid mucopolysaccharides, bound lipids, alkaline phosphatase enzymes, acid phosphatase enzymes and for DNA. The epithelium was poorly differentiated stratified to pseudostratified columnar type in group-I (0-50 days of gestation), stratified columnar to simple columnar in group II (51-100 days of gestation) and simple columnar in group III (101-150 days of gestation). The intensity of reactions increased as age of foeti advanced. The goblet cells of intestinal gland showed moderate reaction for PAS and AMPs in mid prenatal period (group-II) and intense to highly intense reaction in late prenatal period (group-III). The cytoplasm of the epithelial cells exhibited negative to mild reaction for bound lipids in group I, mild to moderate in group-II and mild to intense reaction in group-III. The luminal border showed weak acid phosphatase reaction in group-I and II, and mild to moderate reaction in group-III. The nuclei of the epithelial cells showed mild to moderate Feulgen reaction in group-I and II and intensely positive reaction in group-III.

Funding: DUVASU, Mathura, Uttar Pradesh, India

## 2) IMMUNOLOCALIZATION OF SODIUM-GLUCOSE CO-TRANSPORTERS SGLT1 AND SGLT2 IN BIRDS KIDNEYS

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ABSTRACT: In glucose homeostasis in birds, the kidney is considered the most intriguing organ because the avian kidney efficiently absorbs all the filtered glucose, ensuring that the glucose is not lost in the urine. Although the expression of glucose transporters in avian kidney tissue and also the localization of sodium-dependent glucose co-transporters (SGLT) in mammalian kidney tissue have been determined to some extent, the same cannot be said about the localization of SGLT transport proteins in avian kidney. The aim of the research was to compare the immunolocalization of SGLT1 and SGLT2 in kidney tissue of hen and ostriches chicken. Sixteen three-day-old female chicken participated in the study: eight ostriches (Struthio camelus var. domesticus) and eight hen chicken (Gallus gallus domesticus). Tissue sections 0.5–1.0 cm in diameter were removed from the renal cortex and medulla, fixed in 10% formalin, dehydrated, and embedded in paraffin. 6 µm thick sections were cut, deparaffinized, followed by immunohistochemical staining with polyclonal primary antibodies Rabbit anti-SGLT1 and Rabbit anti-SGLT2 according to the manufacturer's instructions (IHC kit, Abcam, UK). Visually, the localization of SGLT1 and SGLT2 in different bird species appeared to be similar - SGLT1 was detected in the epithelial cells of the straight proximal tubules in the medullary rays and in the outer stripe of the renal medulla, and SGLT2 was observed in the apical part of the epithelial cells of the proximal tubules of the kidney. Comparing the staining intensity of SGLT1 and SGLT2, it was noted that the staining of SGLT2 was more intense compared to that of SGLT1, which may be due to the greater reabsorption of glucose via SGLT2 in the nephron.

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### 3) IMMUNE CELL INFILTRATION OF TESTICULAR GERM CELL TUMORS – WHAT IS KNOWN AND WHICH QUESTIONS ARE UNSANSWERED SO FAR?

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ABSTRACT: Testicular germ cell tumors (TGCT) are the most common tumors in young men (14-44 years) and mostly present as Seminoma (SE) and Embryonic Carcinoma (EC), both arising from germ cell neoplasia in situ (GCNIS). Those cells are thought to "hi-jack" the immune system and may therefore favor TGCT development. T cells are the major component of tumor infiltrating lymphocytes (TIL) but involvement of T cell subtypes such as regulatory (Treg) or follicular helper T (Tfh) cells is poorly understood.

We used a retrospective and prospective patient cohort to analyze immune cells in TGCT compared to controls. We performed immunohistochemistry (IHC) on SE (n=75), EC (n=26), preserved spermatogenesis (n=10), testicular inflammation (n=12), and GCNIS (n=30) and flow cytometry (FC) on SE (n=12) and EC (n=6) using markers for macrophages, dendritic cells (DC), T and B cells. Those approaches using different and heterogeneous patient samples, showed that the immune cell environment is shifted from (resident) macrophages in normal testis to (newly recruited) T cells in TGCTs. Treg and Tfh were most abundant in SE tumor-center sites. Comparing SE and EC, SE (n=33) contained mostly T cells and macrophages whereas in EC tumor-samples (n=10), mostly T cells and DCs were detected. In SE, follicular like structures were visible with increased numbers of B cells and Tfh.

This study describes the complexity of immune cell niches in TGCTs and provides first indications of a potential importance of rare T cell subtypes in the immune environment of TGCT. The prospective clinical database will allow correlations between immune cell patterns and clinical parameters, e.g. localized and metastatic TGCTs. By this comprehensive approach, we aim at deciphering the role of "immune editing" during TGCT development, progression and possible metastatic behavior. Our results will help to identify novel prognostic factors and immune-therapeutic concepts in human TGCTs.

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# 4) *IN VITRO* DIFFERENTIATION OF DENDRITIC CELLS FROM PERIPHERAL BLOOD MONOCYTES IN THE CONTEXT OF CANINE LEISHMANIOSIS

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ABSTRACT: Dendritic cells (DCs) are potent antigen-presenting cells that establish a bridge between innate and adaptive immunity, making them extremely important in cell-based therapy studies. Leishmania infantum is the aetiological agent of canine leishmaniosis (CanL), a parasitic disease that may be fatal if not treated. Due to their immunostimulatory role, DCs constitute key cells in defining the outcome of Leishmania infection. The present study aimed to characterize morphologically and phenotypically canine DCs, obtained from peripheral blood monocytes (moDCs) by differentiation in vitro with a cocktail of immune modulators and analyze their interaction with virulent L. infantum promastigotes. Peripheral blood mononuclear cells from healthy dogs were isolated and cultured with colony-stimulating factor and canine recombinant interleukin-4 for 7 days. Subsequently, the morphology and topography of moDCs were evaluated by optical and scanning electron microscopy. After 7 days of differentiation, cells presented visible changes in their shape and size, with several cytoplasmic projections (dendrites), a morphology compatible with DCs differentiation. To confirm a canine moDC phenotype, expression of the cell surface molecules (CD1a, CD11c, CD14 and CD83) was evaluated by multiparametric flow cytometry, along with cell viability. Most moDCs displayed high viability and expression of CD11c+ CD1a+ CD83+ phenotype and low frequency of CD14+ cells. L. infantum promastigotes, exhibited a strong tropism to moDCs, interacting directly with the cell's membrane allowing them to be internalized and infect the cells. Therefore, viable moDCs were successfully differentiated in vitro and exhibited strong interaction with L. infantum promastigotes, making them strong candidates for future cell-based therapy studies.

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# 5) A GLANCE INTO LEISHMANIA INFECTION

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ABSTRACT: Leishmaniosis is a group of parasitic zoonotic disease diseases endemic in many countries, affecting millions of humans and animals worldwide. Macrophages are the definitive host cells of *Leishmania*. Nevertheless, neutrophils are the first cells to encounter the parasite shortly after its inoculation in the dermis by the phlebotomine sandfly, contributing to parasite destruction. Regardless of the initial control, Leishmania parasites have strategies to evade intracellular effector mechanisms and be transferred to macrophages. The host immune response towards leishmania is mainly organ-specific, and the role of other cells in innate immune response, such as hepatocytes have started to emerge. Hepatocytes constitute the majority of hepatic cells and play a key role in innate immunity against Leishmania as well as distinct macrophage lineages (MØs - blood macrophages and liver macrophages - KC). Taken together, our group studies demonstrated that the host immune response could shift from effector actions based on active phagocytic cells, with capacity to destroy these pathogens, to tolerogenic immune reactions, allowing replication and persistence of the parasite inside the host. Forward-looking research approaches such as the isolation of extracellular vesicles (EVs) produced by Leishmania parasites are under study and are a focus of our group in order to evaluate the immunogenic potential of EVs. Although there is a huge quantity of intricate parameters involved in the success or failure of the immune response and the persistence of the infection, it is paramount to get it along simple lines for a multidisciplinary perspective in one health viewpoint. Main results from the last years of research of our group are presented in scientific illustrations crossed with ultrastructure images. Research of biological and immunological processes related to zoonotic parasitic diseases can clarify complex pathways, which can lead to the development of innovative methodologies that contribute to health promotion and social improvement.

