42nd Annual Meeting
The Korean Society of Developmental Biology

New Perspectives on Development and Organoid: From Aqua to Land Animals

AUG 24-25, 2023
Education Support Center,
Gangneung-Wonju National University
(Gangneung Campus)

주 최 : (사)한국발생생물학회,
강릉원주대학교 동해안생명과학연구원

후 원 : 수중생태기술연구소, 한국오가논, 스펙스,
늘품바이오, 옵티젠, LG화학, 한솔켐바이오텍,
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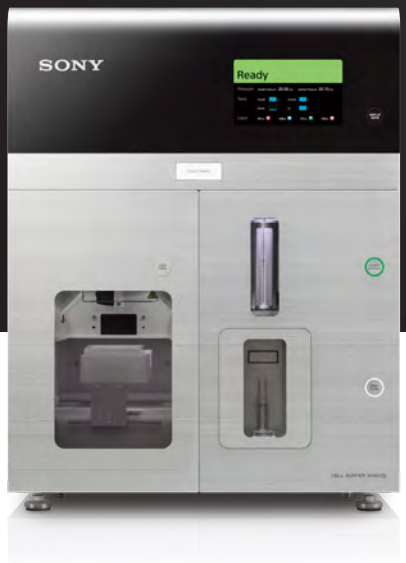

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정기학술대회

New Perspectives on Development and Organoid: From Aqua to Land Animals

2023년 8월 24일(목) ~ 8월 25일(금)
강릉원주대학교 강릉캠퍼스
교육지원센터

주　　최: (사)한국발생생물학회,
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제42회 한국발생생물학회 정기학술대회 학술 프로그램

New Perspectives on Development and Organoid: From Aqua to Land Animals

주최: (사)한국발생생물학회, 강릉원주대학교 동해안생명과학연구원
장소: 강릉원주대학교 강릉캠퍼스 교육지원센터
일자: 2023년 8월 24일(목)~8월 25일(금)

8월 24일 (목) 12:00~18:00			좌장
12:00–13:00	등록		
13:00–13:10	**개회식 (회장 환영 인사, 강릉원주대학교 교학부총장 축사)**		(사회) 학술위원장
13:10–13:50	기조강연 I	기억의 생물학 **강봉균 교수**(서울대학교)	조성진 교수
13:50–14:30	기조강연 II	우리나라 패류양식과 발생생물학적 연구 **허영백 소장**(국립수산과학원 남동해수산연구소)	권준영 교수
14:30–14:40	Coffee Break & Booth Tour		
14:40–17:20 (15:55–16:05 CB & BT)	심포지엄 I	지구의 끝 극지, 그 쿨한 생명의 이야기 **김진형 박사**(한국해양과학기술원 극지연구소)	이현식 교수 조종기 교수 김종한 박사
		수중 미세플라스틱의 존재가 수산생물에 미치는 영향 **김준환 교수**(선문대학교)	
		Current advances in gene and cell therapy for incurable disease **박한슬 교수**(충북대학교)	
		Coffee Break & Booth Tour	
		The 3rd clutch in axoneme: defective sperm motility and male infertility by altered radial spoke **황재연 교수**(부산대학교)	
		Effects of time-restricted feeding on the letrozole-induced mouse model of polycystic ovary syndrome **류기진 교수**(고려대학교)	
		Making of an inner ear **복진웅 교수**(연세대학교)	
17:20–18:00	**포스터 세션**		문성환 교수 류홍열 교수
17:30–18:00	**이사회**		(사회) 총무위원장
18:00	**환영 리셉션**		(사회) 학술위원장

8월 25일 (금) 8:50~12:30			좌장
08:50–10:00	심포지엄 II (젊은과학자 I)	Relation of secondary oocyte spindle location and embryo development **윤솔아 석사과정**(성신여자대학교)	최태영 교수 박태주 교수
		Investigating the role of interleukin-7 in porcine embryos: implications and findings **오동진 박사과정**(충북대학교)	
		The regulation of ribosome-biogenesis related NPM 1 in mouse endometrium **이기완 석사과정**(건국대학교)	
		Characterization of fine particulate matter (PM2.5)-induced developmental and teratogenic defects in *Xenopus* embryos **정은혜 석사과정**(경북대학교)	
		Mebendazole exerts anti-cancer activity by preferentially inhibiting cilia formation **홍주연 석사과정**(울산과학기술원)	
		Signaling of myoinhibitory peptides and its possible role in larval development in Pacific abalone **박성우 석사과정**(강릉원주대학교)	
		Nirmatrelvir induces detrimental effects on sperm function by disturbing the AKT pathway **정은주 석사과정**(경북대학교)	
10:00–10:10	Coffee Break & Booth Tour		
10:10–10:50	심포지엄 III (젊은과학자 II)	Role of Chil3l1 in uterine endometrium during the estrous cycle **김병석 박사과정**(건국대학교)	정한성 교수
		Distribution of dopaminergic neuron of *Octopus minor* brain and dopamine transporter system **이찬준 박사과정**(충북대학교)	
		참돔의 장내 미생물 군집 조성 **강민주 박사과정**(한국해양과학기술원)	
10:50–11:35	심포지엄 IV (신진과학자)	Overview on status and technological advances in tuna aquaculture in Korea **조정현 박사**(국립수산과학원 제주수산연구소)	권우성 교수
		The noncanonical action of MYC promotes tumor development by orchestrating an immunosuppressive tumor microenvironment **이경민 교수**(한양대학교)	
		Neurotrophin-4 in the porcine ovary: its effect on oocyte maturation and developmental competence **김미래 박사**(충북대학교)	
11:35–11:50	Coffee Break & Booth Tour		
11:50–12:30	기조강연 III	동물줄기세포 연구현황과 활용기술 **이창규 교수**(서울대학교)	현상환 교수

8월 25일 (금) 12:30~18:00			좌장
12:30–13:30	Lunch Break		
13:30–15:10	심포지엄 Ⅴ	DNA damage response in mammalian oocytes **오정수 교수**(성균관대학교)	구덕본 교수 이영돈 교수
		Enhancing ovum pick-up productivity through mild FSH stimulation **이준구 교수**(한경국립대학교)	
		국내 두족류 연구현황과 발전방안 **유해균 박사**(국립수산과학원 동해수산연구소)	
		Strategies for mass cultivation of marine algae **선우인영 박사**(한국해양과학기술원 제주바이오연구센터)	
15:10–15:30	Coffee Break & Booth Tour		
15:30–17:10	심포지엄 Ⅵ	Neuropeptide regulation of developmental stage and metabolism in Pacific abalone **손영창 교수**(강릉원주대학교)	전용필 교수 이성호 교수
		Recent studies on anorexia and tissue wasting induced by cancer cachexia **염은별 교수**(경북대학교)	
		Developmental myogenesis and basal proliferation of satellite cells in craniofacial muscles **김은혜 교수**(경상국립대학교)	
		Application of sonic hedgehog signaling-induced *in vitro* oocyte maturation to *Klotho*-knockout pig production **이상훈 교수**(충남대학교)	
17:10–17:30	**시상**		(사회) 학술위원장
17:30–18:00	**총회 및 폐회식**		(사회) 총무위원장

