

Annual reproductive cycle and gametogenesis of the Chinese minnow (*Rhynchocypris oxycephalus*) inhabiting a stream in Seogwipo, Jeju

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Environmental factors generally regulate reproduction in teleost fishes in temperate regions. The Chinese minnow (*Rhynchocypris oxycephalus*), a freshwater cyprinid widely distributed in Northeast Asia, typically shows the gonadal development and spawning in response to increasing water temperature and photoperiod. However, water temperature in water streams in Jeju that populations are inhabiting fluctuated from approximately 15 to 18 °C, suggesting temperature may not be the only regulatory factor of reproductive activity. The present study investigated the annual reproductive cycle and gametogenesis of *R. oxycephalus* inhabiting streams in Jeju to identify the environmental factors influencing reproductive activity. Specimens were collected monthly (10 males and 10 females, respectively) for two years, from May 2024 to April 2026. Gonadosomatic index (GSI) was evaluated, and gonadal histological examinations were conducted to assess the developmental stages of the gonads and reproductive cycle. Female GSI values were consistently higher than those of males and reached a maximum in July (7.1 ± 0.4), indicating the peak spawning period. In females, GSI was significantly decreased after July and again increased in September. Then, the GSI decline gradually through December. Males exhibited the highest GSI in June (3.3 ± 0.4), but the GSI was relatively consistent throughout the year. In female gonads, early vitellogenic oocytes, late vitellogenic oocytes, migratory nucleus oocytes, and post-ovulatory follicle were predominantly observed during maturation and spawning stages, whereas chromatin-nucleolus oocytes, perinucleolar oocytes, cortical alveolus oocytes and oil-droplet oocytes were observed during the winter season. In males, spermatogenesis progressed through spermatogonia, spermatocytes, spermatids, and spermatozoa. Spermatids and spermatozoa were predominant in the spawning season, whereas spermatogonia and spermatocytes were more frequent in winter. The seasonal patterns in gonadal development closely corresponded to the monthly changes in GSI. These findings suggest that the reproductive cycle of *R. oxycephalus* inhabiting streams in Jeju is influenced more strongly by photoperiod than by water temperature. Artificial alterations in light conditions may shift the natural reproductive cycle by modifying the timing of gonadal development and spawning, potentially disrupting the reproductive rhythms of *R. oxycephalus*.

Key words: *Rhynchocypris oxycephalus*, reproductive cycle, gonadosomatic index, photoperiod, artificial alteration

Reproductive cycle and BPG axis-related gene expression in the female temperate wrasse, *Halichoeres tenuispinis*, inhabiting in Jeju

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This study investigated the effects of long-term marine environmental changes on the reproductive characteristics of the temperate wrasse, inhabiting the coastal waters of Jeju. Experimental fish were collected at two-week intervals from the northern coast (Hamdeok) and the southern coast (Bomok) of Jeju between 2023 and 2025, and gametogenesis and reproductive cycles were examined through histological analyses of the gonads. In addition, the expression patterns of genes associated with the brain (Hypothalamus)-pituitary-gonad (BPG) axis were analyzed to investigate endocrine regulation of reproduction. In females, oogenesis proceeded sequentially through oogonia, chromatin-nucleolus oocytes, perinucleolar oocytes, cortical alveolus oocytes, vitellogenic oocytes, germinal vesicle migration and breakdown oocytes, hydrated oocytes, and regressing oocytes. In males, spermatogenesis progressed through spermatogonia, spermatocytes, spermatids, and spermatozoa. Based on the three-year investigation, individuals from both regions exhibited similar seasonal reproductive patterns, consisting of a gonadal development phase in May, a maturation and spawning phase from June to August, and a resting phase from September to October, indicating that this species is a summer-spawning fish. Repeated spawning during the spawning season suggests that this species is an asynchronous multiple spawner. In 2025, analysis of gene expression in the hypothalamus (*gnrh2*) pituitary gland (*fishβ*, *lhβ*), and gonads (*cyp19a*, *cyp19b*) using a one-year sample revealed that seasonal variation patterns generally corresponded with the histologically observed processes of gonadal development and maturation. Spawning characteristics of wrasse may be influenced by water temperature and photoperiod. Monthly frequency analyses indicated an earlier onset of sexual maturation in females from the southern coast in May 2023, which may be associated with relatively higher seawater temperatures recorded in April 2023. Overall, these findings suggest that water temperature and photoperiod may influence sexual maturation and reproductive cycles in wrasse inhabiting the coastal waters of Jeju and provide fundamental information for understanding reproductive adaptation strategies of coastal fish in response to long-term marine environmental changes.

Key words: *Halichoeres tenuispinis*, Reproductive cycle, BPG axis, Gonadal development, Spawning season

Comparison of growth, gonadal development, and reproductive performance of triploid and diploid olive flounder (*Paralichthys olivaceus*) in commercial aquaculture

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This study evaluated growth performance, gonadal development, and reproduction-related gene expressions in diploid and triploid olive flounder (*Paralichthys olivaceus*) at the farm scale. Eggs and milt were collected from sexually mature adults and allowed to fertilize. Half of the fertilized eggs were cold-shocked to induce triploidy, while the other half hatched under normal conditions as diploids. Ploidy was confirmed by flow cytometry. Triplicate tanks per treatment were stocked in May 2023 with 3000 three-month-old juveniles (3.42.2 g) per tank at a commercial farm, and sampling was conducted bimonthly for one year. Diploid fish showed higher body weight and total length compared to triploid fish. Gonadosomatic index of diploid females exhibited a clear annual reproductive cycle, whereas triploids showed no gonadal development. At the onset of spawning season, diploid females showed yolk accumulation, followed two months later by vitellogenesis and residual follicle layers. Ovaries of triploid females were dominated by germ cells. Diploid males showed seasonal variations in gonadosomatic index, and spermatocytes and sperm were dominant in February. Triploid males exhibited limited testicular development, characterized by spermatogonia by April. Triploid fish showed lower follicle-stimulating hormone subunit gene expression compared to diploids. Cytochrome P450 family 19 subfamily A gene expression was lower in triploid females than in diploids. During the spawning season, cholesterol side-chain cleavage enzyme and cytochrome P450 family 11 subfamily C expressions were significantly higher in diploid males than in triploids. Overall, triploidy did not efficiently redirect energy from suppressed gonadal development to somatic growth in olive flounder.

Key words: Olive flounder, *Paralichthys olivaceus*, Triploid, Diploid, Reproductive

Effects of marine heatwaves and marine coldspells on histological alterations and hematophysiological responses in pearl-spot chromis (*Chromis notata*)

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Recent climate change-induced alterations in marine environmental conditions, particularly seawater temperature, have been reported to affect the distribution, species composition, and maturation processes of marine organisms throughout marine ecosystems. Among these changes, the increasing frequency and intensity of marine heatwaves (MHWs) and marine coldspells (MCSs) have emerged as major physical stressors in marine ecosystems and are expected to substantially affect temperate fish species during summer and winter, respectively. This study investigated the histological and hematophysiological responses of pearl-spot chromis (*Chromis notata*), a representative coastal fish species inhabiting the waters around Jeju Island, under artificial marine heatwave (AMHW) and artificial marine coldspells (AMCS) conditions to evaluate the potential impacts of future extreme thermal events.

Two replicate tanks were established for each treatment, with 50 fish (total length: 12.26 ± 0.99 cm; body weight: 27.31 ± 6.45 g) randomly assigned to each tank. For the AMHW treatment, water temperature was increased from 27 °C to 30 °C at a rate of 1 °C per day and maintained for 7 days, while the control group was maintained at 27 °C throughout the experiment. For the AMCS treatment, water temperature was decreased from 14 °C to 11 °C at a rate of 1 °C per day and maintained for 10 days, whereas the control group was maintained at 14 °C. Hematological parameters and histological changes in the gill, muscle, liver, head kidney, and spleen were subsequently analyzed.

All fish exposed to AMHW died by day 7, whereas no mortality was observed in the AMCS group throughout the 10-day experimental period. Histological analysis revealed progressive tissue damage in the gill, muscle, liver, head kidney, and spleen under AMHW conditions. In contrast, under AMCS conditions, histological alterations were primarily limited to the gill and liver, while no remarkable changes were observed in the remaining tissues. Hematological analysis showed decreases in red blood cell count (RBC), hematocrit (Hct), and hemoglobin (Hb) under both AMHW and AMCS conditions. Under AMHW, glucose increased, whereas total protein, cholesterol, and calcium decreased, accompanied by significant increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicating hepatic injury. In contrast, AMCS resulted in decreased glucose and total protein levels and a tendency toward increased cholesterol, while liver enzyme activities and electrolyte concentrations remained unchanged.

Overall, these findings demonstrate that both marine heatwaves and marine cold spells induce substantial physiological stress and tissue damage in *C. notata*, potentially disrupting metabolic processes and reducing survival. The results further suggest that the increasing occurrence of marine heatwaves and marine coldspells associated with climate change may pose a significant threat to temperate marine ecosystems.

Key words: *Chromis notata*, Marine ecosystem, Marine heatwave, Marine coldspells, Temperate zone

Early sex determination of Olive flounder (*Paralichthys olivaceus*) using sex-specific gene expression profiles and molecular markers

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The advancement of sex identification technologies in aquaculture has primarily focused on genetic, histological, and hematological approaches based on morphological and sexual dimorphism. The olive flounder (*Paralichthys olivaceus*) is a commercially important species in the South Korean aquaculture industry, where females exhibit more than 30% higher growth rates than males. Consequently, the development of early sex identification methods is advantageous for increasing productivity in aquaculture. In this study, we aimed to develop a sex identification method based on the relative expression ratios of sex-specific gene transcripts prior to morphological sex differentiation. Female-associated genes included ovarian-type aromatase (cyp19a), forkhead box (foxl2), and zona pellucida 3 and 4 (zp3 and zp4), whereas male-associated genes included cholesterol side-chain cleavage (cyp11a), 11 β -hydroxylase (cyp11b), and gonadal soma-derived factor (gsdf). The results showed that the expression level of cyp19a was higher than that of cyp11a in females. Based on the relative expression ratios, individuals were classified as female when the proportion of female-specific gene expression was $\geq 79.08\%$ and as male when it was $\leq 5.5\%$. To further identify potential molecular markers, differential gene expression (DEG) analysis was conducted using brain tissues from individuals after the sex differentiation stage. However, although several genes exhibited significant differences in expression between sexes, none were directly associated with sex determination or differentiation mechanisms. In addition, considering that olive flounder can undergo masculinization under stress conditions, the expression patterns of stress-related genes, corticotropin-releasing hormone (crh) in brain tissue and glucocorticoid receptor (gr) in gonadal tissue, were analyzed. However, no significant differences in the expression of these genes were observed between males and females. These results demonstrate that sex identification is feasible prior to the morphological sex differentiation stage using relative expression ratios of sex-specific transcripts. In contrast, transcriptomic analysis after sex differentiation and the expression of stress-related genes appear to have limitations as reliable markers for sex identification. Therefore, sex-specific transcripts during the pre-differentiation stage may serve as effective indicators for the development of early sex identification technology in olive flounder.

Key words: *Paralichthys olivaceus*, sex identification, Sex-specific gene, Molecular markers, Aquaculture

Effects of dietary *Haematococcus lacustris* supplementation of heat stress resistance in juvenile *Fenneropenaeus chinensis*

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Fenneropenaeus chinensis is a major aquaculture crustacean cultivated along the western coast of Korea. However, the rising summer water temperatures associated with recent climate change have emerged as a serious concern in *F. chinensis* aquaculture, threatening productivity through impaired growth, reduced immune function, and mass mortality. Water temperatures in earthen pond aquaculture farms have been reported to reach as high as 34.5°C, underscoring the urgent need for management strategies to mitigate heat stress. *Haematococcus lacustris* is a natural carotenoid source capable of accumulating more than 3% astaxanthin on a dry weight basis. However, studies specifically examining the effects of dietary *H. lacustris* supplementation on heat stress tolerance in crustaceans remain limited. Therefore, this study aimed to evaluate the effects of dietary *H. lacustris* supplementation on body carotenoid accumulation and the expression of genes associated with heat stress resistance in juvenile *F. chinensis*. Diets supplemented with *H. lacustris* at various concentrations (0, 100, 200 mg/kg) were administered for three weeks. After the feeding trial, total body carotenoid content was analyzed. The shrimp were then subjected to acute heat stress at 33°C, and changes in the expression levels of MnSOD, CAT, and HSP70 were analyzed in the hepatopancreas. Total body carotenoid content increased significantly in proportion to the dietary *H. lacustris* supplementation level ($p < 0.05$). Following acute heat stress at 33°C, MnSOD and CAT gene expression levels in the *H. lacustris*-supplemented groups were significantly elevated compared to the control ($p < 0.05$), while HSP70 gene expression was significantly decreased ($p < 0.05$). These trends were most pronounced in the 200 mg/kg group. Collectively, these results suggest that dietary *H. lacustris* supplementation promotes body carotenoid accumulation, which in turn enhances the expression of antioxidant defense genes and suppresses the heat shock response, thereby improving heat stress tolerance in juvenile *F. chinensis*.

