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Cover illustration by Pšenica Kovačič depicts a domestic cat and is inspired by the Editorial manuscript - In the spotlight: Can illustration help us understand our cats better? by Erjvec and Kovačič in this issue. See page 181.

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Reflecting and Looking Forward – The Role of Veterinary Scientific Journals in Veterinary Research and Medicine

Refleksija in pogled naprej – vloga veterinarskih znanstvenih revij v veterinarskih raziskavah in medicini

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With this issue, we will close 2023 and it is my distinct pleasure to summarize the continued improvements of the Slovenian Veterinary Research journal we've made this year. To advance authors', reviewers', and readers' experiences, we first updated the Open Journal System. We continuously revise and develop our instructions for authors, reviewers, and editors to streamline the pathway from submission to publication and make it easier for all. We have updated the graphical image of the journal, invited an artist to the Editorial team, and started featuring our manuscripts with illustrated artwork on the cover of the journal (1). With this, we want to emphasize the published work and novel discoveries, but also strengthen the interconnectedness of science and art (1). Furthermore, we emphasize manuscripts published in our journal or work pertinent to the discoveries on disease pathophysiology, therapy, prevention, and public health by short articles, and editorials (1-4). With these articles, which are published in both languages, English and Slovenian, we want to also reach out to veterinary students, educators, and clinicians and bring them relevant novel discoveries, locally and internationally. One of the importance of scientific journals is facilitating communication and collaboration among researchers, practitioners, students, and educators in the veterinary community. In the future, we would like to further strengthen this contribution and

Spoštovani sodelavci, avtorji, bralci, recenzenti in člani uredniškega odbora Slovenskega veterinarskega zbornika!

S to številko smo zaključili leto 2023 in v posebno veselje mi je opozoriti na novosti, ki smo jih opravili v tem letu. Da bi izboljšali izkušnje avtorjev, recenzentov in bralcev, smo posodobili sistem odprtega dostopa. Po potrebi prilagajamo navodila za avtorje, recenzente in urednike, vse z namenom izboljšanja postopkov do objave prispevkov.

Letos smo spremenili tudi grafično podobo revije in v uredništvo povabili akademsko ilustratorko. Z njenimi ilustracijami, ki krasijo naslovnice posameznih številk, predstavljavljamo nekatere objavljene prispevke, s čimer opozarjamo na znanstveno aktualne teme in novejša odkritja, hkrati pa krepimo povezovanje z umetnostjo (1). S kratkimi uvodniki večkrat izpostavljamo posamezne objavljene članke, ki obravnavajo znanstveno tematiko iz področij javnega zdravja, patofiziologije, terapije in preventive bolezni (1-4).

S temi uvodnimi prispevki, ki jih objavljamo v angleškem in slovenskem jeziku, želimo predvsem študentom veterinarske medicine, pedagoškim delavcem in klinikom približati pomembnejša novejša odkritja. Eden od pomenov znanstvenih revij je olajšanje komunikacije in sodelovanja med keep informing about the latest developments, best practices, and emerging issues, and encourage a continuous exchange of ideas to help advance veterinary research and medicine.

With the new image of the Jurnal, we also developed a new logo. We kept the blue color, traditionally associated with veterinary medicine, potentially because it is perceived as calm, soothing, and associated with trust, care, and compassion, all adjectives pertinent to veterinarians. We also included a symbol of veterinary medicine, "Rod of Asclepius", a serpent coiled around a rod with a little artistic touch of our Art editor (2). We started to be active on social media, we are now promoting and sharing our journal and manuscripts to readers through X (formerly known as Twitter), Facebook, and LinkedIn. With all these activities we want to strengthen our mission of fostering the growth and development of the journal to advance comparative research and ultimately veterinary and human medicine to enhance the overall well-being and health of animals and humans.

All of these initiatives and improvements are only possible because our journal is still self-published by the Veterinary Faculty at the University of Ljubljana with the support of the Slovenian Research and Innovation Agency (ARIS), and the big publishing houses can't impose their culture, structure, and systems on our efforts. Being self-published allows us to be innovative and creative, however, it also requires a lot of volunteer, unpaid work, and service from our Editorial Team. We couldn't have done all of these without the amazing Editorial Team, Co-Editors, Assistant to the editor, Executive and Associate Editors, and I would like to thank you all. Due to your passionate and voluntary work, we can continue the more than 60-year-old legacy of our Journal. Slovenian Veterinary Research Journal started in 1961 as "Reports Collection" of the Department for Veterinary Medicine of the Biotechnical Faculty at the University of Ljubljana (5). Much has changed since the launching of the Journal, the Veterinary Faculty became an independent member of the University of Ljubljana, accredited by the EAEVE (European Association of Establishments for Veterinary Education), the Journal changed names and became an international journal in 2004, articles were published in English language (we still translate abstracts to Slovenian language and by doing so, keep them available to local veterinary community), and obtained impact factor in 2010, but the mission stayed the same (5). Since day one, the Journal has served as a scientific communication platform and a vehicle for knowledge dissemination among veterinary medicine researchers, clinicians, students, and educators. Furthermore, the Journal also provides an educational hub for future editors and reviewers who continue its mission. Last but not least, the Journal stimulates interdisciplinary networking and collaboration, central to impactful discoveries that apply to both, animal and human health. The interconnection between people and animals, and their shared environment is now well recognized by the One Health concept (6). Veterinary medicine and comparative

raziskovalci, veterinarji-praktiki, študenti in učitelji v veterinarski skupnosti, kar bi v prihodnosti radi še okrepili in hkrati obveščali o razvoju, dobrih praksah in morebitnih nastajajočih težavah ter spodbujali neprekinjeno izmenjavo idej in tako pospešili napredek v veterinarskih raziskavah in veterinarski medicini.

Z novo podobo revije smo oblikovali tudi nov logotip. Ohranili smo modro barvo, ki je tradicionalno povezana z veterinarsko medicino, predvsem ker je umirjena, pomirjujoča ter povezana z zaupanjem, skrbnostjo in sočutjem; s pridevniki, ki ponazarjajo osebno predanost veterinarskemu poklicu. V logotip smo vključili tudi simbol veterinarske medicine, Asklepijevo palico, ovito s kačo, znotraj črke »V«, ki ga je umetniško oblikovala naša likovna urednica (2). Postajamo aktivni na družbenih medijih, promoviramo in delimo našo revijo in objavljene prispevke z bralci prek spletnih omrežij X (nekdanji Twitter), Facebook in LinkedIn. S temi aktivnostmi želimo okrepiti prepoznavnost revije in prispevati k napredku raziskovalnega dela tako v veterinarski, kot humani medicini ter izboljšanju zdravja živali in ljudi.

Vse te pobude in izboljšave smo lahko izpeljali, ker našo revijo izdaja Veterinarska fakulteta Univerze v Ljubljani ob podpori Agencije za raziskovalno in inovacijsko dejavnost RS (ARIS) in nam tako večje založbe ne morejo vsiljevati njihovih lastnih praks. Izdajanje revije v samozaložbi nam omogoča inovativnost in ustvarjalnost, vendar zahteva tudi veliko prostovoljnega dela naše uredniške ekipe. Vsega tega ne bi mogli storiti brez izredne energije in dela sourednic, sourednika, pomočnice urednice, izvršnih in pridruženih urednic ter urednikov in rada bi se vam vsem zahvalila. Z vašim neutrudnim in prostovoljnim delom lahko nadaljujemo več kot 60-letno tradicijo naše revije. Slovenski veterinarski zbornik je začel delovati leta 1961 kot »Zbornik« Oddelka za veterinarsko medicino Biotehniške fakultete Univerze v Ljubljani (5). Z dolgoletnim izhajanjem se je marsikaj spremenilo. Veterinarska fakulteta je postala samostojna članica Univerze v Ljubljani, kasneje tudi akreditirana pri Evropskem odboru za veterinarsko izobraževanje (European Association of Establishments for Veterinary Education, EAEVE). V tem obdobju se je revija nekajkrat preimenovala in postopno pridobivla vse večji pomen tudi v mednarodni raziskovalni skupnosti. Članke objavljamo v angleškem jeziku, s slovenskimi prevodi izvlečkov pa hkrati skrbimo, da jih približamo lokalnim uporabnikom. V letu 2010 je bil Slovenski veterinarski zbornik umeščen na seznam revij s faktorjem vpliva, kar je še povečalo mednarodni ugled revije, poslanstvo pa je ves čas ostalo enako (5). Slovenski veterinarski zbornik že od vsega začrtka služi kot platforma za širjenje znanja in novih dognanj med raziskovalci, zdravniki, študenti in učitelji, poleg tega omogoča izobraževanje raziskovalcev in bodočih urednikov ter recenzentov, ki nadaljujejo njegovo poslanstvo. Nenazadnje spodbuja interdisciplinarno mreženje in sodelovanje, kot pomembna dejavnika novih odkritij na presečišču zdravja živali in ljudi. Razumevanje naše medsebojne povezanosti z živalmi v skupnem okolju je ključno za doseganje optimalnega zdravstvenega stanja ljudi in živali, s tem pa tudi ciljev iniciative research play an integral role in achieving the One Health goals (6). Not only because animals both impact and are impacted by people and the environment, but also because we learn from understanding similarities and differences between humans and other animals, referred to as zoobiquity, a term coined by Barbara Natterson-Horowitz and Kathryn Bowers (7,8). Furthermore, addressing the health questions through an evolutionary lens brings a novel, out-of-the-box, understanding and opportunities for prevention and therapeutic avenues (9).

Together, veterinary science and journals like ours can help build bridges, cross-disciplinary and cross-continental. We will strive to do that, we have plenty of room for improvement and advancement, including the turn-around time of the manuscripts, manuscripts quality, and expansion of indexing to name a few, as we have plenty of ideas, how on to do it.

Please enjoy this issue, send us your work, and serve as reviewers - together we can contribute to the future of veterinary and human medicine.

»Eno zdravje« (6). Veterinarska medicina je tudi ključnega pomena za primerjalne raziskave, ki igrajo znatno vlogo pri doseganju teh ciljev (6). Ne le zato, ker živali vplivajo na ljudi in okolje in obratno ampak tudi, ker se hkrati učimo iz razumevanja podobnosti in razlik med ljudmi in živalmi, kar sta Barbara Natterson-Horowitz in Kathryn Bowers poimenovali »Zoobiquity« (7, 8). Poleg tega raziskovanje zdravstvenih vprašanj prinaša tudi novejša spoznanja o boleznih in vse boljše možnosti za njihovo preprečevanje in zdravljenje (9).

Slovenski veterinarski zbornik, lahko pomaga graditi meddisciplinarne mostove in prispeva k napredku znanja za vse. Prizadevali si bomo, da postanemo boljši, da skrajšamo čas presoje člankov, da še izboljšamo njihovo kakovost in razširimo indeksiranje.

Uživajte v tej številki, pošiljajte nam svoje prispevke in postanite naši recenzenti – skupaj lahko prispevamo k boljši prihodnosti veterinarske in humane medicine.

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Can Illustration Help us Understand our Cats Better?

Ali nam ilustracija lahko pomaga bolje razumeti naše mačke?

Key words

feline;
facial expression;
cat communication;
welfare;
illustration

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Recent feline behavioral research, spearheaded by Scott and Florkiewicz (1), has made groundbreaking strides in decoding the complexity of cat communication. The study meticulously cataloged 276 facial expressions, each intricately linked to a broad spectrum of the cats' emotional states and communicative intents. This extensive array of expressions, which includes a "play face" akin to those observed in other species, signifies a depth of social interaction previously unacknowledged in domestic cats.

Florkiewicz also employs scientific illustration as an invaluable tool, enhancing the granularity of behavioral analysis (2). Detailed illustrations highlight the subtleties of cats' expressive range, previously obscured by less nuanced observation methods. Finka et al. (3) have demonstrated how such visual articulation can deepen our comprehension of feline social behaviors, transcending the constraints of traditional observational techniques. This innovative study categorizes and correlates expressions with fundamental emotional-motivational systems (4). It distinguishes between affiliative behaviors—indicative of positive, engaging interactions like play—and non-affiliative behaviors, which encompass a spectrum of negative or protective states from anxiety to aggression. The study extends to caregiving and reproductive behaviors, further delineating the emotional complexity of these animals.

Scott and Florkiewicz (1) also probe the broader implications of their findings for feline welfare. They argue that a deeper understanding of feline expressions can significantly

Nedavne raziskave mačjega vedenja, ki sta jih vodili Scott in Florkiewicz (1), so naredile prebojne korake v dešifriranju kompleksnosti komunikacije mačk. Študija je natančno katalogizirala 276 izrazov obraza, vsak tesno povezan s širokim spektrom čustvenih stanj in komunikacijskih namenov mačk. Ta obsežen nabor izrazov, ki vključuje »igralni obraz«, podoben tistim, opaženim pri drugih vrstah, označuje globino socialne interakcije, ki prej ni bila priznana pri domačih mačkah.

Florkiewicz prav tako uporablja znanstveno ilustracijo kot neprecenljivo orodje, ki izboljšuje natančnost analize vedenja (2). Podrobne ilustracije poudarjajo subtilnosti izraznega obsega mačk, prej zakrite z manj niansiranimi metodami opazovanja. Finka in sod. (3) so pokazali, kako lahko takšna vizualna artikulacija poglobi naše razumevanje socialnih vedenj mačk, presegajoč omejitve tradicionalnih tehnik opazovanja. Ta inovativna študija kategorizira in korelira izraze z osnovnimi čustveno-motivacijskimi sistemi (4). Ločuje med afiliativnimi vedenji – ki nakazujejo na pozitivne, vključujoče interakcije, kot je igra – in neafiliativnimi vedenji, ki obsegajo spekter negativnih ali zaščitnih stanj, od anksioznosti do agresije. Študija se razteza na vedenje oskrbe in reproduktivno vedenje, kar dodatno opredeljuje čustveno kompleksnost teh živali.

Scott in Florkiewicz (1) prav tako raziskujeta širše posledice svojih ugotovitev za dobrobit mačk. Trdita, da lahko globlje razumevanje mačjih izrazov znatno izboljša kakovost interakcij med ljudmi in mačkami in s tem dobrobit domačih improve the quality of human-cat interactions, thereby enhancing the welfare of domestic cats. For instance, recognizing expressions of pain or distress allows for more empathetic and effective caregiving. Furthermore, the study illuminates the potential impact of selective breeding on the evolution of feline expressiveness, suggesting that human preferences might inadvertently alter the communicative capabilities of future feline populations. In our ongoing research (5) and collaboration in the project European Researchers' Night, Humanities Rock, Human(e), animal 22/23 (Evropska noč raziskovalcev, Humanistika, to si ti! Človek, žival), we integrate observational rigor with scientific illustration (Figure 1) to delve into feline body language, including facial expressions and cephalic types (brachycephalic, dolichocephalic, mesocephalic). This approach, enriched by considering cats' living styles, aids in understanding the expression of natural feline behaviors, enhancing our insight into their emotional states and physical variations.

mačk. Na primer, prepoznavanje izrazov bolečine ali stiske omogoča bolj empatično in učinkovito oskrbo. Poleg tega študija osvetljuje morebitni vpliv selektivne vzreje na evolucijo mačjega izraznega jezika, kar nakazuje, da bi lahko človeške preference nenamerno spremenile komunikacijske sposobnosti prihodnjih populacij mačk.

V našem tekočem raziskovanju (5) in sodelovanju v projektu Evropska noč raziskovalcev, Humanistika, to si ti! Človek, žival 22/23, tudi združujemo temeljito opazovanja vedenja mačk z znanstveno ilustracijo (Slika 1), da bi poglobljeno raziskali njihovo telesno govorico, vključno z izrazi obraza in tipi glave (brahicefalični, dolihocefalični, in mezocefalični). Ta pristop, dopolnjen z upoštevanjemin poznavanjem življenjskih slogov mačk, pomaga pri razumevanju naravnega izražanja mačjih vedenj in izboljšuje naš vpogled v njihova čustvena stanja in fizične različnosti. Tako naše raziskave kot tudi raziskave Florkiewicza (2) so pokazale pomen

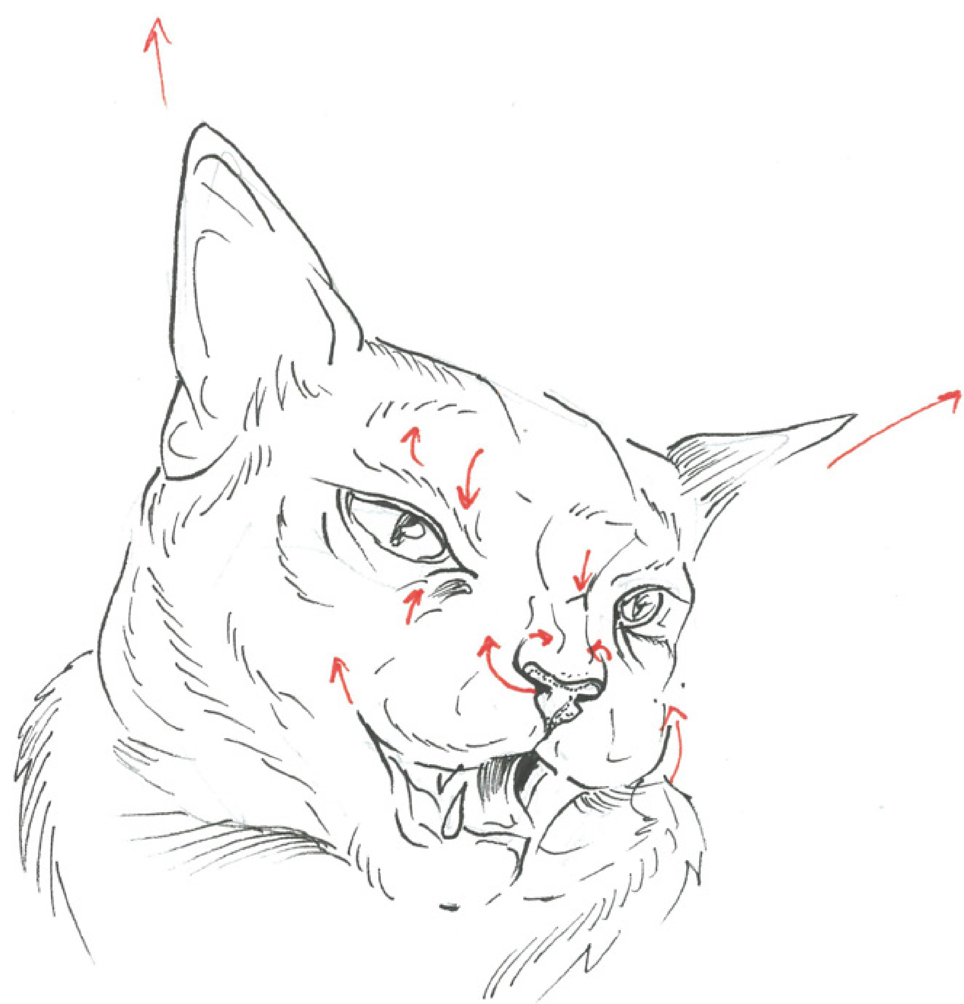

Figure 1: Analysis of facial movement for better understanding (P. Kovačič)

Our findings and Florkiewicz's (2) research underscores the value of blending behavioral science with artistic representation, advancing our knowledge of cat welfare and fostering a deeper human-cat connection.

združevanja vedenjske znanosti z umetniško reprezentacijo, kar napreduje naše znanje o dobrem počutju mačk in spodbuja globljo povezavo med človekom in mačko.