제42회 한국발생생물학회
정기학술대회 초록

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심포지엄Ⅲ: 한국발생생물학회 젊은과학자Ⅱ

심포지엄Ⅳ: 한국발생생물학회 신진과학자

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심포지엄 VI

포스터 초록

기조강연

Dev. Reprod. Vol 27, 2023 (Suppl. 1). pp 3

기억의 생물학:
Memory and engram synapses

Bong-Kiun Kaang

Distinguished Professor, School of Biological Sciences, Seoul National University

Learning is the process by which we obtain the information about the world; memory is the process by which that information is stored. About 100 years ago, Richard Semon coined engram or memory trace that is defined as the lasting physical changes in the brain that occur as a result of an experience. D.O. Hebb who was searching for engram, proposed an idea of cell assembly and Hebbian synaptic plasticity, in 1949. Now it became possible to identify and label engram cells by powerful molecular biological tools and optogenetics. However, we do not know whether memory formation strengthens synapses between engram cells in different brain regions. So, we asked if memory formation strengthens synapses between engram cells, structurally and functionally. In this talk, I will present our recent structural and functional approaches to reveal enhanced structural and functional connectivity between engram cells in the hippocampus during memory acquisition.

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기조강연Ⅱ

우리나라 패류양식과 발생생물학적 연구

허영백

국립수산과학원 남동해수산연구소

(hur0100@korea.kr)

우리나라 패류 양식생산량은 394,710톤으로 중국, 칠레 다음으로 세계 3위(FAO, 2000)이고, 양식산업적 규모는 1조 2천억 원이다. 패류양식산업의 지속적인 발전을 위해 지금까지 다양한 양식 및 종자생산 기술이 개발되었지만, 기후변화 등 서식환경변화와 노령화 등 양식생산 구조변화에 부응하는 기술개발 대응 미흡 및 우량품종 개발 부족 등으로 빈번한 대량 폐사, 성장 둔화 등 품종 열성화 현상이 심화되고 있어 많은 어려움을 겪고 있다. 뿐만 아니라, 최근 기후변화로 인한 해양환경변화는 여름철 고수온, 겨울철 저수온 등 빈번한 이상수온 현상과 함께 연안 기초생산력의 변화에 많은 영향을 미치는 것으로 보고되고 있어, 번식생물학적 측면에서 서식환경의 변화에 의존적인 패류양식의 지속적인 발전을 위해서는 기후변화에 의한 개체군의 서식생태 및 발생생물학적 변동에 미치는 영향을 정확히 이해하는 것은 생태 및 양식생물학적인 측면에서 대단히 중요한 과제 중의 하나이다.

계절 환경에 서식하는 대부분의 수생생물의 번식 특성은 진화생물학적으로 자손세대가 더 많은 먹이와 피난처를 이용하여 생존과 성장 가능성을 높일 수 있도록 상대적으로 먹이 발생량 등, 이들 종의 서식조건이 유리한 계절기간과 번식주기 변화가 동기화되어 있다. 우리나라와 같이 온대 지역에 서식하는 패류는 대부분 봄과 여름이 번식시기이다. 이와 같은 계절적 번식특성은 먹이생물이 풍부한 시기 축적한 에너지 이용 조건뿐만 아니라, 성공적인 번식은 번식시기 전에 발생하는 많은 스트레스 환경을 극복하기 위한 생리적 변화 요구조건에 대처할 수 있는 생물학적 능력도 많은 영향을 미치는 것으로 알려져 있다. 특히 성숙산란기 전이나 생식시기 동안의 가혹한 스트레스 환경 조건은 생체유지 및 기초 대사를 유지하기 위해 많은 양의 에너지를 요구할 수 있다. 이러한 경우 번식과정은 상대적으로 많은 에너지를 요구하기 때문에 암컷은 배우자 생산에 필요한 에너지를 공급하지 않거나, 배우자 생산에 소비되는 에너지를 줄임으로써 생식보다는 생존을 선택할 수 있다. 이러한 결과로 서식환경 조건은 패류를 포함한 해양 무척추동물의 번식주기에 많은 영향을 미칠 수 있는 것으로 보고되고 있어, 패류 등 양식산업에 많은 변화를 야기할 수 있다. 따라서 기후변화 등에 대응하면서 양식생물 자원의 지속적인 생산과 관리 그리고 안정적인 종자생산 기술개발을 위한 기반요소인 환경변화와 발생생물학적 영향을 정확히 이해하는 것은 매우 중요하기 때문에 향후 관련 연구를 돕고자 지금까지 연구현황을 알아보고자 한다.

Keywords: shellfish aquaculture, climate change, shellfish reproduction, seed production

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Current research and applications in animal stem cells: a pig review

Chang-Kyu Lee[1,2]

[1]Department of Agricultural Biotechnology, Animal Biotechnology Major, and Research Institute of Agriculture and Life Science, Seoul National University

[2]Institute of Green Bio Science and Technology, Seoul National University

Stem cells have been employed as a promising tool in the biomedical field including disease modeling and xenotransplantation because they can recapitulate the developmental process *in vitro*. In recent, species-specific developmental features in domestic animals especially pigs have been actively studied in the aspect of comparative development biology. These researches improve the ultimate value of biology and availability of developmental knowledge in domestic animals for agricultural purposes. We have been conducting experiments with pig embryos and stem cells in terms of development to facilitate biomedical and industrial applications. We investigated the genetic network of pluripotent genes and epigenetic features such as genomic imprinting and X chromosome inactivation (XCI) in preimplantation embryo. Further, we developed embryo aggregation method and analyzed metabolism during embryogenesis to improve quality of *in vitro*-produced embryo. Based on these studies, we established various porcine stem cells derived from embryo including embryonic stem cells (ESCs), embryonic germ cells (EGCs) and induced pluripotent stem cells (iPSCs). In particular, the authentic porcine ESCs which exhibited *in vivo* developmental competency could be established by dissecting the porcine species-specific pluripotency signaling pathways. Further, we have performed studies on the mechanism of myogenesis using muscle stem cells, providing developmental insights and expanding the value of stem cells to cellular agriculture. An in-depth understanding of embryology and stem cell biology in pigs will open a new possibility for agricultural biotechnology.