Keywords: *Fenneropenaeus chinensis*, heat shock, *Haematococcus lacustris*, antioxidant

Micro-CT-Based Assessment of Thalidomide Derivative-Induced Skeletal Malformations in the Common Marmoset

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Thalidomide is a well-established human teratogen that causes limb reduction and craniofacial malformations. The common marmoset (*Callithrix jacchus*) is one of the few laboratory animal species that recapitulates these characteristic developmental abnormalities, making it a valuable positive-control model for enhanced pre- and postnatal development (ePPND) studies. However, skeletal evaluation in this species still relies largely on qualitative and destructive Alizarin Red S staining, while quantitative reference data for skeletal assessment remain limited. Therefore, this study piloted micro-computed tomography (micro-CT) as a non-destructive and quantitative approach to complement gross morphological examination. One pregnant common marmoset received the thalidomide derivative EM-12 at 5 mg/kg once daily by oral gavage from gestational day (GD) 50 to GD57. One control dam received DMSO as the vehicle according to the same dosing schedule. The EM-12-exposed dam delivered three stillborn offspring at GD141. The vehicle-control dam delivered three offspring; one died on the day of birth, while two survived to postnatal day (PND) 93 and were subsequently euthanized for evaluation. All neonates underwent gross morphometric examination followed by micro-CT imaging and three-dimensional (3D) reconstruction. The measured parameters included interorbital distance, mandibular length, gonial angle, and long-bone lengths normalized to crown-rump length. All three EM-12-exposed neonates exhibited congenital malformations of varying severity, including bilateral hindlimb agenesis, a 4-mm forelimb bud, single-digit forelimbs, unilateral renal agenesis, and splenic hypoplasia. Micro-CT confirmed appendicular skeletal defects and enabled quantitative assessment of craniofacial features, including interorbital distance, mandibular length, and gonial angle. Importantly, micro-CT preserved specimens for subsequent analyses. Although preliminary and not statistically generalizable due to the inclusion of only one dam per group, these findings demonstrate the potential of micro-CT as a quantitative, non-destructive skeletal assessment tool and establish a measurement framework for the ongoing full-scale study. This study was supported by the Korea Health Technology R&D Project (RS-2023-00266010) through KHIDI, funded by the Ministry of Health and Welfare, Republic of Korea.

Keywords: Common marmoset; Thalidomide; Developmental toxicity; Teratogenicity; Congenital malformation

Periodic reversal of the heartbeat in *Halocynthia* juveniles

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Among animals with open circulatory systems, many arthropods and some annelids exhibit a characteristic pattern in which the direction of heartbeat periodically reverses. Among deuterostomes, tunicates are highly unusual in exhibiting this phenomenon. In this study, we investigated the onset of the heartbeat and its periodic reversal in the tunicate *Halocynthia roretzi* juveniles. In both adult and juvenile tunicates, the heart is located at the base of the body, beneath the digestive tract. At a water temperature of 13°C, the heartbeat was first observed 10 days after hatching, whereas at 9°C, it was first detected 13 days after hatching. The heartbeat continuously for several minutes, temporarily stopped, and then resumed beating. Upon resumption, the direction of blood flow was consistently reversed. The heartbeat was slower at 9°C than at 13°C and tended to decrease in frequency as the juveniles developed. The duration of heartbeat cessation tended to be longer at 9°C than 13°C, but no clear age-dependent difference was observed. Heartbeat could be observed until approximately four months after hatching at 13°C, when pigment deposition began to occur on the tunic of the juveniles. In future studies, we plan to conduct comparative analyses of heartbeat characteristics among different tunicates species to gain further insight into the mechanisms regulating heartbeat and its periodic reversal.

Key words: tunicate, juvenile, heartbeat, periodic reversal.

Innovations in Health Management for Sustainable Aquaculture: From Nutritional Physiology to AI-Based Disease Prediction and Diagnosis

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Sustainable aquaculture requires an integrated health management strategy that simultaneously addresses productivity, disease resistance, environmental stress, and resource efficiency. Nutritional physiology provides a fundamental basis for this strategy because balanced nutrient supply influences growth, digestive function, immune competence, stress tolerance, early development, and disease susceptibility in cultured aquatic animals. Species-specific determination of nutrient requirements, feeding rates, and stocking densities, together with the use of functional ingredients and alternative protein sources, can therefore improve animal health while reducing dependence on fishmeal and raw fish-based feeds. In addition, integrated multi-omics approaches combining histopathology, digestive physiology, microbiome profiling, transcriptomics, and metabolomics can provide a more comprehensive understanding of complex diseases such as emaciation disease in olive flounder.

However, nutritional and biological information alone is insufficient for the early management of rapidly emerging disease events under fluctuating farm conditions. Recent advances in artificial intelligence enable the integration of water-quality sensors, farm management records, and disease-associated images into a unified health-monitoring framework. In the proposed approach, standardized environmental data are subjected to quality control and time-series analysis to predict temperature and water-quality changes and detect anomalous events. Image segmentation, active learning, and vision-language models are subsequently applied to identify and interpret disease-associated lesions. An AI-agent workflow further integrates visual observations with environmental and management data to generate explainable diagnostic reports and practical response recommendations.

Together, nutritional physiology, multi-omics analysis, and multimodal AI establish a continuum from health promotion and disease prevention to early warning and assisted diagnosis. This integrated framework can reduce dependence on experience-based decision-making, facilitate timely farm responses, and support environmentally responsible and data-driven aquaculture. The convergence of biological knowledge and digital technologies will be essential for improving fish health, farm resilience, and the long-term sustainability of aquaculture production.

Keywords: sustainable aquaculture; fish health management; multimodal artificial intelligence; disease prediction and diagnosis

Establishment, characterization and myogenic differentiation of a novel muscle cell line from Korean rockfish (*Sebastes schlegelii*)

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The establishment of an in vitro skeletal muscle cell model represents a reliable and efficient cellular tool for investigating growth mechanisms and other physiological functions in fish. However, the current understanding of the molecular mechanisms underlying muscle growth and differentiation in teleost fish remains limited. Also, further research is required to develop and characterize fish muscle cell lines to enhance the understanding of myogenesis and determine species-specific responses. Hence, the present study aimed to establish a myoblast cell line, characterize its cell growth and morphology, and examine myogenic differentiation capacity under in vitro conditions. For primary culture, epaxial muscle was excised and cells were isolated using collagenase type 1 (300 U/mL, 1 h, 24°C), seeded in 100-mm cell culture dish with Leibovitz's L-15 (L-15) media supplemented with 20% fetal bovine serum (FBS), 1% penicillin/streptomycin, 1% antibiotic/antimycotic, 10 ng/mL fibroblast growth factor (bFGF), and incubated in 24°C. Cell passaging was performed when confluency reached 80–90% using 0.25% EDTA-trypsin. The cells were continuously sub-cultured for more than 60 passages over 10 month-period, and named Korean rockfish muscle *Sebastes schlegelii*-1 (KRMSS-1) cell line. Cell line was myoblast to fibroblast-like morphology with stabilized doubling time at ~36 h, and high cell viability (>90%). Cells were successfully cryopreserved in 5% dimethyl sulfoxide, with 85-90% recovery rate. Bacterial screening showed mycoplasma-free culture. PCR amplification of CO1 gene provided supporting evidence of rockfish origin of the cell line. Best culture conditions were at 24°C temperature, 20% FBS inclusion, and 10 ng/mL bFGF. Myotube formation was induced by 2% horse serum and differentiated for 12-day period, resulting in myocyte alignment and cell fusion. Phase distribution of G0/G1 phase significantly increased at day 3 post-induction, indicative of cell cycle withdrawal. The protein expression of Pax7 and MyoD expression decreased while myogenin, desmin and myosin heavy chain increased during the 12-day differentiation. Intracellular signaling associated with differentiation program was similarly assessed. Differentiation was associated with increase in the expression of PI3K/Akt/mTor pathway while MEK/ERK pathway exhibited independent response. p-MEK/MEK ratio increased while p-MEK/MEK ratio showed biphasic pattern. The demonstration of cell proliferation capacity and myogenic phenotypes underscores normal cellular functionality and highlights its valuable application in studies related to muscle cell physiology and molecular biology.

Keywords: *Sebastes schlegelii*, muscle cell line, cell differentiation, PI3k/Akt/mTor pathway

Effects of replacing *Undaria pinnatifida* with underutilized seaweeds on growth-related endocrine responses and edible tissue quality of abalone (*Haliotis discus hannai*)

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The Pacific abalone (*Haliotis discus hannai*) is one of the major high-value aquaculture species widely cultured in Asian countries, including Korea, China, and Japan. In abalone aquaculture, *Undaria pinnatifida* is commonly used as a seaweed ingredient in formulated diets due to its high palatability and nutritional value. However, the stable supply of *U. pinnatifida* as a feed ingredient is limited by its extensive use for human consumption and food processing, while its seasonal production may also lead to fluctuations in supply and price. Therefore, the development of alternative ingredients is needed to reduce dependence on this species. Possible alternatives were *Ulva australis* and laver by-products. Macroalgae *U. australis* occur in large quantities along the Korean coast, causing environmental problems through excessive proliferation and accumulation. However, their use as feed ingredients for herbivorous abalone is not fully implemented. Meanwhile, laver by-products are leftover seaweed tissues from harvesting or preprocessing, regarded as waste material. These two alternatives could provide substantial value in terms of the efficient utilization of underutilized resources and may help in feed sustainability for abalone aquaculture. Hence, this study aimed to evaluate the practical applicability of formulated diets containing underutilized seaweed and to assess their potential as substitutes for conventional *U. pinnatifida*. Three experimental diets were prepared: a control diet (Control) containing 30% *U. pinnatifida* meal; Diet 1, in which 75% of the *U. pinnatifida* meal was replaced with a 1:1 mixture of *Ulva* spp. and laver by-products; and Diet 2, in which 100% of the *U. pinnatifida* meal was replaced with *Ulva* spp. These diets were fed to abalone (43.07 ± 0.22 mm, 8.21 ± 1.05 g) for 4 months in a commercial nursery facility. At the end of the feeding trial, survival was statistically highest in both Diet 1 and Diet 2 than the control diet. Also, Diet 2-fed abalones had the highest specific growth rate but not statistically significant from other dietary treatments. Cell-free hemolymph growth hormone levels did not differ significantly among the dietary treatments. Texture analysis showed that hardness was significantly higher in Diet 2 than in the control group, whereas springiness, cohesiveness, chewiness, and shear force did not differ significantly among treatments. Electronic tongue analysis revealed no clear separation among the dietary treatments in the overall taste profile; however, umami intensity was significantly higher in Diet 1 and Diet 2 than in the. The results of this study demonstrated that replacing *U. pinnatifida* with underutilized seaweed resources, including *Ulva* spp. and laver by-products, had no adverse effects on the somatic growth or the overall quality characteristics of the edible portion of abalone. Therefore, *Ulva* spp. and laver by-products may serve as alternative seaweed ingredients capable of replacing *U. pinnatifida* in formulated abalone diets while maintaining physiological responses and edible-part quality.

Key words: *Haliotis discus hannai*, underutilized seaweeds, growth performance, textural properties, taste characteristics

Cellular origin shapes adipogenic differentiation and lipid accumulation in seven-band grouper (*Epinephelus septemfasciatus*) cells

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Adipogenic differentiation and lipid accumulation are important determinants of tissue composition and quality in cultivated seafood. In this study, we investigated differences in adipogenic responses between muscle-derived satellite cells (GMSC) and visceral preadipocytes (GPA) isolated from seven-band grouper (*Epinephelus septemfasciatus*). Under the same adipogenic conditions, both cell types showed lipid accumulation and activation of adipogenesis-related factors, whereas the extent and temporal patterns of differentiation differed substantially. GPA displayed a rapid adipogenic response accompanied by transient cell-cycle progression followed by cell-cycle arrest and activation of mTOR/p70S6K and PPAR γ -associated signaling. Regulation of the INSIG-1/SCAP/SREBP-1 pathway was also closely associated with lipid deposition in GPA, as inhibition of this pathway markedly suppressed triglyceride accumulation. However, extended adipogenic induction resulted in metabolic stress and a pronounced reduction in GPA viability. In comparison, GMSC underwent a slower and less pronounced adipogenic transition while maintaining relatively high cell viability throughout the induction period. Collectively, these results indicate that the adipogenic capacity and metabolic response of seven-band grouper cells are strongly influenced by their cellular origin. Understanding these lineage-specific characteristics may facilitate the selection and combination of muscle- and adipose-derived cells for controlling lipid content and tissue composition in cultivated seafood.