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Original Research Article

Expression of Insulin, Glucagon, Somatostatin, and Pancreatic Polypeptide in the Pancreas of the Eurasian Moorhen (*Gallinula chloropus*)

Key words

pancreas;
islet;
insulin;
glucagon;
somatostatin;
polypeptide;
diabetes

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Abstract: The pancreas is a complex gland that possesses both endocrine and exocrine functions. The present study investigated the gross anatomy, histochemical features, and immunohistochemical expression of insulin, glucagon, somatostatin, and pancreatic polypeptide in the pancreas of the Eurasian moorhen. Grossly, the pancreas consisted of three duodenal lobes and unpaired splenic and gastric lobes. The pancreatic capsule appeared thin with no distinct lobulation pattern. Three islet types were observed namely alpha, beta, and mixed types. The alpha-type islets formed mainly of glucagon- and somatostatin-expressing cells. The beta-type islets appeared relatively smaller than the two other islet types and formed predominately of insulin-expressing cells with a limited number of other endocrine cells. The mixed islets were formed by almost equal proportions of insulin-, glucagon- and somatostatin-expressing cells. A higher number of alpha-type islets was observed in the splenic lobe than in other pancreatic lobes. Unlike other pancreatic endocrine cells which appeared oval or triangular in shape, somatostatin-expressing cells appeared with irregular outlines with cytoplasmic processes contacting each other's forming a meshwork within the islet. The results of this study revealed species-specific features of the endocrine and exocrine pancreas in the Eurasian moorhen and could suggest pancreatic functional differences depending on feeding habits.

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Introduction

The pancreas is a glandular organ associated with the gastrointestinal tract with both digestive (exocrine) and hormone-secreting (endocrine) functions. The avian pancreas consists of 2-3 duodenal lobes and a single splenic lobe, all located mainly inside the U-shaped coil formed by the duodenum in these species (1). The exocrine pancreas is formed by pancreatic acini and ducts. The latter structures are presented in a progressive order and act mainly to drain the secretion of the pancreatic acini into the duodenum. The endocrine pancreas is made up of clusters of endocrine cells termed the pancreatic islets (of Langerhans). The latter structures are considered as micro-organs and produce several hormones including insulin (beta cells), glucagon (alpha cells), somatostatin (delta cells) and pancreatic polypeptide, pancreatic polypeptide (PP cells) (2).

In contrast to the mammalian pancreas which contains only a mixed type of islets, the avian pancreas contains more than one islet type. The three main types of islets reported in the avian species are alpha islets (formed predominately of alpha cells), beta islets (formed predominately of beta cells), and mixed type islets (contains equal proportions of alpha and beta cells) (3). Different types of pancreatic islets present in the avian pancreas act cooperatively to orchestrate the blood glucose levels, a major source of bird energy. Failure of the endocrine pancreas to maintain sufficient levels of pancreatic hormones in blood is associated with incidence of diabetes mellitus. Indeed, diabetes mellitus with an absence of insulin expression has been reported in pet birds (4, 5). The avian pancreas has been reported to be a target for pathogenic viruses, including avian affluenza and Newcastle disease viruses, that commonly infect birds (6, 7).

The Eurasian moorhen (*Gallinula chloropus* - Linnaeus, 1758), also called the marsh hen or the black gallinule, is a principal member of the family Rallidae that has been shown to withstand harsh environmental conditions. It is the most popular member of the family Rallidae around the world (8). Moorhens are distributed across several areas of the world including the Middle East, South and Central Asia, Europe, and North and South America (9, 10). Their distribution is regularly affected by seasonal changes as they usually migrate during the winter towards the southern parts of their range (11). The meat of moorhens is also famous for its great taste and is often consumed by people in their vicinity.

Understanding the functional anatomy of the pancreas is essential for interpreting interspecies-specific differences in diet and lifestyle. In the present study, the anatomy, microstructure, and distribution of different types of pancreatic islets in various regions of the moorhen pancreas were investigated for the first time. Furthermore, the relative proportions of hormone-expressing cells were compared among the pancreatic islets, the exocrine pancreas, and the pancreatic ducts.

Materials and methods

Animals and sampling

Ten adult black gallinules (*Gallinula chloropus*) from both sexes collected from vegetated areas near the Nile Delta of Dakahliya Governorate (Egypt) were obtained from a local distributor. The birds were transported in suitable cages to the Anatomy Laboratory at the Faculty of Veterinary Medicine at Mansoura University. The animal study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine at Mansoura University (Code no. R.41), agreed with the ARRIVE guidelines, and was done in accordance with the National Institutes of Health guide for use of animals in research (NIH Publications No. 8023, revised 1978). All birds were euthanized by cervical disarticulation followed immediately by complete blood exsanguination. Seven birds were utilized for identifying different pancreatic lobes and characterizing the histological and histochemical features of the pancreas. The whole pancreas of the three remaining birds was extracted and different lobes were processed for serial sectioning and subsequent screening of islet composition and distribution within the whole organ using immunohistochemistry (12). Pancreases were collected as soon as the birds became completely immobile, trimmed from surrounding tissues, and fixed in 10% neutral buffered formalin. Following their fixation, pancreatic tissue samples were dehydrated, cleared, and embedded in paraffin.

Histological and histochemical analyses

Paraffin sections of 4-µm thickness were cut and allowed to dry at 56 °C for 1 hour. After that, the sections were dewaxed, rehydrated, and the visualizations of the exocrine and endocrine components of the pancreas were enhanced using the following stains: hematoxylin and eosin (H&E), Masson's trichrome, alcian blue-PAS (AB-PAS), and AB-aldehyde fuchsin following the protocols adopted by Suvarna et al. 2018 (13). AB-aldehyde fuchsin staining was employed to better differentiate the pancreatic islets from the surrounding exocrine tissue (14).

Immunohistochemistry

Following their dewaxing and rehydration, tissue sections selected for the immunohistochemical procedure were prepared as previously described (15, 16). Briefly, antigenic epitopes were retrieved via boiling the sections in citrate buffer (pH = 6) using a microwave at 750 watts for 20 minutes. To minimize nonspecific binding of antibodies, tissue sections were incubated with 5% bovine serum albumin (BSA) for 1 hour before their incubation with the primary antibodies. The optimal working dilution for each primary antibody, that achieved strong positive signals with minimal background, was determined and used. Incubation of sections with primary antibodies was done for 3 hours at room temperature. Details of primary antibodies used in

Table 1: Primary antibodies used in the present study

Target protein	Clone	Host	Code #	Company	Dilution
Insulin	Monoclonal anti-Insulin (EPR17359)	Rabbit	ab181547	Abcam (Cambridge, MA, USA)	1:100.000
Glucagon	Monoclonal anti-Glucagon (ICACLS)	Mouse	14-9743-80	Invitrogen (Waltham, MA, USA)	1:500
Somatostatin	Monoclonal anti-Somatostatin (ICACLS)	Mouse	14-9751-80	Invitrogen (Waltham, MA, USA)	1:200
Pancreatic Polypeptide	Polyclonal anti-Pancreatic polypeptide (1973)	Goat	NB100-1793	Novus Biologicals, (Littleton, CO, USA)	1:200

the present study are shown in Table 1. For negative control sections, primary antibodies were omitted and replaced with the blocking buffer. Next, all sections were washed thoroughly in PBS containing 0.05% Tween-20 (PBST) and further incubated with corresponding biotinylated secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA) for 30 minutes.

The VECTASTAIN Elite ABC-Horseradish Peroxidase (HRP) kit (PK-6100, Vector Laboratories, Burlingame, CA, USA) was applied for an additional 30 minutes to allow binding to the secondary antibody. The latter step was preceded by covering the sections with 0.3% H2O2/PBS for 20 minutes to quench the endogenous peroxidase activity. Bound antibodies were visualized by incubating the stained sections with diaminobenzidine solution (SK-4103, Vector Laboratories) for 2-10 minutes. All sections were finally counterstained with hematoxylin, dehydrated, and cleared (17, 18).

Photomicrography, morphometry, and cell quantification

All stained sections were analyzed and photographed using a Leica DM3000 light microscope. For the histological and histochemical analyses, three pancreatic sections 500 μm apart were examined per bird. For the immunohistochemical study, 12 sections 100 μm apart spanning the whole pancreas were evaluated. Islet diameter was estimated using the measure tool of the ImageJ2 software (19). The number of cells expressing insulin, glucagon, somatostatin, and pancreatic polypeptide per every 100 cells was quantified within and outside (acinar and ductal epithelium) the pancreatic islets as described previously (15).

Statistical analysis

Data were analyzed using the GraphPad Prism 7 (GraphPad Software, CA, USA). Differences in islet diameter and distribution of each type of the hormone-expressing cells were determined using one-way ANOVA followed by Tukey`s multiple comparison test. P-values ≤0.05 were considered significant.

Results

Grossly, the pancreas of the Eurasian moorhen appeared to consist of three duodenal lobes, two ventral and one dorsal, a single gastric lobe, and a single splenic lobe (Figure 1). The nomenclature of these lobes is based on their relation to the duodenal loop, the gizzard, and the spleen respectively (Figure 1A-C). The dorsal duodenal lobe is the largest of all lobes. It was located dorsal and parallel to the left ventral duodenal lobe and continued cranially with the gastric and splenic lobes (Figure 1B,C,D).

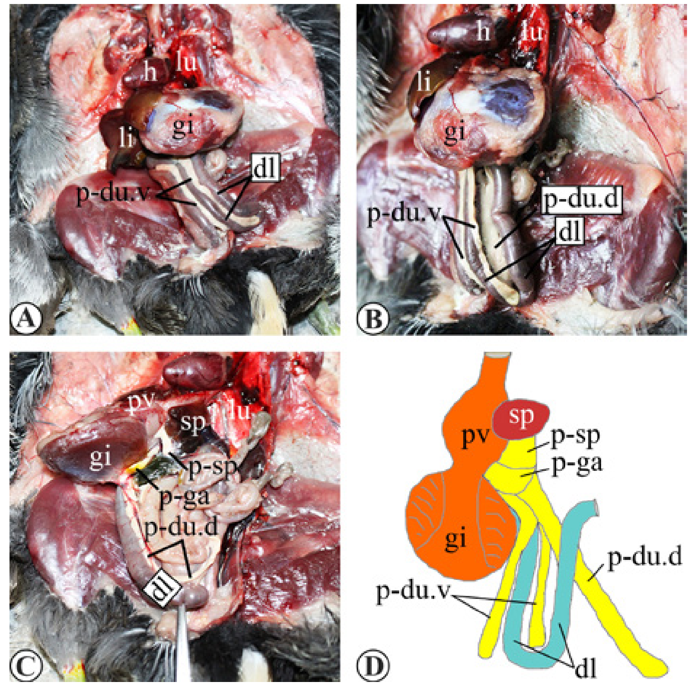

Figure 1: Gross anatomic appearance of the pancreas in the Eurasian moorhen. Photographs A-C) and schematic representation D) of ventral views of the celomic cavity showing different parts of the pancreas and their relations to surrounding organs. The paired ventral duodenal lobes (p-du.v) are displaced to show the single dorsal duodenal lobe (p-du.d) in B. The gizzard (gi) is also right-shifted to reveal the gastric (p-ga) and splenic (p-sp) parts of the pancreas. Abbreviations: dl, duodenal loop; h, heart; li, liver; lu, lungs; pv, proventriculus; sp, spleen

Microscopically, in both H&E- and AB-aldehyde fuchsin-stained sections, the pancreatic islets appeared as lightly stained cellular clusters permeated by a large number of blood vessels and surrounded by the acini of the exocrine pancreas (Figure 2A,B,F). Lymphoid aggregations were occasionally seen in the vicinity of a number of islets (Figure 2B). Large-sized pancreatic islets (> 200 μm in diameter) were seen within the dorsal duodenal and the splenic lobes (Figure 2E). The pancreas was invested by a thin connective tissue capsule through which pancreatic blood vessels find their way to the pancreatic parenchyma (Figure 2C-E). The lobulation of the pancreas appeared indistinct and the parenchyma of each lobe formed a single entity. The pancreatic ducts appeared of variable sizes and secretory activities based on their position within the pancreatic ductal tree. The wall of the pancreatic ducts appeared thicker, and their lumen became irregular towards the ductal exit from the pancreas (Figure 2F-I). The pancreatic secretion is collected by two main pancreatic ducts that were situated near the midparts of the three duodenal lobes (Figure 2G). These secretions showed moderate reactions for alcian blue-PAS staining (Figure 2I).

Analysis of insulin-expressing cells within the Eurasian moorhen pancreas revealed the presence of three islet types: alpha, beta, and mixed (Figure 3 A-L). The alpha-type pancreatic islets lacked insulin expression, though few cells could be observed in a number of serially sectioned islets (Figure 3 A). These islets consisted mainly of glucagon-,

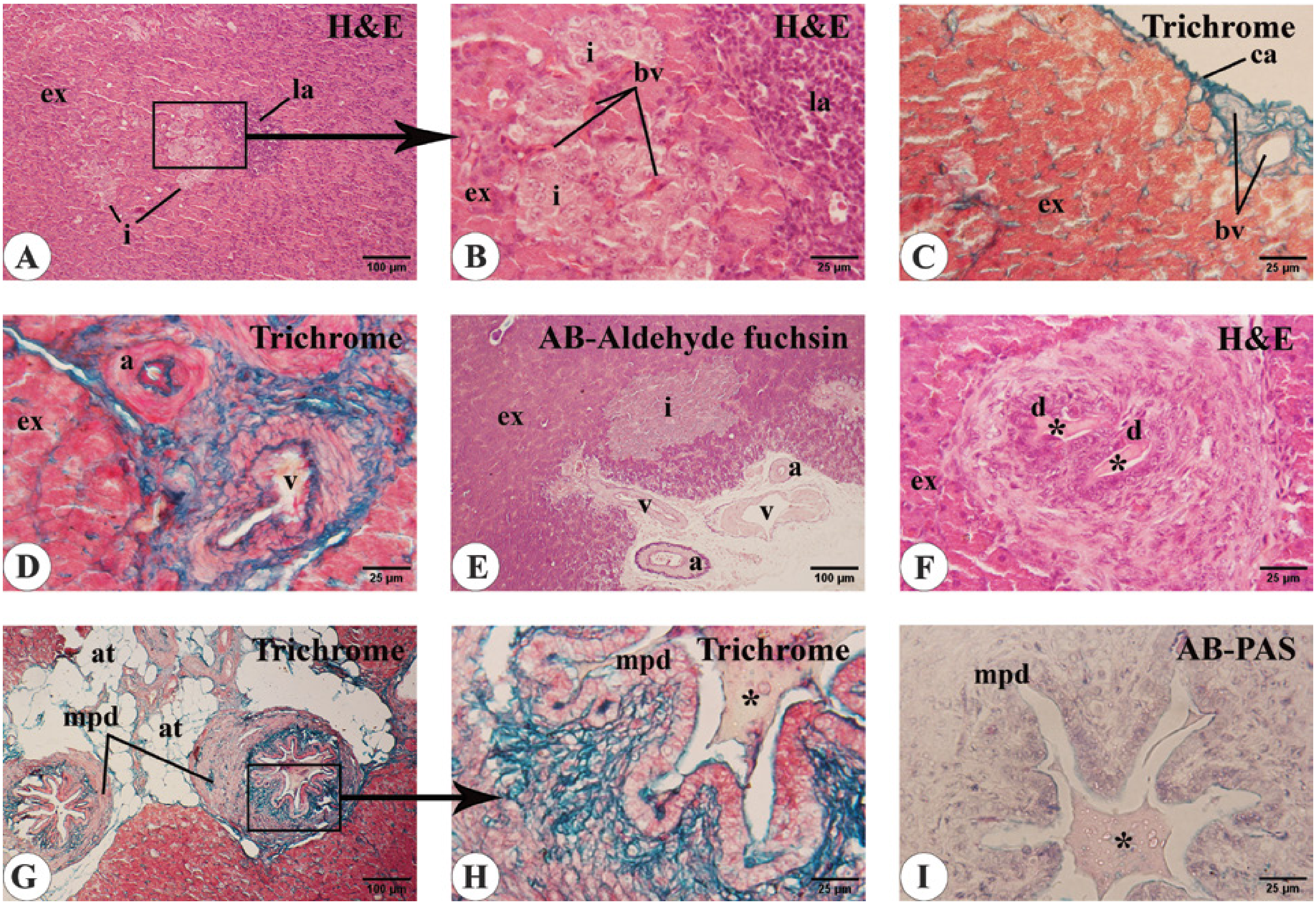

Figure 2: Microscopic architecture of the pancreas in the Eurasian moorhen. Pancreatic islets (i) appeared as lightly stained cellular clusters surrounded by the acini of the exocrine pancreas ("ex" in A,B,E,F). The connective tissue fibers are distributed mainly within the capsule (C) and the wall of the blood vessels (D) and large pancreatic ducts (G,H). Note the secretion within the pancreatic ducts ("*" in F, G-I). Abbreviations: a, artery; at, adipose tissue; bv, blood vessel; ca, capsule; d, duct; la, lymphoid aggregation; mpd, main pancreatic duct; v, vein

somatostatin-, and PP-immunoreactive cells of decreasing proportions (Figure 3 B-D, Table 2). The mixed-type islets contained almost equal proportions of the insulin- and glucagon-expressing cells in which the former type of cells tended to have a centric position within the islet (Figure 3 E,F). The beta-type pancreatic islets appeared of smaller diameter (38 ± 2.9 µm) compared to the alpha- (183 ± 55.4 µm, $P < 0.001$) and mixed-type (72 ± 15.6 µm, $P < 0.05$) islets and formed predominately of insulin-expressing cells (Figure 3I-L). Although the majority of insulin-expressing cells were found within the islets especially those of beta and mixed types (Figure 4 A-C), a fraction of these cells was also detected within or near the wall of pancreatic ducts especially those having small size (Figure 4 D, E). The specificity of these immunoreactions was confirmed by their absence in pancreatic sections incubated with no primary antibody (Figure 4 F).