Keywords: pig, embryology, stem cells, pluripotency, cellular agriculture

심포지엄 I

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지구 끝 극지, 그 쿨한 생명의 이야기

***김진형**

한국해양과학기술원 극지연구소

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남극반도를 둘러싼 남극해는 연평균 빙점(–1.9℃)에 이르는 차가운 해수온과 더불어 고염분, UVR 등 극지 특이적 환경을 지닌다. 남극어류는 현재까지 123종이 보고되었으며, 이중 남극암치아목(Notothenioidei) 어류가 남극해 전체 어류 생물량의 약 90%를 차지한다. 이 어류들은 척박한 극지 환경에 적응해 살아남기 위해서 종 특이적인 적응기작을 통해서 진화해 왔다.

극지연구소는 2016년부터 남극 세종과학기지 및 장보고과학기지 연안에서 채집된 극지 어류를 유지 배양하기 위하여 과학기지 및 국내 극지연구소에 극지 해양생물 전용 아쿠아리움을 구축하였다. 현재 남극 검정암치, 남극대리석무늬암치, 남극에메랄드암치 등 지금까지 10종의 남극 어류를 국내로 이송하여 장단기에 걸쳐 유지하였고, 다양한 사육조건 및 먹이 선호도를 조사하여 장기간 유지에 필요한 호조건을 찾고 있다. 이를 통해 극지 어류의 환경 적응기작을 이해하고, 극지방에서 일어나고 있는 급격한 환경변화에 따른 생물 및 생리반응을 이해하기 위한 다양한 실험을 수행하고 있다. 또한, 극지 어류 만이 특이적으로 가지게 된 특성을 기반으로 유전자원의 활용 및 기능 유전자를 발굴하기 위한 연구를 수행하고 있다. 전 세계적으로 극지 어류의 생식 및 인공번식에 관해서는 거의 알려진 바가 없다. 따라서 만약 남극 어류의 인공 산란을 유도하여 인공번식을 한다면 지속가능한 많은 흥미로운 결과를 얻을 수 있을 것으로 기대한다.

Keywords: 남극 어류, 남극암치아목, 극지, 적응기작, 인공번식

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Toxic effects of microplastic exposure on fish and future research directions

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Plastics are used not only as packaging material in our daily lives but also in construction, automobiles, electronics and electrical products, clothing, and agriculture, and industry. In 2018, global plastic production reached approximately 360 million tons, of which 80,000 tons are estimated to flow into the aquatic environment. Approximately 10% of plastic waste produced globally is found in the marine environment (9.5 million tons of plastic waste annually), representing a major hazardous substance. Plastics are divided into various types, such as polyethylene (PE), polystyrene (PS), polyvinylchloride (PVC), polyethylene terephthalate (PET), polyamide (PA), polypropylene (PP) and ethylene vinyl acetate (EVA) depending on the material used and structure, and have different characteristics and usage. Evidence of freshwater and marine fish consuming MPs has been widely documented. MP consumption has physical and chemical toxic effects, including mechanical injury. Other adverse effects include energy disturbance, decreased reproduction/growth, oxidative injury, metabolic disorders, cellular lesions, endocrine disruption, decreased immunity, neurotransmission disorder and genotoxicity, also potentially leading to mortality.

MPs primarily accumulate in the gills and intestines of fish, with particles subsequently moving to and accumulating in other major tissues via the circulatory system. The size of MP particles, rather than exposure routes (waterborne or dietary exposure) and environment (freshwater or seawater), influence the extent of accumulation. Accumulation of MPs in the tissues of fish affects the circulatory system, impacting various hematological parameters related to fat metabolism, immune defense, blood coagulation, osmotic pressure and molecular transport. Exposure to MPs influences ROS production in fish, stimulating or inhibiting antioxidant reactions, and disturbing glutathione and its dependent cycle reactions. In addition, exposure to MPs caused immune toxicity in fish, stimulating or inhibiting immune responses. MP exposure also inhibited AChE in fish, causing behavioral and cognitive disorders. In the future, in-depth research is needed on on the relationship between toxicity when exposed to MP and other toxicants in combination is also considered necessary.

Keywords: microplastics, toxicity, bioaccumulation, oxidative stress, immuno-neuro toxicity

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Current advances in gene and cell therapy for incurable disease

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Gene and cell therapies hold great promise in the treatment of neurodegenerative diseases. Neurodegenerative diseases are characterized by the progressive loss of structure and function of neurons in the brain or spinal cord. Here is an overview of research on gene and cell therapy approaches for Alzheimer's disease (AD). For gene therapy, CRISPR-mediated gene therapy has been performed in AD. CRISPR-mediated gene therapy is a potential therapeutic strategy. However, *in vivo* gene editing via CRISPR remains a challenge, limiting its therapeutic applications for AD. We developed a CRISPR nanocomplex that efficiently targets AD-related genes in the brain *in vivo*, demonstrating its potential for use in novel therapies. For cell therapy, we performed single-cell RNA sequencing analysis and uncovered a previously undiscovered disease-associated subpopulation of oligodendrocytes during the course of AD. Aberrant Erk1/2 signaling has been identified to be associated with disease-associated oligodendrocyte activation in the AD brain. Specifically, inhibition of Erk1/2 signaling in disease-associated oligodendrocytes rescued impaired myelination and Aβ-related pathology in an AD mouse model. Our results suggest that disease-associated oligodendrocyte molecular profiling is a promising new therapeutic strategy for AD treatment. Acknowledgement: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) No. 2021R1C1C1006551.