Keywords: *Epinephelus septemfasciatus*, Adipogenic differentiation, Cellular origin, SCAP/SREBP pathway

Development of a Functional 3D Bone Scaffold Incorporating a Nanotechnology–Based Periosteum

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Large bone defects remain challenging to reconstruct because effective regeneration requires both structural support and a biological environment that promotes cellular activity and tissue integration. This study aimed to develop a functional 3D-printed bone scaffold incorporating a nanotechnology-based periosteum and stem cell-derived exosomes and to evaluate its biocompatibility, osteogenic potential, and structural stability in vitro and in a small-animal subcutaneous implantation model. Three-dimensional PCL/OCP composite scaffolds were fabricated at a 70:30 ratio with a 500- μ m pore size, surface-modified with dopamine, loaded with low (10 μ g/mL) or high (100 μ g/mL) concentrations of exosomes, and wrapped with a nanotechnology-based periosteum-like membrane. In vitro responses were evaluated using LV-SEM, LIVE/DEAD staining, CCK-8, BCA protein-release, alkaline phosphatase (ALP), and Alizarin Red S (ARS) assays. LV-SEM confirmed cell attachment in all groups and favorable cell–scaffold interactions on the modified surfaces. Among the exosome-treated scaffolds, the low-exosome group showed the most favorable responses in LIVE/DEAD, CCK-8, and ARS analyses, indicating superior cell viability, proliferation, and mineralization. Both low- and high-exosome groups demonstrated sustained protein release through day 21 and increasing ALP activity over time, although ALP activity at day 14 remained lower than that of the control groups. For in vivo evaluation, scaffolds were implanted subcutaneously and collected at 2 and 4 weeks for gross, histological, and Micro-CT assessment. Histologically, the scaffolds demonstrated generally favorable biocompatibility without widespread severe inflammation, necrosis, or tissue injury. Scaffold–tissue contact, tissue infiltration, and collagen-rich connective tissue formation further indicated progressive tissue integration and an environment compatible with osteogenic potential. Micro-CT showed favorable structural preservation in the exosome-treated groups. At 4 weeks, both low- and high-exosome groups exhibited significantly greater object volume and object volume fraction than the control, while the high-exosome group additionally showed significantly greater structure thickness and connectivity density. Overall, dopamine modification, exosome loading, and nanotechnology-based periosteum wrapping produced a functional scaffold with favorable cellular compatibility, osteogenic activity, tissue integration, and structural stability. The low-exosome group demonstrated the most consistent in vitro biological response, whereas the high-exosome group showed particularly favorable structural preservation in vivo.

Keywords: PCL:OCP, 3D-printed bone scaffold, Exosomes, Nanotechnology-based periosteum, Bone regeneration

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Development of a Swim-up-Based Sperm Cryopreservation Protocol for the Common Marmoset (*Callithrix jacchus*)

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The common marmoset (*Callithrix jacchus*) is widely used as a nonhuman primate model because of its small body size, relatively short generation time, and suitability for reproductive and genetic studies. These characteristics are also advantageous for the production of gene-edited and transgenic marmoset models. As the use of these animals increases, methods for preserving valuable genetic resources are becoming more important. In particular, sperm cryopreservation can provide a means of storing the genetic material of selected males and may support assisted reproductive techniques such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). One practical difficulty in working with marmoset semen is its variable and often incomplete liquefaction. Previous studies have described the persistent viscosity of marmoset semen, and in our preliminary experiments, the degree of liquefaction and separation of the sperm-containing fraction also differed among ejaculates. This variability can make it difficult to prepare a consistent sperm population for freezing. We therefore examined a pre-freeze sperm preparation approach in which motile sperm are collected by swim-up before cryopreservation. The aim is to reduce the influence of differences in semen liquefaction and to obtain a more uniform population of motile sperm for freezing.

Two commercially available freezing media, SpermFreeze Solution™ (Vitrolife) and Quinn's Advantage Sperm Freezing Medium™ (SAGE), were included in the protocol development and compared under the same freezing and thawing conditions. To evaluate the cryopreservation process, key parameters such as sperm concentration, total motility, progressive motility, and post-thaw recovery were selected and are currently under investigation.

This study aims to establish a practical sperm cryopreservation method for the common marmoset by using pre-freeze swim-up selection to reduce variation associated with incomplete semen liquefaction. The resulting protocol may support sperm banking and future reproductive applications, including IVF and ICSI, as well as the preservation of genetically valuable, gene-edited, and transgenic marmoset lines. This study was supported by the Korea Health Technology R&D Project (RS-2023-00266010; RS-2025-16063082) through KHIDI, funded by the Ministry of Health and Welfare, Republic of Korea.

Key words: Common marmoset, sperm cryopreservation, swim-up, semen liquefaction, genetic resource preservation

Temporal Dynamics of Pulmonary ILC3 Frequency Accompanying Bacterial Clearance in a Murine Model of *Acinetobacter baumannii* Pneumonia

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Acinetobacter baumannii is a leading cause of multidrug-resistant nosocomial pneumonia, yet the innate immune mechanisms governing pulmonary defense remain incompletely defined. Among innate immune cells, group 3 innate lymphoid cells (ILC3s), ROR γ t-expressing innate lymphocytes, contribute to host defense against several respiratory bacteria, but their dynamics during *A. baumannii* lung infection are unknown. This study aimed to analyze the kinetics of pulmonary ILC3s following *A. baumannii* infection and to assess their relationship with bacterial burden and infection-associated weight loss. Six-week-old female C57BL/6J mice were inoculated intranasally with 1×10^9 CFU of *A. baumannii* or sterile PBS as a control. Lungs were harvested at days 1, 2, 4, 6, and 8 post-inoculation, and ILC3s were quantified by flow cytometry as a percentage of CD45⁺ cells. Bacterial burdens in the lung, spleen, and blood, as well as body weight, were monitored in parallel. *A. baumannii*-infected mice showed maximal body weight loss at day 3 and recovered by days 6–8. Pulmonary bacterial burden was highest at day 1 and became undetectable by day 6. A lower but detectable splenic bacterial burden was observed at days 1–2, whereas bacteremia was not detected. Compared with PBS-treated controls, pulmonary ILC3 frequency was significantly reduced at days 1, 2, and 4 after infection and returned to control levels by days 6–8, paralleling bacterial clearance and recovery of body weight. *A. baumannii* infection induced a transient decline in pulmonary ILC3 frequency during the acute phase, which resolved as bacteria were cleared. These findings suggest that lung ILC3s are dynamically regulated during the early innate response to *A. baumannii*. Future studies assessing ILC3 absolute numbers and cytokine production are needed to define their functional contribution.

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Key words: *Acinetobacter baumannii*, Group 3 innate lymphoid cells, Pneumonia, Innate immunity, Flow cytometry

Scirrhous Hepatocellular Carcinoma in a Dog: Diagnostic Challenges and Postoperative Sorafenib Administration

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Scirrhous hepatocellular carcinoma (HCC) is a rare histologic subtype of HCC characterized by abundant fibrous stroma, and its clinical behavior, diagnostic features, and optimal management in dogs remain poorly understood. Its diagnosis is particularly challenging due to the predominance of fibrous tissue in biopsy specimens, which may lead to misinterpretation as benign fibrosis, and due to its atypical immunohistochemical profile. This case report describes the diagnostic challenges and postoperative sorafenib administration in a dog with a hepatic neoplasm most consistent with scirrhous HCC. A 14-year-old neutered male Maltese dog with pituitary-dependent hyperadrenocorticism was found to have a hepatic mass during routine ultrasonographic monitoring. Initial Tru-Cut biopsy revealed fibrosis without evidence of malignancy, but subsequent enlargement prompted surgical resection. Histopathology revealed poorly demarcated polygonal neoplastic cells within abundant dense fibrous stroma. Immunohistochemistry revealed diffuse cytokeratin 19 positivity and absent hepatocyte paraffin 1 immunoreactivity. Together with staging findings, these results were most consistent with scirrhous HCC. Postoperative sorafenib tosylate (Nexavar®, Bayer Korea, Seoul, Korea) was initiated at 2 mg/kg PO q12h. After nine months, mild leukopenia developed and resolved following temporary discontinuation. Sorafenib was resumed at 2 mg/kg PO q24h for an additional three months. Follow-up ultrasonography showed no new hepatic masses or obvious progression of remaining nodules. This case highlights diagnostic challenges posed by fibrous stroma and immunohistochemical overlap with biliary or ductular neoplasms. To our knowledge, this is the first report describing postoperative sorafenib administration in a dog with a hepatic neoplasm most consistent with scirrhous HCC, providing preliminary information on its feasibility and tolerability.

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Keywords: Canine, Hepatocellular carcinoma, Scirrhous HCC, Sorafenib

Pulmonary Inflammation and NET-Associated Responses Following Acute *Acinetobacter baumannii* Infection

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Acinetobacter baumannii is a multidrug-resistant opportunistic pathogen that causes pneumonia and sepsis. Neutrophil extracellular traps (NETs) are important innate immune structures involved in antibacterial defense. However, whether NET formation occurs in vivo during *A. baumannii* lung infection remains poorly understood. This study aimed to investigate whether intranasal *Acinetobacter baumannii* infection promotes NET-associated responses in vivo and to characterize the accompanying bacteriological, cellular, and histopathological changes in the lungs. Female C57BL/6J mice were intranasally infected with *A. baumannii* and euthanized at 6, 24, and 48 h post-infection. Body weight, bacterial burden, bronchoalveolar lavage fluid (BALF) differential cell counts, blood smear findings, myeloperoxidase (MPO)-DNA complex levels, lung histopathology, and immunohistochemical expression of MPO and citrullinated histone H3 (citH3) were evaluated. Infected mice showed significant body weight loss from 24 to 48 h post-infection. Pulmonary bacterial loads were high at 6–24 h and significantly decreased at 48 h. BALF analysis revealed marked neutrophil recruitment in infected mice. NET-like filamentous structures in blood smears, increased MPO-DNA complex levels, and elevated pulmonary MPO and citH3 expression were observed, with the strongest NET-associated changes detected at 24 h. Histopathology showed peribronchial and perivascular inflammatory infiltration with alveolar disruption, which was most prominent at 24 h and persisted through 48 h. *A. baumannii* infection induced acute pulmonary inflammation with neutrophil recruitment, lung tissue injury, and increased NET-associated markers. These findings suggest that NET formation is promoted during the early phase of *A. baumannii* lung infection. Further studies using lung-isolated neutrophils and transcriptomic analysis are needed to clarify the molecular mechanisms regulating NET formation during *A. baumannii* lung infection.

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Key words: *Acinetobacter baumannii*, neutrophil extracellular traps, pulmonary inflammation, neutrophils, mouse model

Clinical presentation and short-term outcome of clear cell adnexal carcinoma of the skin in a dog

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Clear cell adnexal carcinoma is a rare cutaneous adnexal neoplasm in dogs, characterized by a lack of definitive differentiation toward apocrine, sebaceous, or follicular lineages. Although regarded as a low-grade neoplasm with a favorable outcome following surgical excision, its characteristics remain incompletely characterized because of its rarity. This case describes the clinicopathological features and short-term outcome of cutaneous clear cell adnexal carcinoma in a dog. A 15-year-old neutered female English Cocker Spaniel was presented with enlarging masses on the right third digit (28.9×29.43 mm) and thoracic region (38.84×29.79 mm), both of which were bleeding. Laboratory tests, radiographs, and ultrasonography revealed no remarkable findings or evidence of metastasis. Fine-needle aspiration and impression cytology of both masses were nondiagnostic, showing predominantly inflammatory components. Punch biopsies of both masses were subsequently performed; impression cytology of the digital lesion demonstrated malignant epithelial cells, whereas the thoracic lesion revealed only inflammatory cells and erythrocytes. Histopathology of the digital mass revealed clear cell adnexal carcinoma featuring multilobulated nests of epithelial cells with abundant vacuolated cytoplasm. Thoracic biopsy demonstrated dermal and subcuticular hemorrhage without evidence of neoplasia. Given the favorable prognosis of surgical excision, surgical treatment was performed; the third digit was amputated and the thoracic mass was excised. Antineoplastic treatment was not performed, and no recurrence or metastasis was observed during the 8-week follow up. Further immunohistochemical staining is currently underway to characterize the tumor. This case highlights an exceptionally rare cutaneous adnexal carcinoma in a dog, describing its diagnosis and adding valuable clinical data.