Regarding the glucagon-expressing cells within the Eurasian moorhen pancreas, they revealed widespread expression within the pancreatic islets (Figure 5 A, B), the exocrine pancreatic acini (Figure 5C), and the pancreatic ducts (Figure 5 D, E). Their numbers markedly exceeded those of insulin-expressing cells, especially those located within the exocrine pancreas and pancreatic ducts (Table 2). They revealed the highest abundance within the alpha-type islets especially those of the dorsal duodenal and splenic lobes (Figure 5B). The extra insular glucagon-expressing cells appeared with regular outlines without any cytoplasmic branching (Figure 5 D).

The somatostatin-expressing cells revealed a branched morphology especially in the alpha-type pancreatic islets (Figures 3C, 6 A-D). The cytoplasmic processes of these cells formed an interconnected network surrounding the other types of islet cells (Figure 6 C, D). The frequency of these cells was observed to be proportionally related to that of the glucagon-expressing cells within the islet (Figure 6 A, B).

The pancreatic polypeptide-expressing cells were found mainly toward the periphery of alpha- and mixed-type islets (Figure 3 D, H). They revealed oval or triangular shapes and their frequency appeared higher within the small (< 50 um in diameter) and medium-sized (> 50 to 100 um in diameter) islets compared to the large-sized (> 100 um in

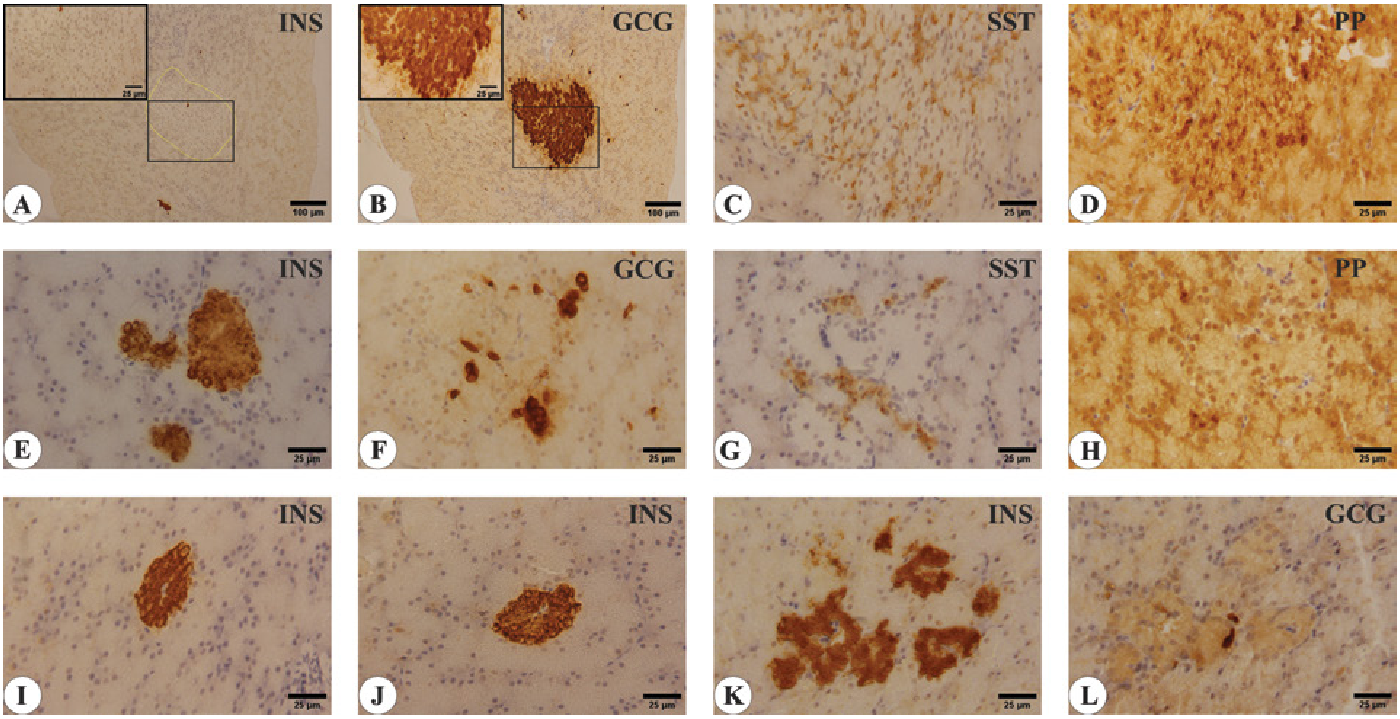

Figure 3: Representative photomicrographs for the distribution of hormone-expressing cells within the three different types of pancreatic islets of the Eurasian moorhen pancreas. A-D) Alpha islets. E-H) Mixed islets. I-L) Beta islets. The islet perimeter is indicated by a dotted yellow line in Figure 3A. INS, insulin; GCG, glucagon; SST, somatostatin; PP, pancreatic polypeptide

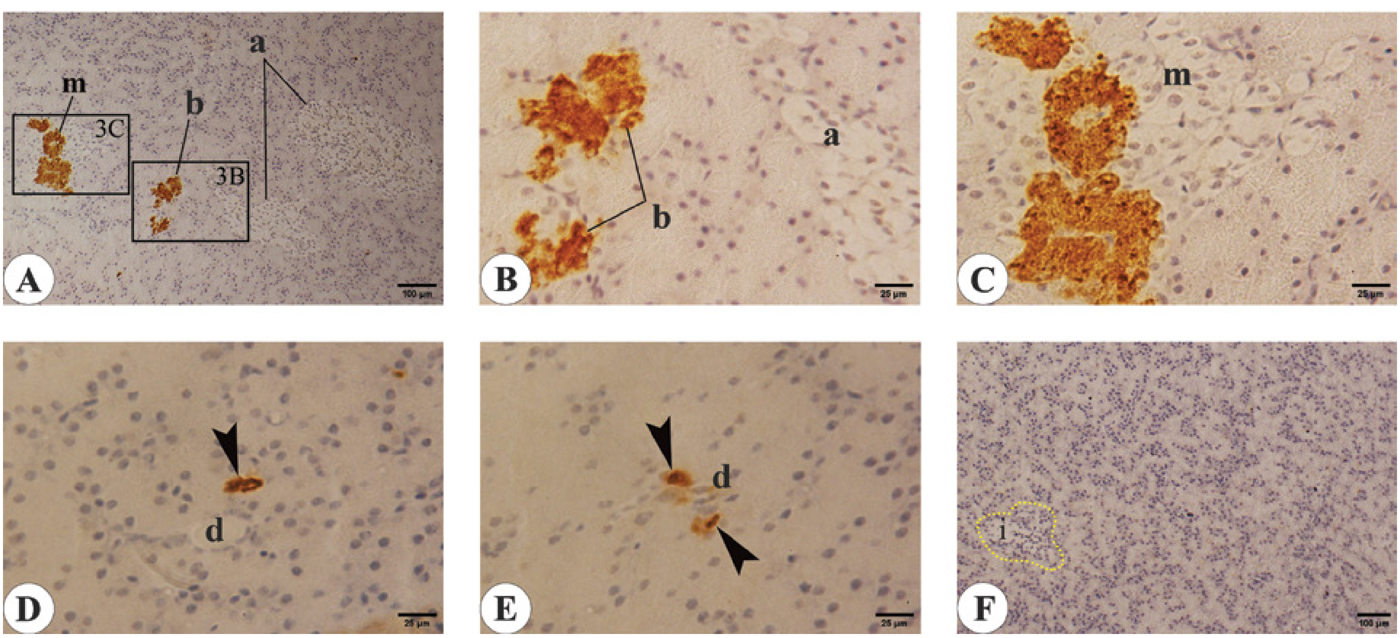

Figure 4: Representative photomicrographs for insulin expression in the dorsal duodenal lobe of the Eurasian moorhen pancreas. A-C) Insulin-expressing cells were observed within beta (b) and mixed (m) type islets but were absent within the alpha-type islets (a). D,E) Insulin-expressing cells (arrowheads) were occasionally seen close to the wall of small pancreatic ducts (d). F) Negative control. The islet (i) perimeter is indicated by a dotted yellow line in Figure 4F

diameter) ones (Figure 7 A-C). A number of these cells was also seen within or near the lining epithelium of pancreatic ducts (Figure 7 D, E). The specificity of such immunoreactions was verified by their absence in stained pancreatic sections in which the primary antibody was omitted (Figure 7F).

A schematic representation for the distribution of various types of islets in different lobes of the Eurasian moorhen pancreas is shown in Figure 8. Based on data from complete analyses of pancreases from three adult moorhens, the splenic and gastric lobes contained a higher number of alpha-type islets and fewer beta- and mixed-type islets than the paired ventral and single dorsal duodenal lobes.

Table 2: Distribution of endocrine cells in the Eurasian moorhen pancreas

Part	Pancreatic islet type			Exocrine pancreas	Ductal epithelium
Hormone	**Alpha**	**Beta**	**Mixed**		
Insulin	+/- (1.9±0.3^{Cc})	++++ (98.7±9^{Aa})	+++ (45.2±5.6^{Ab})	+/- (0.5±0.01^{Bd})	+/- (0.3±0.07^{Cd})
Glucagon	+++ (74.7±9.6^{Aa})	+/- (0.5±0.0^{Be})	+++ (46±13^{Ab})	+ (8.6±0.55^{Ac})	+ (5.2±0.60^{Ad})
Somatostatin	++ (21±5.0^{Ba})	+/- (0.8±0.1^{Bc})	+ (7.0±1.4^{Bb})	+/- (0.1±0.00^{Cd})	+/- (0.2±0.05^{Cd})
Pancreatic polypeptide	+ (2.4±0.7^{Ca})	- (0.0±0.0^{Cc})	+/- (1.8±0.9^{Ca})	+/- (0.2±0.00^{Cb})	+ (2.3±0.55^{Ba})

The number of hormone-expressing cells per each 100 cells was used to generate the following scheme: +/-, < 2 cells; +, 2-10; ++, 10.1-45; +++, 45.1-80; ++++, > 80. Different superscript letters (uppercases for columns; lowercases for rows) indicate statistical significance. $P < 0.05$.

Discussion

The composition of the endocrine pancreas is influenced by several physiological and pathological conditions that include fasting, and pancreatectomy. The pancreatic response to damaging insults varied according to the dietary regime. For instance, complete removal of the endocrine pancreas resulted in lethal hypoglycemia in granivorous birds, but it caused severe hyperglycemia and diabetes mellitus in carnivorous birds (20).

Differences in islet cell composition among different vertebrate species may also represent a further endocrine pancreatic adaptation. Unlike pancreatic islets of raptors which were dominated by insulin-expressing cells, the cellular composition of the three types of islets observed in the moorhen pancreas revealed a higher abundance of glucagon-expressing cells than any other type of endocrine cells which is the case for a number of granivorous birds including chickens and ducks (21). Another similarity between the pancreas of moorhen and that of chicken is the presence of a large number of glucagon-expressing cells in the splenic lobe of the moorhen`s pancreas. The presence of this large number of glucagon-secreting cells close to the spleen which is known for richness in blood possibly accelerates the delivery of the secreted glucagon to the systemic circulation. Tomita et al. noted that alpha-type islets constituted more than 75% of all islets present in the splenic lobe of chicken pancreas (22). Such enrichment of chicken pancreas with glucagon-expressing cells is usually reflected by their extremely high blood glucagon levels, ~ 10-fold more than those of mammals (20). Differences in body metabolic requirements between birds of prey and other herbivorous or omnivorous birds account for such

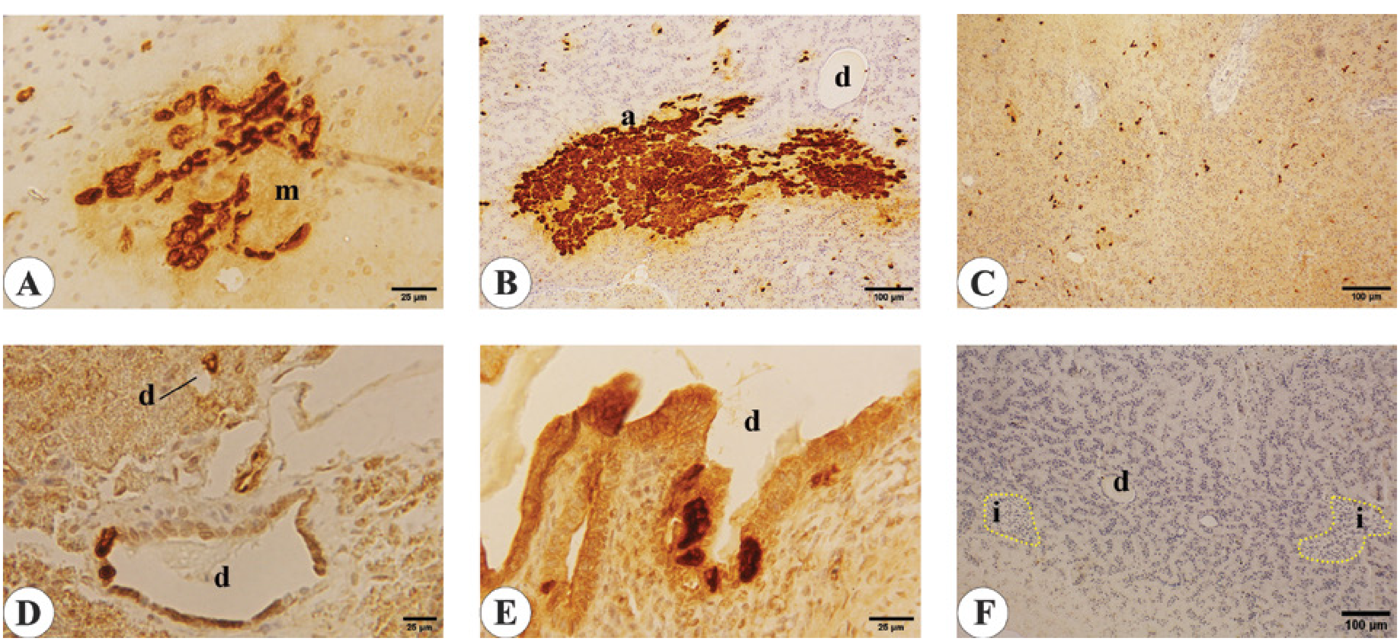

Figure 5: Representative photomicrographs for glucagon expression in the ventral duodenal (A, C-E) and splenic (B) lobes of the Eurasian moorhen pancreas. Glucagon-expressing cells were observed within mixed type islets (A), alpha type islets (B), exocrine pancreas (C), and pancreatic ductal epithelium (D,E) but were absent within the negative control section (F). The islet (i) perimeter is indicated by a dotted yellow line in Figure 5F. Abbreviations: a, alpha type islet; d, duct; m, mixed type islet

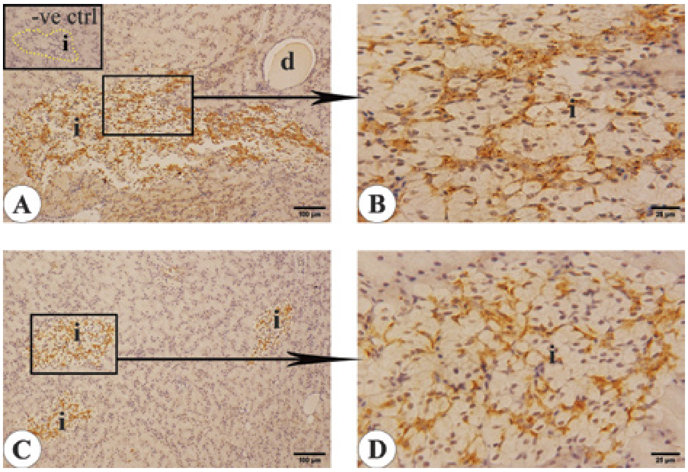

Figure 6: Representative photomicrographs for somatostatin expression in the splenic (A, B) and dorsal duodenal (C, D) lobes of the Eurasian moorhen pancreas. Somatostatin-expressing cells appeared with irregular outlines with interconnected cytoplasmic processes. The islet (i) perimeter is indicated by a dotted yellow line in the negative control (-ve ctrl) inset of Figure 6A. Abbreviations: d, duct; i, pancreatic islet

variation. Avian insulin has a strong anabolic effect; thus, it is required for building the body muscles required for hunting and grasping the prey. Conversely, glucagon is a potent catabolic hormone, and it is high levels in the herbivorous or omnivorous birds work to prevent hyperglycemic episodes via maintaining steady levels of blood glucose (23).

The pancreatic polypeptide-expressing cells appeared as the lowest abundant islet cell type within the Eurasian moorhen`s pancreas. The functional roles and biological relevance of the pancreatic polypeptide-expressing cells in the avian pancreas remain poorly understood (24). The islet numbers of these cells were higher within the duodenal lobes of the pancreas than in the splenic lobes. This corresponds to their distribution pattern in the pancreas of other vertebrates including humans (25) and chickens (22).

Somatostatin-expressing cells or delta cells produce somatostatin hormone which suppresses the secretion of both glucagon and insulin from alpha and beta cells respectively (26). These cells continuously work to balance both the anabolic and catabolic activities of birds (27). Recently, reciprocal feedback from alpha and beta cells has been suggested (28). The juxtaposition of delta cells to other types of islet cells is fundamental for the proper interplay between these cells in terms of hormonal control. In accordance with a such important role, somatostatin-expressing cells were observed in all types of islets of moorhen`s pancreas with a higher presence in the alpha-type islets by the present study and others (21). In contrary to islets of humans and rodents in which somatostatin-expressing cells appear with regular outlines (29), the somatostatin-expressing cells appeared branched in moorhen`s pancreas suggesting a role for cell-cell contact alongside the classical paracrine mode of signaling in the studied avian species (30).