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# 6) ESTABLISHING AN IN VITRO MODEL FOR HUMAN TESTICULAR GERM CELL TUMORS: ANALYSIS OF JUNCTION-ASSOCIATED PROTEINS IN FS1 SERTOLI CELLS AND TCAM-2 SEMINOMA-LIKE CELLS.

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ABSTRACT: Testicular germ cell tumors including seminoma are common tumors in young men, but their unique pathogenesis is still unclear. Particularly, the lack of representative animal models and the limited availability of testicular tissue hamper research. Recently, a human cell culture model using FS1 Sertoli cells (SCs) and TCam-2 seminoma-like cells was developed, which may offer a new approach for research on seminoma progression and the role of somatic SCs in this process. Within the normal seminiferous epithelium, SC-junctions form the blood-testis-barrier, enabling intercellular communication, functional tissue organization and spermatogenesis. Additionally, junction-associated proteins are involved in various intracellular processes like cell differentiation and proliferation, suggesting a link to tumorigenesis. Thus, in the present study, the occurrence of selected proteins involved in the formation of adherens, gap, and tight junctions was investigated in FS1 and TCam-2 cells, using RNA-microarrays, RT-PCR, Western blot, immunocytochemistry and immunofluorescence, and compared with human testicular biopsies. Moreover, dye-transfer experiments were performed. In FS1 SCs, N-cadherin, connexin-43, connexin-45, claudin-11, ZO-1 and ZO-2 were clearly synthesized, mostly displaying a membrane-associated localization. Furthermore, Lucifer Yellow was able to diffuse between SCs, indicating functional intercellular communication *in vitro*. Thus, it can be assumed that cultured FS1 SCs – like differentiated SCs within the seminiferous epithelium – possess functional connexin-based gap junctions, and also adherens and tight junctions, providing a prerequisite for establishing an intercellular permeability barrier. The same proteins were also detectable in TCam-2 cells, with the exception that expression/synthesis of connexin-43 and claudin-11 was weak to non-detectable. Since both proteins are known to inhibit proliferation, a relationship to seminoma progression might be assumed. In vitro results largely matched with corresponding biopsies, arguing for the cell lines' representativeness. Building on this study's outcome, further direct and indirect coculture experiments could help evaluating the role of SC junctional proteins in context with seminoma development.

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# 7) HISTOLOGICAL AND HISTOCHEMICAL EXAMINATION OF THE LACRIMAL GLAND IN THE EURASIAN WOLF (*CANIS LUPUS LUPUS* LINNAEUS, 1758)

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ABSTRACT: The Eurasian wolf is a subspecies of the gray wolf, a predatory mammal from the Canidae family. It inhabits northern, central and eastern Europe (small populations in the countries of western and southern Europe) and a large area of western and central Asia. According to the IUCN, the Eurasian wolf is a species of Least Concern (LC). Samples for the tests were taken from eight adult individuals from the Wrocław Zoological Garden (2 wolves) and 6 wolves were obtained as a result of traffic accidents throughout the country. All research material was collected from 2017 to 2022/2023 years. The post mortem acquisition of tissues for research and the conduct of research do not require the consent of the Local Ethical Committee for Animal Experiments. The Wrocław University of Environmental and Life Sciences obtained permission from the General Directorate for Environmental Protection in Warsaw (Poland) to collect dead wolves. The following stains were made: H&E, mucicarmine, PAS, AB pH 1.0, AB pH 2.5, AB pH 1.0/PAS and HDI. The lacrimal gland of the Eurasian wolf was a multilobar complex alveolar gland that produces a serous secretion. It was surrounded by a thick connective tissue capsule with numerous fat cells, which formed partitions dividing the stroma of the gland into numerous large and small lobes. Very numerous fat cells were also observed within the interlobular septa and lobes. The vesicles had a small lumen and consisted of tall conical secretory cells with basophilic cytoplasm. Numerous leading wires were lined with single-layer cubic epithelium. Mucicarmine, PAS and HDI stains revealed a negative (-) reaction within the vesicles, a weak (-/+) reaction visible in the secretory segments in the AB pH 1.0 staining and a middle reaction (++) in the AB pH 2.5 and AB pH 2.5/ PAS (magenta color dominant and blue color sparse).

### 8) HISTOLOGICAL ANALYSIS OF THE UPPER EYELID, LOWER EYELID AND THIRD EYELID OF THE SPARROW HAWK (ACCIPITER NISUS LINNAEUS, 1758) (AVES: ACCIPITRIDAE)

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ABSTRACT: The sparrow hawk is a species of medium-sized bird of prey in the Accipitridae family. It is the most numerous representative of the clawed order in Eurasia. It is a species of Least Concern (LC) according to the IUNC. Samples for testing were taken from two individuals that died of natural causes and were collected post-mortem. The post mortem acquisition of tissues for research and the conduct of research do not require the consent of the Local Ethical Committee. The following stains were made: H&E and picro-Mallory trichrome. The anterior surface of the upper and lower eyelids was covered with stratified keratinized squamous epithelium composed of 2 to 5 layers of nucleated cells. Beneath the epithelium was a thin lamina propria made of loose connective tissue. The stroma of both eyelids consisted of dense fibrous connective tissue with irregular weave and numerous clusters of melanocytes. In the lower eyelid there was a terraceal plate made of dense fibrous connective tissue of regular weave with numerous fibrocytes. The posterior surface of both eyelids consisted of two parts - marginal zone and facies conjunctiva. The marginal zone was covered with non-keratinized stratified squamous epithelium containing 5 to 10-12 layers of nucleated cells. Pigment cells were found in the basal marginal zone in both eyelids. The conjunctival surface of both eyelids was covered with a columnar stratified epithelium containing 4 to 5 layers of cells. There was a single conjunctival lymphoid follicle in the area of the ocular zone of the upper eyelid. The third eyelid had 9 to 10-11 conjunctival folds. Conjunctival surface and bulbar surface of the third eyelid was covered by a stratified columnar epithelium and contained two to four layers of the nucleated cells. In the third eyelid the single lymphoid follicle was observed.

# 9) LIGHT AND SCANING ELECTRON MICROSOPIC STRUCTURE OF THE *PECTEN OCULI* IN HAMERKOP (*SCOPUS UMBRETTA* GMELIN, 1789) (AVES: SCOPIDAE)

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ABSTRACT: The pecten in birds is pigmented and highly vascular structure arises from retina, at the ovoid exit point of the optic nerve. There are three types of the *pecten oculi* in birds: *pecten* plicatus oculi, pecten vanellus oculi and pecten conicus oculi. For histology one pecten oculi was placed in 4% buffered formaldehyde for at least 72 hours and then rinsed in running water for 24 hours. Then samples were processed in a vacuum tissue processor – ETP (RVG3, Intelsint, Italy) and embedded in paraffin. The specimens were cut using a Slide 2003 (Pfm A.g., Germany) sliding microtome into 4 µm sections. The samples were then stained with H&E. For SEM study the second pecten oculi was fixed in 4% glutaraldehyde in PBS and stored for one day at room temperature. After fixation, in order to dehydration the samples were transferred to 50% ethanol and stored at room temperature for 2h. The process was repeated with 60%-100% ethanol. Dehydrated tissues allowed to air-dry overnight. Samples were coated with gold using ScanCoat6 (Edwards) and observed using a SE1 detector, at 10kV filament tension (SEM, Zeiss Evo LS15). The pecten oculi in hamerkop was a pleated type and was formed by 20-22 thin and rectilinear pleats (folds). This pecten oculi was composed of many capillaries and two parallel vessels - arteriole and venule, whose caliber ranges between 130-240 µm (arteriole) and 20-90 µm (venule). The capillary vessels luminal diameter was very small – between 5 µm and 20 µm on average. The hamerkop had numerous clusters of melanocytes which were located in the bridge and pleats of the pecten oculi. In the SEM pecten oculi was formed by rib-like folds originated from the base and attached the apical part. The surface of the pecten folds was irregular (pectineal capillaries). The capillary vessels were observed around arteriole and venule.

# 10) HISTOLOGICAL AND HISTOCHEMICAL EXAMINATION OF THE HARDERIAN GLAND IN THE EUROPEAN GREEN WOODPECKER (*PICUS VIRIDIS* Linnaeus, 1758) (Aves: Piciformes)

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AFFILIATION: Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland.