Keywords: CRISPR, gene therapy, OPC, cell therapy, Alzheimer’s disease

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The 3rd clutch in axoneme: defective sperm motility and male infertility by altered radial spoke

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Radial spokes (RSs) are multiprotein complexes in axoneme of motile cilia and flagella. RS1, RS2, and RS3 are repeated along the axoneme to modulate ciliary and flagellar movement. RS substructures are distinct in spermatozoa from other cells harboring motile cilia in mammals. Here, we report a leucine-rich repeat-containing protein, LRRC23, is a RS head component indispensable for the RS3 head assembly and flagellar movement in mammalian sperm. From a family with infertile males by reduced sperm motility, we identified a LRRC23 mutation that truncates LRRC23 at the C-terminus. In mouse model mimicking the mutation, the truncated LRRC23 protein is only produced in testis but not detected from sperm tail, causing severe sperm motility defects and male infertility. Purified recombinant human LRRC23 does not interacts with a head protein, RSPH9, which is abolished by the C-terminus truncation of LRRC23. Cryo-electron tomography and sub-tomogram averaging visualized that the RS3 head and sperm-specific RS2-RS3 bridge structure is missing in the LRRC23 mutant sperm. Our study provides new insights into RS3 structure and function in mammalian sperm flagella. This work was supported by start-up funds from Yale University School of Medicine and National Institute of Child Health and Human Development (R01HD096745) to Jean-Ju Chung; National Institute of General Medical Sciences (R35GM142959) to Kai Zhang; Pakistan Academy of Sciences (PAS-171) to Wasim Ahmad; and the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. RS-2023-00210046) to Jae Yeon Hwang.

Keywords: male infertility, WES, sperm flagella, radial spoke, Cryo-ET

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Effects of time-restricted feeding on the letrozole-induced mouse model of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a common female reproductive disorder characterized by hyperandrogenemia, chronic anovulation, cystic ovarian follicles, and luteinizing hormone (LH) hyperpulsatility. Recent studies have shown that continuous exposure of sexually developing mice to the aromatase inhibitor letrozole results in the characteristic features of PCOS. Time-restricted feeding (TRF), a regimen that allows eating only during a specific period in the normal circadian feeding cycle without calorie restriction, improves several metabolic conditions, including obesity, insulin resistance, and metabolic syndrome. This study aimed to investigate whether TRF ameliorates metabolic and reproductive phenotypes in a letrozole-induced PCOS mouse model. Female C57BL/6N mice were subcutaneously implanted with letrozole or placebo pellets at 4 weeks of age. Starting at 5 weeks of age, the letrozole-treated mice were randomly assigned to different feeding regimens: 1) TRF for 4 h (ZT12–ZT16) or 2) *ad libitum* diet. After 4 weeks of dietary intervention, estrous cycles were determined with daily vaginal smear examination for 10 days, and serial tail-tip blood sampling was performed at 5-min intervals for 2 h to measure the LH pulse frequency, amplitude, and mean LH levels in the diestrus cycle stage. Serum testosterone and estradiol levels were measured after mice were euthanized. Ovaries were histologically examined to determine the number of cystic follicles. As results, letrozole-treated mice in the *ad libitum* group demonstrated multiple PCOS-like phenotypes including anovulation (estrous cycle arrest), elevated serum testosterone levels, increased body weight, and polycystic ovaries. Furthermore, compared to the controls, letrozole-treated mice exhibited more rapid and elevated LH pulsatility, with increased pulse frequency, amplitude, and mean levels in the diestrus stage. After TRF for 4 weeks, elevated testosterone levels, LH pulse frequency, amplitude of LH pulse, mean LH levels, and increased cystic follicle number reverted to normal levels in letrozole-treated mice. However, the percentage of diestrus cycles, which indicate the arrest of estrous cycling, did not differ between the TRF and *ad libitum* groups. In conclusion, letrozole-treated mice showed several apparent phenotypes that are seen in women with PCOS, including increased serum testosterone levels and markedly hyperactive LH pulse secretion, which were partly restored to normal ranges after 4 weeks of TRF for 4 h/day. Our findings indicate that TRF may have a therapeutic effect on both the metabolic and reproductive phenotypes of PCOS. However, further studies with diverse TRF regimens are necessary to confirm these findings and reveal the underlying physiological mechanisms.

Keywords: polycystic ovary syndrome, time-restricted feeding, letrozole, luteinizing hormone

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Making of an inner ear

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The inner ear consists of two different sensory organs, the vestibule and the cochlea, which are responsible for balance and hearing, respectively. The inner ear develops from a ball-shaped primordial structure called the otocyst. Sonic hedgehog signals emanating from the ventral midline, such as the floor plate and notochord, have been shown to specify the dorsoventral axis of the otocyst. Once the dorsoventral axis is established, the dorsal half of the otocyst develops into the vestibule, and the ventral half forms the cochlea. Removal of Sonic hedgehog signaling early in development results in the complete absence or truncation of the cochlea. The cochlea then elongates and coils into its unique spiral shape. The mechanosensory hair cells are arranged in a special way so that the hair cells located at the basal end of the cochlea detect high frequency sounds and the hair cells at the apical end detect low frequencies. Gradual anatomical and physiological changes along the cochlea facilitate frequency selectivity. This special arrangement is known as tonotopy. However, how tonotopy is established is only beginning to be understood. In this talk, I will discuss how the tonotopy is established during cochlear development.

Keywords: inner ear, development, cochlea, tonotopy, frequency selectivity

심포지엄 II
한국발생생물학회 젊은과학자 I

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Location of second oocyte spindle and ART outcome

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One of the causes of ART failure is aneuploidy. Aneuploidy can happen during biological process and the artificial reproductive technology. However, the relationship between the location of the oocyte meiotic spindle and embryo development including chromosomal abnormality has yet to be well-studied in the *in vitro* fertilization (IVF) cycles, although most of intracytoplasmic sperm injection (ICSI) is following the popular method. Therefore, this study investigated the association between the angle of meiotic spindle deviation and embryo development and euploidy in PGT-A cycle patients. Morphokinetic analysis was performed with a time-lapse monitoring system. This study was conducted on 2,441 oocytes from 303 patients who underwent Polscope and PGT-A from March 2022 to January 2023. Spindle in oocytes was observed using Polscope before ICSI, and oocytes were divided into 4 groups according to the angle of meiotic spindle deviation from the polar body position: A1 (beneath; 0～5°), A2 (adjacent; 5～15°), A3 (away; >15°), and A4 (non-visible). The embryos were single-cultured and monitored using the time-lapse system. Fertilization rate was significantly low in the non-visible spindle A4 group compared with other groups. The good quality embryo (GQE) rates were not different between them. Among groups with a spindle, only A1 and A2 showed significant high in the GQE rate compared with the non-visible group. Also, the groups having a spindle showed statistically higher blastocyst rates of biopsy than those in the group without a spindle on Day 5 or 6. There was a significant difference between spindle angles in A1 and A2 at tPNf and t2in the euploid embryo group. Put together, the results suggest that the position of the oocyte meiotic spindle with morphokinetic parameters may provide essential information to identify euploid embryos with the highest developmental potential.