Despite the small portion of the pancreas occupied by pancreatic ducts, their contribution to the proper functioning of both exocrine and endocrine parts of the pancreas is substantial. Pancreatic ducts convey the enzymatic secretion of the exocrine pancreas towards the duodenum. Also, their lining epithelium produces a large amount of bicarbonate for neutralizing the acidity of pancreatic juice and stomach brought chyme. In addition to this, the ductal epithelium has been suggested as a potential source for endocrine

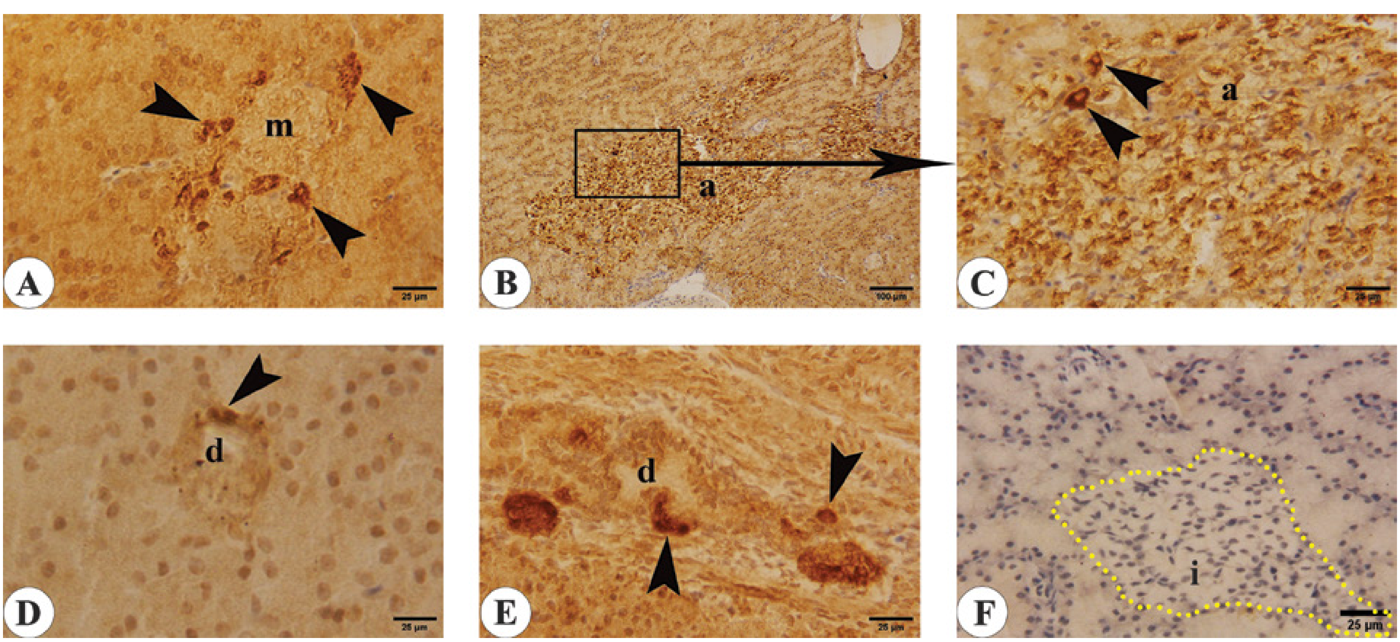

Figure 7: Representative photomicrographs for pancreatic polypeptide expression in the splenic lobe of the Eurasian moorhen pancreas. Pancreatic polypeptide-expressing cells (arrowheads) were observed within mixed type islets (A), alpha type islets (B,C), and pancreatic ductal epithelium (D,E) but were absent within the negative control section (F). The islet (i) perimeter is indicated by a dotted yellow line in Figure 7F. Abbreviations: a, alpha type islet; d, duct; m, mixed type islet

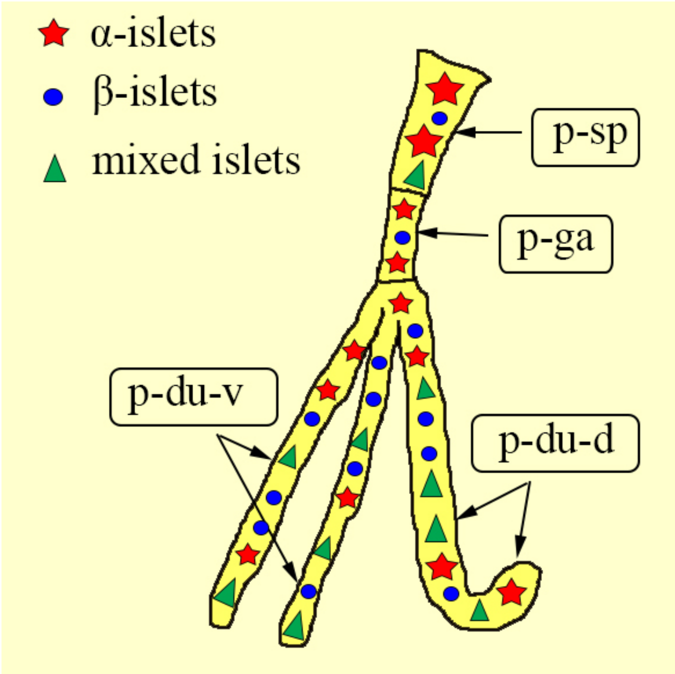

Figure 8: Schematic representation for the distribution of various types of islets in different lobes of the pancreas in the Eurasian moorhen. Abbreviations: p-du.d, dorsal duodenal lobe; p-du.v, ventral duodenal lobes; p-ga, gastric lobe; p-sp, splenic lobe

cells during development and regeneration (31). This role is further highlighted by the lack of a definite stem cell niche within the pancreas (32). The presence of hormone-expressing cells among and close to the ductal epithelium of the moorhen pancreas confirms the role of the pancreatic ducts in the neogenesis of endocrine cells and suggests an evolutionarily conserved role for these structures.

Taken together, the results of the present study provided a complete picture of the macroscopic and microscopic anatomy of the pancreas in the Eurasian moorhen. Although the gross anatomy of the Eurasian moorhen pancreas revealed several shared similarities with other domestic birds, the immunohistochemical analysis of the expression of insulin, glucagon, somatostatin, and pancreatic polypeptide revealed a species-specific expression pattern that suggested unique endocrine signaling pathways. The large extent and the high glucagonic nature of the moorhen pancreas could represent an adaptation to its omnivorous feeding behavior via sustained and controlled release of digestive enzymes and glucose-sensing hormones. The developmental mechanisms governing the arrangements of these hormone-expressing cells into three different types of definitive islets warrant further investigations and will give insights into the adaptation of the vertebrate pancreas to various metabolic and ecological conditions.

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Izražanje insulina, glukagona, somatostatina in pankreatičnega polipeptida v trebušni slinavki evrazijskega zelenonoge tukalice (*Gallinula chloropus*)

A. M. Abdellatif

Izvleček: Trebušna slinavka je kompleksna žleza z endokrino in eksokrino funkcijo. V tej študiji smo preučevali splošno anatomijo, histokemične značilnosti in imunohistokemično izražanje inzulina, glukagona, somatostatina in pankreasnega polipeptida v trebušni slinavki evrazijske zelenonoge tukalice. Makroskopsko je bila trebušna slinavka sestavljena iz treh dvanajstnikovih režnjev ter neparnih vraničnega in želodčnega režnja. Kapsula trebušne slinavke je bila tanka, brez izrazitega vzorca lobulacije. Opazni so bili trije tipi otočkov - alfa, beta in mešani. Otočki tipa alfa so bili sestavljeni predvsem iz celic, ki so izražale glukagon in somatostatin. Otočki tipa beta so bili relativno manjši od drugih dveh tipov otočkov in sestavljeni pretežno iz celic, ki so izražale inzulin ter omejenega števila drugih endokrinih celic. Mešani tipi otočkov so bili sestavljeni iz enakega deleža celic, ki izražajo inzulin, glukagon in somatostatin. V vraničnem režnju je bilo opazno več otočkov tipa alfa kot v drugih režnjih trebušne slinavke. V primerjavi z drugimi endokrinimi celicami trebušne slinavke, ki so bile ovalne ali trikotne oblike, so bile celice, ki izražajo somatostatin, nepravilnih obrisov s citoplazemskimi izrastki, ki so se stikali med seboj in tvorili mrežo znotraj otočka. Rezultati te študije so razkrili vrstno specifične značilnosti endokrine in eksokrine trebušne slinavke pri evrazijski zelenonogi tukalici in bi lahko nakazovali funkcionalne razlike v delovanju trebušne slinavke glede na prehranjevalne navade.

Ključne besede: trebušna slinavka; otoček; inzulin; glukagon; somatostatin; polipeptid; sladkorna bolezen

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Original Research Article

Identification of Best Growth Curve Model for Anatolian Black Cattle

Key words

Anatolian Black cattle;
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Abstract: The aim of this study was to identify the model that best describes the growth trajectory from birth to 24 months of age in Anatolian Black Cattle (ABC) raised for conservation purposes. A total of 493 weight records of 113 animals at birth, 3, 6, 12, 18, and 24 months were collected. Six different non-linear models were used to describe the growth curve of animals: 2nd degree polynomial, 3rd degreee polynomial, Logistic, Brody, Von Bertalanffy, and Gompertz models. In the study, R^2 values of the models were: 0.997, 0.999, 0.953, 0.979, 0.924, and 0.862; corel values (correlation between the observed and estimated curves) were 0.994, 0.998, 0.989, 0.993, 0.961, and 0.703; Residual Standard Deviations (RSD) were 3.216, 1.388, 11.533, 3.561, 14.736, and 27.141, respectively. Given these values, it was found that the 3rd degree polynomial model was the best to describe the growth curve of ABC. As a result of the analyses, it was noticed that the values predicted by this model deviated by 1-3 kg from the observed values in all periods and in all environmental factors examined (sex, dam age, parity, birth year and birth, season). It was found that these differences increased up to 4-5 kg only in the 18-month period. The results also showed that ABC continued to grow after 24 months of age. As a result, traits such as age at sexual maturity, breeding age, and slaughter age can be easily predicted by identifying the model that best describes growth and development in herds.

Introduction

Cattle breeding has an important place in Turkey's animal husbandry; while there are around 18 million cattle in Turkey, approximately 8% of this is local breeds (1). Domestic cattle breeding is important in terms of rural employment and development, rural sociology and the use of poor pasture areas. Anatolian Black Cattle (ABC) is one of the breeds that is most widely grown and spread among the domestic breeds in Turkey. This breed is grown especially in the Central Anatolian Region of Turkey and is mostly grown for meat and milk yield by breeders living in rural areas. It has adapted to these conditions since it has grown in unfavourable conditions in this region for many years. They have gained resistance to harsh winters, drought, hunger, thirst, and diseases (2, 3). Growth and development values in Turkey's domestic cattle breeds are generally slower than those of developed cattle breeds. In studies conducted with the ABC breed in Turkey, live weights at birth, 3, 6 and 12 months of age were found to be 14.85 kg, 49.37 kg, 81.22 kg, and 97.29 kg, respectively (2). In other studies with the same breed, live weights from birth to 12 months of age were the following: 16.97- 21.35 kg, 63.21 - 68.18 kg, 101.04 - 110.33 kg, and 152.16 kg- 184.57 kg, respectively (3, 4, 5).

Growth in cattle is a function that continues throughout the life of the animal, from embryonic stages to adulthood, and can be explained mathematically by growth curve models (6). The change in any of the examined features over a certain period is defined as the growth curve (7). The growth curve shows the statistical relationship between the weight and time or age of animals, which is shaped under the influence of genetic potential and environmental factors (8). The growth of living organisms does not progress at a

Figure 1a: Anatolian Black cows and calves

Figure 1b: Animal weighing

constant rate throughout their lives (9). In the case of constant growth, linear models are used, and when the growth rate occurs at different times depending on age, nonlinear models (such as Negative Exponential, Brody, Logistics, Gompertz, Bertalanffy, Richards, and Weibull) are used (6, 10). The fact that living things have different growth rates in some periods necessitated the use of nonlinear models, which were more comprehensive models (8). There is still a need to investigate whether the most commonly preferred non-linear models are sensitive to the length of the growth period prior to truncation of the data (11).

The aim of this study was to evaluate non-linear models of the growth curve in ABC cattle taken at individual weights from birth to 24 months and to determine the model that best explains growth. For this purpose, 2nd degree polynomial, 3rd degree polynomial, Logistic, Brody, Von Bertalanffy, and Gompertz models were analysed.

Materials and methods

Animals

The animal material of this study consisted of Anatolian Black Cattle (ABC) grown in the "International Center for Livestock Research and Training" (39°97' N, 33°10' E; elevation 826 m) located in Ankara. This breed has been conserved within the scope of the project "Conservation of Domestic Genetic Resources and Sustainable Use" conducted by the General Directorate of Agriculture Research and Policies. The study was carried out on a total of 113 heads of ABC born between 2015 and 2020.

ABC calves were raised with their dams from birth, and they were allowed to suckle their dams freely. The cows were not milked on the farm. Feeding of ABC bred cows was two meals a day, morning and evening, ad libitum in the form of total mixed feed. ABC cows were given 80% barley bales and 20% dry meadow grass as roughage.

Table 1: Descriptive statistics of body weight at different ages in Anatolian Black Cattle

Statistics	BW	3MW	6MW	12MW	18MW	24MW
N	113	93	96	98	35	58
Minimum (kg)	13.00	37.00	52.50	89.00	144.00	178.00
Maximum (kg)	30.00	99.00	153.00	283.00	332.00	444.00
Female (kg)	17.25	61.70	90.42	144.30	188.65	225.34
Male (kg)	19.55	67.66	103.08	162.75	245.50	303.90
Mean (kg)	18.57	65.10	98.33	155.60	217.89	264.64
Standard Error	0.312	1.340	2.120	3.830	7.970	7.910
Coefficient of Variation (CV%)	17.86	19.88	21.17	24.40	21.64	22.78

Notes: BW=birth weight, 3MW=3 month weight, 6MW=6 month weight, 12MW=12 month weight, 18MW=18 month weight, 24MW=24 month weight.

Table 2: Non-linear models used to describe the growth of Anatolian Black Cattle

Model	Equation	Reference
2nd Degree Pol.	$y_t=\beta_0 + \beta_1 t + \beta_2 t^2$	12
3rd Degree Pol.	$y_t=\beta_0 + \beta_1 t + \beta_2 t^2 + \beta_3 t^3$	12
Logistic	$y_t=A\ (1 + be^{-kt})^{-1}$	13
Brody	$y_t=A\ (1 - be^{-kt})$	14
Von Bertalanffy	$y_t=A\ (1 - be^{-kt})^3$	15
Gompertz	$y_t=A\ \exp(-be^{-kt})$	16

yt = observed BW at age t (kg); β_0, β_1, β_2, β_3: regression coefficients of 2nd and 3rd degree polynomial models; A: the asymptotic limit of the BW when age t approaches infinity (kg); b: the integration constant, related to the initial weights of the animal and without a well-defined biological interpretation; k: ratio of the relative intensity of growth (maturation rate); t: time (month).

Figure 1a shows Anatolian Black cows and calves, while Figure 1b shows the weighing of an animal.

Data set

In this study, birth weight, 3, 6, 12, 18, and 24 month live weights of 113 calves born between 2015 and 2020 were used. These values were determined by weighing them with precision scales up to 200 g. The characteristics of the data are presented in Table 1. In addition, information on sex, dam age, parity, birth year, and month was also recorded.

Predicting the growth curve

In the study, six different non-linear models were used in the estimation of growth curves, and these models are presented in Table 2.

In the study, R^2 (coefficient of determination), RSD (Residual Standard Deviation), and corel (correlation) between the observed and estimated growth curves were used to compare the models.

Statistical analysis

Statistical analyses were carried out using the Proc Nlin in SAS (17). Growth curve models were fitted for each animal separately, and then the best-fitted model parameters and the other phenotypic data were analyzed using the Proc Glm in SAS (17). For analysis, sex (female, male), age of dam (2-3, 4-7, 8-10, 11+), parity (1, ...7), birth year (2015, ... 2020), and birth season (winter, spring, summer, autumn) were included in the model, and these were taken as fixed effects in the GLM analyses. To determine the differences between groups, the Tukey test was used.

Results and discussion

The growth curve parameters as derived from 6 different models using 493 weight records for ABC are presented in Table 3. The 3rd degree polynomial model showed the highest R^2 and corel and lowest RSD, indicating the best goodness of fit. On the other hand, the Gompertz model was the least fitted to estimate the ABC weight based on its lowest value of R^2. That is, the 3rd degree polynomial model with four parameters (β_0, β_1, β_2, β_3), which has the highest R^2 and smallest RSD value, best explains the change in live weight

Table 3: Model comparison for growth of Anatolian Black Cattle

Model	β_0	β_1	β_2	β_3	R^2	RSD	corel
2nd degree polynomial	21.80±0.619	0.44±0.0132	0.01±0.001	-	0.997	3.216	0.994
3rd degree polynomial	18.72±0.361	0.33±0.099	0.01±0.002	0.01±0.001	0.999	1.388	0.998
	A	**b**	**k**	t_i			
Logistic	206.09±8.417	-	-1.79±1.788	8.84±8.839	0.953	11.533	0.989
Brody	225.56±15.625	0.83±0.027	0.01±0.001	-	0.979	3.561	0.993
Von Bertalanffy	169.96±15.650	-0.19±0.124	0.01±0.005	-	0.924	14.736	0.961
Gompertz	129.05±4.530	2.751±0.079	0.40±0.000	-	0.862	27.141	0.703

β_0, β_1, β_2, β_3: regression coefficients of 2nd and 3rd degree polynomial models; A: the asymptotic limit of the BW when age t approaches infinity (kg); b: the Integration constant, related to the initial weights of the animal and without a well-defined biological interpretation; k: ratio of the relative intensity of growth (maturation rate); t: time (month).