ABSTRACT: Green woodpecker is a species of medium-sized bird from the woodpecker family (Picidae) inhabiting almost all of Europe and western Asia. It is a resident bird larger than the green woodpecker. According to the IUCN, the green woodpecker is a species of Least Concern (LC). Samples for testing were taken from two individuals that died of natural causes and were collected for analysis on the basis of Permission from the Regional Director of Environmental Protection in Wrocław for the transport, collection and possession of dead specimens of species occurring in the Lower Silesian Voivodeship No.: WPN.6401.83.2021.MH. Directive 2010/63/EU of the European Parliament and of the European Council of 22 September 2010 on the protection of animals used for scientific purposes indicates that the post mortem acquisition of tissues for research do not require the consent of the Local Ethical Committee for Animal Experiments. The following stains were made: H&E, Azan trichrome, Movat-pentachrome, MGP-Y, PAS, AB pH 2.5 and HDI. Harderian gland in the green woodpecker was multilobar tubuloacinar, which is consistent with the first type of Burns (1992) classification. The lobes emptied into a large and irregular lumen of a primary ducts lined with columnar epithelium cells of varying highest. The secondary and tertiary ducts were lined by cuboidal cells with small lumen. The acini were lined with columnar epithelium and were located at the periphery of the lobes. Movat-Pentachrome and Azan trichrome stainings showed positive reaction (++) of the acini. MGP-Y stain showed population of plasma cells in the interstitium of the gland. Histochemical analysis confirm negative reaction visible in secretory segments in PAS staining, while average (++) positive reaction in AB pH 2.5 and HDI staining.

# 11) MORPHOLOGICAL VARIATIONS OF THE INTERATRIAL SEPTUM IN ADULT HORSE HEARTS.

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ABSTRACT: The fossa ovalis (FO), a membranous depression in the interatrial septum, represents the postnatal foramen ovale. It develops by the fusion of septum primum and secundum to form the floor of the FO and its limbus, respectively. In humans/pigs/sheep, the adult interatrial septum shows variations in appearance, often featuring a right and/or left septal pouch (RSP/LSP). Detailed morphological knowledge of the equine FO is important for minimally-invasive cardiac techniques such as transseptal puncture, where the interatrial septum is punctured to access the left atrium, for example to perform ablation.

In the cadaveric hearts of 12 adult warmblood horses, the FO size and thickness were measured and the presence of a RSP/LSP was recorded. The foramen ovale patency was evaluated. Per horse, a 10mm sample of the FO was examined histologically.

On its right side, the FO appeared macroscopically smooth and oval in all horses, showing a craniocaudal length between 10-30mm and a height of 8-27mm. A prominent limbus and RSP were present in all cases. The RSP was mostly (58%) craniocaudally elongated with a depth of 7-34mm. The remaining 42% showed a dorsoventral elongation of 9-29mm. The left surface appeared smooth in 84%, netlike in 8% and showed a ridge in 8% of the horses. A LSP was observed in 25% of the hearts, with a craniocaudal depth of 4-10mm and a dorsoventral height of 2-10mm. No patent foramen ovale was found. The thickness of the FO was 4-6mm. Histologically, a muscular layer surrounded by fibro-adipose tissue was located midway between the right and left endocardium.

In conclusion, the interatrial septum showed individual variations with predominantly a smooth, oval shaped FO and a RSP. Knowledge of the FO morphology allows to select the proper device and location to puncture the interatrial septum.

Funding: No external funding.

# 12) AGE-RELATED DISTRIBUTION OF LYMPHOCYTES IN THE INTESTINAL MUCOSA OF CHICKENS.

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ABSTRACT: The chicken has served as an important animal model in developmental biology and immunology and is one of the most intensively vaccinated domestic animal species. While we have a comprehensive understanding of the development of the adaptive immune system during embryogenesis, very little is known about the temporal-spatial development of the gut immune system post hatch. To provide the basic information for advanced functional studies of the gut associated lymphoid tissue, we set out to follow the development of the intraepithelial and the lamina propria lymphoid and myeloid compartment from hatch to 8 weeks of age by immunohistology and flow cytometry (FACS).

Tissue samples were taken from duodenum, jejunum, ileum, caecum and rectum from line M11 white leghorn chicken from day 1 to 8 weeks after hatch and subjected to staining with cell line specific monoclonal antibodies (mab) for leucocytes (CD45), T-cells (TCR-1 and -2), B-cells (AV20, 2C4) and myeloid cells (KUL01).

One day old chicken showed numerous CD45+ cells in all parts of the intestinal tract which were mainly KUL01+ myeloid cells with very few lymphoid cells. Cell numbers increased until 6-8 week after hatch but showed no further increase thereafter. This was due to an increase in lymphoid cell numbers which first appeared at the end of the first week after hatch. T-cells expressing the  $\alpha\beta$  T-cell receptor (TCR2+) were largely restricted to the lamina propria, while  $\gamma\delta$  T-cells receptor (TCR1+) expressing cells were abundant in the epithelium (intra-epithelial lymphocytes, IEL) but also frequent in the lamina propria. Interestingly, approximately 50% of intra-epithelial  $\gamma\delta$  T-cells co-expressed the B6 antigen identified by mab Av20 as revealed by two colour immune-fluorescence staining and FACS analysis. This antigen was previously discussed as a pure B-cell marker but based on our data also stains subpopulations of macrophages and  $\gamma\delta$  T-cells.

# 13) LOOKING FOR THE BEE'S KNEES – (ULTRA-)STRUCTURE OF SELECTED HONEYBEE ORGANS INFECTED WITH CHRONIC BEE PARALYSIS VIRUS.

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ABSTRACT: Honeybees are ranking third in the importance of production animals in Germany especially because of their performance as pollinators. Despite of this there are no specific pharmacotherapies established for most of their diseases. This project aims for morphologic examinations of organs form honeybees infected with chronic bee paralysis virus (CBPV) to better understand the pathogenesis as a prerequisite for developing new and optimizing existing therapies treatment.

Samples of CBPV-infected (confirmed by PCR) honeybee colonies as well as pathogen free colonies were dissected in insect saline solution. Brain, ventriculus and hypopharyngeal glands were transferred into fixation buffer and embedded for light and transmission electron microscopy. For light microscopy, 1 µm slides of the organs were H&E stained. Furthermore, samples were incubated with anti-caspase-3-antibody, counterstained with DAPI, and examined using a confocal microscope.

Preliminary results of light microscopic evaluation showed virus-unspecific degenerative findings, such as slightly peripheral nucleic hyperchromasia, karyopyknosis, and karyorrhexis. Caspase-3-activity could be demonstrated in some cells in brain and midgut tissue showing some apoptotic cells. Initial transmission electron microscopic examination identified the virus encapsulated in CBPV-positive samples of the ventriculus, whereas it could not detectable in negative controls.

Light microscopic findings will be corroborated by ongoing examination and compared to control samples. Further analysis with the transmission electron microscope is necessary to detect the virus in other organs and investigate possible alterations in the cell organelles.

Funding: This work was funded by a grant from the Leipzig veterinary junior scientist support programme financed by the "Freundeskreis Tiermedizin", the Faculty of Veterinary Medicine, and by Ceva Santé Animale as well as the "Universitätsstiftung", university foundation of Leipzig University.

# 14) ANATOMICAL AND HISTOLOGICAL INVESTIGATION OF THE MASSETER FASCIA IN A CAT (FELIS CATUS) – A PRELIMINARY STUDY.

#### AUTHORS: Rozwadowska A., Janus I.

AFFILIATION: Wrocław University of Environmental and Life Sciences, Norwida Str. 25, 50-375 Wrocław, Poland

ABSTRACT: Last two decades of research on fascial system have proved that the function of fascia is not only to protect internal organs and skeletal muscles or to supply them with blood vessels and nerves. Fascia plays an important role in movement coordination and biomechanics, proprioception, integration and in pain perception.