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Key words: Clear cell adnexal carcinoma, Histopathology, Immunohistochemistry, English Cocker Spaniel

Urine protein electrophoresis identifies distinct urinary protein patterns in two dogs with lymphoma

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Proteinuria is common in dogs with lymphoma, but routine tests quantify protein loss without characterizing its molecular-weight distribution. This case report evaluated whether urine protein electrophoresis (UPE) could provide complementary information on proteinuria in two dogs with lymphoid malignancies. We analyzed urine proteins by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis after protein normalization and Coomassie Brilliant Blue staining. Banding patterns were interpreted according to molecular-weight regions associated with glomerular and tubular protein loss. Case 1, a 10-year-old neutered male Maltese with stage Vb high-grade B-cell lymphoma, had severe proteinuria (urine protein-to-creatinine ratio, 42) and concurrent Gram-negative bacteriuria. Banding in the 45–60 kDa region suggested tubular or mixed proteinuria rather than a pattern dominated by free immunoglobulin light chains. Case 2, a 9-year-old intact female Jindo with stage V cytotoxic T-cell lymphoma or leukemia, had marked proteinuria, hypoalbuminemia, and increased symmetric dimethylarginine. Broad, predominant banding at approximately 35–60 kDa, together with bands at approximately 60–70 kDa and 20–35 kDa, supported mixed glomerular–tubular proteinuria. These findings demonstrate that severe proteinuria in dogs with lymphoid malignancies may exhibit heterogeneous electrophoretic patterns and should not be attributed solely to Bence-Jones proteinuria. UPE may help guide further renal and urinary evaluation and longitudinal monitoring after consideration of pre-renal and post-renal causes. However, the absence of immunofixation and renal histopathology precluded definitive identification of specific proteins or underlying renal lesions. Both dogs died before serial UPE reassessment, precluding evaluation of longitudinal or prognostic utility. Larger prospective studies are warranted to establish the clinical utility of UPE in tumor-associated proteinuria.

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Key words: Dog, Lymphoma, Proteinuria, Urine protein electrophoresis, Renal protein loss

Perioperative Changes in Peripheral Blood Myeloid-Derived Suppressor Cells in Dogs with Solid Tumors: A Preliminary Study of Two Cases

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Myeloid-derived suppressor cells (MDSCs) are immunosuppressive cell populations involved in tumor immune evasion, progression, and metastasis, and increased peripheral blood MDSCs have also been reported in dogs with cancer compared with healthy dogs. However, most veterinary studies to date have focused on cross-sectional assessment of MDSC levels at a single time point, and little is known about the dynamic changes in MDSCs associated with changes in tumor burden following surgical resection. In human cancer patients, MDSCs may transiently increase immediately after surgery in response to tissue injury, inflammation, and neuroendocrine stress, followed by a decline during recovery after tumor removal. This preliminary study aimed to determine whether similar perioperative changes in peripheral blood MDSC phenotypes could be observed in dogs with solid tumors.

Two dogs undergoing surgical resection of solid tumors were serially evaluated before surgery, during the early postoperative period, and during the recovery period. Peripheral blood MDSC populations, including total MDSCs, polymorphonuclear MDSCs (PMN-MDSCs), and monocytic MDSCs (M-MDSCs), were assessed using flow cytometry. Histopathologic examination confirmed a clear surgical margin in one dog and an incomplete margin in the other. In both dogs, the proportion of total MDSCs increased during the early postoperative period. In the dog with complete tumor resection, total MDSCs increased from 25.25% preoperatively to 54.24% in the early postoperative period and subsequently decreased to 35.26% during recovery. In contrast, in the dog with an incomplete surgical margin, total MDSCs increased from 44.82% preoperatively to 46.32% in the early postoperative period and further increased to 71.44% during recovery. This late postoperative increase was predominantly associated with a marked increase in the PMN-MDSC population, from 17.51% to 67.51%. Thus, although both dogs showed an early postoperative increase in MDSCs, their subsequent changes during recovery were divergent, with a decrease after complete resection and a continued increase after incomplete resection.

These preliminary observations suggest that dogs with solid tumors may exhibit an early postoperative increase in circulating MDSCs similar to that reported in human cancer patients. Furthermore, postoperative MDSC kinetics during the recovery period may potentially reflect the completeness of tumor removal or residual tumor burden. However, because this study included only two cases, the effects of tumor type, systemic inflammation, and interindividual differences in baseline MDSC levels could not be excluded, and no causal relationship between surgical margin status and MDSC changes can be established. Further prospective studies with larger populations and standardized perioperative sampling are warranted to investigate the association of MDSC kinetics with surgical margins, metastasis, recurrence, and survival outcomes.

Key words: Myeloid-derived suppressor cells, canine cancer, surgery, tumor burden, flow cytometry

Diagnostic Utility of Hematologic Inflammatory Indices for Differentiating Idiopathic and Structural Epilepsy in dogs

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Background: Neuroinflammation contributes to epilepsy, but biomarkers differentiating idiopathic epilepsy (IE) from structural epilepsy (SE) in dogs are limited.

Objectives: To compare hematologic inflammatory indices among healthy, IE, and SE dogs and assess the ability of distinguish SE from IE.

Material and Methods: Records of 179 dogs (62 healthy, 72 IE, and 45 SE) were reviewed. The SE group included 21 dogs with meningoencephalitis of unknown etiology (MUE), 14 with hydrocephalus, 8 with brain tumors, and 2 with ischemic stroke. Hematologic counts were used to calculate the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), systemic inflammation response index (SIRI), and aggregate index of systemic inflammation (AISII). Groups were compared using Kruskal–Wallis tests with Dunn’s post hoc tests. Receiver operating characteristic analysis evaluated discrimination between IE and SE.

Results: All five indices differed among the healthy, IE, and SE groups (all overall $P < 0.001$). NLR, SII, SIRI, and AISII were higher in SE than in healthy and IE dogs (all adjusted $P < 0.001$). PLR was also higher in SE than in healthy ($P = 0.005$) and IE dogs ($P < 0.001$). No index differed between healthy and IE dogs. Dogs with MUE had higher values for all indices than healthy and IE dogs. Areas under the curve were 0.764 for NLR, 0.727 for PLR, 0.767 for SII, 0.712 for SIRI, and 0.727 for AISII. SII had the numerically largest area under the curve (0.767; 95% confidence interval, 0.679–0.855), with 80.0% sensitivity and 61.1% specificity at a cutoff > 968.1 .

Conclusions: Hematologic inflammatory indices, particularly SII, were increased in dogs with SE, with the most consistent elevations in MUE. These indices may help identify dogs more likely to have structural brain disease and prioritize advanced diagnostic evaluation, but their diagnostic utility is limited when used alone.

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Key words: canine; hematologic inflammatory indices; idiopathic epilepsy; structural epilepsy; systemic immune-inflammation index (SII)

Development of an Artificial Intelligence–Based Quantitative Analysis System for Feline Thyroid Scintigraphy

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Feline hyperthyroidism is one of the most common endocrine disorders in older cats, and thyroid scintigraphy is useful for identifying the location, distribution, and functional activity of hyperfunctioning thyroid tissue. Quantitative scintigraphic parameters, including the thyroid-to-salivary gland ratio (TSR), thyroid-to-background ratio (TBR), and thyroid volume, may provide additional objective information for disease assessment and treatment planning. However, these measurements are generally performed manually and may be affected by operator-dependent variation. The aim of this study was to develop an artificial intelligence-based system for automated quantitative analysis of feline thyroid scintigraphy and to investigate the associations of scintigraphic parameters, including TSR, TBR, and thyroid volume, with clinical indicators of hyperthyroidism, including serum total thyroxine concentration and the extent of clinical signs.

Thyroid scintigraphic images obtained from cats examined at Chungbuk National University Veterinary Medical Center were retrospectively collected. Thyroid regions were manually annotated to construct a dataset for model development. Quantitative analysis was performed using the original DICOM images, and the analysis workflow was designed to calculate TSR, TBR, and thyroid volume based on tracer activity within the detected thyroid regions. Automated thyroid segmentation and quantitative measurements generated by the model were compared with manually obtained results to assess agreement and analytical performance. The applicability of the automated approach was also evaluated across different scintigraphic patterns, including unilateral and bilateral thyroid lesions. This study is expected to provide a standardized and reproducible approach for quantitative evaluation of feline thyroid scintigraphy and to clarify the clinical relevance of scintigraphic parameters by investigating their associations with disease severity and clinical characteristics in cats with hyperthyroidism.

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Key words: feline hyperthyroidism, artificial intelligence, thyroid scintigraphy, quantitative analysis, clinical relevance

Assessment of Serum MMP-9 and TIMP-1 Levels in Canine Idiopathic Epilepsy

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Idiopathic epilepsy (IE) is a frequent neurological disorder in dogs, with neuroinflammation implicated in seizure activity and disease progression. Matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) modulate extracellular matrix remodeling and blood-brain barrier permeability, and both have been associated with seizure-induced inflammatory pathways. This prospective observational study aimed to quantify and compare serum MMP-9 and TIMP-1 concentrations between 27 client-owned dogs with IE and 16 clinically healthy control dogs using enzyme-linked immunosorbent assay (ELISA). Nonparametric statistical methods revealed that dogs with IE showed higher serum TIMP-1 concentrations (median, 76.82 ng/mL vs. 25.37 ng/mL; $P = 0.0004$) and lower MMP-9/TIMP-1 ratios (median, 0.322 vs. 0.896; $P = 0.0035$) than healthy controls. Serum MMP-9 concentrations did not differ significantly between IE dogs and controls (median, 26.28 ng/mL vs. 19.85 ng/mL; $P = 0.0615$). However, among dogs with IE, those with status epilepticus lasting ≥ 5 minutes had higher serum MMP-9 concentrations than those with shorter seizure events (median, 33.03 ng/mL vs. 22.96 ng/mL; $P = 0.0125$), whereas no other subgroup differences were identified (all $P > 0.05$). In conclusion, alterations in circulating TIMP-1 and the MMP-9/TIMP-1 ratio occur in canine IE, and elevated MMP-9 is observed in dogs with prolonged status epilepticus, indicating an involvement of matrix regulation in seizure-related neuroinflammatory responses.

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Key words: canine idiopathic epilepsy, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, neuroinflammation, blood-brain barrier

Epidemiologic Features and Treatment Patterns of Feline Hyperthyroidism

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Feline hyperthyroidism (FHT) is a common endocrinopathy of older cats, but information regarding its clinical characteristics and treatment patterns in Asian populations remains limited. This study investigated the epidemiologic and clinical features of hyperthyroid cats undergoing radioiodine therapy (RAIT) in South Korea. Medical records of 152 cats treated with RAIT at Chungbuk National University Veterinary Teaching Hospital between 2018 and 2025 were retrospectively reviewed. Data regarding signalment, housing environment, clinical signs, disease duration, route of RAIT selection, previous antithyroid drug (ATD) treatment, methimazole formulation and adverse effects, serum total thyroxine (tT4) concentration, and thyroid scintigraphic findings were evaluated. Relationships between disease duration and clinical, treatment-related, and scintigraphic variables were also assessed. The median age at diagnosis was 12 years (interquartile range [IQR], 10–13 years), and 19.7% of cats were diagnosed before 10 years of age. All cats were strictly housed indoors. The median interval from diagnosis to RAIT was 5 months (IQR, 3–10 months), and only 4.6% had a disease duration exceeding 2 years. Unilateral thyroid involvement was observed in 50.7% of cats, whereas severe hyperthyroidism (tT4 $\geq$ 19.4 μ g/dL) was identified in 10.5%. Selection of RAIT was initiated by owners in 59.9% of cases and recommended by veterinarians in 40.1%. Among cats previously treated with ATDs, human-licensed or generic methimazole was used more commonly than veterinary-licensed methimazole (84.0% vs 18.1%). Pruritus occurred more frequently in cats receiving human-licensed or generic formulations than in those receiving veterinary-licensed methimazole (20.7% vs 3.8%; $P = 0.047$). Longer disease duration was associated with higher serum tT4 concentrations, greater thyroid tumor volume, increased thyroid-to-salivary gland and thyroid-to-background ratios, a greater frequency of refractory responses to ATDs, and an increased proportion of bilateral thyroid involvement ($P < 0.05$). These findings characterize the clinical and treatment-related features of hyperthyroid cats receiving RAIT in South Korea and indicate that RAIT was commonly selected relatively soon after diagnosis. The observed increase in biochemical and scintigraphic disease severity with longer disease duration further supports routine thyroid screening and early consideration of definitive treatment in affected cats.