R^2: coefficient of determination; RSD: Residual Standard Deviation; corel: correlation between observed and estimated growth curves

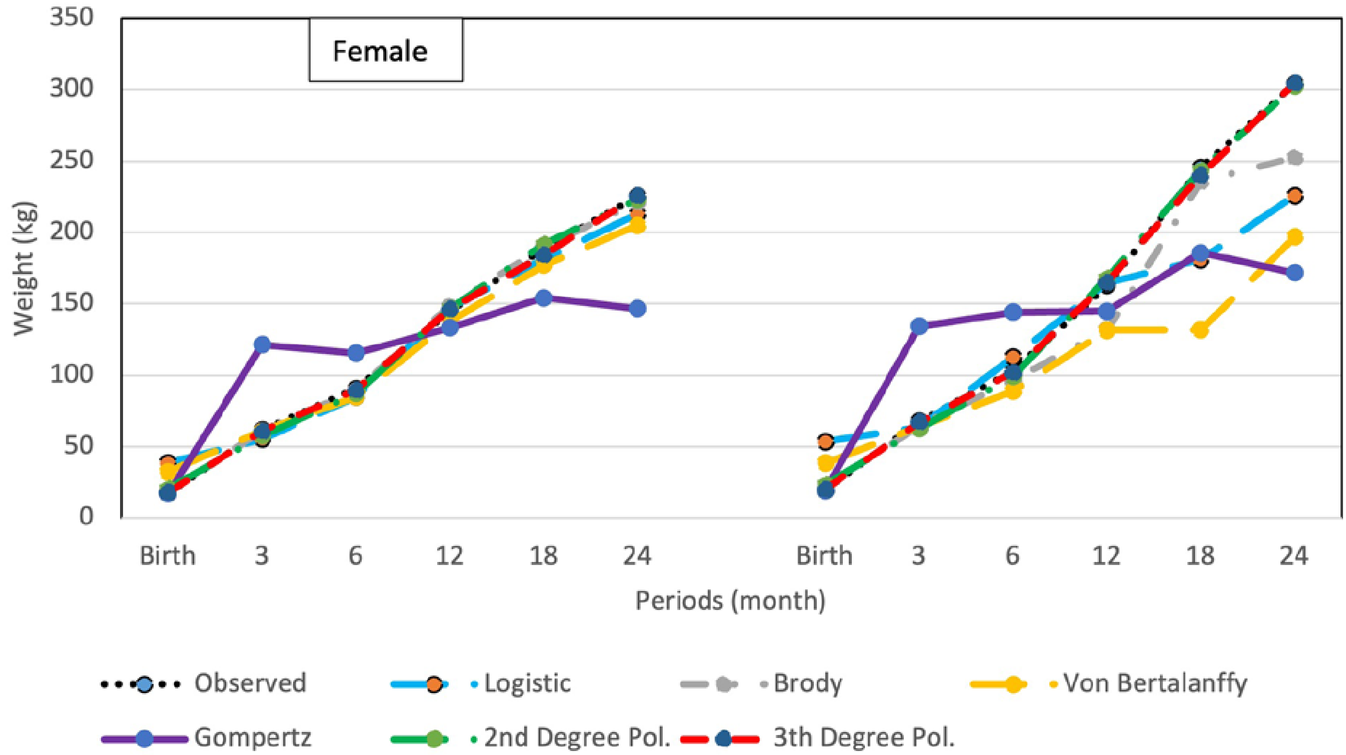

Figure 2: Observed and estimated growth curve of females and males by the models and sex of animals

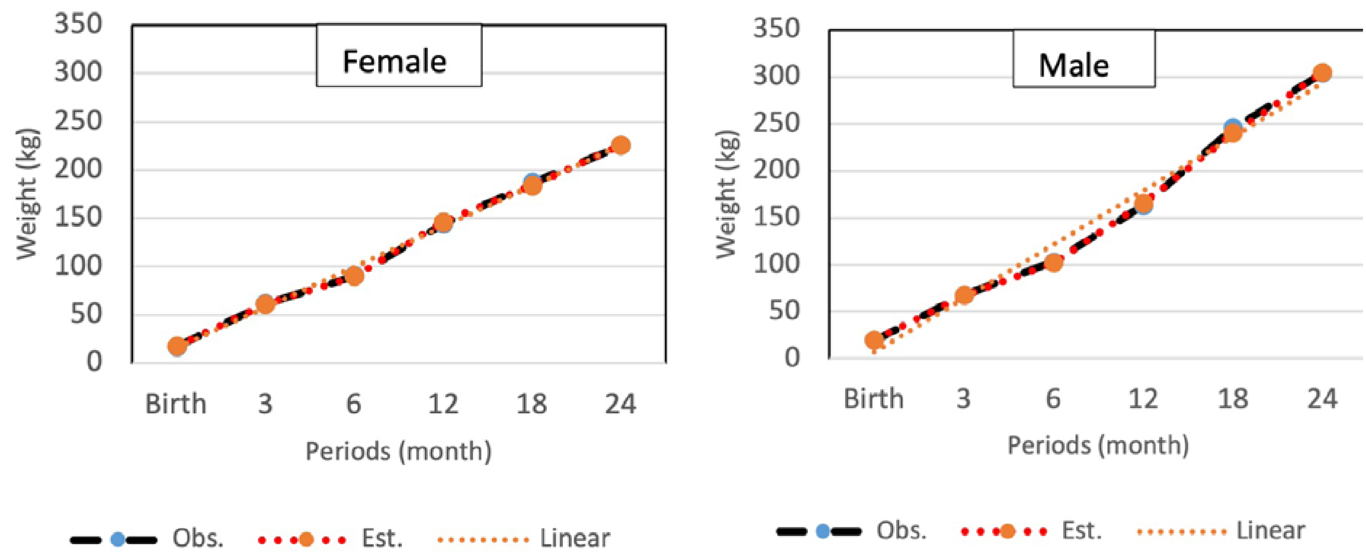

Figure 3: Growth curve with 3rd degree polynomial model by sex

according to age in ABC. In Figure 2, the growth curve of the observed weights by gender and estimated according to the models is presented. As can be seen in Figure 2, the model most compatible with the values observed in ABC from birth to 24 months is the 3rd degree polynomial model.

In similar studies, the 3rd degree polynomial model was determined to be the most suitable model in the Holstein breed by Heinrichs and Hargrove (12); in the Ayrshire, Brown Swiss and Shorthorn breeds by Heinrichs and Hargrove (18); and in the Holstein and Brown Swiss breeds by Akbulut (19). On the other hand, the most suitable models were the Gompertz and Von Bertalanffy model (R^2=0.70) in Madura breed by Hartati and Putra (6); Richards model (R^2=0.999) in Holstein by Tutkun (10);the Logistic model in the pre-weaning period; the Gompertz and Richards models in the post-weaning period in the Holstein breed by Koşkan and Özkaya (20); Richards model (R^2=0.968, 0.960) in Brown-Swiss and Holsteins by Bayram and Akbulut (21); the Richards model (R^2=0.976) in Anatolian Buffaloes by Şahin et al. (22). The most suitable models differ in studies conducted with different breeds and environmental conditions. In practice,

Table 4: Least square means and standard errors (SE) of the 3rd degree polynomial model parameters by different environmental factors

Factor	Group	n	β_0±SE	β_1±SE	β_2±SE	β_3±SE
Sex	Female	48	17.61±0.608	0.01±0.182	0.011±0.0028	0.0009±0.0004
	Male	65	20.25±0.573	0.35±0.171	0.005±0.0027	0.0005±0.0003
Dam Age	2-3	31	21.18±1.773	-0.52±0.530	0.006±0.0083	-0.0006±0.0011
	4-7	46	17.45±0.797	0.80±0.238	0.002±0.0037	0.0006±0.0005
	8-10	17	18.12±1.037	0.17±0.310	0.006±0.0048	0.0007±0.0006
	11+	19	18.99±0.979	0.26±0.293	0.019±0.0046	0.0020±0.0006
Parity	1	29	14.40±1.704	0.92±0.510	0.010±0.0080	0.0023±0.0010
	2	24	18.00±0.985	-0.45±0.295	0.016±0.0046	0.0012±0.0006
	3	19	19.95±0.995	-0.34±0.297	0.018±0.0046	0.0008±0.0006
	4	15	19.48±1.084	-0.39±0.324	0.012±0.0050	-0.0001±0.0007
	5	12	20.90±1.189	0.13±0.355	0.003±0.0055	-0.0002±0.0007
	6	8	20.33±1.511	0.63±0.452	-0.002±0.0071	0.0001±0.0009
	7	6	19.47±1.683	0.73±0.503	-0.002±0.0079	0.0005±0.0010
Year	2015	18	17.81±1.026	0.49±0.307	0.003±0.0047	0.0005±0.0006
	2016	23	19.03±0.876	0.15±0.262	0.007±0.0041	0.0001±0.0005
	2017	17	18.39±0.967	0.17±0.289	0.003±0.0045	-0.0001±0.0006
	2018	24	19.70±0.784	0.21±0.234	0.008±0.0036	0.0011±0.0005
	2019	10	19.27±1.147	-0.10±0.343	0.012±0.0054	0.0016±0.0007
	2020	21	19.39±1.041	0.14±0.311	0.013±0.0047	0.0009±0.0006
Season	Winter	17	17.49±0.960[b]	-0.15±0.287	0.179±0.0044[a]	0.0019±0.001
	Spring	55	19.78±0.696[ab]	0.37±0.208	0.002±0.0032[b]	0.0002±0.000
	Summer	26	20.58±0.777[a]	0.41±0.232	0.004±0.0036[b]	0.0001±0.000
	Autumn	15	17.88±1.038[b]	0.08±0.310	0.008±0.0048[b]	0.0006±0.001

[a,b] The means with the different superscripts within the factor in the same column are different (P<0.05).

β_0, β_1, β_2, β_3: regression coefficients of 3rd degree polynomial model

determining the weight-age relationship of cattle requires a lot of expense and time (21). In order to make reliable estimations in different regions and different breeds and to use the obtained parameters for selection purposes, first the identification of the appropriate model is necessary.

In the rest of this paper, the results of the 3rd degree polynomial model were presented since this model was determined to be the best fitted to real measurements obtained from animals. Table 4 reflects the least square means and their corresponding standard errors of β_0, β_1, β_2, β_3 parameters by environmental factors. As a result of the analysis, β_0 values vary between 17.45-21.18 (except for parity 1) in all environmental factors. The differences between these values were found to be statistically significant only in the seasonal group (P<0.05). In addition, β_2 values

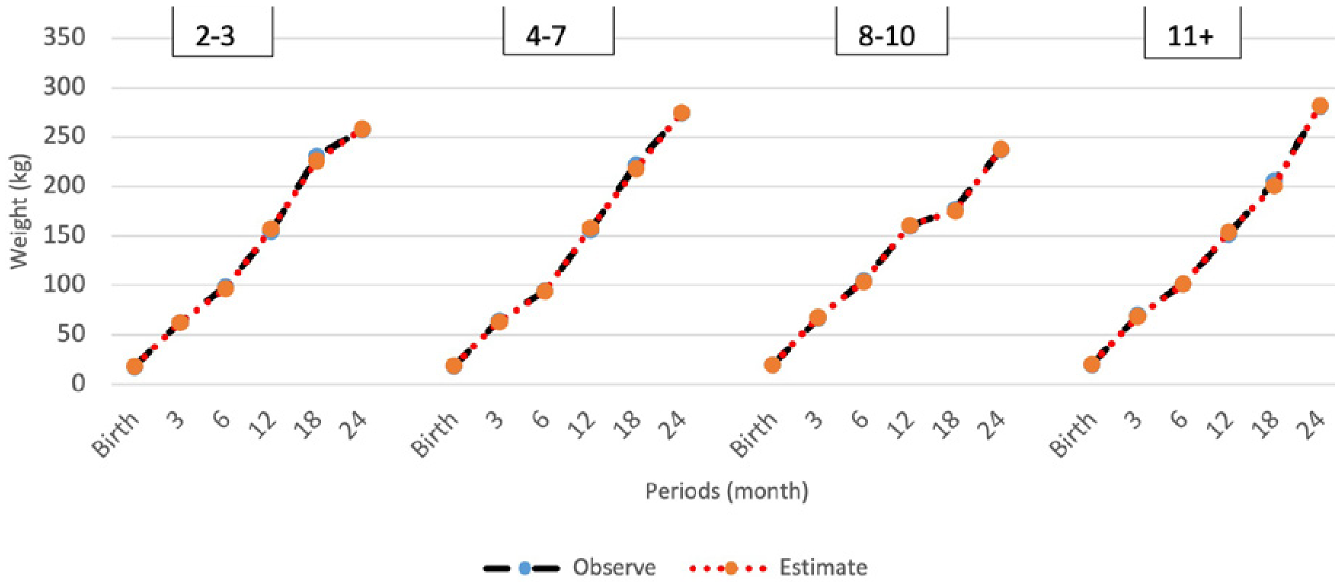

Figure 4: Growth curve with 3rd degree polynomial model by age of dam

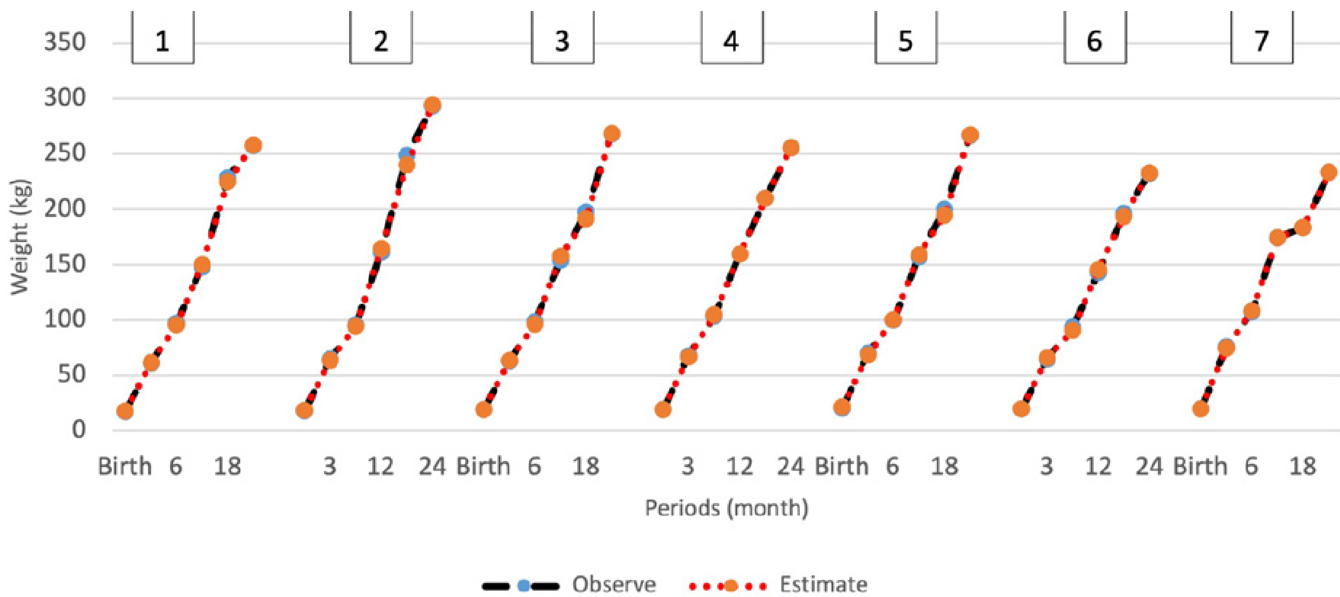

Figure 5: Growth curve with 3rd degree polynomial model by parity

were found to be statistically significant between seasons (P<0.05).

In Figures 3, 4, 5, 6, and 7, the growth curves of animals according to sex, dam age, parity, birth year, and birth season are presented using the 3rd degree polynomial model. As Figure 3 is examined, it has been determined that males have a higher weight than females in both observed and predicted values in all periods. Sahin et al. (22) found that adult live weight was higher in males in all models (Logistic, Gompertz, Richards, Brody) examined in Anatolian buffaloes. Hartati and Putra (6) reported that the animals had similar growth characteristics in all models (Logistik, Gompertz, Von Bertalanffy) which were examined in both sexes in Madura cattle. Growth in both males and females in the study continued until the age of 24 months, which can be clearly seen in the linearly plotted growth curve in Figure 3. Akbulut (19), using the 3rd degree polynomial model, determined that the growth in Holstein and Brown Swiss breeds continued linearly up to 18 months.

When Figures 4 and 5 were examined, the differences according to dam age and parity became more pronounced after 18 months of age. According to the chosen model, it was estimated that calves from the 11+ dam age group had higher live weights in periods BW, 3M, and 24M. It was also estimated that the 8-10 dam age group had higher live weights in periods 6M and 12M, while the 2-3 dam age group had higher live weights in the 18M period. When live weights measured in different periods were examined according to parity, it was determined that the animals born from the 5th parity cows had a higher live weight in the birth