Post-mortem dissection of masseter muscles and fasciae of 5 skeletally-matured domestic cats was performed. In each cadaver, one randomly chosen muscle was used for anatomical investigation, the contralateral muscle with fascia was obtained with covering skin for histological analysis. Samples were post-fixed in 4% buffered formalin and embedded in paraffin. 4µm sections were cut and stained with haematoxylin and eosin, Weigert's resorcin fuchsin for elastic fibers, Picrosirius red and Masson-Goldner trichrome. Sections were examined in terms of general structure of the superficial layer of the masseter fascia: collagen fibers arrangement and direction, presence and distribution of elastic fibers, blood vessels and nerves presence, caliber and distribution.

At anatomical dissection presence of 4 separate layers of masseter fascia was revealed, each of them with different direction of collagen fibers – superficial and deep layer, fibrous layer in the angle between ramus of the mandible and zygomatic arch and fibrous layer at the mandibular ramus. The superficial layer of the masseter fascia was cover with the subcutaneous tissue composed of loose connective tissue with collagen fibers running in different directions, aggregates of adipose tissue and numerous, torturous blood vessels and nerves. The superficial masseter fascia was build of 3 distinct layers – thin external and internal with thick middle layer of packed, compact bundles of collagen fibers oriented perpendicular to the direction of muscle fibers. The compact middle layer received branches of blood vessels and nerves from the external and internal layer. Only few sparse elastic fibers were present running parallel to collagen fibers.

Funding: Statutory research and development activity funds of the Wrocław University of Environmental and Life Sciences

# 15) THE IN OVO AND EX OVO CAM MODEL AND ITS POTENTIAL IN (VETERINARY) ONCOLOGY RESEARCH

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ABSTRACT: The chicken embryonic chorio-allantoic membrane (CAM) model is a promising, rapid and cost-effective model to investigate multiple hallmarks of cancer in both humans and animal settings. The CAM is a highly vascularized extra-embryonic membrane which enables xenografting of tumor cells, and can be used to study metastasis and tumor angiogenesis. Furthermore, the CAM allows for rapid tumor development, accelerating the rate of obtaining results compared to other *in vivo* models.

Different variations of the CAM model exist; they can be largely divided in *in ovo* or *ex ovo* models. *In ovo* models require making a small window in the egg, allowing the embryo to grow in its natural environment, but limiting manipulation space and *in vivo* imaging possibilities. *Ex ovo* models consist of growing the embryos outside the egg, in a recipient, allowing for easy manipulation, visualization and providing a large space on the CAM to perform experiments. However, not much is known about the differences in tumor growth dynamics between these two experimental settings.

By comparing the growth of GFP-labelled HT1080 cells (human fibrosarcoma) in both *in ovo* and *ex ovo* settings, we observed in preliminary trials that xenografting in the *in ovo* CAM model was more successful and the tumor grew larger compared to in the *ex ovo* CAM model. Additionally, the mortality in *ex ovo* chicken embryos after inoculation of HT1080 cells was higher.

These findings can lead towards a standardization of the use of the *in ovo* or *ex ovo* CAM model in oncological experiments.

Funding: NA

### 16) GLYCAN PROFILES OF THE MALE DONKEY URETHRA

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ABSTRACT: The urethra of the male has a dual function, acting as a route for urine and semen. The physicochemical properties of the urethral epithelium surface are of paramount importance in the prevention of bacterial colonization (Alm et al., 1982). Despite its considerable importance, scanty studies report the molecular composition of the urethra of non-human species, including Equides. The presence of glycoconjugates in the pelvic urethra of male donkeys has been found by Alcian Blue-PAS staining (Abou-Elhamd et al., 2019), but in this species as well as in other equids an in-depth characterization of the glycan profiles is still lacking. In this study, the glycan pattern of the pelvic and penile urethra of the male donkey was investigated by lectin histochemistry. Fragments from the pelvic and penile urethra of healthy adult male donkeys were fixed in 4% (w/v) PBS-buffered paraformaldehyde, embedded in paraffin wax, and stained with a panel of twelve lectins. The results demonstrated a complex glycopattern in both the pelvic and penile urethral segments which also differed in the glycan composition. Briefly: i) the lumen of the pelvic urethra contained much more Nlinked glycans but fewer sialoglycans and neutral O-linked glycans compared to the penile urethra; ii) the luminal surface of the pelvic urethra expressed more N-linked glycans but no fucoglycans than the penile urethra; iii) goblet cells of the penile urethra secreted neutral and sialylated O-glycans as well as few fucoglycans. These findings suggest that the donkey pelvic and penile urethra are constituted of different glycopatterns and secrete glycans that modify the urethral milieu, thus protecting the urethra against pathogens and regulating the composition of the seminal plasma glycoproteins, which in turn have an impact on male fertility. The results could be a reference point for evaluating the pathological changes of the urethra of this endangered species.

Funding: none.

# 17) ADRENAL CORTEX AFTER PROLONGED EXPOSURE TO MODERATE HEAT: STEREOLOGICAL AND HORMONAL STUDY.

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ABSTRACT: Elevated ambient temperature, an anticipated consequence of global warming, provokes different responses of the hypothalamic-pituitary-adrenal (HPA) axis, depending on the temperature intensity and its duration. Concerning the effect of prolonged exposure to moderate heat, insufficient data exist regarding stereological investigations of the adrenal gland, as a component of the HPA axis. Therefore, the aim of this study was to elucidate stereological and hormone-secreting characteristics of adrenocortical cells after prolonged exposure to moderate heat. Adult male Wistar rats (n=7) were continually kept at  $35\pm1^{\circ}$ C and the control group (n=7) was kept at room temperature ( $20\pm2^{\circ}$ C), for 14 consecutive days. Adrenal glands were embedded in paraffin, serially cut (5µm thick sections), and stained with H&E. A multipurpose test system M42 was used for stereological analyses, and serum corticosterone was measured. Compared to the control group, the body mass, adrenal weight, and absolute adrenal volume were decreased (p<0.05) by 16.4, 14.0% and 15.2%, respectively. The absolute volumes of the adrenal cortex and zona fasciculata (ZF) were decreased (p<0.05) by 12.5%, and 20.5%, while no significant changes in the volumes of the remaining two cortical zones were found, in comparison with controls. Changes in the total number of adrenocortical cells were evidenced only in ZF, showing a decrease (p<0.05) by 26.7%, which corresponds with the registered decrease of serum corticosterone by 24.4%. The nuclear volume of the adrenocortical cells increased only in the ZF by 14.7% (p<0.05), while no changes were registered in other zones, compared to the controls. We hypothesize that this increase, together with the presence of dark cells in the ZF, is a result of a compensatory mechanism due to their significantly reduced number. Our results, reflecting a reduction of vast adrenocortical features only in ZF, indicate its decreased activity.

Funding: /

# 18) COMPARISON OF KIT STAINING PATTERNS BETWEEN PRIMARY CANINE MAST CELL TUMOURS AND DRAINING OR METASTATIC LYMPH NODES: A PRELIMINARY STUDY

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The CD117 tyrosine kinase receptor (KIT) is localized on the cell membrane and is frequently investigated in canine mast cell tumours (MCTs) which are by far the most common skin neoplasms in dogs. Aberrantly localized KIT receptors have been linked to a worse biological behavior of MCTs. For KIT, three immunohistochemical staining patterns exist, ranging from the benign membrane pattern I over the focally cytoplasmic pattern II, to the malignant generalized cytoplasmic pattern III; combinations of membrane and cytoplasmic patterns also exist. The expression of KIT patterns in lymph nodes from dogs with cutaneous MCTs, have not yet been investigated. The goal of this study was to compare the KIT patterns from primary MCTs with the corresponding lymph nodes. To do so, immunohistochemical staining of 15 primary MCTs and 17 corresponding lymph nodes with a polyclonal rabbit anti human CD117 antibody (Agilent, Santa Clara, United States; 1:400) was performed. All samples were graded by a board-certified veterinary pathologist according to the consensus of Berlato et al. (2021). Samples consisted of 1 Grade I/Low-grade, 4 Grade II/Low-grade, and 4 Grade III/High-grade MCTs. Histopathological results of the remaining 6 MCTs and 2 lymph nodes are still pending. Out of 15 lymph nodes, 3 were considered as reactive, 1 as draining and 11 as metastatic. Remarkably, in 10/15 cases, the KIT pattern in the primary MCT did not correspond to the pattern in the lymph node(s). Whether the KIT patterns in the lymph nodes can be used as a prognostic marker for biological behavior will be further investigated.