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Key words: feline hyperthyroidism, radioiodine therapy, methimazole, thyroid scintigraphy, epidemiology

Clinical responses to mesenchymal stem cell therapy in two cats with feline atopic syndrome

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Feline atopic syndrome (FAS) is a hypersensitivity disorder influenced by genetic and environmental factors. Mesenchymal stem cells (MSCs) are multipotent stromal cells derived from various tissues, including adipose and uterine tissues, with immunomodulatory effects that may reduce FAS-associated inflammation and pruritus. This case series describes MSC therapy in two cats with FAS. Case 1, a 5-year-old neutered male mixed-breed cat, presented with ear pruritus and abdominal excoriations. FAS was diagnosed after excluding other skin diseases through diagnostic testing. Despite conventional treatment with dietary management, antibiotics, antifungals, anti-inflammatory agents, topical therapy, and immunotherapy, the clinical course remained refractory; therefore, adipose-derived MSCs were administered intravenously at 1×10^6 cells/kg weekly for four weeks. Response was assessed using the Visual Analog Scale for feline pruritus (VAScat), Feline Dermatitis Extent and Severity Index (FEDESI), Scoring Feline Allergic Dermatitis (SCORFAD), and transepidermal water loss (TEWL), all improving through week 16. No adverse events occurred, and clinical improvement persisted for three months without pruritus recurrence. Case 2, a 5-year-old neutered male Abyssinian cat, presented with inguinal pruritus and hindlimb excoriations and was diagnosed with FAS after the same exclusions. Despite conventional treatment, including immunotherapy, the clinical course remained refractory. Therefore, uterine-derived MSCs were administered at the same dose and route weekly for eight weeks. All measures improved without adverse events, and clinical improvement persisted for four months without recurrence. This case series highlights sustained improvement following MSC therapy in two cats refractory to conventional treatment, supporting its potential as a therapeutic option for FAS.

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Key words: feline atopic syndrome, mesenchymal stem cells, immunomodulation, pruritus, refractory disease

Identifying Districts with Suspected Underreporting of Severe Fever with Thrombocytopenia Syndrome during the Early Surveillance Period, Republic of Korea, 2013–2015

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Emerging vector-borne diseases are a growing concern in the Republic of Korea, where severe fever with thrombocytopenia syndrome (SFTS) became a nationally notifiable disease in 2013. Infections were retrospectively confirmed in specimens predating formal surveillance, and case fatality rates declined from 47.2% in 2013 to 26.6% in 2015, then stabilized after clinical practice guidelines were disseminated in 2016, implying limited sensitivity during the early surveillance period. We therefore developed a model of district (si-gun-gu)-level SFTS occurrence from a period of relatively stable surveillance (2017–2024). Applying this model to 2013–2015, we regarded districts in which SFTS was predicted to occur but no case was notified as having suspected underreporting. We analyzed all 250 districts, defining the outcome as the presence or absence of a notified case per district-year. Multicollinearity screening retained 21 predictors including satellite-derived environmental, meteorological, and community-level socioeconomic variables. We employed logistic regression, random forest, gradient boosting machine, XGBoost, and their ensemble, with province (si-do) or district as a random intercept in the logistic models and province as a predictor in the tree-based models (11 models total). Under an expanding-window design, models were trained on 2020–2024, tuned on 2019, validated on 2018, and evaluated once on 2017. The ensemble incorporating province was selected as the final model, with an evaluation AUC of 0.707 (95% CI 0.643–0.771) at a classification threshold of 0.506. For 2013–2015, districts predicted positive but unreported numbered 97, 77, and 68, respectively, whereas those predicted positive and reported numbered 19, 28, and 32, respectively. The declining trend persisted across thresholds of 0.3–0.7. Early surveillance for a newly introduced vector-borne disease thus appears to have been insensitive in many districts. Identifying where case reporting is weakest can provide a basis for strengthening preparedness ahead of future introductions, and further work will examine which risk factors for underreporting shape early surveillance sensitivity.

Keywords: Severe fever with thrombocytopenia syndrome; Underreporting; Surveillance sensitivity; Vector-borne disease; Machine learning

Economic Evaluation of Potential Layer Vaccination against Highly Pathogenic Avian Influenza in Korea Using a Metapopulation Transmission Model

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Highly pathogenic avian influenza (HPAI) continues to cause recurrent outbreaks and preemptive culling in Korean poultry farms, generating substantial disease-control and production losses. Although vaccination has been considered as a complementary control measure, quantitative evidence on the economic value of vaccinating layer chickens under Korean epidemic conditions remains limited. This study evaluated the potential epidemiological and economic consequences of layer vaccination during the 2022/23 and 2023/24 HPAI seasons.

We developed a three-group metapopulation transmission model comprising layers, other chickens, and ducks. Weekly number of farm-level incidence from the Korea Animal Health Integrated System (KAHIS) were fitted using a Poisson likelihood. The model incorporated within- and between-group transmission, wild-bird-derived external infection pressure, removal of infected farms, and preemptive culling. Vaccine effectiveness was assumed to be 70% at both the farm and individual levels, and within-farm vaccination coverage was assumed to range from 0% to 100% in 10% increments. Three scenarios were compared: no-vaccination baseline with existing preemptive culling, vaccination with preemptive culling maintained, and vaccination with layer farms exempted from preemptive culling. Economic analysis included culling-related costs, layer production-interruption losses, outbreak-control costs, and vaccination costs; 300 KRW per bird was used as the base case, with 100, 200, and 300 KRW examined in sensitivity analysis.

The results with 70% coverage showed that predicted outbreak farms decreased by 26.7-45.4% and model-estimated total culled farms by 24.1-57.1%, depending on season and control scenario. At 300 KRW per vaccinated bird, estimated total cost savings were 0.44-6.03 billion KRW relative to baseline across seasons and scenarios. Outbreak reduction generally increased with coverage, whereas cost savings varied nonlinearly with vaccination cost and coverage. Our results implied that layer vaccination could complement existing HPAI control by reducing outbreak and culling burdens. However, because the model parameters are not evident, further studies are required to reduce the uncertainty regarding vaccination costs, production losses, compensation costs, outbreak-control costs, and other benefits.

Key words: highly pathogenic avian influenza, layer chickens, vaccination, metapopulation transmission model, economic evaluation

Diagnostic utility of shear wave elastography combined with B mode ultrasonography in feline hyperthyroidism

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Feline hyperthyroidism, most commonly caused by benign adenomatous hyperplasia, is the most common endocrine disorder in senior cats. Although thyroid scintigraphy is considered the diagnostic gold standard, its clinical use is limited by cost, availability, and radiation safety requirements. This prospective case-control study evaluated the diagnostic utility of B-mode ultrasonography, 2D shear wave elastography (SWE), and the distance between the common carotid artery and the subisthmic trachea (DCSIT) in feline hyperthyroidism. This prospective case control study compared ultrasonographic characteristics of the thyroid glands between 30 clinically healthy client-owned cats and 30 cats with scintigraphy-confirmed hyperthyroidism. Thyroid glands were evaluated with B-mode ultrasonography for size, echotexture, echogenicity, vascularity, and parenchymal lesions. DCSIT was measured at the thyroid level, and tissue stiffness was quantified using 2D SWE. Before scintigraphy, cats were blindly classified by ultrasonography as having unilateral, bilateral, or multifocal disease, and these classifications and thyroid size measurements were subsequently compared with scintigraphic findings. Hyperthyroid cats showed significantly increased thyroid width, height, and volume compared with healthy cats (all $p < 0.01$), whereas thyroid length did not differ significantly. SWE stiffness was also significantly higher in hyperthyroid cats than in healthy cats, and DCSIT was significantly altered due to displacement of the common carotid artery ($p < 0.001$). Optimal cut-off values for predicting hyperthyroidism were thyroid height > 3.86 mm, SWE stiffness > 24.18 kPa, and DCSIT > 3.69 mm. Combining these three parameters achieved a sensitivity of 97.7%, and ultrasonographic classification showed excellent agreement with scintigraphy (Cohen's kappa 0.88), with strong concordance in thyroid measurements (ICC 0.87). Multiparametric ultrasonography incorporating thyroid height, SWE stiffness, and DCSIT provides a reliable, non-invasive, and radiation-free approach for screening and assessing feline hyperthyroidism and may serve as a practical complement to thyroid scintigraphy.

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Keywords: Distance between the carotid artery and the subisthmic trachea, Feline hyperthyroidism, Shear wave elastography, Thyroid height, Ultrasound

AI-based triple phase CT radiomics for differentiating hepatocellular carcinoma from hepatocellular adenoma in dogs

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This study aims to compare the diagnostic performance of conventional CT assessment with radiomics-based artificial intelligence (AI) analysis using triple-phase CT in dogs, ultimately developing an AI model for the differential diagnosis of hepatocellular carcinoma (HCC) and hepatocellular adenoma (HCA). The study included 75 dogs with histologically confirmed HCC or HCA that had undergone triple-phase CT scanning. Conventional CT evaluation incorporated both qualitative and quantitative analytical methodologies. For the radiomics approach, regions of interest (ROI) for the hepatic masses were manually segmented across all three imaging phases, leading to the extraction of 1,316 distinct radiomics features. Shapley additive explanations (SHAP)-based feature selection was then applied to identify the most critical variables, which were subsequently evaluated across multiple AI modeling frameworks. The results demonstrated that AI analysis significantly improved diagnostic discrimination between the two tumor types, particularly when utilizing random forest and boosted tree-based models. In the arterial phase, the most influential features were primarily characterized by intensity and statistical descriptors derived from wavelet and GLCM transformations. The portal phase was uniquely characterized by texture heterogeneity descriptors, with wavelet-based GLSZM features exhibiting the strongest diagnostic performance. During the delay phase, features derived from washout-related intensity and multiscale texture descriptors, specifically Laplacian of Gaussian and wavelet transforms, proved to be the most informative. Ultimately, the integration of these multiphase features achieved the highest overall classification accuracy. In conclusion, radiomics-based AI analysis of triple-phase CT demonstrates superior diagnostic value over conventional CT assessments by quantitatively capturing sub-visual tumor heterogeneity that remains undetectable through standard evaluation

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Key Words: AI, Hepatocellular adenoma, Hepatocellular carcinoma, Radiomics, Triple phase CT

Development of a Deep Learning–Based Liver Size Assessment Model Using Canine Abdominal Radiographs

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Liver size is a key diagnostic indicator in small animal practice, but abdominal radiographic assessment remains subjective and reader-dependent, and existing deep learning research in this area has been largely confined to CT. This study developed a deep learning model to classify liver size into three categories—micro, normal, and hepatomegaly—using 2,713 usable lateral abdominal radiographs pooled from three institutions (Chungbuk National University Veterinary Teaching Hospital, Sungsim Animal Medical Center, and Time Animal Medical Center). A square-crop baseline preprocessing pipeline was compared with an enhanced pipeline incorporating marker removal, CLAHE, and brightness correction; although no statistically significant difference was found, Grad-CAM analysis showed more clinically relevant attention to the region of interest with the enhanced pipeline, which was adopted for subsequent experiments. Four architectures (EfficientNetV2-S, DenseNet-121, ConvNeXt-Tiny, Swin-T) were compared via stratified 5-fold cross-validation, then trained with Focal Loss and multi-objective Optuna optimization (minimizing validation loss while maximizing macro F1-score). Multi-objective optimization produced the best-performing model: EfficientNetV2-S achieved a macro F1-score of 0.851 on the held-out test set ($n = 407$). Multi-model ensembling offered no meaningful gain over this single model, so EfficientNetV2-S was selected as the final model. These results support plain abdominal radiography as a viable first-line screening tool for objective liver size assessment, with external validation across additional institutions planned to confirm generalizability.

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Keywords: Deep learning, Hepatomegaly classification, Hyperparameter optimization, Multi-institutional Study, Radiographic screening

Effects of Saffron-Derived Extract on Bovine Oocyte Maturation and Subsequent Embryonic Development : A Dose-Optimization Study

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Natural plant extracts have gained interest as reproductive culture supplements to mitigate oxidative stress; however, the physiological threshold and efficacy of saffron-derived extract during bovine in vitro maturation (IVM) remain to be established. This study evaluated the dose-dependent effects and preliminary cytotoxicity of extract to determine its optimal concentration for bovine IVM. Preliminary screening revealed that high-dose supplementation (2.5 mg/mL) exerted severe cytotoxic effects, substantially arresting embryonic development. Consequently, a lower concentration range was tested by supplementing cumulus-oocyte complexes (COCs) with 0, 10, 50, or 100 µg/mL of extract during IVM. Following in vitro fertilization, subsequent embryonic developmental competence was assessed, focusing on cleavage, blastocyst (BL) formation, and hatched blastocyst (HB) rates. While a high cytotoxic dose suppressed embryonic development, supplementation within the lower concentration range (10–100 µg/mL) supported in vitro culture conditions, showing concentration-dependent trends in blastocyst and hatching rates. Quantitative gene expression analyses of quality- and stress-related markers are currently under further investigation to establish cellular mechanisms. In conclusion, these findings define the preliminary toxicity threshold of extract in bovine IVM and suggest that low-dose ranges may support subsequent embryonic development, warranting further mechanistic validation.