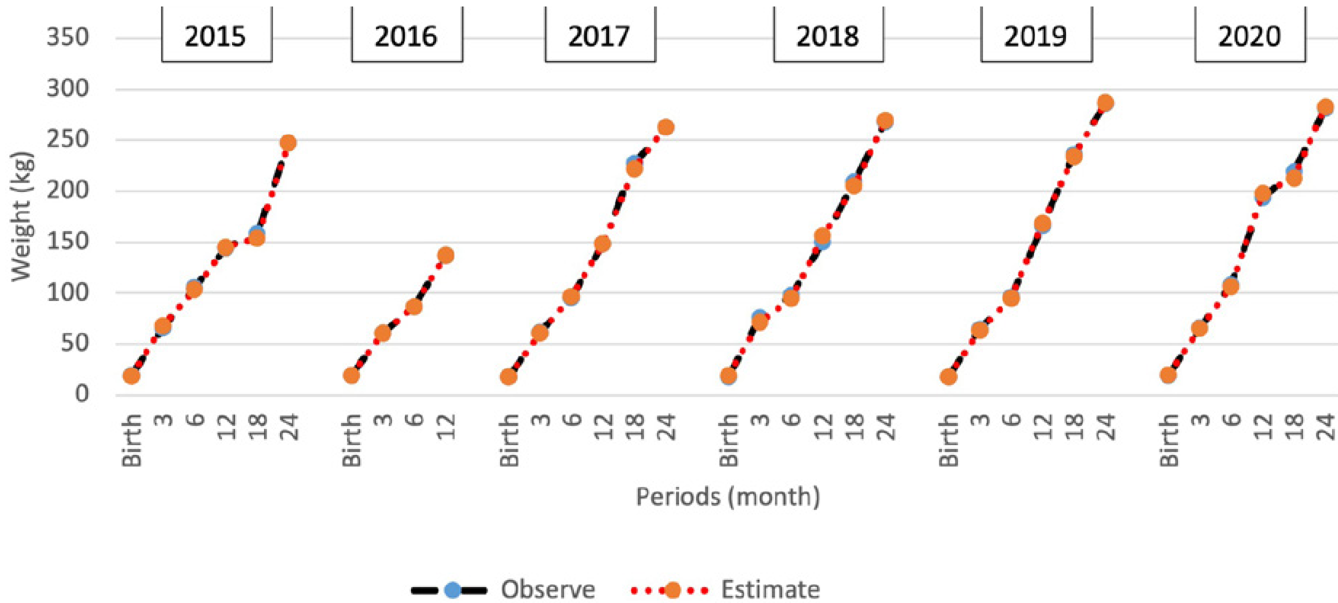

Figure 6: Growth curve with 3rd degree polynomial model by birth year

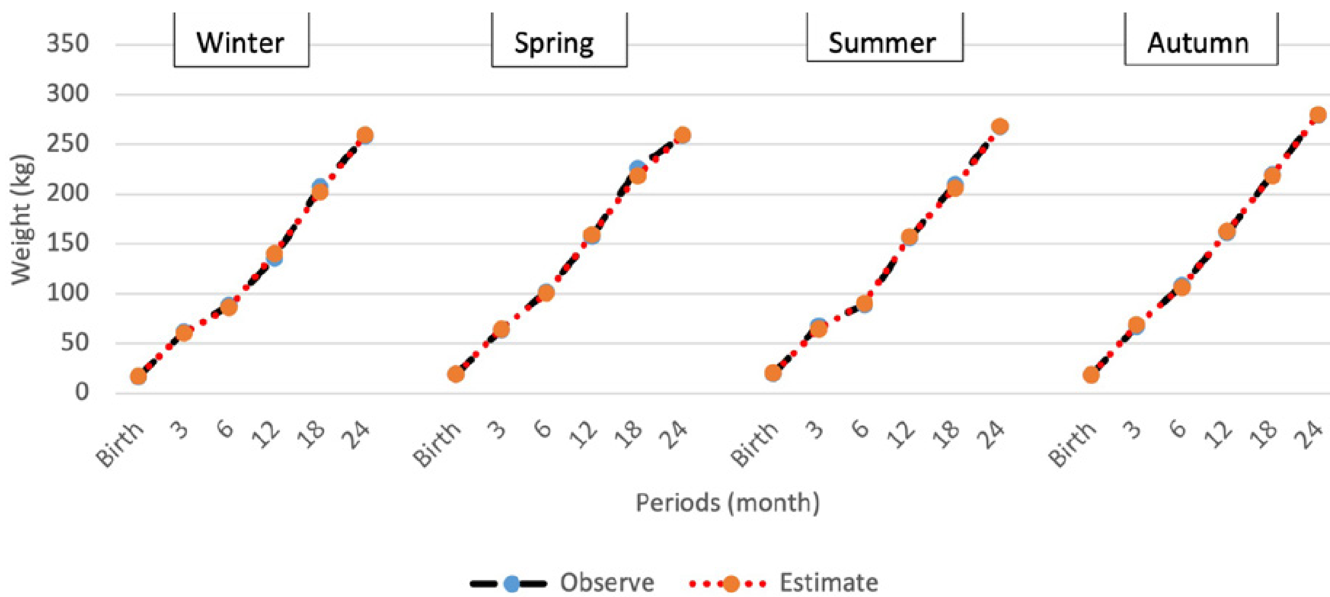

Figure 7: Growth curve with 3rd degree polynomial model by birth season

period. In addition, while the animals born from the 7th parity cows had a higher live weight in the 3M, 6M, and 12M periods, the animals born from the 2nd parity cows had a higher live weight in the 18M and 24M periods.

When Figures 6 and 7 are examined, the differences according to the year of birth and season of birth begin to appear mostly in 6M. While studying the differences in live weights by years, it was observed that the calves born in 2020 had higher live weights at birth, 6M, and 12M periods. Additionally, those born in 2018 had higher live weights in the 3M period, and those born in 2019 had higher live weights in the 18th and 24M periods. When the differences in live weights according to the seasons were examined, it was found that the animals born in the summer season had higher weights at birth and 3M periods. It was also observed that while the animals born in the spring season had higher live weights at the 6M and 12M periods, the animals born in the autumn season had higher live weights at the 18M and 24M periods.

According to this model, the estimated values showed a deviation of around 1-2 kg in female and male animals at all periods compared to the observed values, while in males they showed a deviation of 3-5 kg only in the 12M and 18M periods (Figure 3). In other graphs (Figure 4-7), the differences between the generally estimated values and the observed values are between 1-3 kg, and the differences were found to be around 4-5 kg only in 18M periods. This indicates that the 3rd degree polynomial model is the most appropriate model for the growth values of ABC.

Monitoring the growth and development of animals during some periods in the growth process will be of great benefit to the farms in terms of herd management, care, and feeding regulation (22). In order to obtain reliable estimates of the growth curve parameters, it may be necessary to collect growth data until the point when the growth curve starts to flatten or the growth rate slows down (11). Changes in body weight in animals reflect the influence of environmental factors and management systems, particularly nutrition (23). In addition, by monitoring the growth of the animals, early intervention can be made for animals that have a problem in their development.

Conclusions

According to the results of the study, the 3rd degree polynomial model was determined to be the most suitable model in ABC according to the R^2, RSD, and corel values. By using the 3rd degree polynomial model on the farm, the general growth and development of the animals can be followed, and conditions such as sexual maturity age, breeding age, and appropriate slaughter age can be easily predicted. Examining the growth curve is important for breeders to decide on the optimum body weight of the animals, the appropriate age, and the ideal weight. Growth curve parameters can be successfully applied to animals and may benefit the development and design of selection strategies.

Acknowledgements

The population of the study consisted of Anatolian Black Cattle breeds practiced in connection with the "Conservation of Domestic Genetic Resources and Sustainable Use". Therefore, the authors kindly acknowledge the contribution of the General Directorate of Agricultural Research and Policies (Ministry of Agriculture and Forestry) of the Republic of Turkey, which has given the necessary permission to provide the animal material used in the study.

The author of the study kindly declares no competing interest.

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Določitev najboljšega modela krivulje rasti za anatolsko črno govedo

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Izvleček: Namen te študije je bil določiti model, ki najbolje opisuje potek rasti od rojstva do 24 mesecev starosti anatolskega črnega goveda (ABC), vzrejenega za namene ohranjanja. Zbranih je bilo 493 podatkov o telesni teži 113 živali ob rojstvu ter pri 3, 6, 12, 18 in 24 mesecih starosti. Za opis krivulje rasti živali je bilo uporabljenih šest različnih nelinearnih modelov, in sicer polinom 2. stopnje, polinom 3. stopnje, logistični model ter modeli Brody, Von Bertalanffy in Gompertz. V študiji so bile vrednosti R^2 modelov naslednje: 0,997, 0,999, 0,953, 0,979, 0,924 in 0,862; vrednosti correl (korelacija med opazovanimi in ocenjenimi krivuljami) so bile 0,994, 0,998, 0,989, 0,993, 0,961 in 0,703; ostanki standardnih odklonov (RSD) so bili 3,216, 1,388, 11,533, 3,561, 14,736 in 27,141. Glede na te vrednosti je bilo ugotovljeno, da je polinomski model 3. stopnje najbolje opisal krivuljo rasti ABC. Na podlagi analiz je bilo ugotovljeno, da so vrednosti, ki jih je napovedal ta model, za 1-3 kg odstopale od ugotovljenih vrednosti v vseh obdobjih in pri vseh preučevanih okoljskih dejavnikih (spol, starost matere, paritete, leto rojstva in sezona rojstva). Ugotovljeno je bilo, da so se te razlike povečale na 4-5 kg le v 18-mesečnem obdobju. Rezultati so tudi pokazali, da se je ABC še naprej povečevala po 24 mesecih starosti. Posledično je mogoče lastnosti, kot so starost ob spolni zrelosti, plemenska starost in klavna starost, enostavno napovedati z določitvijo modela, ki najbolje opisuje rast in razvoj v čredah.

Ključne besede: anatolsko črno govedo; živa teža; krivulja rasti; nelinearni modeli

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The Diagnostic Accuracy of Radiographic Cardiac Indices in the Assessment of Cardiomegaly and Left Atrial Enlargement in Rats With Dilated Cardiomyopathy: An Experimental Study

Key words

dilated cardiomyopathy;
doxorubicin;
VHS;
RLAD;
VLAS;
rat

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Abstract: The purpose of this study was to determine the diagnostic value of the vertebral heart score (VHS), radiographic left atrial dimension (RLAD), and vertebral left atrial size (VLAS) in the radiographic evaluation of doxorubicin-induced dilated cardiomyopathy (DCM) in rats. The animals were allocated into two experimental groups, a DCM group (n=100), which received weekly injections of doxorubicin (2 mg/kg per dose intraperitoneally [ip]) over nine weeks, and a control group (n=18) receiving an appropriate volume of 0.9% saline ip. Radiographic cardiac indices (VHS, RLAD, VLAS) were measured two weeks after the final injection, and cardiac morphological parameters (heart weight [HW], heart weight:body weight ratio [HBW], and heart weight:tibial length ratio [HTL]) were determined after sacrifice in all surviving rats (17 control and 54 DCM). Correlations were calculated between the indices and parameters, as well as their sensitivity and specificity in detecting cardiomegaly and left atrial enlargement (LAE) with the three classifiers (HW≤0.96 g, HBW≤235, and HTL≤201). A powerful negative correlation was observed between the indices and parameters (rs≥-0.711, P<0.001). Area under the curve values were 0.989 for RL-VHS, 0.992 for DV-VHS, 0.971 for RLAD, and 0.948 for VLAS. The sensitivity and specificity of these indices in detecting cardiomegaly and LAE at the optimal cut-off values were 94.4% and 100%, respectively, at 8.8 vertebrae (v) for RL-VHS and DV-VHS, 96.3% and 94.1% at 1.4v for RLAD, and 94.4% and 88.2% at 2.1v for VLAS. Radiographic cardiac indices offer an accurate and repeatable method for predicting cardiomegaly and LAE in rats with DCM.

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Introduction

Exotic animal practice is one of the fastest-growing disciplines in veterinary medicine (1). As with other pet animals, specific medical tests, including diagnostic imaging, are now both a requirement in small exotic mammals and also specifically requested by their owners. Similar to other exotic animals (2), heart diseases are also observed in pet rats (3). Left ventricular hypertrophy, left atrial enlargement (LAE), diastolic dysfunction, and heart failure with pulmonary congestion can be seen in addition to spontaneous cardiomyopathy associated with the presence of aging-related left atrioventricular thrombus in rats (4, 5). Dilated cardiomyopathy (DCM) has been reported in pet rats (6).

DCM is a primary myocardial disease mainly characterized by cardiac chamber dilation, resulting in impaired systolic and diastolic function. The use of the anthracycline drug doxorubicin as a chemotherapeutic agent frequently induces myocardial toxicity, making it an optimal and widely used experimental model for DCM (7, 8). The reference model of chronic doxorubicin-induced DCM was initially described in rabbits (9). This model has also been used in rats and has shown to be successful in inducing not only morphological changes but also cardiac remodeling and systolic and diastolic left ventricular dysfunction (8).

Although echocardiography is a well-established method for assessing cardiac structure and function in both human and veterinary medicine (10), it is not used routinely in pet rats (11). Heart size and left atrial dimension can be evaluated using radiographic cardiac indices including vertebral heart score (VHS) (12), vertebral left atrial size (VLAS) (13), and radiographic left atrial dimension (RLAD) (14). While these indices have also been described for healthy rats (11, 15), they have not to date been reported for rats with DCM.

The purpose of this study was to determine the diagnostic value of VHS, RLAD, and VLAS in the radiographic evaluation of doxorubicin-induced DCM in rats.

Materials and methods

Animals

One hundred eighteen adult male Wistar albino rats (n=118, weight 280-360 g) were housed in the Akdeniz University Experimental Research and Application Center (Türkiye) in groups of four to six animals at 50-60% humidity and 20-21°C in a 12-h dark/light cycle. Standard rat chow and free access to water were provided. The study was conducted in compliance with the national guidelines for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Akdeniz University animal care ethics committee (no. B.30.2.AKD.0.05.07.00/111). The rats were randomly divided into two groups; control (n=18) and DCM (n=100), before drug or saline injection.

Experimental protocol

Adriamycin (doxorubicin chloride) was purchased from Saba (Istanbul, Türkiye) and dissolved in saline (10 mg/100 ml). It was then injected (2 mg/kg per dose) weekly via the intraperitoneal (ip) route over a period of nine weeks (cumulative dose 18 mg/kg) for the induction of DCM (7, 8). A control group was constituted consisting of animals of matched body weight receiving an appropriate volume of 0.9% saline ip over nine weeks. All surviving rats (17 control and 54 DCM) were subjected to radiographic examination two weeks after the last drug or saline injection.

Radiographic procedures

All surviving rats were then anesthetized using a combination of ketamine (75 mg/kg, Ketasol, Richter Pharma-Interhas, Türkiye) and xylazine HCl (5 mg/kg, Xylazine Bio, Bioveta-Interhas, Türkiye) administered ip.

RL contrast radiographs were obtained by means of a bolus injection of 0.5 ml of non-ionic opaque contrast agent (300 mg I/ml Iohexol, Omnipaque®, Opakim, Türkiye) from the tail vein. Exposure was performed as soon as the injection of the contrast agent was completed (15). For DV projection, the animal was immediately placed onto another cassette in the sternal position, and the thoracic region was imaged under the same exposure conditions.

Radiographic images obtained using a computed radiography reader (FCR Prima T2®, FujiFilm, Tokyo, Japan) were stored for subsequent use. These radiographic images were anonymized and randomized, and then evaluated by two observers using commercially available computer software (Image Intelligence™, FujiFilm, Tokyo, Japan). The observers, both with more than 20 years' veterinary experience, were blinded to the groups, and the body weight (BW), heart weight (HW), and tibial length (TL) of each rat. They were able to manipulate the images as required, including by changing the window width, window level, and magnification, and the radiographic cardiac indices were measured. Intra-observer agreement for all radiographic measurements was determined with one observer (MK) performing measurements on two separate occasions on 10 randomly selected rats from each group. Inter-observer variability was determined by two observers (MK and MAÇ) completing all radiographic measurements for the same 20 rats.

Measurements of radiographic cardiac indices

VHS was measured as described by Buchanan (2000) (16). On RL view, the cardiac long (L) axis was measured from the tracheal bifurcation to the cardiac apex. The cardiac short (S) axis was determined by measuring the distance from the intersection of the caudal border of the heart with the dorsal border of the CaCV to the cranial border of the heart. Commercially available computer software was used to apply 90° rotation between the L and S axes (Figure 1A1 and 1B1). On DV view, the L axis was determined by measuring the distance from the intersection of the right mediastinal border with the silhouette of the heart to the apex. The S axis was defined as the widest measurement obtained perpendicular to the L axis (Figure 1A2 and 1B2). These two axes were then repositioned over the thoracic vertebrae from the cranial edge of T4, parallel to the vertebral column, and each length was then expressed in terms of the number of thoracic vertebrae (v), to the nearest 0.1v. The sums of vertebral numbers on the L and S axes obtained from RL and DV views were used as RL-VHS and DV-VHS, respectively.

RLAD was obtained as described by Sanchez Salguero et al. (2018) (14). A line bisecting the 90° angle at the junction of the RL-VHS, and L and S axes was extended from there to the radiographic projection of the dorsocaudal edge of the LA. Computer software was employed in order to establish a 45° angle between this line and the junction of

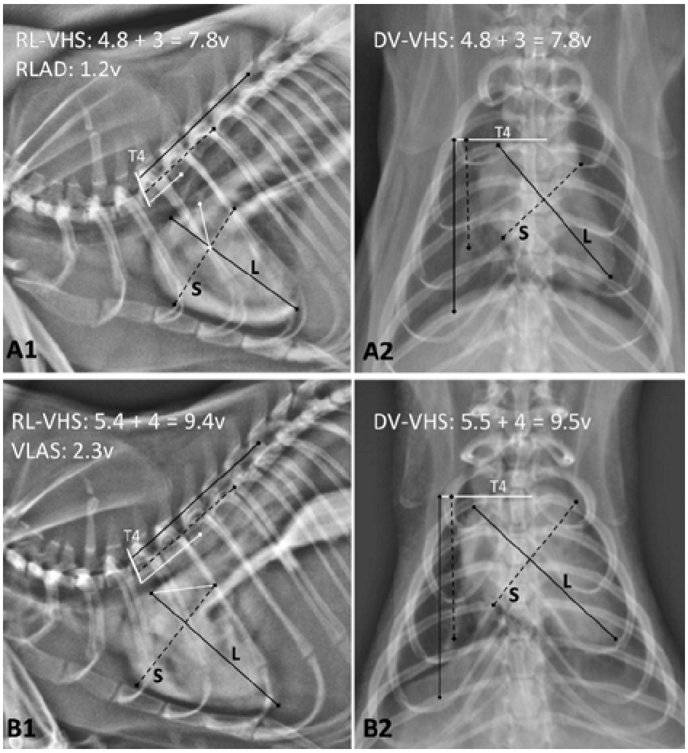

Figure 1: Contrast right lateral (RL) and dorsoventral (DV) thoracic radiographs (parameters: 65 kVp, 8 mA, 0.1 s, 30-cm film-focus distance) at 2 weeks after the last DOX dose or saline injection from two rats in the control (A) and dilated cardiomyopathy (B) groups. A and B- The black line (L) represents the long axis of the heart, and the dashed black line (S) represents the short axis. The vertebral heart score (VHS) was obtained from the total of S and L converted into vertebral values (v) by moving from the cranial margin of the 4th thoracic vertebra in a caudal direction. A1- The radiographic left atrial dimension (RLAD) was obtained by converting the measurement (white line) from the intersection of the L and S axes to the dorsal wall of the left atrium (LA) at a 45o angle into a vertebral value, as in VHS. B1- The white line represents the distance from the ventral of the tracheal bifurcation to the intersection of the caudal edge of the left atrium with the caudal vena cava. Similarly to RLAD, vertebral left atrial size (VLAS) was obtained by converting this measurement into a v value

the L and S axes (Figure 1A1). Similar to the VHS, the same line was then positioned over the thoracic vertebrae beginning, at the cranial edge of T4, and the number of vertebral units was estimated to the nearest 0.1 vertebral body length (Figure 1A1).

VLAS was obtained as described by Malcom et al. (2018) (13). A line was measured from the tracheal bifurcation to the most caudal aspect of the LA at the junction with the dorsal border of the CaVC. The same line was normalized to v, beginning from the cranial edge of T4, similar to VHS and RLAD (Figure 1B1).

Cardiac morphological parameters

Once the radiographic cardiac indices had been measured, all rats under ketamine-xylazine anesthesia were weighed and sacrificed via a thoraco-abdominal incision. The heart was removed, washed in ice-cold saline, and then weighed. Tibial length (TL) was measured from the mediolateral view of the right tibia. HW was divided by the BW and TL to yield heart weight:body weight ratio (HBW) and heart weight:tibial length ratio (HTL) values for each rat.