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Investigating the role of interleukin-7 in porcine embryos: implications and findings

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Interleukin-7 (IL-7), a vital factor that affects cell development, proliferation, and survival, plays an important role in mammalian oocyte maturation. Despite this knowledge, its role in embryonic development remains unknown. Therefore, we aimed to investigate the effects of IL-7 supplementation on *in vitro* culture (IVC) of porcine embryos after parthenogenetic activation (PA) based on characteristics such as cleavage, blastocyst formation rate, total cell number, apoptosis rate, and cell lineage specification in blastocysts. Immunofluorescence revealed that IL-7 and its receptor, IL-7Rα (IL-7R) localized in the cytoplasm of porcine parthenote embryos. By supplementing the IVC medium with various concentrations of IL-7, we observed that an optimal concentration enhances embryonic development, expression of the inner cell mass (ICM) marker SOX2, phosphorylated STAT5 levels in blastocysts, and reduces blastocyst apoptosis. Next, we investigated whether IL-7 supplementation affects blastocyst formation through PI3K/Akt signaling pathway and further influences the efficiency of porcine embryonic stem cell (pESC) establishment. During IVC after PA, we treated IL-7 and the PI3K inhibitor, wortmannin (Wort.), to determine whether IL-7 and PI3K/Akt correlate with porcine blastocyst formation, apoptosis, and gene expression. Then, we demonstrated that treatment with IL-7 and Wort. during IVC affected the SOX2+ cells, ICM ratio, and pAKT expression in blastocysts. Finally, we assessed the efficiency of pESC establishment using blastocysts from each group on day 7 after PA. The establishment of pESC using IL-7-treated blastocysts showed significantly higher colony formation and expression of the core pluripotency markers compared to control blastocysts. In conclusion, IL-7 supplementation could enhance embryonic development and ICM ratio via the PI3K/Akt signaling pathway during porcine embryonic development *in vitro*, potentially assisting the establishment and stable core pluripotency of pESC.

Keywords: *in vitro* culture, porcine embryos, embryonic development, interleukin-7, PI3K/AKT signaling pathway

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The regulation of ribosome-biogenesis related NPM1 in mouse endometrium

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Endometrial cancer is the sixth most common cancer in women, with 85% of cases falling under Type I, which is influenced by estrogen. In endometrial cancer, NPM1 expression is regulated by estrogen, and this regulation affects the severity of the cancer. To address endometrial cancer and other NPM1-associated diseases effectively, understanding the regular regulation and functions of NPM1 in its normal state is crucial. However, the regulation of NPM1 in the normal state of the uterus remains unknown. Therefore, we investigated the changes in NPM1 expression due to estrogen treatment in mouse uterus and aimed to explore the expression patterns and functions of NPM1 through bioinformatics analysis. The results revealed that NPM1 in the uterus responds to estrogen and its expression is regulated by estrogen receptor alpha. Additionally, bioinformatics analysis to uncover the functions of NPM1 showed that estrogen-treated uterus exhibited activated ribosome biogenesis, which might be regulated by MYC. Thus, this study revealed that NPM1 expression in the uterus might be regulated by estrogen-induced MYC and could have a role in ribosome biogenesis. This study discovered the functions of NPM1 in the uterus and shed light on the possibility of MYC being involved in the pathway that regulates NPM1 expression by estrogen. The findings hold significant implications for understanding the functions of NPM1 in the uterus and unveiling new possibilities that MYC mediates the regulation of NPM1 expression.

Keywords: NPM1, ribosome biogenesis, MYC, endometrial cancer, estrogen

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Characterization of fine particulate matter (PM2.5)-induced developmental and teratogenic defects in *Xenopus* embryos

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Particulate matter 2.5 μm (PM2.5) is an indicator of air pollution introduced into the atmosphere by various natural and human activities. It is known that it can stay suspended in the atmosphere for a long time and travel long distances, causing various diseases such as respiratory diseases, cardiovascular diseases, and eye diseases such as glaucoma. To date, there are many studies on respiratory and cardiovascular diseases caused by PM2.5, but there is a lack of research on the eyes, which are in direct contact with PM2.5. In this study, we investigated the adverse effects of PM2.5 to organogenesis including the eyes using a frog embryo teratogenesis assay-*Xenopus* (FETAX). For morphological analysis, *Xenopus laevis* embryos were exposed to PM2.5 at various concentrations, and for genetic analysis, embryos at the affected concentrations were subjected to real-time qPCR and whole-mount *in situ* hybridization (WISH). Embryos exposed to 70 μg/mL developed malformations including reduced length, edema, small eye, gut miscoiling, and cardiac edema. Genetic analysis revealed that PM2.5 reduced the expression of eye-related marker genes such as *rx1*, *pax6*, as well as various organ marker genes such as *darmin*, and *ldlrap1*. We also checked cell migration to see if it was involved in the teratogenesis, but migration was not affected. In conclusion, our study shows that PM2.5 affects gene expression during *Xenopus* embryonic development, leading to developmental toxicity and teratogenicity.

Keywords: *Xenopus*, PM2.5, FETAX, teratogenicity, eye development

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Mebendazole exerts anti-cancer activity by preferentially inhibiting cilia formation

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The cilium, a microtubule-based organelle, plays a pivotal role in embryonic development and maintaining physiological functions in the human body. Cilia function as sensors, transducing a diverse range of extracellular signals, including growth factors, fluid flow, and physical forces. Moreover, cilia are intricately involved in regulating cell cycle progression and preserving DNA integrity, as their formation and resorption dynamics are tightly controlled during the cell cycle. Consequently, numerous studies have linked defects in specific ciliary proteins to the DNA damage response.

In this study, we investigated the impact of Mebendazole, an anthelmintic drug renowned for its microtubule growth inhibition, on ciliogenesis. By utilizing *Xenopus laevis* embryos as a model system, we discovered that Mebendazole treatment significantly hindered ciliary formation and induced DNA damage. Remarkably, primary cilium-bearing cancer cells exhibited heightened vulnerability to the combined treatment of Mebendazole and conventional anti-cancer drugs. These findings shed light on the specific influence of Mebendazole on cilia, rather than cytosolic microtubules, in triggering DNA damage and potentially elucidate an unidentified mechanism underlying Mebendazole-mediated cancer therapy.