### 19) EFFECT OF MECHANICAL FLUID SHEAR STRESS AND239 HYPOXIA ON THE MYOGENIC DIFFERENTIATION PARAMETERS OF SKELETAL MUSCLE-DERIVED STEM CELLS.

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ABSTRACT: Skeletal muscle-derived stem cells (MDSCs) play the fundamental role in muscle regeneration. The mechanical and oxidative stresses on the muscle alter its physiological performance as well as regenerative capacity. This study investigates the influence of various stimuli including cyclic (CFSS) or periodic for 1h per day (PFSS) mechanical fluid shear stress and oxidative stress by means of hypoxia (3% O<sub>2</sub>) on myogenic differentiation (MD) capacity of MDSCs. C2C12 mouse myoblasts were differentiated under CFSS, PFSS and hypoxia for up to 7 d using a mechanical rocker and O<sub>2</sub> controlled chamber. MD parameters including assessment of cell proliferation, evaluation of cell differentiation using a morphometric analysis of indicative myotubes and expression of MD markers were carried out. Both CFSS and PFSS altered the cell morphology, abundant of cytoplasmic processes and enhanced cell proliferation at 72h compared to control. An enhanced cell viability indicated by increase of number and size of cell colonies under PFSS compared to both CFSS and control cultures. The number of Myogenin positive cells indicative for MD was increased under PFSS at 72h compared to other experimental conditions. Both FSS conditions increased myotube formation and upregulated *MyoD*, *Myogenin*, and contractile protein marker expression at day 7. Hypoxia enhanced cell viability up to 72h compared to normoxia. Hypoxia impairs MD of MDSCs indicated by downregulation of Myogenin and mTOR expression and reduced myotube formation. Hypoxia induced nitric oxide production in the proliferating cells up to 72h in contrast, the concentration was reduced in the differentiated cells at day7. An upregulated VEGF expression up to 72h was detected under hypoxia. The data provide evidence of improved MD capacity under mechanical stress. In contrast, cultivation under hypoxia reduced MD parameters but alternatively promotes angiogenesis. The data revealed the response of myogenic precursors under the influence of mechanical and oxidative stressors.

Funding: DFG: grant number (AR 333/11-1).

# 20) THE EPITHELIA OF THE BIRDS BEAK CAVITY – STRUCTURAL, FUNCTIONAL AND PRACTICAL ASPECTS

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ABSTRACT: The mucous membrane of the beak cavity is covered with two types of keratinized epithelium, i.e. ortho- and parakeratinized epithelium, which have evolved depending on mechanism of food intake. Examination of the structure of these epithelia, the way of keratinization process and renewal is important as basic knowledge and in veterinary practice during the evaluation of pathological changes in oral cavity. Therefore, complex micro- and ultrastructural studies on the ortho- and parakeratinized epithelium of the beak cavity in the domestic duck by using LM, SEM and TEM. Molecular analysis of the specific for birds alpha- and beta-keratin, proteins of the cornification process: keratin-associated proteins (KAPs) i.e. filaggrin and loricrin and transglutaminase 1 (TGM-1) as well as connexin in the intercellular gap junctions were done by IHC, Western blot, and Raman spectroscopy. The obtained results reveal important differences in both types of epithelia in the microstructure of cornified layer, the diverse cellular arrangement of keratin fibres, and exfoliation patterns. The orthokeratinized epithelium, which forms the lingual nail on the apex and mechanical papillae involved in food intake by pecking, grazing and filter-feeding, is characterized by a stronger expression with KAPs and TGM-1 and a higher percentage amount of beta-keratin than parakeratinized epithelium, which determines a formation of a more effective protective mechanical barrier. Studies on the distributions of connexins revealed that gap junctions are present, especially in the intermediate layer of both types of epithelia, ensuring transport of metabolites and synchronisation of the cornification. The pattern of connexins of parakeratinized epithelium may indicate a potential faster epithelial renewal of the dorsal surface of the tongue, where so-called "under- or over-tongue transport" occurs. Unlike mammals, in the beak cavity of birds, the presence of parakeratinized epithelium not indicates pathological changes called *parakeratosis*. This knowledge should be introduced more widely in veterinary practice.

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### 21) INVESTIGATING THE IMPACT OF ORIENTATION OF A SUBCUTANEOUS SENSOR TO ITS FOREIGN BODY REACTION AND FIBROTIC CAPSULE THICKNESS IN PIGS USING HISTOLOGICAL ANALYSIS.

AUTHORS: Carlier J1, De Bels L1, Asins Aznar E2, Huysmans H2, Vlaminck L1, De Spiegelaere W1

AFFILIATION: 1University of Ghent (UGent), Merelbeke, Belgium. 2Indigo Diabetes N.V., Zwijnaarde, Belgium.

ABSTRACT: The fibrotic capsule evoked by a foreign body reaction against subcutaneously implanted devices potentially hampers their detection accuracy. We designed an experiment to investigate whether the orientation of the devices' sensing cavity, axially (towards the body) or abaxially (towards the skin), makes a difference in fibrotic capsule thickness. Secondly, we evaluate whether the cavity is completely filled by vascularized tissue. Therefore, we implanted ten discoidshaped prototypes in the subcutis of one Göttingen minipig, distributed over both the pig's flanks by making a subcutaneous pocket. They are made of a polyetheretherketone core encapsulated by a polydimethylsiloxane silicone polymer and feature a central cavity of 2.2 mm deep (7.5 mm x 5 mm large), both on the axial and on the abaxial side of the implant. After explantation, samples were stained for histological analysis using hematoxylin & eosin and Masson's trichrome to measure the thickness of the fibrotic capsule and its ingrowth into the sensors' cavity. Preliminary results show a difference in thickness of the fibrotic capsule at the level of the cavity between axial and abaxial orientation. This indicates that the orientation of subcutaneously implanted medical devices influences the capsule thickness and potentially the detection accuracy of the device. Overall, there was only minimal to no tissue ingrowth in the sensing cavity. Future steps could feature the application of ingrowth stimulating materials such as surgical meshes to improve ingrowth of vascularized tissue in the sensing cavity.

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## 22) ISOLATION AND CHARACTERIZATION OF CANINE EXTRACELLULAR VESICLES FROM MEDICINAL SIGNALING CELLS.

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ABSTRACT: Extracellular vesicles (EVs) are nano-sized membrane-bound vesicles released into the extracellular space by most cell types, including MSCs. They are an important mechanism of intercellular communication and are therefore involved in a variety of physiological and pathological processes. EVs can range in size from 30 nm to 1 µm. They have become the focus of scientific attention in recent years. EVs are composed of a lipid bilayer membrane and contain a variety of molecules, including proteins, nucleic acids such as miRNA, carbohydrates and lipids. Due to their ability to transport various information, their therapeutic use is of highest interest, since they offer the advantage of a cell-free option for the transfer of therapeutic material to recipient cells compared to classical stem cell treatment. In particular, the regeneration of damaged tissues such as cartilage and the use in cancer therapy are in the focus of interest. For a possible therapeutic use of extracellular vesicles in dogs, EVs from medical signaling cells, previously referred to as mesenchymal stem cells, were successfully isolated using different methods (ultracentrifugation, ultrafiltration), concentrated and identified using appropriate markers as well as Western blot and immunogold labeling in transmission electron microscopy. Based on these studies, the exact composition of canine EVs in terms of miRNA and transported proteins as well as a biological effect on recipient cells can be investigated.

Funding: Society for the Promotion of Cynological Research e.V. (gkf) Germany

# 23) TIE2 RECEPTOR LOCALIZATION IN GLOMERULAR PODOCYTES USING SUPER RESOLUTION MICROSCOPY AND FLUORESCENCE LIFETIME IMAGING MICROSCOPY

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ABSTRACT: The TIE2 receptor (tyrosine kinase with immunoglobulin-like and epidermal growth factor homology domains-2), a member of the tyrosine kinase receptor family, was initially discovered and studied in endothelial cells. Recent literature shows that this protein is also present on, but not exclusively, pericytes, macrophages or various cancer lines. By using immunogold labeling, Western Blot and qPCR (quantitative polymerase chain reaction), our group has previously observed this molecule on glomerular podocytes. Here we show immunofluorescent localization of TIE2 in mouse kidney glomeruli with Stimulated Emission Depletion Microscopy (STED) and Fluorescence Lifetime Imaging Microscopy (FLIM).