Statistical methods

Statistical analysis was performed on commercial software (IBM SPSS Statistics 22.0, SPSS Inc., USA). Descriptive statistics were generated, and the Shapiro-Wilk test was applied to evaluate the normality of the distribution of continuous data. BW, HW, HBW, TL, HTL, RL-VHS, DV-VHS, RLAD, and VLAS values are presented as median and interquartile ranges (IQR). Comparisons between the control and DCM groups were performed using the Mann-Whitney U test. Spearman's rank-order correlation coefficient (rs) was applied to evaluate correlations between cardiac morphological parameters (HW, HBW, and HTL) and radiographic cardiac indices (RL-VHS, DV-VHS, RLAD, and VLAS). The following indices were used to evaluate this relationship for this analysis: an rs value from -1.0 to -0.7 was regarded as indicating strong negative correlation, values from -0.7 to -0.3 negative correlation, values from -0.3 to +0.3 small correlation or no association, values from +0.3 to +0.7 weak positive correlation, and values from +0.7 to +1.00 strong positive correlation. ROC curves, and the area under the curves (AUC) with 95% confidence intervals (CI) were generated for each radiographic cardiac index using HW ≤ 0.96 g, HBW ≤ 235, and HTL ≤201 as the classifiers. The AUC values of the four measurements were compared using Delong's method (17) (MedCalc® Statistical Software version 20.115, 2022, MedCalc Software Ltd., Ostend, Belgium). Sensitivity and specificity for each radiographic cardiac index were determined with the Youden index in order to determine optimal cut-off values. Intra- and inter-observer variabilities were assessed for each radiographic cardiac index by means of intraclass correlation coefficient (ICC) estimates and 95% confidence intervals based on a single rater, absolute agreement, and a two-way random (inter-observer) and mixed (intra-observer) effect. ICC values >0.9 were regarded as excellent, 0.75 to 0.9 as good, 0.5 to 0.75 as moderate, and values <0.5 as poor (18). P values <0.05 were regarded as statistically significant.

Results

The mortality rate in the DCM group was 46% (n=46/100), while one animal from the control group (n=1/18, 5.6%) died.

Significant decreases in terminal BW and cardiac morphological parameters (HW, HBW, and HLT) and significant increases in radiographic cardiac indices (VHS, RLAD, and VLAS) were observed in the DCM group compared to the control group (*P*<0.001). The change in TL was not statistically significant (*P*=0.246) (Table 1).

The degrees of association between the radiographic cardiac indices and cardiac morphological parameters for all rats enrolled in the study are presented in Table 2. A strong

Table 1: Descriptive data for the radiographic cardiac indices (VHS, RLAD, and VLAS) and cardiac morphological parameters (HW, HBW, and HTL) between the control and DCM groups

Variables	Groups			
	Control		DCM	
	Median	IQR	Median	IQR
Terminal BW (g)	483	447-521	425*	355-460
HW (g)	1.17	1.09-1.27	0.9*	0.84-0.96
HBW ([mg/g]100)	244	232-259	211*	193-235
TL (cm)	4.59	4.30-4.81	4.51^{ns}	4.25-4.79
HTL (mg/cm)	260	245-278	198*	185-201
RL-VHS	8	7.5-8.9	9.1*	8.7-9.6
DV-VHS	8.2	7.6-9	9.2*	8.7-9.9
RLAD	1.2	1-1.5	1.7*	1.5-2.1
VLAS	1.9	1.6-2.2	2.3*	2.1-3.1

Data are presented in median and interquartile ranges (IQR) unless stated otherwise. ns, not significant P=0.246.

*Values within a row differ significantly (P<0.001) from that of the control group. Abbreviations: DCM, dilated cardiomyopathy; BW, body weight; HW, heart weight; HBW, heart weight:body weight ratio; TL, tibial length; HTL, heart weight: tibial length; RL-VHS, right lateral vertebral heart size; DV-VHS, dorsoventral vertebral heart size; RLAD, radiographic left atrial dimension; VLAS, vertebral left atrial size

negative correlation was determined between these indices and parameters ($r_s \geq -0.711$, $P<0.001$).

Diagnostic accuracy, cut-offs, and likelihood ratios for RL-VHS, DV-VHS, RLAD, and VLAS in the radiographic prediction of cardiomegaly and LAE as determined by the three classifiers (HW≤0.96 g, HBW≤235, and HTL≤201) in the 71 rats are summarized in Table 3. Since two animals from the control group and one from the DCM group did not meet all these three classifiers, these were not included in the ROC analyses (15 control, 53 DCM). The ROC curve, AUC, and cut-off values for the radiographic cardiac indices are shown in Figure 2. The ROC analyses indicated that all radiographic cardiac indices were useful in identifying rats with cardiomegaly and LAE due to DCM. AUC values were similar for RL-VHS (0.989), DV-VHS (0.992), RLAD (0.971), and VLAS (0.948). The optimal cut-off values for VHS, RLAD, and VLAS with the greatest sensitivity and specificity for detecting cardiomegaly and LAE were 8.8v, 1.4v, and 2.1v respectively. Sensitivity and specificity for both RL-VHS and DV-VHS were 94.4% and 100%, compared to

Table 2: Spearman correlation coefficients (rs)* quantifying the degree of association between radiographic and cardiac morphological variables in 71 rats

Radiographic variable	Cardiac morphological variable		
	HW	HBW	HTL
RL-VHS	-0.830	-0.755	-0.790
DV-VHS	-0.825	-0.711	-0.782
RLAD	-0.868	-0.731	-0.810
VLAS	-0.841	-0.727	-0.784

Abbreviations: HW, heart weight; HBW, heart weight:body weight ratio; HTL, heart weight:tibial length ratio; RL-VHS, right lateral vertebral heart size; DV-VHS, dorsoventral vertebral heart size; RLAD, radiographic left atrial dimension; VLAS, vertebral left atrial size, * All P<0.0001

Table 3: Receiver operating characteristic curve analyses for determining the diagnostic accuracy of the radiographic variable cut-offs for radiographic prediction of cardiomegaly and LA enlargement due to dilated cardiomyopathy when heart weight ≤0.96 g, heart weight:body weight ratio ≤235, and heart weight:tibial length ratio ≤201 were adopted as criteria in 68 rats

Radiographic variables	AUC (95% CI)	z and P values	Cutoff (vertebrae)	Sn (%)	Sp (%)	+LR	-LR	Youden Index J
RL-VHS	0.989±0.001 0.929-1.00	51.467 < 0.0001	> 8.8	94,44	100.00	n/a	0.056	0.944
DV-VHS	0.992±0.008 0.934-1.00	64.981 < 0.0001	> 8.8	94.44	100.00	n/a	0.059	0.7706
RLAD	0.971±0.019 0.901-0.996	25.135 < 0.0001	> 1.4	96.30	94.12	16.37	0.039	0.9041
VLAS	0.948±0.031 0.867-0.986	14.503 < 0.0001	> 2.1	94,44	88.24	8.03	0.063	0.8268

Abbreviations: AUC, area under the curve; CI, confidence interval; Sn, sensitivity; Sp, specificity; +LR, positive likelihood ratio; −LR, negative likelihood ratio; RL-VHS, right lateral vertebral heart size; DV-VHS, dorsoventral vertebral heart size; RLAD, radiographic left atrial dimension; VLAS, vertebral left atrial size

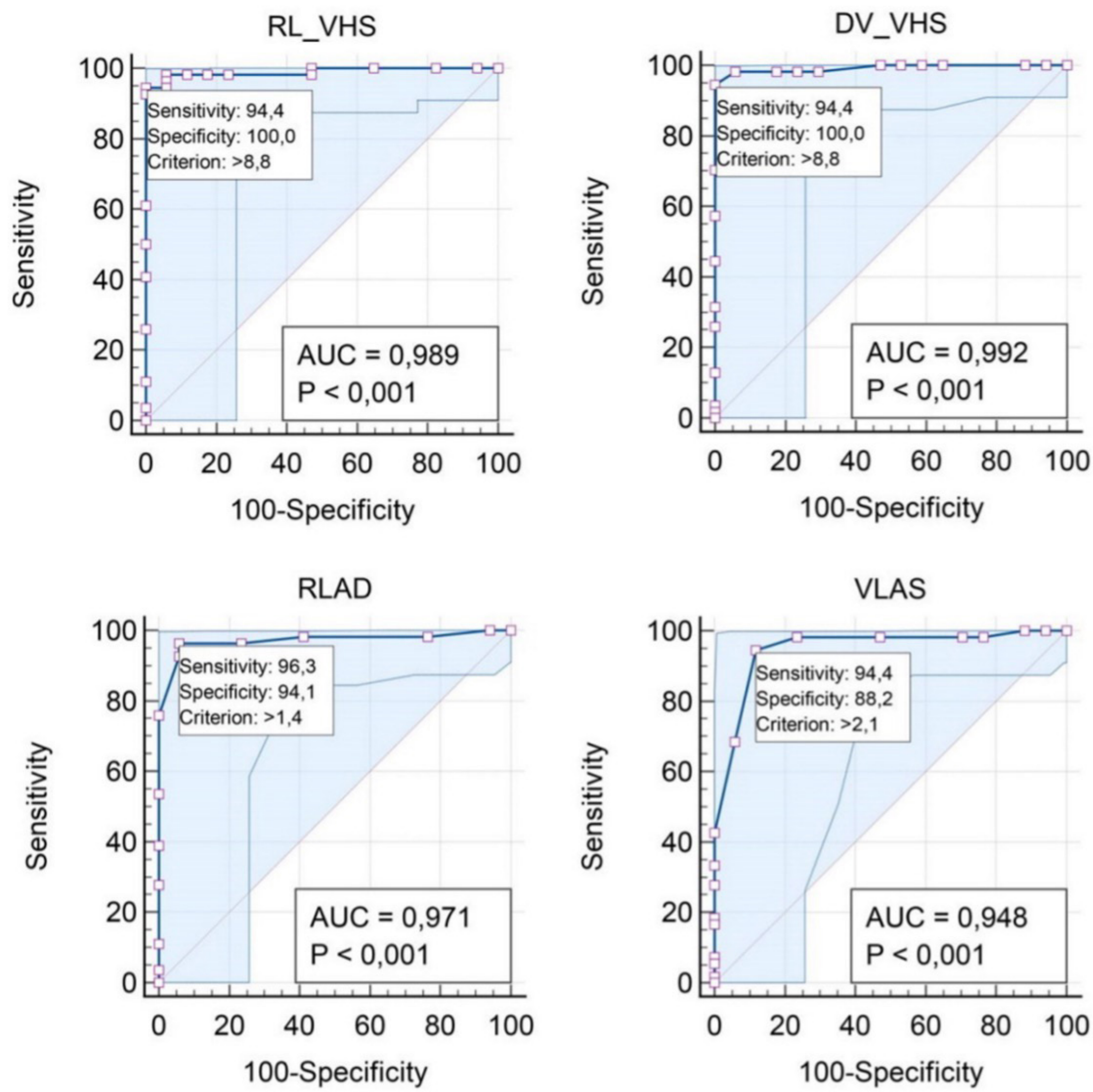

Figure 2: Receiver-operating characteristic curves and areas under the curve (AUC) for variables right lateral (RL)-vertebral heart score (VHS), dorsoventral (DV)-VHS, radiographic left atrial dimension (RLAD), and vertebral left atrial size (VLAS) to differentiate rats with the heart weight ≤0.96 g, heart weight:body weight ratio ≤235, and the heart weight:tibial length ratio ≤201. The RL-VHS, DV-VHS, RLAD, and VLAS optimal cutoff values which provided the greatest sensitivity and specificity along their respective curves are shown

96.3% and 94.1% for RLAD and 94.4% and 88.2% for VLAS, respectively.

Both intra- and inter-observer variabilities assessed using ICC values demonstrated good to excellent agreement for all radiographic cardiac indices (ICC>0.75, P<0.05) (Table 4).

Discussion

This study describes objective measurements (VHS, RLAD, and VLAS) for estimating cardiomegaly and LAE in rats with DCM using thoracic radiographs.

The heart silhouette from thoracic radiographs in animals can be objectively evaluated by means of VHS. VLAS and RLAD have been employed in the radiographic diagnosis of LAE in dogs in recent years. However, the cranial cardiac border on lateral thoracic plain radiographs in rats is unclear due to the opacity of the soft tissue in the cranial mediastinum. This radiographic feature in rats can lead to erroneous VHS values being obtained, especially by inexperienced operatives. Lateral thoracic contrast radiography, a simple and complication-free method, has been shown to be more effective than thoracic plain radiography, and that the heart can be evaluated with radiographic indices independently of the heart silhouette (15). Lateral thoracic contrast radiographs were therefore employed in the present study.

Short-term (19, 20) and long-term (7-9) doxorubicin injection models are generally used for the induction of DCM and heart failure. Although cardiac dysfunction and irregularity in cardiac functions have been documented using the Langendorff preparation of myocardial contractility in a short-term model in which high-dose anthracyclines were used for less than two weeks, functional and structural cardiac changes, cardiac function and remodeling consistent with DCM have not been confirmed with in vivo imaging techniques. Nonetheless, both changes in cardiac morphology and also cardiac remodeling and left ventricular systolic dysfunction have been shown to be induced in rats in long-term injection models over an 8-12 week period (8). A long-term injection model was therefore employed in this study. However, since doses exceeding 1 mg/kg result in heart failure due to cardiotoxicity associated with the dose of doxorubicin used, they yield the classic symptoms of DCM, but similarly to the present study (46%), they also cause mortality rates of between 32% and 82% (8, 9, 19, 20). Mortality is associated with acute heart failure resulting from cardiotoxicity, although other factors reducing survival rates include nephrotoxicity, hepatotoxicity, and severe gastrointestinal bleeding (8, 21, 22).

A decrease in cardiac morphological parameters (HW, HBW, and HTL) is widely regarded as indicating cardiac atrophy in experimental studies involving doxorubicin (23-25). At the same time, a powerful correlation exists between HW and the echocardiographic left ventricular mass index (26). In addition, the cardiac morphological parameters used in the present study decreased significantly compared to the control group, and a strong negative correlation was determined between these parameters and the radiographic cardiac indices (*P*<0.001) (Table 2). The three cardiac morphological parameters (HW≤0.96 g, HBW≤235, and HTL≤201) were thus used as classifiers for ROC curve and AUC analysis of the radiographic variables. In our previous study (32), when HBW≥293 was employed as a criterion for radiographic prediction of cardiomegaly and LEA in rats developing eccentric cardiac hypertrophy due to volume overload, the AUC and cut-off values obtained were similar to the results of the present research (Table 3). Compared with our results, other studies using echocardiographic the left atrial-to-aortic root ratio (LA:Ao≥1.6) and normalized left ventricular end-diastolic dimension (LVIDDN≥1.7) as cardiomegaly and LAE criteria in dogs with degenerative mitral valve disease (13, 14, 27-29) have reported higher cut-off values (≥10.7 to >11.7 for VHS, ≥1.7 to ≥1.8 for RLAD, and 2.3 to ≥2.4 for VLAS) despite lower AUC values (0.81 to

Table 4: Intra- and inter-observer agreements for RL-VHS, DV-VHS, RLAD, and VLAS in rats

Radiographic variable		Intra-observer agreement			Inter-observer agreement		
		ICC	95% CI	P value	ICC	95% CI	P value
RL-VHS	Control	0.92	0.58-0.98	<0.01	0.87	0.48-0.97	<0.01
	DCM	0.83	0.48-0.92	<0.01	0.78	0.40-0.93	<0.01
DV-VHS	Control	0,90	0.59-0.93	<0.01	0.82	0.55-0.97	<0.01
	DCM	0.88	0.36-0.94	<0.01	0.86	0.60-0.97	<0.01
RLAD	Control	0.90	0.71-0.95	<0.001	0.88	0.68-0.93	<0.001
	DCM	0.86	0.55-0.95	<0.001	0.90	0.59-0.96	<0.001
VLAS	Control	0.95	0.79-0.98	<0.001	0.91	0.74-0.97	<0.001
	DCM	0.91	0.77-0.97	<0.001	0.90	0.72-0.96	<0.001

Abbreviations: DCM, dilated cardiomyopathy; RL-VHS, right lateral vertebral heart size; DV-VHS, dorsoventral vertebral heart size; RLAD, radiographic left atrial dimension; VLAS, vertebral left atrial size

0.94 for VHS, 0.93 to 0.99 for RLAD, 0.84 to 0.95 for VLAS). In contrast to dogs, such echocardiographic criteria have not been reported in either experimental cardiological rat studies, or in pet rats. Although cardiac morphological parameters are regarded as useful in experimental DCM studies, compared with echocardiography, magnetic resonance imaging, or computed tomography (30-33), they are not a true gold standard method in determining radiographic cardiac index cut-off values. In addition, there is a small limit to our study in that only adult males from one rat strain are used; therefore, the cut-off values may differ in females, juveniles, or other strains. Nevertheless, our results should constitute a useful tool for comparison in future studies with both more advanced diagnostic methods and gender, age, and strain of rats, and can also be used to predict cardiomegaly and LAE in rats with DCM.

Inter-observer variability has been described as one of the factors impacting on radiographic cardiac indices (34, 35). Consistent with previous studies (11, 15, 28, 29, 36), good to excellent agreement was observed in intra- and inter-observer variabilities for all radiographic cardiac indices in the present research (Table 4).

Conclusions

Radiographic cardiac indices exhibiting powerful negative correlation with the cardiac morphological parameters employed as a marker of cardiac atrophy exhibited high sensitivity and specificity in predicting cardiomegaly and LAE resulting from DCM in rats. The recommended cut-off values (8.8v for VHS, 1.4v for RLAD, and 2.1v for VLAS) can be used for this purpose in both pet rats and in rat models of DCM.