Keywords: cilium, mebendazole, DNA damage, cancer therapy, microtubules, *Xenopus laevis*

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Signaling of myoinhibitory peptides and its possible role in larval development in Pacific abalone *Haliotis discus hannai*

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In invertebrates, myoinhibitory peptides (MIPs) exert their effects locally as hormones, neurotransmitters, and neuromodulators for various physiological functions, including juvenile hormone signaling, gut muscle contraction, larval development, growth, metabolism, and reproduction. Insect MIPs are repetitive proneuropeptides that contain multiple paracopies of a conserved $W(X)_{5-8}W$-amide motif, which stabilizes its beta-turn conformation. The evolutionary conserved MIP family members are potent ligands for sex peptide receptors (SPRs) in yellow fever mosquito *Aedes aegypti*, fruit fly *Drosophila melanogaster*, and the sea slug *Aplysia californica*. However, MIP signaling has not yet been investigated in mollusks except for *A. californica*. MIP signaling and functions in gastropod mollusks may provide in-depth information on the molecular conservation of MIP signaling systems in invertebrates. In this study, we identified a MIP precursor and a full-length transcript coding SPR in the Pacific abalone *Haliotis discus hannai* (Hdh), characterized Hdh-MIP signaling, and examined the role of Hdh-MIP in larval development. Similar to the insect MIP precursors, we found a total of eight paracopies for Hdh-MIPs, harboring MIP-specific $W(X)_{5-8}W$-amide motifs, except for Hdh-MIP2 with a substituted S residue instead of C-terminal W. Luciferase reporter assays demonstrated that all examined Hdh-MIPs but not Hdh-MIP2 significantly activate intracellular cAMP accumulation in Hdh-SPR-expressing mammalian cells. When we explored the contribution of MIP to the regulation of metamorphosis in *H. discus hannai* veliger larvae, Hdh-MIPs inhibited the attachment and metamorphosis of the swimming larvae at 2 to 8 days post-fertilization. In contrast to this result, a MIP treatment induced the settlement of a marine Annelida *Platynereis dumerilii* post-larvae. Our findings provide novel insight into the molecular basis of MIP signaling and larval metamorphosis in marine gastropods.

Keywords: myoinhibitory peptide, sex peptide receptor, invertebrate

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Nirmatrelvir induces detrimental effects on sperm function by disturbing the AKT pathway

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As COVID-19 broke out, several therapeutic agents have been applied. Nirmatrelvir (NMV) is a recently developed oral antiviral drug for adults at high risk of progression to severe COVID-19, and it selectively inhibits Sars-Cov-2 main protease. Despite the wide use of NMV, the study of its male reproductive toxicity is still lacking. On the other hand, several antiviral drugs have been reported to suppress the phosphorylation of protein kinase B (AKT). Especially, ritonavir used together with NMV as one of the COVID-19 treatment ingredients has been known to suppress sperm functions by altering the AKT phosphorylation. Therefore, the present study was performed to investigate the male reproductive toxicity of NMV and how it affects the phosphorylation of AKT in spermatozoa during the capacitation. For the experiment, Duroc boar spermatozoa were incubated with various concentrations of NMV (0, 0.1, 1, 10, 50, and 100 μM). Then, sperm motility, motion kinematics, capacitation status, intracellular ATP level, and cell viability were evaluated. In addition, expression levels of PKA, tyrosine-phosphorylated substrates, AKT, and phospho-AKT (Thr^{308} and Ser^{473}) were measured by western blotting. Our results showed that sperm motility and motion kinematic parameters were significantly decreased at the highest concentration (100 μM). The ratio of capacitation was decreased from 50 μM, and the intracellular ATP level was decreased from 1 μM significantly, while there was no significant difference in the ratio of acrosome reaction and cell viability. The PKA activation was significantly decreased while protein tyrosine phosphorylation was not significantly altered. In addition, AKT and phospho-AKT (Thr^{308} and Ser^{473}) expression levels were significantly increased. Taken together, it could be estimated that NMV has detrimental effects on sperm functions by altering the AKT phosphorylation. Therefore, caution needs to be taken for reproductive toxicity when prescribing and taking NMV.

Keywords: nirmatrelvir, spermatozoa, capacitation, AKT

심포지엄 Ⅲ

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Expression of Chi3l1 in the mice uterine epithelial cell according to the estrogen receptor alpha

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In the uterus, unlike other organs, dynamic modification occurs in response to ovarian hormones such as estrogen and progesterone. Chitinase-like proteins, CLPs, which belong to the glycoside hydrolase family 18 of proteins are expressed differently across the species due to mutations in the enzymatic active site of chitinase. Chitinase-3 like 1, Chi3l1, which is expressed in both humans and mice, is the most studied protein among CLPs and is known to play a role in host defense and immune regulation. Although many studies have been conducted in respect of pathology like asthma and cancer with Chi3l1, nothing has been revealed about the expression pattern of Chi3l1 in the physiology of the uterus and estrogen. In this study, according to microarray data, we confirmed that the Chi3l1 mRNA expression in the uterus was induced by 17β-estradiol treatment in the ovariectomized wild-type mice not in estrogen receptor alpha (ERa) knock-out mice. In addition, the expression of Chi3l1 mRNA was not promoted in the uterus-specific Era conditional knock-out mice with the injection of estradiol. In the uterus of mice, Chi3l1 mRNA and protein expression was significantly regulated during the estrous cycle. The expression was highest in the proestrus stage and gradually decreased until the diestrus stage. Moreover, Chi3l1 mRNA and protein expression was dramatically increased in the uterus of ovariectomized mice by administration of 17β-estradiol, especially 24 hours after. As microarray data, the expression of Chi3l1 and CHI3L1 via 17β-estradiol was inhibited through treatment of ICI, estrogen receptor alpha specific antagonist. Progesterone could not induce the expression of Chi3l1 in the uterus. We also identified the cell type specific expression of Chi3l1 in mouse uterus through the isolation and culture of uterine primary cell. The Chi3l1 expression was only identified in epithelial cell not in stromal cell after 17β-estradiol treatment. These results support the bioinformatic data analysis, Chi3l1 was exclusively localized in the luminal and glandular epithelial cells of the uterus. Our study revealed that an increase in Chi3l1 mRNA and protein of uterine epithelial cells can be induced by estrogen. It is known that Chi3l1 also plays the role in epithelial-mesenchymal transition and angiogenesis. This represents a possibility that Chi3l1 has a crucial role in regulating the vigorous changes in the uterus with estrogen.