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Keywords : Saffron extract, Bovine IVM, Cytotoxicity, Embryo development

Protective Effects of Gold Nanoparticles on Vitrified Bovine In Vitro-Produced Blastocysts

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Bovine in vitro-produced (IVP) embryos are valuable resources for genetic preservation, embryo transfer, and reproductive biotechnology. However, cryopreservation remains limited by cryo-warming injury caused by osmotic imbalance, cryoprotectant-associated toxicity, oxidative stress, cellular structural disruption, and ice crystallization- or recrystallization-associated physical damage. Although vitrification minimizes ice crystal formation by inducing a glass-like state, insufficient stability during warming may impair membrane integrity, cytoskeletal organization, and trophectoderm function, reducing developmental competence. This study evaluated whether threonine-functionalized gold nanoparticles (AuNPs) could mitigate cryo-warming injury in bovine IVP blastocysts. AuNP physicochemical properties and stability in vitrification and warming-related media were assessed. Bovine IVP blastocysts were assigned to control and AuNP-treated groups, vitrified, warmed, and evaluated for morphological survival, re-expansion, developmental progression, and degeneration. Cellular and molecular assessments related to oxidative stress, apoptosis, cytoskeletal stability, and trophectoderm integrity were further performed. This study provides an integrated evaluation of AuNP-based cryoprotection as a physical and chemical strategy to improve bovine embryo cryopreservation.

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Keywords : Bovine IVP embryo, Vitrification, Gold nanoparticle, Cryopreservation, Developmental competence

학생 참여형 혈액세포 형태학 교육과 라벨 데이터 축적을 통합한 Human-in-the-Loop 웹 플랫폼 개발

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혈액도말 세포의 형태를 판별하는 능력은 반복 관찰과 전문가 피드백을 통해 형성되며, 학습 과정에서 생성되는 학생 응답은 검수 가능한 라벨 데이터로 전환될 수 있다. 본 연구에서는 세포 형태학 교육과 데이터 축적을 연결하는 웹 기반 C.E.L.L. 플랫폼을 개발하였다. 학생은 정답과 인공지능 예측을 확인하지 않은 상태에서 세포를 분류하고, 진도와 정오답 및 혼동행렬을 통해 학습 결과를 확인한다. 분류 체계는 대상 동물 종과 교육 목적에 따라 새로운 세포 클래스를 유동적으로 추가하거나 수정할 수 있도록 구성하였다. 교수·전문가는 학생 응답 분포와 오류 양상을 검토하여 최종 기준 라벨을 확정하며, 확정된 라벨은 종 정보 및 원본 영상의 좌표와 함께 데이터셋으로 축적된다. 또한 기존 연구에서 수집한 학생별 응답 자료를 활용하여, Dawid-Skene(DS) 모형으로 학생별 혼동행렬과 응답 신뢰도를 추정하고 인공지능의 분류 확률을 사전정보로 반영한 maximum a posteriori(MAP) 추론을 통해 의사 라벨(pseudo label)을 집계하는 실증 분석을 수행한다. 전문가 확정 라벨을 기준으로 단순 다수결, 인공지능 단독 예측 및 DS 기반 MAP 집계 결과의 정확도와 신뢰도 및 자동 확정 가능 비율을 비교하며, 특히 인공지능의 판단 신뢰도가 낮거나 인공지능과 학생 응답이 불일치하는 사례에서 DS 기반 집계가 의사 라벨의 신뢰성을 높이고 전문가 검수가 필요한 표본을 선별하는 데 유용한지를 평가한다. 이를 통해 C.E.L.L. 플랫폼은 세포 형태학 교육, 학습자별 성취도 진단, 집단지성 기반 라벨 추론 및 전문가 검수를 연결하는 human-in-the-loop 데이터 구축 환경으로 활용될 수 있다.

Key words: 혈액세포 형태학, 생물학 교육, human-in-the-loop, 데이터 라벨링

Erythropoietin supplementation during in vitro culture enhances embryonic development and attenuates oxidative stress in porcine parthenogenetic embryos

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Erythropoietin (EPO) is best known as a hematopoietic cytokine that regulates erythropoiesis; however, we previously demonstrated that EPO supplementation during in vitro maturation enhances the cytoplasmic maturation of porcine oocytes. Nevertheless, its role during preimplantation embryonic development remains unclear. This study investigated whether EPO supplementation during in vitro culture (IVC) improves the developmental competence and redox status of porcine parthenogenetic embryos. To determine the optimal concentration, parthenogenetically activated embryos were cultured with EPO at 0, 10, 100, or 1000 mU/mL for 7 days, and blastocyst formation was evaluated. Among the tested concentrations, 100 mU/mL EPO significantly increased blastocyst formation compared with the control group (40.95% vs. 25.12%, $p < 0.05$), whereas 10 and 1000 mU/mL EPO did not significantly improve blastocyst development. Based on these results, 100 mU/mL EPO was selected for further analysis. At the cleavage stage, intracellular reactive oxygen species levels were significantly decreased in EPO-treated embryos ($p < 0.001$), whereas glutathione levels and mitochondrial membrane potential were not significantly altered. In Day 7 blastocysts, intracellular reactive oxygen species levels were also significantly reduced following EPO treatment ($p < 0.05$). Furthermore, mitochondrial superoxide levels, assessed by MitoSOX staining, were significantly decreased in EPO-treated blastocysts ($p < 0.05$). Collectively, these findings demonstrate that EPO supplementation during IVC enhances blastocyst development in porcine parthenogenetic embryos and attenuates oxidative stress during both cleavage-stage and blastocyst-stage development.

Keyword: porcine, embryo development, antioxidant, Blastocyst

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A Simplified Protocol for Directly Generating Vascularized Porcine Brain Organoids from Embryonic Stem Cells

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Vascularizing brain organoids remains a central challenge, as the absence of an intrinsic vascular network limits nutrient and oxygen delivery and leads to necrotic cores. Multiple strategies have been developed—including assembloid fusion with blood-vessel organoids, endothelial cell premixing or co-culture, ETV2-driven genetic engineering, in vivo engraftment, microfluidic devices, and factor-based co-differentiation. However, these approaches have been established almost exclusively in human systems, and many require exogenous cell sources, genetic modification, host transplantation, or specialized hardware. The pig, with its gyrencephalic, human-comparable brain size and physiology, is a valuable translational model, yet no vascularized porcine brain organoid system has been reported. Here we adapt a factor-based co-differentiation strategy to porcine embryonic stem cells (pESCs), generating vascularized porcine brain organoids in a single continuous protocol from one starting cell population. Following embryoid body formation, organoids were sequentially guided through mesoderm induction (CHIR99021), vascular progenitor specification (BMP4, VEGF, bFGF), Matrigel embedding, and neural maturation (N2/B27 with VEGF). Two initial seeding densities (4,500 and 9,000 cells) were compared, and both produced reproducible organoid morphology across the differentiation timeline (Day 0–Day 48). Immunofluorescence confirmed the co-emergence of neural and vascular compartments within a single organoid: GFAP⁺ astrocytes and TUJ1⁺ neurons developed alongside CD31⁺, CD34⁺, and CD146⁺ vascular networks distributed throughout the tissue. These results demonstrate that a single induction scheme can simultaneously specify neural and vascular fates, providing a simplified large-animal platform for translational neurodevelopmental and neurovascular research.

Keywords: Vascularized brain organoid, Porcine embryonic stem cell, Neurovascular co-differentiation

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Establishment of Porcine Embryonic Stem Cell-Derived Striatal Organoid Model

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Pigs share key anatomical and physiological similarities with humans, particularly in the brain, making them valuable models for studying neurodevelopmental diseases. However, an in vitro platform that recapitulates early porcine neurodevelopment remains lacking. The striatum is a key component of neural circuits and is vulnerable to degeneration in Huntington's disease. Here, we aimed to develop a porcine striatal organoid model that overcomes ethical and reproducibility limitations of in vivo systems. Porcine embryonic stem cells (pESCs) established from the inner cell mass of IVF-derived porcine blastocysts were used to generate embryoid bodies (EBs). The optimal pESC number for EB formation was determined by evaluating EBs generated with varying initial cell numbers. Neural induction was performed via dual SMAD inhibition and confirmed by the formation of neural rosette structures. Treatment with 100 ng/mL BMP inhibitor and 10 mM TGF- β inhibitor resulted in the highest neural induction efficiency. To induce ventral forebrain patterning, WNT and SHH signaling pathways were modulated using IWP-2 and SAG, respectively, thereby recapitulating in vitro the morphogen concentration gradients that regulate early brain development in mammalian embryos. Flow cytometric analysis showed that treatment with 2.5 μ M IWP-2 and 10 nM SAG tended to increase the proportion of striatal neurons. Immunofluorescence and RT-qPCR analysis confirmed the expression of GSX2 and DARPP32, specific markers of LGE progenitor and striatum, in Day 16 porcine striatal organoids (pStrO) at both the protein and mRNA levels. Single-cell RNA sequencing and electrophysiological assessments will validate transcriptional identity and functional maturation. Collectively, this study establishes a preclinical porcine striatal organoid platform for neurodegenerative disease modeling.

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Key words : Porcine embryonic stem cell, Striatum, Neurodevelopment, Organoid, Patterning

Myo-Inositol Enhances Porcine Oocyte Quality and Developmental Competence Through Improved Cellular and Metabolic Function

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Myo-inositol (Myo-Ins) has been suggested to improve oocyte quality; however, its effects on porcine oocyte maturation and subsequent developmental competence remain unclear. This study investigated the effects of Myo-Ins supplementation during in vitro maturation (IVM) on oocyte quality and embryonic development following somatic cell nuclear transfer (SCNT). Porcine cumulus-oocyte complexes (COCs) were matured with 0, 5, 10, or 20 mM Myo-Ins and evaluated for mitochondrial membrane potential (MMP), ATP levels, endoplasmic reticulum (ER) integrity, cytoplasmic and mitochondrial Ca^{2+} levels, early apoptosis, cortical granule distribution, and meiotic maturation-related factors. Myo-Ins supplementation enhanced MMP, ATP levels, and ER integrity, increased cytoplasmic and mitochondrial Ca^{2+} levels, and reduced early apoptosis. Cortical granule distribution was also improved following Myo-Ins treatment. Moreover, the mRNA expression of CCNB1, CDK1, and MAPK1 was significantly increased, while Western blot analysis revealed significantly higher Cyclin B1 and CDK1 protein levels in the 20 mM Myo-Ins group compared with the control. Oocytes matured with 5 mM Myo-Ins also showed an increased blastocyst formation rate after SCNT, with reduced apoptosis in the resulting blastocysts. Collectively, these findings indicate that Myo-Ins supplementation during IVM improves porcine oocyte quality by supporting mitochondrial and ER function, modulating intracellular Ca^{2+} levels, reducing apoptosis, and promoting meiotic maturation, thereby enhancing developmental competence following SCNT.

Keywords: Myo-inositol; Porcine oocyte; In vitro maturation; Somatic cell nuclear transfer; Mitochondrial function

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Effects of Acute Heat Stress on Sperm Motility and Phosphorylation Signaling in Capacitated Hanwoo Spermatozoa

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Capacitation is an essential process for the acquisition of fertilization ability and is regulated by intracellular phosphorylation. Although Heat stress is known to impair sperm functions, the effects of acute heat stress on capacitated Hanwoo (Korean native cattle) spermatozoa have not been fully identified. This study investigated changes in sperm motility, capacitation status, and phosphorylation signaling in response to acute heat stress. After thawing, Hanwoo spermatozoa were divided into three groups: before capacitation (BC), after capacitation under incubation at 37 °C (AC37), and at 41 °C (AC41) for 30 min. Sperm motility was assessed using computer-assisted sperm analysis (CASA). Capacitation status was evaluated by Hoechst 33258/chlortetracycline fluorescence (H33258/CTC) dual staining. Phosphorylation of PKA substrates and tyrosine-phosphorylated proteins were examined by western blotting. Compared with BC, AC37 showed no significant differences in overall sperm motility, whereas AC41 exhibited significantly reduced motility compared with both BC and AC37, including total motility (MOT), progressive motility (PRG), rapid motility (RPD), curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity (LIN), beat-cross frequency (BCF), mean angular displacement (MAD), dance (DNC), dance mean (DNM), and mean amplitude of head lateral displacement (ALH) ($P < 0.05$). H33258/CTC dual staining showed no significant differences in capacitation status patterns among the three groups, suggesting that acute heat stress did not alter the overall capacitation pattern. In contrast, PKA substrate phosphorylation was altered by heat stress, with both increased and decreased phosphorylation patterns observed at specific bands in AC41 compared with AC37. Tyrosine-phosphorylated protein showed a significant decrease in AC41 compared with AC37 ($P < 0.05$). Taken together, acute heat stress at 41 °C does not alter capacitation status but significantly reduced sperm motility and downstream phosphorylation signaling, suggesting a potential negative impact on the fertilization competence of Hanwoo spermatozoa.