Kidneys from wildtype mice were subjected to a tissue clearing and expansion protocol, followed by immunolabeling with two different polyclonal goat anti-TIE2 antibodies (R&D Systesm AF762 and AF313) and a donkey anti-goat STAR RED secondary antibody (abberior STRED). The three-dimensional localization of TIE2 was assessed using STED and FLIM in the glomeruli and adjacent peritubular capillaries.

TIE2 immunolabeling, when assessed with STED, revealed a fluorescent signal on the endothelial cell layer of both the glomerular and peritubular capillaries as well as on the podocyte cells. The FLIM analysis revealed additional information, showcasing a similar fluorescent lifetime between the glomerular and peritubular capillaries, but a different, longer, lifetime coming from the podocytes TIE2. The distinct fluorescence lifetimes of the STAR RED antibody can be due to the different microenvironment compartments in which the capillary endothelial cells and podocytes reside in. It can also be the consequence of different concentrations or a different conformation of the receptor on these cells.

Funding: No external funding was used.

# 24) IMMUNOHISTOCHEMICAL INVESTIGATION INTO PROTEIN EXPRESSION PATTERNS OF FOXO4, IRF8 AND LEF1 IN CANINE OSTEOSARCOMA.

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ABSTRACT: Osteosarcoma (OSA), is the most common type of primary bone malignancy in people and dogs. OSA has an aggressive nature, with incidence rates ranging from 13.9-27.2 cases per 100,000 dogs, and there have previously been limited experimental model systems. These factors have resulted in few advances in patient outcomes in recent decades. Recent developments have identified similarities in the aetiology and progression of human and canine OSA, highlighting canine OSA as an appropriate and useful model system. The model used in the present study was a naturally occurring, spontaneous OSA, with samples taken with owner permission from patients presenting at clinics for veterinary treatment. Rottweiler post-mortem OSA tissue (n = 32) was obtained from Bridge Pathology. All animal tissue was approved by the Ethics committee at the University of Nottingham School of Veterinary Medicine and Science and complied with national ethics procedures (permission number - UG 20331). Our molecular comparisons of canine OSA against healthy bone, using RNA sequencing and qRT-PCR, have resulted in the identification of proteins aberrantly expressed in OSA, suggesting their involvement in tumour progression. Abnormal RNA expression was found in forkhead box protein O4 (FOXO4), interferon regulatory factor 8 (IRF8), and lymphoid enhancer binding factor 1 (LEF1). The present study utilises immunohistochemistry to characterise cellular localisation and expression patterns of these proteins within canine OSA tissue. H-scoring, a well-established semi-quantitative protein expression technique, was used to assess nuclear, cytoplasmic and stromal staining. Qualitative data was also recorded to contextualise staining locations. These investigations allow further understanding of the roles played by these proteins within their respective molecular pathways, including oxidative stress response, immune regulation and Wnt pathway activation. Deeper understanding of the mechanisms involved in OSA are essential contributions towards the development of novel diagnostic, prognostic and treatment options in human and veterinary medicine contexts.

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# 25) HISTOLOGICAL STUDY OF THE THREE MAJOR SALIVARY GLANDS OF THE RED PANDA (*AILURUS FULGENS F.*)

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ABSTRACT: The secretion of major and minor salivary glands plays an important role in moisturizing the food pieces, which makes swallowing easier. In addition, saliva components start the initial digestion of carbohydrates, limit the growth of bacteria in the oral cavity, as well as saliva is involved in the immunological response through salivary immunoglobulin A (IgA). The type of diet as well as habitat influence the size of glands of oral cavity and their structure. So far, the histological structure of salivary glands in the red panda (Ailurus fulgens f.), whose diet is dominated mainly by young leaves and shoots of bamboo, has not been analyzed. Thus the aim of this study was the histological examination of three salivary glands in this species. The mandibular, parotid and monostomatic sublingal glands (major salivary glands) were collected post-mortem from three adult red pandas – two males and one female between 2020-2022 from Wrocław Zoological Garden. The samples of glands were analyzed histologically (hematoxylin&eosin, Masson-Goldner trichrome and Azan trichrome staining methods) with using a Zeiss Stemi 2000-C microscope (Carl Zeiss, Jena, Germany). The parotid and sublingual glands were surrounded by a thin connective tissue capsule, while the mandibular gland was surrounded by a thick connective tissue capsule. The stroma of parotid gland was formed by thin connective tissue septa which divided the gland into many lobes with many ducts. The shape of the lobes varied and the type of gland was compound multilobar and branched. The mandibular gland stroma was composed of the large lobes with a small amount of interlobar tissue. It was compound branched tubular gland. The sublingual gland was composed of mostly mucous acini. Although red panda (Ailurus fulgens f.) belongs to Carnivora order some differences in the structure of three major salivary glands were detected. However individual features cannot be excluded.

Funding: There is no external funding for this study.

Ethics Committee permission: According to Polish law and European law, studies on tissues obtained *post-mortem* do not require the approval of the Ethics Committee (Journal of Laws of the Republic of Poland, the Act of January 15, 2015, on the protection of animals used for scientific or educational purposes).

# 26) ANATOMY AND HISTOLOGY OF THE BALL PHYTON (*PHYTON REGIUS*) WITH CLINICAL IMPLICATIONS

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ABSTRACT: Throughout the world the number of exotic pets is increasing, including various species of snakes. They are appealing as companion animals because they are easy to handle and care for and look attractive with their color combinations of different morphs. The anatomical and histological structure of reptiles, including snakes, has been studied to a greater extent at the anatomical level rather than the histological level, and many studies investigate single organ systems or organs and have not focused on the individual species. In the case of the Ball python (*Phyton regius*), one of the most common pet species, neither a comprehensive histological study nor a study with a larger number of male and female Ball pythons has been conducted. No sex-specific differences for the various organ systems have been described in the available literature.

In our study, we aimed to examine the anatomy and histology of *Phyton regius* in detail using specimens from 24 *Phyton regius*. Samples for gross and histological examination were obtained *postmortem* from Golob d.o.o., Clinic for Small, Wild and Exotic Animals, Muta Slovenia, under permit number U34443-6/2917/2, as animal by-products according to Regulation (EC) No. 1069/2009. We histologically examined the structures of major organ systems in male and female animals. In addition, we focused on the topographic anatomy of males and females to assist veterinarians and clinicians in determining locations for blood sampling, endoscopy, and ultrasound, particularly with regard to the location of internal organs. Schematic drawings of the topographical anatomy of the organs were made to provide veterinarians and clinicians easy and professional access to the organs in preparation for examinations.

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### 27) COLLAGEN REMODELING IN EQUINE EXUBERANT GRANULATION TISSUE CHARACTERIZED BY PICROSIRIUS RED STAINING.

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ABSTRACT: Exuberant granulation tissue (EGT) is a disorder of second intention wound healing at distal limbs of horses. EGT is characterized by hyperproliferating granulation tissue. During the proliferative phase, myofibroblasts produce the immature collagen III which is later replaced by the mature collagen I in the remodeling phase. Here, we investigated the collagen deposition and maturation in EGT versus experimentally induced wounds. According to the fibers' thickness, absorbance and their birefringence, Picrosirius red staining enables differentiation of green, immature and yellow-red, mature collagen fibers under polarized light. Archived formalin-fixed paraffinembedded biopsies were used from a previous study approved by the Ghent University ethical committee (approval number 2014/183). In total, 12 clinical cases displaying EGT and eight experimentally induced wounds were analyzed at different sampling time points. Picrosirius red staining was analyzed with QuPath. We quantified the ratio of green vs. red fibers which facilitates the creation of groups of early (up to 14 days old) and late stages (more than 14 days old) of wounds based on the clinical report of wound age. Both in the experimental and the field samples, the average ratio of immature to mature collagen of EGT wounds at early stages is higher than in older ones (early:  $\overline{X} = 0.67$  [±0.56]; late:  $\overline{X} = 0.32$  [±0.49]), which is equally in the experimental induced wounds (early:  $\overline{X} = 0.7549$  [±0.1446]; late:  $\overline{X} = 0.228$  [±0.1603]). A microscopical affirmation of the previously reported age of the EGT wound based on the range of the control wounds was successful in seven samples.