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Natančnost diagnostike radiografskih kazalcev srca pri ocenjevanju kardiomegalije in povečanja levega preddvora pri podganah z dilatativno kardiomiopatijo: Eksperimentalna študija

M. Kaya, M. A. Çetinkaya

Izvleček: Namen te študije je bil določiti diagnostično vrednost vertebralne srčne ocene (VHS), radiografske dimenzije levega atrija (RLAD) in vertebralne velikosti levega atrija (VLAS) pri radiografski oceni z doksorubicinom povzročene dilatativne kardiomiopatije (DCM) pri podganah. Živali so bile razdeljene v dve eksperimentalni skupini: skupino z DCM (n=100), ki je devet tednov prejemala tedenske injekcije doksorubicina (2 mg/kg na odmerek intraperitonealno [ip]), in kontrolno skupino (n=18), ki je prejemala ustrezno količino 0,9 % fiziološke raztopine ip. Radiografske kazalnike srca (VHS, RLAD, VLAS) smo izmerili dva tedna po zadnji injekciji, morfološke parametre srca (teža srca [HW], razmerje med težo srca in telesno težo [HBW] ter razmerje med težo srca in dolžino goleni [HTL]) pa smo določili po žrtvovanju vseh preživelih podgan (17 kontrolnih in 54 DCM). Izračunane so bile korelacije med kazalniki in parametri ter njihova občutljivost in specifičnost pri odkrivanju kardiomegalije in povečanja levega atrija (LAE) s tremi klasifikatorji (HW≤0,96 g, HBW≤235 in HTL≤201). Med kazalniki in parametri je bila ugotovljena močna negativna korelacija (rs≥-0,711, P<0,001). Vrednosti površine pod krivuljo so bile 0,989 za RL-VHS, 0,992 za DV-VHS, 0,971 za RLAD in 0,948 za VLAS. Občutljivost in specifičnost teh kazalnikov pri odkrivanju kardiomegalije in LAE pri optimalnih mejnih vrednostih sta bili 94,4 % oziroma 100 % pri 8,8 vretencu (v) za RL-VHS in DV-VHS, 96,3 % oziroma 94,1 % pri 1,4 v za RLAD ter 94,4 % oziroma 88,2 % pri 2,1 v za VLAS. Radiografski kazalniki srca omogočajo natančno in ponovljivo metodo za napovedovanje kardiomegalije in LAE pri podganah z DCM.

Ključne besede: dilatativna kardiomiopatija; doksorubicin; VHS; RLAD; VLAS; podgana

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Case Report

Aggressive Behavior in a Bitch After Deslorelin Implant Insertion

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Key words

bitch;
aggression;
behavior;
deslorelin implant;
long-lasting GnRH

Abstract: In male dogs, long-acting gonadotropin-releasing hormone (GnRH) analogs are widely accepted as an alternative to surgical castration but are also used to suppress and induce estrus in bitches (off label). Behavioral changes reported in bitches in association with the use of long-acting GnRH analogs have included male-like behavior associated with triggered estrus, increased food intake, enlargement of mammary glands and milk production, pseudopregnancy, and urinary incontinence. In male dogs, intra-species and rarely inter-species aggression may occur during the flare-up effect. However, at the time of downregulation, this behavior should no longer occur if it is testosterone dependent.

An intact, three-year-old female cocker spaniel with Addison's disease was admitted to our clinic for consultation on spaying options because of pseudopregnancies followed by mastitis, which had to be treated with antibiotics, after her heat cycles. The owners were hesitant about surgical sterilization and therefore, deslorelin implant was inserted. Approximately one month after implantation, owners observed the onset of aggressive behavior, including incessant barking, extreme irritability, and aggression toward other dogs and towards family members. The behavior problems started to escalate and the owners were not able to handle her anymore. After removal of the implant, the observed aggression ceased, and the bitch returned to normal behavior.

Although aggressive behavior toward other dogs and sometimes even toward owners has been observed after neutering or insertion of a deslorelin implant, this is the first report of extremely aggressive behavior toward owners and other dogs in a female dog after insertion of a 4.7 mg deslorelin implant.

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Introduction

Aggression is the most common and serious behavioral problem in dogs. It is also the most common reason pet owners seek professional help from veterinarians and behaviorists (1). Aggression in dogs can be directed toward familiar people, toward strangers, or toward other dogs. Aggressive behavior in dogs is a common problem in society and a frequent cause of dog-human bites (2), surrender of adult dogs to shelters, and euthanasia of adult dogs (3).

Gonadectomy is commonly used to treat and prevent behavior problems, including aggressive behavior in dogs and it is advocated by veterinarians as a way to make dogs better-behaved companions (4). However, researchers provide conflicting information. In a study by Hopkins et al. (5), it was found that in gonadectomized dogs, aggressiveness toward other dogs decreased by 62%, but territorial and fear-induced aggressive behavior remained unchanged. The same observations were made in another study in which neutering had an effect on all behavioral problems related to aggression except aggressive behavior toward strangers (6). In contrast, other studies suggest no discernible pattern of changes in aggression (7, 8). Some studies note that gonadectomized dogs of both sexes are significantly more likely than intact dogs to bark, growl, lunge, snap, open their eyes wide, and raise or curl their lips when approached by an unknown human or dog (9, 10). A recent study of 15, 370 dogs concluded that no single factor is responsible for aggressive behavior in dogs, but that there are multiple environmental and genetic factors that contribute to aggressive behavior. Neutering does not result in a predictable decrease in aggressive behavior in all male and female dogs, although it may be effective in some (4). This supports the previous 2013 recommendation from the Society for Theriogenology and the American College of Theriogenology Board of Directors that gonadectomy should be decided on a case-by-case basis (11).

Gonadotropin-releasing hormone (GnRH) is a key regulator of reproductive function. It is released in a pulsatile fashion from hypothalamic neurons. The peptide binds to specific GnRH receptors on pituitary gonadotrophs. Its activation leads to synthesis and secretion of LH and FSH. In females, gonadotropin secretion from the pituitary gland is modulated by estradiol and progesterone. These feedback effects of ovarian steroids initiate the LH surge and lead to suppression of GnRH release in the luteal phase. The pulsatile release of GnRH from the hypothalamus is essential for the maintenance of ovarian function (12). Slow-release GnRH agonist implants are widely accepted as an alternative to surgical castration in male dogs and cats. Although deslorelin implants are approved in the EU for male dogs and, as of June 2022, for male cats and prepubertal bitches (13), numerous studies have been conducted in the adult bitch to investigate its use as a contraceptive and/or method of estrus induction (14, 15, 16). The first step in the mechanism of action is a flare-up effect with an increase in gonadotropin synthesis. Such increased gonadotropin synthesis leads to estrus induction in females, when treated in anestrus (14, 15). This was indeed observed when females in anestrus were treated with GnRH analog (14). However, when females in diestrus with P4 > 5 ng/ml were treated, the first report concluded that there is no flare-up effect (17). Later studies showed that the initial flare up effect is associated with estrus induction when deslorelin is administered to bitches both in anestrus as well as (although more rarely) in diestrus (18). Most authors have confirmed that all adult bitches respond in the same manner regardless of size and age; however, depending on the stage of the cycle, the bitch's response may vary (19, 20, 21). After the flare-up effect desensitization of the GnRH receptors occurs, resulting in a transient, long-term, and fully reversible down-regulation of reproductive endocrine functions in dogs (21, 22, 23). These promising results led to the use of the implants for estrus induction and suppression in bitches predisposed to the side effects of sterilization (off-label use). Although many adult bitches do well when treated with deslorelin, some signs such as persistent oestrus, pyometra, urinary incontinence, ovarian tumors, and minor behavioral and physical changes may occur (24).

Case

An intact, three-year-old female cocker spaniel was admitted to our hospital for consultation on spaying options. The dog was diagnosed with Addison's disease at one year of age and was well controlled with a combination of oral hydrocortisone (Hydrocortisone Roussel, Sanofi Winthorp Industrie, France) at a dosage of 0.4 mg/kg/12h and subcutaneous desoxycorticosterone pivalate (Zycortal, Dechra, UK) at a dosage of 1.3 mg/kg/30 days. Based on clinical response to therapy and biannual laboratory checks, her disease was stable. Her first heat occurred at 9 months of age, and since then she has had regular cycles every 6 months. After the fourth cycle, she developed pseudopregnancy followed by mastitis, which had to be treated with antibiotics. The owners wanted to stop the cycles and prevent the pseudopregnancy but were hesitant about surgical sterilization because of the risk of anesthesia, due to her condition. Moreover, they feared irreversible consequences of gonadectomy, especially weight gain. They decided to use a deslorelin implant because its effect is reversible and can be implanted without surgical intervention.

Deslorelin (Suprelorin 4.7 mg implant for dogs, Virbac, France) was implanted subcutaneously in the umbilical region on day 23 after the onset of visible signs of proestrus. Diestrus was confirmed by vaginal cytology, but progesterone was not measured. Approximately one month after implantation, owners observed the onset of aggressive behavior, including incessant barking, extreme irritability, and aggression toward other dogs. Such behavior was not normal for this previously very well-mannered dog. The aggression escalated rapidly with her becoming aggressive also toward people, firstly toward strangers but later toward family members as well, culminating in a bite attack requiring medical intervention on one of the owners. The change in behavior was so extreme that the owners were unable to physically interact with her any more as she attacked anyone who approached her. About 70 days after implantation signs of pseudopregnancy became visible with enlarged mammary glands but no milk production and lethargy. Another side effect of the implant was polyphagia and about 10% weight gain.

Approximately two and a half months after administration, the implant was removed. All signs of aggressive behavior ceased within the next few months, and according to the owners, she returned to the gentle dog she was prior to implantation. Within two weeks, she also lost 600 g in weight despite being fed the same diet. In the two years after the implant removal, once resolved, the aggressive behavior never reoccurred although her heat cycles returned to

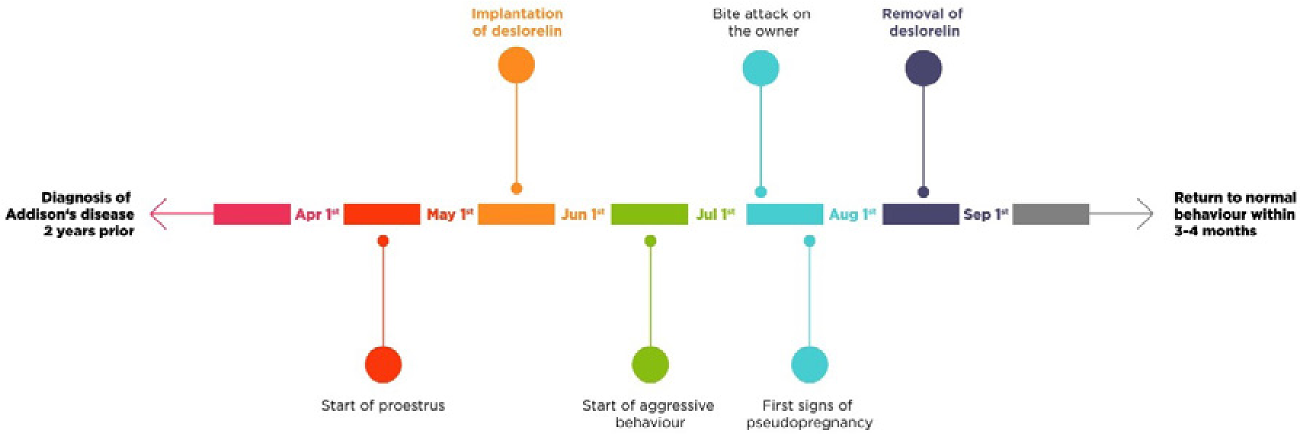

Figure 1: The exact course of events that took place from the insertion of the implant to its removal

normal as before deslorelin implantation. The exact course of events are shown in Figure 1.

Discussion

The present case is the first report of the occurrence of severe aggression in a bitch after the insertion of a deslorelin implant. Surgical sterilization is often suggested to the owners to alleviate unwanted and aggressive behavior in some dogs (25). Because of their ability to suppress sex steroid hormones, GnRH agonist-releasing implants may also be useful in modifying or minimizing testosterone-related behaviors. In castrated male dogs and dogs with deslorelin implants, improvements have been noted in sexually dimorphic male behaviors described as libido, hypersexuality, intermale conflict, and excessive urine marking (26). Therefore, it has been suggested that implants releasing GnRH agonists are an alternative for clients seeking to alter testosterone-mediated behavior in their male dogs without having to perform castration or as a trial to what could be achieved by permanent sterilization (27). On the other hand, recent studies have shown that the likelihood of undesirable behavior increases significantly in neutered animals. Neutered dogs were more aggressive, more fearful, more restless, more difficult to train, and less responsive to signals (2, 4, 23). In addition, and of bigger importance, there is a risk of an increase in dominance aggression toward family members (28), with owners (2) and children (29) being bitten more likely by neutered animals. Another study found that these effects were more pronounced when animals were neutered before puberty (27).

The behavioral aspect of GnRH agonist implants in bitches has not yet been described, but it is generally expected that the effects should be similar to those of sterilization. However, there is a major difference between surgical and medical castration, namely that gonadotropin levels are very high after surgical castration (30) whereas they are likely to be low to basal during deslorelin treatment because of the inhibitory effect of deslorelin on gonadotropin release. In general, it is not known whether such a change may have an impact on disease and possibly on behavior. In the present case, severe aggressive behavior toward other dogs and strangers was initially observed. Subsequently, the aggression toward family members escalated and the dog became so aggressive that the owners could no longer safely handle her. Most studies have found that the risk of unwanted aggressive behavior after sterilization is higher in dogs that already showed behavioral problems before gonad removal (31). However, in our case, no aggression was observed in this bitch before implant placement, and the aggression resolved after implant removal.

We must mention that the dog suffered from Addison's disease and was treated with a physiological dose of corticosteroids and mineralocorticoids. Exogenous corticosteroids are synthetic analogues of natural steroid hormones and are used in both human and veterinary medicine mainly for their anti-inflammatory and immunosuppressive effects. The synthetic analogues of cortisol have increased glucocorticoid activity (32). Exogenous corticosteroid treatments have been reported to cause negative emotional states in human patients and laboratory animals, with similar changes noted in domestic dogs (33). Behavioral changes associated with exogenous corticosteroid treatments in dogs have been reported in only a few studies (33, 34), showing increased anxious behavior and avoidance of contact with humans. This may include a greater tendency to retreat when approached or attempts to snap or bite under these circumstances. It has been reported by owners that dogs treated with corticosteroids tended to react aggressively when petted or even approached, and that their dogs also appeared to be significantly more prone to avoid people or situations (34, 35, 36). It was also found that dogs receiving corticosteroids after being referred for behavioral problems were significantly more likely to exhibit negative behavior compared to dogs not treated with these medications (33). Thus, aggressive behavior in this bitch could potentially be caused by treatment with corticosteroids. However, it should be mentioned that the bitch had been taking these medication for more than two years before implantation of deslorelin and returned to her normal behavior after the implant was removed despite continued treatment with the same dose and type of corticosteroids.

In humans, it has recently been found that Addison's disease can cause psychiatric symptoms that often accompany the cardinal symptoms of adrenal insufficiency and are related to the severity of the disease (37). Therefore, untreated and uncontrolled Addison's disease in dogs could cause similar problems and lead to aggressive behavior. However, as mentioned earlier, the bitch had been treated with medication for more than two years and was very well controlled. Because both corticosteroids and gonadectomy produce behavioral changes in some animals, it is possible that the corticosteroid treatment with the implant increased the behavioral changes. Despite physiologic doses of corticosteroids used in this case such scenario can not be fully excluded.

A possible mechanism to investigate may be that deslorelin treatment somehow stimulated minor adrenal function by stimulating ACTH-secreting neurons of the pituitary gland, thereby increasing adrenal cortisol production and causing a hyperadrenocortical state (due to concomitant exogenous corticosteroid administration). A recent article (13) reported the development of pituitary carcinoma causing a Cushing-like syndrome in a bitch chronically treated with deslorelin for urinary incontinence. A relationship between the pituitary tumor and the clinical signs could not be confirmed, but the possibility that deslorelin causes a minor stimulation of the pituitary corticotrophs should not be discounted. From this point of view, it would have been interesting to know the status of Na-K balance in this bitch during the period of increased aggressiveness, but we do not have these data.

This case also raises the question of the risk of taking multiple endocrine drugs at the same time and their possible interactions on general health and behavior. Since inappropriate behavior is the most common cause of animals ending up in shelters or abandoned by their owners, it is very important to inform owners before sterilization about possible behavioral side effects which, although rare, are irreversible following surgical gonadectomy. The use of GnRH agonist implants to determine the effects of gonad removal is therefore beneficial and strongly recommended for male dogs and cats, perhaps even prepubertal females, but we cannot say with certainty at this time that such treatment is also recommended for adult females, given all the reported side effects. The veterinary profession should increase awareness about treatments that alter animal behavior and the importance of monitoring and reporting behavioral side effects as adverse events. Drug-drug interactions should be discussed more frequently, as they can affect the effectiveness of the medication and treatment, cause unexpected side effects, or enhance the effects of a particular medication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Agresivno vedenje psice po vstavitvi implantata deslorelina

M. Zakošek Pipan, D. Pavlin

Izvleček: V veterinarski praksi so dolgo delujoči analogi gonadotropin-sproščujočega hormona (GnRH) široko sprejeta alternativa kirurški kastraciji psov. Uporabljajo se tudi pri psicah bodisi za supresijo ali indukcijo estrusa, pri čemer gre za neoznačeno uporabo zdravila. Pri uporabi dolgo delujočih analogov GnRH so pri psicah opazili vedenjske spremembe, kot so moško obnašanje, povečan apetit, rast mlečnih žlez, laktacija, navidezna brejost in urinska inkontinenca. Pri pasjih samcih pa se lahko v začetnem obdobju, ko implantat spodbudi izločanje testosterona, pojavlja agresivnost do drugih psov ali redko drugih živali.

Na naši kliniki smo obravnavali nesterilizirano, tri leta staro psico pasme koker španjel z diagnosticirano Addisonovo boleznijo. Lastniki so želeli nasvet glede možnosti kontracepcije, saj je pri psici v preteklih ciklusih prihajalo do navidezne brejosti in posledičnega mastitisa, ki je zahteval antibiotično zdravljenje. Lastniki so bili zadržani do kirurške sterilizacije, zato so se odločili za vstavitev deslorelinskega implantata. Približno mesec dni kasneje so pri psici opazili začetek agresivnega vedenja, ki je vključevalo stalno lajanje, izjemno razdražljivost in agresijo do drugih psov ter do družinskih članov. Neželeno vedenje se je stopnjevalo do te mere, da lastniki niso mogli več obvladovati svoje psice. Po odstranitvi implantata je opažena agresija umirila, in psica se je vrnila v normalno stanje.

Čeprav je agresivno vedenje do drugih psov in včasih tudi do lastnikov opisano po kirurški kastraciji ali vstavitvi deslorelinskega implantata pri psih, je ta prispevek prvi, ki opisuje izjemno agresivno vedenje do psov in lastnikov pri psici po vstavitvi 4,7 mg deslorelinskega implantata.

Ključne besede: psica; agresija; vedenje; deslorelinski implantat; dolgo delujoči GnRH

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