Keywords: uterus, chitinase like protein, chitinase-3 like 1, estrogen, estrogen receptor alpha

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Distribution of dopaminergic neuron of *Octopus minor* brain and dopamine transporter system

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Dopamine (DA) is the most important catecholamine in the brain. The involvement of DA as a neurotransmitter in the regulation of physiological functions in the central nervous system (CNS) has been studied well. DA-related gene mutations or changes in the amount of DA in the CNS cause diseases such as Parkinson's disease, attention deficit hyperactivity disorder (ADHD) and depression. It has been known that DA is conserved not only in vertebrates but also in invertebrates. According to studies using *Drosophila*, Mollusk and *Caenorhabditis elegans* as model systems, DA classical pathway and the sequence of DA receptors are conserved in invertebrates. However, in the Mollusca CNS, presence of DA core genes such as DA decarboxylase (DDC), D1-like receptor and DA transporter (DAT), and location of dopaminergic neuron are unclear. In this study, we identified the DA core gene in the *Octopus minor* genome through phylogenetic analysis. In addition, the location of DA neurons and receptors in the CNS were visualized by *in situ* hybridization or immunohistochemistry. This study will provide basic information for understanding the DA system in Mollusca.

Keywords: cephalopod, *Octopus minor*, central nervous system, dopamine

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Microbiome of groupers: effect of microbial dietary to ameliorate in health

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This study on the microbiome of groupers: the whole-body microbiome of groupers larvae (red-spotted grouper *Epinephelus akaara* ♀ × ♂, RG; red-spotted grouper ♀ × giant grouper *E. lanceolatus* ♂, RGGG; kelp grouper *E. bruneus* ♀ × ♂, KG; kelp grouper ♀ × giant grouper ♂, KGGG; convict grouper *Hyporthodus septemfasciatus* ♀ × ♂, CG), and the effect of microbial feed additives on 1-year-old RGGG. The type of feed changed the microbial composition of the RGGG larvae. The dominant species of RGGG larvae with chlorella was *Dulcicalothrix* spp. (21.78%), and those with rotifer were *Carnobacterium* spp. (38.90%) and *Vibrio* spp. (38.27%). The five species of groupers larvae with rotifer had a dominant phylum, Pseudomonadota in common, and *Vibrio* spp. were the largest proportion among them. The α-diversity of groupers larvae with rotifer was even higher in KG and KGGG than in RG, RGGG, and CG. The 1-year-old RGGG fed dietary with added prebiotics, *Lactobacillus plantarum*, *Bacillus subtilis*, *B. licheniformis*, and synbiotics, respectively, for eight weeks. The specific growth rate and weight gain of RGGG with synbiotics were significantly higher than those of the control group and RGGG with *L. plantarum*, and *B. licheniformis* ($p<0.05$). After eight weeks, the width of the intestinal villi of RGGG with microbial supplements was significantly wider than that of the control group ($p<0.05$). The number of goblet cells in their intestine was significantly lower than that of the control group ($p<0.05$). A further analysis of this study in progress will help understand the connection between the microbiome and diet, and contribute to developing techniques to improve groupers' growth, immunity, and disease resistance while replacing antibiotics in the aquaculture industry.

Keywords: microbiome, groupers, microbial feed additives, growth performance, intestinal villi

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Overview on status and technological advances in tuna aquaculture in Korea

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[연구의 경과] 한국의 참다랑어 양식 연구는 일본이나 호주, 지중해 연안국보다 20～35년 정도 늦은 2005년에 시작되었다. 가장 먼저 해상가두리와 육상수조 등 사육시설을 구축했고, 이어서 자연산 종자를 채포해 어미까지 양성하는 관련 연구를 수행했다. 연구 개시 이후, 국내 해상가두리와 육상수조에서 양성 중이던 어미로부터 2015년과 2020～2021년에 각각 30만 개와 44만 개의 수정란을 생산한 바 있으며, 몰타와 크로아티아에서 채집한 수정란 180여만 개를 8회에 걸쳐 수송해 종자생산연구에 활용하기도 했다. 확보한 수정란으로 자치어기 감모 예방을 위한 영양학적 연구와 기초 생리학적 연구 등의 연구를 수행하는 한편, 중간육성 단계인 참다랑어 유어까지 양성하였다.

[현주소] 최근에는 양성 중인 어미로부터 수정란을 안정적으로 생산하기 위한 산란유도 기술 개발을 연구 중이다. 그 일환으로 해상가두리에서는 5～6세 어미의 생식주기를 구명해 한국 남해 해역에서 양성 중인 어미의 산란 가능성을 확인했고, 육상수조(직경 25 m, 수심 8 m)에서는 수온, 광주기 등의 환경조절 프로그램과 성성숙 유도 호르몬 투여법을 구축해 적용하면서 재현성 연구를 수행 중이다.

[연구의 당위] 남획 등 지속불가능한 어업 관행이 개체수의 급감을 불러왔고, 방치할 경우 공유재의 비극(tragedy of the commons)으로 이어진다는 점에서, 그리고 기후변화에 따른 해양온난화(marine warming)가 미치는 영향에 대한 연구가 주목받고 있는 현실에서, 참다랑어 양식에 대한 세계 각국의 연구는 전 지구적 공유자산자원(open-access resources)의 회복 및 유지를 위해 반드시 필요하다. 한국 국내적으로는 양식 선진국과의 종자생산 기술 격차를 해소해 세계 수준의 양식 기술을 확보함으로써 점증하는 수요에 대응해야 한다는 당위가 존재한다.

[미래 어젠다] 그러나 참다랑어 양식기술 개발, 특히 전주기 양성에 있어서, 현실적으로 두 가지 벽에 부딪혀 있는 실정이다. 하나는 채포한 자연산을 어미까지 양성하는 것은 가능하지만, 그 어미로부터 수정란을 확보하는 것은 불안정하다는 것이다. 또 하나는 수정란에서 유어까지 양성하는 것은 가능하지만, 그 유어를 어미까지 양성하는 데는 실패를 거듭해 왔다는 것이다. 향후 한국의 참다랑어 양식연구의 어젠다는 이 두 가지 문제의 해결에 있다고 할 것이다.