In summary, collagen remodeling in EGT mostly resembles experimentally induced wounds, with the difference that the latter were performed under almost sterile conditions. The next step of this ongoing work is to substantiate our findings by investigating more samples and additionally immunohistochemical staining for Collagen I and Collagen III.

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# 28) IMPROVING COLOR PERCEPTION IN HISTOLOGICAL IMAGES INTERPRETATION.

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ABSTRACT: Spectral colors and their hues given by the absorption degree of light are perceived differently by the human eye. In histology, their interpretation will vary depending on context due to the different integration of information between the two brain hemispheres. Through analyzing colored images of different organs, tissues and cells using the microscope, first year veterinary students who are experiencing this for the first time will interpret the structures according to the colors they perceive. The ways of understanding histological images through colors can vary greatly, oftentimes leading to incorrect interpretations of structures. This aspect has been known for a while both for histology but especially for histopathology. In time, it has been attempted to fix this, by evidencing more so wrong interpretations based on different color perception, less so the way in which this could be fixed. Moreover, the same image perceived under a microscope and then transposed on other technical devices (video projector, TV set, tablet, mobile, laptop) can be interpreted differently. In order to hone this ability we first tried analyzing student feedback based on a questionnaire with questions targeted in three directions. We were interested in understanding, for the post-pandemic generation, how different color perception is for basic first year histological structures; how the way in which colors are understood can be explained and which would be the best way to detail staining techniques. Data has led to building a new model of spreadsheet representation of the main 15 stains often used in histology, and then a statistic analysis of the opinions of participants to this study, as well as differentiating relevant and irrelevant aspects which could lead to an improvement of image interpretation and efficiency of the cognitive process. The obtained model is adjustable to other categories of information based on color interpretation.

# 29) THREE-DIMENSIONAL AND IMMUNOELECTRON MICROSCOPIC STUDIES OF THE PORCINE MYOCARDIAL TELOCYTES.

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ABSTRACT: The initial documentation of cardiac telocyte (TC) ultrastructure in the porcine myocardial tissue adds to the growing proof of TC's existence in human and rodent species and other less-studied biomedical models. The study intended to verify previous reports substantiating the presence of myocardial TCs concerning other interstitial cell types which display similar ultrastructure, particularly on longitudinal sections. Hearts were collected from 4 to 10 weeks old piglets and processed for electron and light microscopic investigations. Scanning block-face electron microscopy (SBF-SEM) revealed the unique three-dimensional architecture of telocyte as established by the (1) absence of lumen across cutting planes, (2) the wide and veil-shaped appearance of the telopodes, and (3) the presence of pores in these elongations. Notably, cell profiles first identified as TC in a single 2D image were other cells type based on further 3D reconstruction analysis. Detailed comparisons of TC, cardiomyocyte, blood/lymphatic endothelial cells, pericyte, immune cell, and fibroblast structures allowed this study to propose various telocytic 3D models based on the selected planes and orientation of tissue sectioning. Immunogold staining on myocardial Tokuyasu cryosections supported these observations, as revealed by detecting CD31 and LYVE-1 expression among endothelial cells. Interestingly, several TCs or segments of telopodes were also found to be either less or equally immunoreactive to these microvascular markers. To complement these findings, immunohistochemistry and single immunofluorescence of serialized myocardial sections revealed the presence of CD34+/LYVE-1+ cells suggesting TC phenotypes, CD34-/CD31+ blood endothelial cells, and CD34-/LYVE+ lymphatic capillaries. In conclusion, the study's results strongly demonstrate telocyte as a distinct cellular resident of the porcine myocardium. Nevertheless, careful attention should be exercised when interpreting telocytes in 2D.

# 30) COMPARATIVE HISTOCHEMICAL ANALYSIS OF THE LINGUAL GLANDS OF LAC ALAOTRA BAMBOO LEMUR (*HAPALEMUR ALAOTRENSIS*) AND L'HOEST'S MONKEY (*ALLOCHROCEBUS LHOESTI*)

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ABSTRACT: The aim of this study was histochemical analysis of the mixed lingual glands and von Ebner's glands in the tongues of the selected two captive primate species: the Alaotra lemur (Hapalemur alaotrensis) from Lemuridae family and the L'Hoest's monkey (Allochrocebus *lhoesti*) from Cercopithecidae family. According to the IUCN Red List of Threatened Species the L'Hoest's monkey is vulnerable one, while Alaotra lemur is critically endangered species. The lingual glands samples were collected from 4 tongues of adult Alaotra lemur and 2 tongues of adult L'Hoest's monkey (all animals came from Wrocław Zoo). Then samples were analyzed histologically (hematoxylin&eosin, Azan trichrome staining methods) and histochemically (PAS (for neutral mucin detection), AB pH 2.5 (for acid mucins detection), PAS/AB pH 2.5 (for acid and neutral mucins detection) and HDI methods (for carboxylated and sulfated mucopolysaccharides and glycoproteins)). A Zeiss Stemi 2000-C microscope (Carl Zeiss, Jena, Germany) was used to evaluate the results. The mixed lingual glands of both species contained serous and mucous acini with dominance of the last one, while serous acini within von Ebner's glands. Histochemical analysis showed strongly positive reaction in mucous acini of L'Hoest's monkey, while mildly positive reaction in mucous acini of Alaotra lemur (PAS and AB pH 2.5 methods). Negative reaction was confirmed in serous acini of lingual glands of both primate species. Strongly positive reaction was observed in mucous acini of L'Hoest's monkey, while positive reaction in Alaotra lemur using HDI staining. Mild positive reaction was observed in serous acini of L'Hoest's monkey, while positive reaction in mucous acini PAS/AB pH 2.5 method was exposed in this primate. The obtained results confirm the dominance of acid mucins in lingual glands secretion in Alaotra lemur in comparison to L'Hoest's monkey. The different composition of the secretion of the lingual salivary glands may result from the differences in the type of food taken, especially papyrus and reeds in Alaotra lemur.

Funding: There is no external funding for this study.

Ethics Committee permission: According to Polish law and European law, studies on tissues obtained *post-mortem* do not require the approval of the Ethics Committee (Journal of Laws of the Republic of Poland, the Act of January 15, 2015, on the protection of animals used for scientific or educational purposes).

# **31) PRELIMINARY INVESTIGATION OF TELOCYTE CULTURES FROM THE PORCINE HEART.**

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ABSTRACT: The differential adhesion method based on fibroblast isolation is currently available to obtain a culture of telocytes (TC) developed from murine hearts. In this technique, adherent cells, after 60/90 minutes of incubation (37.5°C in 5% CO2), are considered fibroblasts, while the nonadherent cells, the TCs, are collected and replated. Using the technique, the study aimed to describe porcine TCs preliminarily based on morphology and antigenicity. Cells were isolated from the fresh heart tissues of 4 to 10 weeks old piglets following the modified isolation protocol of Li et al. (2016). Primary cultures and subcultures were subjected to morphological and immunohistochemical investigations. Morphological analysis of ventricular and atrial cell cultures revealed a heterogeneous population of cells based on phenotype. Telocytes became more evident in >10 days post-plating for primary cultures and subsequent passages. They were small, prominent cells with refractile cell bodies that were irregular, spindle, or rhombic in shape. Telopodes were also noted for their long elongations with alternating thin (podomer) and dilated (podom) sections. Transmission electron microscopy further demonstrated the ultrastructure of this moniliform appearance, exhibiting the presence of mitochondria in the podoms. Mitochondrial activity in these segments was also observed through Janus Green B staining of fixed cells. Interestingly, four other cellular profiles with phenotypes closely related to what was reported in the literature were also detected. Additionally, live cell imaging showed TC behavior concerning neighboring fibroblasts. TC interactions with these cells through on-and-off extensions suggest telopode importance in communication. Finally, immunofluorescence revealed CD34 positivity in TC cell bodies and Tps. CD31 and podoplanin (PDPN) expressions were absent. In conclusion, this research confirms that the differential adhesion method can be applied for TC harvesting from porcine heart tissues. Further standardization of the isolation and passaging protocol must address morphological variability, which can be tantamount to antigenic heterogeneity.