Keywords: Hanwoo bull, Heat stress, Capacitation, Sperm motility, Phosphorylation signaling

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A Simplified Protocol for Directly Generating Vascularized Porcine Brain Organoids from Embryonic Stem Cells

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Vascularizing brain organoids remains a central challenge, as the absence of an intrinsic vascular network limits nutrient and oxygen delivery and leads to necrotic cores. Multiple strategies have been developed—including assembloid fusion with blood-vessel organoids, endothelial cell premixing or co-culture, ETV2-driven genetic engineering, in vivo engraftment, microfluidic devices, and factor-based co-differentiation. However, these approaches have been established almost exclusively in human systems, and many require exogenous cell sources, genetic modification, host transplantation, or specialized hardware. The pig, with its gyrencephalic, human-comparable brain size and physiology, is a valuable translational model, yet no vascularized porcine brain organoid system has been reported.

Here we adapt a factor-based co-differentiation strategy to porcine embryonic stem cells (pESCs), generating vascularized porcine brain organoids in a single continuous protocol from one starting cell population. Following embryoid body formation, organoids were sequentially guided through mesoderm induction (CHIR99021), vascular progenitor specification (BMP4, VEGF, bFGF), Matrigel embedding, and neural maturation (N2/B27 with VEGF). Two initial seeding densities (4,500 and 9,000 cells) were compared, and both produced reproducible organoid morphology across the differentiation timeline (Day 0–Day 48).

Immunofluorescence confirmed the co-emergence of neural and vascular compartments within a single organoid: GFAP⁺ astrocytes and TUJ1⁺ neurons developed alongside CD31⁺, CD34⁺, and CD146⁺ vascular networks distributed throughout the tissue. These results demonstrate that a single induction scheme can simultaneously specify neural and vascular fates, providing a simplified large-animal platform for translational neurodevelopmental and neurovascular research.

Keywords: Vascularized brain organoid, Porcine embryonic stem cell, Neurovascular co-differentiation

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Biophysical 3D Cues Unlock Extraembryonic Divergence In Porcine Expanded Potential Stem Cells

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Porcine peri-implantation development remains difficult to study *in vivo*, and stem-cell-based blastoid models offer a scalable alternative for investigating early lineage specification. Expanded potential stem cells (EPSCs), which retain competency toward both embryonic and extraembryonic lineages, are a promising yet unexplored source for porcine blastoid generation.

Here, we established porcine EPSC lines from *in vitro* fertilization (IVF)- and somatic cell nuclear transfer (SCNT)-derived blastocysts and characterized their behavior under three-dimensional (3D) suspension culture. Both lines self-organized into compact aggregates within 48 hours. By day 7, quantitative PCR showed that the epiblast marker NANOG, trophectoderm markers CDX2 and GATA3, and the hypoblast marker SOX17 were expressed in aggregates from both origins, with SOX17 expression significantly elevated in the SCNT group ($n = 3$).

Although formation efficiency was initially comparable, SCNT-derived aggregates progressively decreased in size and underwent extensive cell death by day 7, indicating markedly compromised viability relative to IVF-derived counterparts.

These findings demonstrate that 3D structural cues trigger extraembryonic divergence in porcine EPSCs while revealing a pronounced origin-dependent difference in developmental competence and lineage fidelity. Overall, this work establishes porcine EPSCs as a candidate cell source for livestock blastoid engineering and lays the groundwork for dissecting how cell-of-origin influences early mammalian embryogenesis.

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Keywords: porcine blastoid, expanded potential stem cells, extraembryonic lineage, somatic cell nuclear transfer

Gestational Benzyl Butyl Phthalate Exposure Disrupts Sperm Function and Capacitation–Associated Signaling in F1 Male Offspring

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Benzyl butyl phthalate (BBP) is extensively used as a plasticizer, raising concerns regarding its potential adverse effects on human health. Nevertheless, its effects on offspring reproductive function remain unclear, particularly regarding sperm function in F1 male offspring following gestational exposure. Therefore, this study examined the effects of gestational BBP exposure on various sperm functions, including PKA activity and tyrosine phosphorylation. Pregnant ICR mice (F0) received BBP orally at doses of 100, 250, or 500 mg/kg/day from gestation day 7 through parturition. Without additional BBP exposure after birth, male F1 offspring were maintained until postnatal day 78 and subsequently subjected to reproductive assessment. Sperm function was characterized by analyzing motility and kinematic parameters, morphology, capacitation status, cell viability, intracellular ATP and ROS levels, PKA activity, and protein tyrosine phosphorylation. Gestational BBP exposure significantly reduced body weight, testicular volume, and anogenital distance in F1 male offspring. Sperm motility and the proportion of morphologically normal sperm were also significantly decreased. In addition, intracellular ATP levels and capacitation status were significantly reduced, whereas cell viability and intracellular ROS levels were not significantly altered. Furthermore, BBP exposure induced abnormal alterations in PKA activity and tyrosine phosphorylation. Collectively, these findings demonstrate that gestational BBP exposure impairs sperm function and disrupts capacitation-associated signaling in adult F1 male offspring. These results further support the reproductive toxicity of BBP and emphasize the importance of considering its potential effects on offspring reproductive health in safety assessments.

Keywords: Benzyl butyl phthalate, Gestational Exposure, Sperm function, Capacitation, F1 Offspring

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Runx2 Repression as a Potential Gene Therapy for Cartilage Degeneration

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Osteoarthritis (OA) is a degenerative joint disease characterized by progressive cartilage breakdown, in which the transcription factor RUNX2 might play a critical role by upregulating catabolic enzymes and repressing cartilage matrix genes. In this study, we investigated whether expression of Runx2 is linked to catabolic phenotype in both human patient cartilage public data and murine primary chondrocytes. The results show that upregulated RUNX2 in human patients is linked to cartilage degeneration and targeted silencing of Runx2 in murine primary chondrocytes using the CRISPR/dCas9-KRAB system could restore anabolic gene expression in primary murine chondrocytes. Lentiviral vectors encoding dCas9-KRAB and Runx2-targeting sgRNAs were transduced into primary mouse chondrocytes, and gene expression changes were assessed via quantitative real-time PCR. Runx2 repression led to a significant increase in Col2a1 expression, indicating enhanced extracellular matrix synthesis. Concurrently, key catabolic mediators such as Mmp13 and Adamts5 were significantly downregulated. These transcriptional shifts suggest that CRISPR/dCas9-KRAB-mediated Runx2 inhibition reprograms chondrocytes toward a cartilage-preserving, anabolic phenotype. Safranin-O stain and immunofluorescence showed upregulation of cartilage matrix synthesis in vitro. These findings provide evidence that epigenome editing might modulate disease-relevant transcription factors and restore homeostatic gene expression in osteoarthritis. This approach may offer a novel therapeutic strategy for modifying the progression of OA at the transcriptional level. Future studies will focus on in vivo validation to assess long-term efficacy, safety, and translational potential of this gene-targeted intervention.

Key words : CRISPR/dCas9, Osteoarthritis, RUNX2, Gene Therapy

Simulated microgravity impairs early development of porcine parthenogenetic embryos through oxidative stress

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Assessing the impact of altered gravitational conditions on mammalian reproduction is essential for determining the viability of prolonged space missions. Although existing research has largely centered on rodent models, how embryos from large-animal species respond developmentally to microgravity remains poorly understood. In the present study, we examined the impact of simulated microgravity on early embryogenesis by culturing porcine parthenogenetic embryos in a 3D clinostat. Relative to both static and rotational controls, simulated microgravity led to a marked decline in blastocyst formation and a higher incidence of developmental arrest. Blastocysts formed under microgravity conditions displayed fewer total cells and a decreased frequency of full expansion, suggesting diminished developmental potential. During cleavage, embryos exposed to simulated microgravity showed elevated intracellular reactive oxygen species alongside reduced glutathione levels, indicative of oxidative dysregulation at an early stage. By day 6, these embryos exhibited heightened apoptosis and compromised trophectoderm differentiation, as evidenced by reduced trophectoderm cell counts and attenuated expression of junctional proteins CDH1, OCLN, and ZO-1. Taken together, our results indicate that simulated microgravity disrupts early porcine embryogenesis via oxidative stress-driven cellular damage, underscoring the susceptibility of the trophectoderm as a principal barrier to reproductive success under microgravity.

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Key words: Simulated microgravity, Porcine parthenogenetic embryo, Oxidative stress, Apoptosis, Trophectoderm integrity

Tiron supplementation improves the cryosurvival of boar sperm

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The efficiency of boar sperm cryopreservation is often limited by oxidative damage generated during freezing and thawing, leading to impaired sperm function and reduced fertilizing capacity. Tiron, a membrane-permeable antioxidant with strong superoxide-scavenging activity, may alleviate cryoinjury by regulating mitochondrial redox balance. This study investigated the effects of Tiron supplementation on the quality and oxidative status of cryopreserved porcine sperm. Semen samples were cryopreserved in extenders containing 0, 5, 50, or 500 nM Tiron and evaluated after thawing. Supplementation with 50 nM Tiron significantly improved sperm motility, progressive motility, viability, and acrosome integrity compared with untreated controls ($P < 0.05$). Although 500 nM Tiron further enhanced plasma membrane stability, excessive supplementation adversely affected sperm motility and viability. Apoptotic sperm populations were reduced in all Tiron-treated groups, with the lowest level observed at 50 nM ($P < 0.001$). Intracellular ROS accumulation was also markedly suppressed by Tiron treatment, indicating effective protection against cryo-induced oxidative stress. Analysis of oxidative stress-related genes demonstrated increased expression of antioxidant defense genes, including SMCP, CAT, Cu/ZnSOD, and GPX1, whereas the expression of oxidative stress- and apoptosis-associated genes such as ROMO1, SMOX, KEAP1, and NOX5 was reduced in the 50 nM group. In addition, the BAX/BCL2 ratio was decreased, suggesting attenuation of apoptosis-related signaling pathways. These findings demonstrate that Tiron enhances post-thaw sperm quality by limiting oxidative damage and improving cellular resilience during cryopreservation. The results support the use of Tiron as a promising antioxidant additive for porcine semen cryopreservation, with 50 nM identified as the optimal concentration under the conditions tested.

Porcine sperm; Cryopreservation; Tiron; Oxidative stress; Cryosurvival

Canonical Notch inhibition maintains oocyte–cumulus cell communication by preserving transzonal projection integrity during porcine oocyte maturation

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Bidirectional communication between oocytes and cumulus cells (CCs) is essential for oocyte maturation and developmental competence. Although Notch signaling is implicated in ovarian follicular development, its role in regulating oocyte–CC communication during porcine oocyte maturation remains unclear. This study aimed to determine the role of Notch signaling in regulating oocyte–CC communication during porcine oocyte maturation. Cumulus–oocyte complexes were treated with the γ -secretase inhibitor RO4929097 (RO) during in vitro maturation, followed by parthenogenetic activation to assess embryonic developmental competence and investigate the functional role of Notch signaling. Oocyte–CC communication was evaluated by assessing transzonal projection integrity, gap junction protein expression, calcein-AM transfer, and intracellular ATP levels. Notch signaling components were detected throughout porcine follicular development, with DLL1 and NOTCH1 dynamically expressed in follicles from the primordial stage to the antral stage. RO treatment significantly reduced CC expansion, oocyte nuclear maturation, and subsequent embryonic development. Mechanistically, RO disrupted transzonal projection integrity and reduced their number, which were essential for direct communication between oocytes and CCs. In addition, RO decreased the expression of the gap junction proteins Cx37 and Cx43, which was accompanied by impaired calcein-AM transfer from CCs to oocytes and reduced intracellular ATP levels in oocytes. To our knowledge, this study provides the first evidence that Notch signaling is directly involved in maintaining TZP integrity during porcine oocyte maturation, establishing a mechanistic link between Notch signaling and oocyte–CC communication. Our findings further demonstrate that Notch signaling supports the structural and functional coupling between oocytes and CCs by maintaining TZP integrity and gap junction-mediated communication, thereby facilitating metabolite transfer and ultimately promoting oocyte maturation and developmental competence in pigs.

Key words: Notch signaling, Oocyte–cumulus cell communication, Transzonal projection, Gap junction, Oocyte maturation

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Polymethoxyflavones-enriched citrus leaf extract suppresses metabolic stress-induced ELR⁺ CXC chemokine responses in hepatocytes

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Metabolic dysfunction-associated steatohepatitis (MASH) is characterized by hepatic inflammation driven by interactions between metabolically stressed hepatocytes and infiltrating immune cells. We investigated hepatocyte inflammatory responses to palmitic acid (PA) and the protective effects of a polymethoxyflavone (PMF)-enriched citrus leaf extract. PA induced dose- and time-dependent expression of the ELR⁺ CXC chemokines IL-8, CXCL1, and CXCL2, with induction evident as early as 3 h and accompanied by increased chemokine secretion. In contrast, CCL2 was undetectable, suggesting a preferential ELR⁺ CXC chemokine response to metabolic stress. PMF-enriched citrus leaf extract markedly suppressed PA-induced expression and secretion of these chemokines and reduced TNF α expression. These findings suggest that PMF-enriched citrus leaf extract attenuates hepatocyte inflammatory responses to metabolic stress, supporting its potential as a functional ingredient for mitigating MASH-associated hepatic inflammation.