# **32) IMMUNOCYTOCHEMICAL CHARACTERIZATION OF EUROPEAN ROE DEER (CAPREOLUS CAPREOLUS) OVARIES**

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#### ABSTRACT:

Introduction: Little is known about the morphology of European roe deer (Capreolus capreolus) ovaries specially folliculogenesis during fetal development and different stages of pregnancy.

Methods: Ovaries from European roe deer fetuses and adults were collected from the hunting association Weihenstephan. Using immunohistochemistry (cytokeratin 5 (CK5), cytokeratin 8 (CK8), cytokeratin 14 (CK14), cytokeratin 18 (CK18), cytokeratin 19 (CK19), vimentin-, laminin-, S 100-, Estrogen receptors alpha- and beta proteins, detailed morphological characterization of the follicles was made.

Results: Primordial follicles to antral follicles were seen in different time frames in fetal ovaries. The morphology of the different follicular stages of pregnant roe deer is very similar to that of cattle. S 100-, vimentin-, laminin-, Estrogen receptors alpha- and beta proteins showed a different pattern in (pre-) granulosa cells, oocytes, surface epithelial cells and stroma cells. A characteristic pattern of cytokeratins was found during prenatal development as well as in adult ovaries during differentiation of different cell types.

Conclusions: In conclusion we found that Estrogen receptors alpha- and beta proteins play also different roles in prenatal and adult follicular development. The development and differentiation of the (pre-) granulosa cells, oocytes, surfaces epithelial cells and stroma cells is accompanied by a distinct change in the expression of intermediate filament proteins.

Funding: The Norwegian Research Fund, grant number 3840272.

# 33) ROLE OF TRPA1 RECEPTOR IN GASTRIC PROTECTION.

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ABSTRACT: Transient potential receptors (TRP) constitute a large family of receptors in which TRPA1 is among the most important. TRPA1 is activated by plethora of factors, including compounds of natural spices, as allyl isothiocyanate present in horseradish. Although natural medicines and spices are known for years in several cultures to play a gastroprotective role, different experiments reported divergent, and sometimes even opposite effects for some of them. Stomach as a reservoir of food is directly exposed to disruptors ingested by humans and animals. Ethanol, a common content of beverages ingested by human populations, disturbs the homeostasis of the stomach luminal environment, leading to gastric lesions.

To evaluate the role of TRPA1 receptor in gastric reaction to ethanol we have infused ethanol into the stomachs of wild-type animals and mice lacking TRPA1 receptor and evaluated stomach reaction by macro- and microscopic analysis of the stomach mucosa; quantification of mRNA expression for selected proinflammatory cytokines and studied receptor expression in the stomach tissue and finally determined its localization in the gastric wall by confocal laser microscopy.

Results obtained with all techniques applied revealed significant gastroprotective role of TRPA1 receptor for ingested ethanol. Interestingly, the expression of TRPA1 in the gastric tissue was influenced by the administrated ethanol in both, wild-type animals and TRPA1-knockouts, and additionally, in wild-type animals the receptor's expression changed over time, indicating tissue adaptative regulations.

Double immunofluorescence combined with confocal laser microscopy indicated the immunoreactivity for TRPA1 in neuronal and non-neuronal structures of the stomach wall, suggesting their possible role in stomach reaction to ethanol.

The presented study demonstrated the gastroprotective role of TRPA1 receptor against ingested alcohol.

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# **34**) Application of Thyl YFP in analyses of cell fates during embryonic development and in vitro differentiation

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ABSTRACT: The main goal of our study was to describe development, migration and differentiation of neuronal precursors by applying transgenic line B6.CgTg(Thyl YFP)16Jrs/J, which under the control of Thyl gene promoter, expresses YFP. Neural stem cells were isolated from the forebrain of 14.5 old mouse embryos (El4.5), cultured as neurospheres and after one passage differentiated to mature neurons. Immunocytochemistry, qPCR and WB were performed after cells differentiation. Histological slices of embryonic tissue, starting from El2.5 till newbom mice were prepared for immunohistochemistry and stained with specific markers for neuronal precursors, neurons and glia. Confocal images were captured by Olympus FV 3000 microscope and analysed by IMARIS software. In our previous work we described expression of ThyYFP positive cells (Alić 2016, Mitrecić 2017) and transplantation into the mouse brain affected by stroke. However, in this study we were focused on a single YFP positive cells, and we analysed cell fate and cell migration from ventricular zone to the marginal zone of neural tube, developing spinal cord and developing brain in later stage of development. In our study we used specific cell markers (Nestin, SOX2, P AX6, TBR2, TBRI, Ctip2, SATB2, MAP2, TUBB3) and traced individual cells during differentiation and maturation. With IMARIS software we analysed branching of individual neurons, spines and here we described in details cell morphology during embryonic development, and we compared it to differentiation in vitro. Fluorescent data were validated by WB and qPCR. Our data suggest, that B6.CgTg(Thyl YFP) 16Jrs/J is an excellent model for cell tracing, analyses of marker expression and cell morphology. Moreover, we obtained many valuable information about differences in cell differentiation pattern between cells in embryonic brain and in in vitro system.

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# 35) "Embryo-maternal interactions at the time of birth in the Tammar wallaby, Macropus eugenii"

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ABSTRACT: The reproductive strategy of marsupials is based on a very short gestation, followed by a long lactation. Pregnancy is usually shorter than a "non-pregnant" ovarian cycle. The marsupial uterus duplex enables a permanent pregnancy with one uterus hosting a free-floating diapausing blastocyst while in the contralateral uterus "active" pregnancy begins with reactivation of the blastocyst and subsequent implantation. In the Tammar wallaby, the "active" gestation period is 26.5 days. Only shortly before birth does the foetus come into direct contact with the mother through the formation of a yolk sac placenta. The "maternal recognition of pregnancy" manifests itself in an increased vascularisation of the pregnant uterus as well as an increased secretion of the uterine glands. While in eutherian placental animals the maternal immune response is suppressed during implantation, the eutherian prepartum phase is characterised by an inflammatory reaction in the sense of maternal rejection. The extent to which inflammatory processes play a role in marsupial birth is largely unknown. In this study we explore samples of pregnant and non-pregnant peri-partum uteri of animals, in which embryonic development has been closely followed by non-invasive ultrasound using a transcutaneous approach via the pouch. Immunohistochemical methods will be employed to investigate whether signs of an inflammatory reaction are present in the peripartum endometrium and foetal extraembryonic membranes. Local vascularization of the endometrium will be compared between pregnant and non-pregnant uteri of the same animal. Another focus lies on the characterization of cytokines and immune cells, which might be involved in the induction of the birthing process.

Funding: n.n

# **36) ARTIFICIAL INTELLIGENCE APPLICATION IN THE QUANTIFICATION OF ANGIOGENESIS**

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ABSTRACT: Intravenous drug administration might cause endothelial damage and disturb angiogenesis. These effects can be quantified through several methods. Recent advances in artificial intelligences (AI) offer an opportunity to develop new methods in medical research, with increased sample sizes and reproducibility. The aim of our study was to compare the application of AI with a manual method to morphologically quantify in vitro angiogenesis.

Co-cultures of human dermal microvascular endothelial cells and dermal fibroblasts were exposed to iodinated contrast media and labelled with CD31. An AI-software (NIS.ai, Nikon, Japan) was trained for detection and measurements of endothelial capillaries on light microscope images; number of capillaries and their branchings were counted, and their lengths, widths, and total area measured. Number of capillaries and their branchings and width were manually assessed for comparison. Pearson correlation coefficient (r) and linear regression ( $r^2$ ) were used to evaluate the correlation and variability between both methods.

119 images were used. The AI was trained in 40 hours. The images were analyzed faster with the AI than manually (3 minutes vs 45 minutes per image). Using the AI, systematically more capillaries (median 1449 vs 902) and branchings (median 675 vs 305) were counted than with the manual method. Both methods had a strong linear relationship for counting of capillaries and branchings (r-capillaries = 0.88, r-branching = 0.86) and the actual AI values fitted well with the predictive values ( $r^2$ -capillaries = 77%,  $r^2$ -branching = 74%). No correlation was found for measurements of the width (r-width = 0.17,  $r^2$ -width = 3%).

The use of AI reduces the time required for quantitative analysis of angiogenesis and offers a wider variety of evaluation parameters, without interobserver variability and observer bias. Further training and development will open new perspectives. Future analysis will determine whether the manual method or the AI is more reliable.

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