Key words: Metabolic dysfunction-associated steatohepatitis (MASH); Polymethoxyflavones; Citrus leaf extract; ELR⁺ CXC chemokines; Hepatic inflammation

Polymethoxyflavones–Enriched Citrus Leaf Extract Improves Glucose Homeostasis by Suppressing Macrophage–Adipocyte Inflammation in Obesity

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Adipose tissue regulates systemic energy homeostasis through lipid storage and secretion of bioactive factors. Chronic caloric excess disrupts adipose homeostasis, promoting macrophage-driven inflammation and insulin resistance. Polymethoxyflavones (PMFs), citrus-derived flavonoids, have anti-inflammatory and metabolic benefits. We developed a PMF-enriched citrus leaf extract from Jeju citrus cultivars and evaluated its effects in in vivo and in vitro models. In high-fat diet (HFD)-fed mice, chronic administration reduced fasting glucose, improved glucose tolerance, and attenuated hepatic injury without affecting body weight. The extract enhanced glucose uptake and insulin signaling in adipocytes and suppressed macrophage inflammatory responses by inhibiting TLR4/NF- κ B signaling. It also restored adipocyte insulin responsiveness impaired by conditioned medium from inflammatory macrophages. These findings suggest that PMF-enriched citrus leaf extract improves glucose homeostasis by attenuating macrophage–adipocyte inflammatory crosstalk and may serve as a functional ingredient for obesity-associated metabolic dysfunction.

Key words: Polymethoxyflavones; Citrus leaf extract; Insulin resistance; Adipose inflammation; Macrophage–adipocyte crosstalk

Hepatocyte TonEBP promotes metabolic stress-induced hepatic fibroinflammation involving transcriptional activation of ELR⁺ CXC chemokines

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Metabolic dysfunction-associated steatohepatitis (MASH), a progressive stage of metabolic dysfunction-associated steatotic liver disease (MASLD), is characterized by liver inflammation, fibrosis, and hepatocyte injury. Despite its clinical relevance, the molecular mechanisms linking metabolic stress to hepatic fibroinflammation is not fully understood. In this study, we identify Tonicity-Responsive Enhancer-Binding Protein (TonEBP) as a key stress-responsive transcription factor connecting metabolic overload to liver inflammation in non-malignant hepatocytes. Hepatocyte-specific TonEBP deletion reduces liver injury, inflammation, and fibrosis in MASH and steatosis models. Mechanistically, TonEBP recruits nuclear factor- κ B (NF- κ B) to ELR⁺ CXC chemokine gene promoters, promoting neutrophil and macrophage recruitment. These findings establish the hepatocyte TonEBP/NF- κ B axis as a critical driver of immune cell infiltration and fibroinflammation in MASLD progression. By highlighting this axis, we provide mechanistic new insight into the transition from steatosis to steatohepatitis and underscore the importance of TonEBP in regulating inflammatory pathways within hepatocytes.

Key words: MASH; MASLD; Fibroinflammation; TonEBP; ELR⁺ CXC chemokines

Comparative Single-Cell Analysis of Primate PBMCs to Characterize the Common Marmoset and Its Relevance to Human Immune Responses

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The common marmoset (*Callithrix jacchus*) is an important nonhuman primate (NHP) model, but its immune responses have not been extensively compared with those of humans and other primates at the single-cell level. Secukinumab cross-reacts with primate IL-17A but not rodent IL-17A. This provides a rationale for comparing IL-17A-related responses among primate species. In this study, we performed single-cell RNA sequencing (scRNA-seq) of peripheral blood mononuclear cells (PBMCs) from humans, cynomolgus monkeys (*Macaca fascicularis*), and common marmosets to compare their transcriptional responses under inflammatory conditions. For 48 hours treatment comparisons, PBMCs were analyzed under control conditions, after stimulation with zymosan A (100 µg/mL), or after co-treatment with zymosan A and secukinumab (10 µg/mL), an IL-17A-neutralizing antibody. Using high-confidence one-to-one ortholog matching, we integrated 9,564 shared genes across 64,815 single cells (27,316 human; 12,759 cynomolgus monkey; 24,740 common marmoset). Major lymphoid and myeloid cell populations were identified in all three species, allowing direct comparison of cellular and transcriptional responses across species. Zymosan A stimulation altered immune cell composition and inflammatory gene expression, but the extent and pattern of these changes differed among species. Initial analyses showed a more prominent IL-17A-associated transcriptional response in marmoset PBMCs than human and cynomolgus monkey PBMCs. Responses following secukinumab treatment also appeared to vary among species and cell populations. Further analyses of differential gene expression, pathway enrichment, cytokine responses, and cell-cell interactions are ongoing. These analyses will be used to determine which immune responses are shared among the three primate species and which are species-specific. This comparative single-cell analysis will help characterize the immune features of the common marmoset and determine how closely its inflammatory responses resemble those of humans. This study was supported by the Industrial Strategic Technology Development Program (RS-2022-KT218049) funded by the Ministry of Trade, Industry & Energy (MOTIE, Korea) and the Korea Health Technology R&D Project (RS-2025-16063082) through KHIDI, funded by the Ministry of Health and Welfare, Republic of Korea.

Key words: Common marmoset, single-cell RNA sequencing, PBMC, cross-species comparison, inflammatory response

Improving Medical Image Segmentation under Limited Annotation through Cross-Domain Prediction Refinement

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The use of artificial intelligence for quantitative analysis of biomedical images often depends on pixel-level annotations, which are costly to obtain and typically require expert involvement. This limitation becomes particularly important when a segmentation model needs to be applied to a new imaging condition with only a small amount of labeled data. We examined whether information learned from segmentation failures in other medical imaging domains could be reused to improve prediction quality in such data-limited settings. A general-purpose Segment Anything Model (SAM) was used as a fixed initial segmenter, while a compact auxiliary model learned to refine its outputs from the original image and the predicted segmentation. Experiments were conducted using five heterogeneous medical imaging datasets representing endoscopy, breast ultrasound, dermoscopy, echocardiography, and chest radiography. In each evaluation, one imaging domain was excluded from prior training and used as a new target, while information from the other four domains was used to initialize the refinement model. The model was then adjusted with only a small number of labeled target images. When only one labeled target image was available, the average Dice score increased from 0.486 with target-only training to 0.570 with cross-domain initialization. With five labeled images, the corresponding score increased from 0.605 to 0.701. Improvements were also observed with larger annotation sets of 10, 20, and 50 images. These findings indicate that segmentation failures observed across different medical imaging conditions can provide useful prior information for refining predictions in a previously unseen domain. Such a strategy may be useful for biomedical imaging applications in which extensive manual annotation is impractical, including future applications involving cellular or tissue images.

Key words:biomedical image analysis, medical image segmentation, limited annotation, prediction refinement, Segment Anything Model

Patient-Derived Tumor Spheres With Cancer Stem-Like Properties From Canine Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is the most prevalent primary malignant hepatic neoplasm in dogs, yet effective systemic treatment options for unresectable or recurrent disease remain limited. Cancer stem-like cells (CSCs), characterized by self-renewal capacity and therapeutic resistance, have been implicated in tumor initiation, recurrence, and progression. Although cancer stem-like populations in canine HCC have been primarily studied using established cell lines, patient-derived sphere models directly established from spontaneous canine HCC tumors remain scarce. Among three surgically resected canine hepatic tumors, histopathological re-evaluation confirmed HCC in two cases (HCC_N1 and HCC_N3), and the corresponding tumor tissues were enzymatically dissociated for primary culture establishment. Primary canine HCC cells were successfully established under adherent 2D conditions, exhibiting significantly elevated mRNA expression of SOX2, PCNA and MCL1 relative to normal canine fibroblast controls, thereby confirming the baseline stemness potential, hyper-proliferative capacity, and apoptotic resistance of the isolated tumor cells. To selectively enrich for functional cancer stem-like populations, these cells were transferred to serum-free non-adherent conditions, to induce primary sphere formation, with a 3-day primary culture duration proving sufficient to sustain secondary sphere-forming efficiency upon serial passaging. Furthermore, confocal immunofluorescence of the resulting secondary sphere sections confirmed robust protein expression and spatial localization of the CSC markers EpCAM and CD44, validating the successful enrichment of CSC-like phenotypes through 3D sphere culture. Overall, these findings establish a patient-derived 3D canine HCC platform for streamlined CSC-like enrichment. While validation in additional cases is required, this standardizable framework provides high-quality phenotypic and therapeutic data to drive AI-assisted tumor characterization and personalized treatment prediction.

Key words: Canine hepatocellular carcinoma, Cancer stem-like cell, Patient-derived model, Sphere formation, Comparative oncology

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FGF2/BMP4-Induced Extraembryonic Priming of Porcine Expanded Potential Stem Cells Enables to a Cardiac Fate in 3D Culture

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Extraembryonic lineages play important roles in early embryonic development and may influence cardiac differentiation. However, the relationship between extraembryonic priming and cardiac differentiation in large-animal stem cells remains poorly understood. Here, we investigated whether FGF2/BMP4-induced extraembryonic priming of porcine expanded potential stem cells (pEPSCs) facilitates cardiac differentiation and three-dimensional organoid formation.

2F-treated cells were cultured in AggreWell™ 400 plates under cardiac differentiation conditions: CM (cardiomyocyte-promoting), NB_CM (neurobasal-enriched CM), and DP_CM (developmentally primed CM). On day 3, control pEPSCs remained as aggregates without spontaneous beating, whereas 2F-treated cells formed distinct beating core regions under CM and NB_CM. In contrast, DP_CM-cultured organoids exhibited a reticular architecture with synchronized global contractions.

RT-qPCR analysis revealed increased expression of chamber-associated cardiomyocyte markers (MYL7, MYH6, MYL2, and MYH7), although distinct chamber-like structures were not observed. Interestingly, fibroblast-associated marker (VIM) and neural markers (PAX6, NESTIN) were also detected, suggesting multilineage differentiation. Immunofluorescence analysis identified cTnT⁺ cardiomyocytes, α -SMA⁺ smooth muscle cells, and vimentin⁺ mesenchymal-like cells in all conditions. Notably, CD31⁺ endothelial cells were observed in both CM and NB_CM, but not in DP_CM. The presence of endothelial cells in CM and NB_CM may help address the lack of vascular components in conventional cardiac organoid models.

Taken together, 2F-treated cells can self-organize into multicellular cardiac organoids comprising multiple cardiac-associated cell types and endothelial cells. These cardiac organoids could provide a novel model for investigating the contribution of extraembryonic cells to cardiac development.

Key words: Pig, Expanded potential stem cells, Extraembryonic priming, Cardiac differentiation, Organoid

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Differential effects of 6PPD and 6PPD–quinone on human endometrial stromal cell decidualization

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Decidualization of endometrial stromal cells is essential for endometrial receptivity and the establishment of early pregnancy. The tire rubber–derived chemical N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and its oxidative derivative 6PPD-quinone (6PPDQ) are ubiquitous environmental contaminants that are frequently detected in human biospecimens. However, their effects on uterine stromal function remain poorly understood. In this study, we investigated whether 6PPD and 6PPDQ influence decidualization-associated responses in an immortalized human endometrial stromal cell line (tHESC). Both chemicals exhibited dose-dependent reductions in cell viability of tHESCs, and exposure conditions that did not significantly reduce cell viability were selected for subsequent analyses. Under these conditions, 6PPD and 6PPDQ exerted distinct effects on decidualization-associated stromal responses. Phenotypic analyses showed that the two chemicals altered decidualization-associated morphometric parameters in different directions. At the transcript level, 6PPD increased the expression of classical decidualization markers but also downregulated several stromal regulatory genes. In contrast, 6PPDQ produced a broader suppression of decidualization-associated gene expression. Together, these findings indicate that 6PPD and 6PPDQ differentially perturb morphometric and transcriptional decidualization-associated responses in an immortalized human endometrial stromal cell line. Specifically, 6PPD exhibited an internally discordant pattern of program-level dysregulation, whereas 6PPDQ showed a more consistently suppressive profile under decidualizing conditions. These results provide insights into the distinct effects of 6PPD and 6PPDQ on stromal decidualization-associated responses. This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. RS-2025-00559353).

Keywords: 6PPD; 6PPDQ; Endometrial stromal cells; Decidualization; Endometrial receptivity

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