Keywords: 참다랑어, 양식기술, 생식주기, 종자생산

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The noncanonical action of MYC promotes tumor development by orchestrating an immunosuppressive tumor microenvironment

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The proto-oncogene *MYC*, frequently amplified in various solid cancers, has been demonstrated to promote tumor development. Mechanistically, c-Myc which is encoded by *MYC* acts as a global transcription factor, which in turn, induces transcription of genes associated with cell proliferation, survival, migration, metabolism and etc. Recently, its role in orchestrating an immune-cold tumor microenvironment (TME) has been revealed, however, precise mechanisms are not fully understood. Here, we identified 30 genes that are predicted to be directly repressed by c-Myc through the integrated analysis of RNA- and chromatin immunoprecipitation (ChIP)-seq data. Among these genes, low expression of SID1 transmembrane family member 2 (*SIDT2),* which encodes a transmembrane RNA transporter protein, was significantly correlated with a lack of tumor-infiltrating lymphocytes in breast cancer and melanoma. Furthermore, the low level of *SIDT2* expression was associated with poor response to immune checkpoint inhibitors (ICIs). Mechanistically, c-Myc directly suppresses *SIDT2* transcription, resulting in the inhibition of the dsRNA-mediated immune response. Consequently, this leads to the reduced expression of *CXCL9*, *CXCL10*, *CXCL11* and *CCL5*. Finally, we validated that the ablation of *SIDT2* caused a significant reduction in the migration of T cells into cancer cells. Together, these suggest that *MYC* impedes the dsRNA-mediated immune response by repressing *SIDT2* expression, thereby promoting immune evasion of cancer cells.

Keywords: *MYC*, antitumor immunity, SIDT2, cytosolic dsRNA

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Neurotrophin-4 in the porcine ovary: its effect on oocyte maturation and developmental competence

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Neurotrophin-4 (NT-4) is a neurotrophic factor that plays an essential role not only in neural development but also ovarian development. However, only a few studies have investigated the major functional roles of NT-4 in mammalian ovarian development. Therefore, this study aims to investigate the physiological roles of NT-4 in porcine oocyte maturation and embryonic development. We identified the localization of NT-4 and its receptors (TrkB and $p75^{NTR}$) in porcine ovaries, cumulus cells, and oocytes. We demonstrated that NT-4 supplementation during *in vitro* maturation (IVM) enhances nuclear and cytoplasmic maturation of porcine oocytes. NT-4 promotes the maturation of cumulus-oocyte complexes (COCs) by upregulating *NFKB1*, *GDF9*, *BMP15*, *CD9*, and *DNMT3A* transcripts. The level of phosphorylated extracellular signal-regulated kinase 1/2 (ERK1/2) was significantly higher in NT-4-treated cumulus cells compared to control. Both total and phosphorylated ERK1/2 levels were significantly higher in NT-4-treated oocytes compared to control. Thus, NT-4 promotes to oocyte maturation and cumulus expansion by regulating the ERK1/2 signaling pathway in porcine COCs during IVM. Moreover, 10 ng/mL NT-4 supplementation during IVM improved subsequent embryonic development after parthenogenetic activation, *in vitro* fertilization, and somatic cell nuclear transfer. Our study demonstrated for the first time that the physiological roles of NT-4 in the porcine female reproductive system are to enhance oocyte maturation, promote cumulus cell expansion, and improve subsequent embryonic developmental potential.

Keywords: neurotrophin-4, cumulus cells, oocytes, *in vitro* maturation, pig

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심포지엄 V

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DNA damage response in mammalian oocytes

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Meiosis, a process crucial for reducing ploidy, involves two consecutive cell divisions without an intervening S-phase. In the first meiotic division (meiosis I), homologous chromosomes pair and then segregate from each other, whereas sister chromatids segregate during the second meiotic division (meiosis II). A unique characteristic of meiosis in female mammals, not seen in any other cell type, is prolonged arrest at the prophase of meiosis I, which is characterized by the presence of the germinal vesicle (GV). This extended arrest makes oocytes acutely susceptible to the accumulation of DNA damage. Thus, it is critical to have a robust surveillance mechanism to ensure DNA damage repair and maintain oocyte and embryo quality. Surprisingly, however, fully grown oocytes lack a robust G2/M DNA damage checkpoint. Therefore, oocytes with DNA damage resume meiosis.

While interphase cells effectively respond to DNA damage by activating cell cycle checkpoints and DNA repair pathways, mitotic cells are refractory to DNA damage and fail to mount DNA damage-induced cell cycle arrest. Instead, mitotic cells recruit MDC1 and TOPBP1 to damaged sites, forming a filamentous structure that bridges and tethers broken chromosome ends. This allows mitotic cells to mark DNA damage sites for repair in the subsequent G1 phase. Unlike mitotic cells, oocytes have been shown to repair DNA damage during meiosis, implying that the DNA damage response (DDR) in oocytes differs from that in somatic cells.

Here, I present why oocytes do not mount a robust G2/M DNA damage checkpoint and how to repair DNA damage during meiosis.

Keywords: oocyte, DNA damage, checkpoint, meiosis

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Enhancing ovum pick-up productivity through mild FSH stimulation

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Given the global demand for high-quality cattle, it is necessary to develop effective techniques for producing oocytes from high-quality cows. Ovum pick-up (OPU) methods play a significant role in oocyte collection worldwide and are the most efficient means of enhancing genetic advancement through maternal lines in cattle. This study aimed to establish an efficient OPU-derived transferable embryo production system. Oocytes were collected from 20 control and 15 follicle-stimulating hormone (FSH)-treated female Hanwoo. A combination of decreasing doses of FSH (36, 36, 24, and 24 mg, 12 h apart), progesterone, estrogen, and prostaglandin was administered to synchronize and mildly stimulate the animals. *In vitro* blastocysts were generated by *in vitro* maturation, fertilization, and culture. The FSH-treated group (1,125 oocytes) and the control group (1,022 oocytes) exhibited a higher proportion of Grade A and B oocytes (88.2%) than other grades ($p<0.05$), with the majority of them in the germinal vesicle 2 stage (64.0%). Moreover, the FSH group had a significantly higher blastocyst rate (44.7%) than the control group (31.1%) ($p<0.01$). After vitrification and *in vitro* culture warming, the embryos of the FSH group exhibited higher re-expansion rates (Grade 1: 86.9% and Grades 2 and 3: 57.9%) than the control group ($p<0.01$). FSH treatment also reduces working hours, making it an efficient method for embryo production, freezing, and preservation.

Keywords: follicle-stimulating hormone, ovum pick-up, synchronization, vitrification

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두족류 연구현황과 발전방안

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두족류는 연체동물문 두족강에 속하는 동물을 총칭하며, 문어, 오징어, 앵무조개 등이 포함된다. 전세계적으로 약 800종의 살아있는 두족류가 확인되고 있으며, 크기는 10 mm에서 14 m까지 다양하다. 국내에는 약 38종의 두족류가 알려져 있으며, 이 중에서도 문어류(대문어, 참문어 등)와 오징어류(살오징어, 참갑오징어 등)가 주요 상업적으로 이용되고 있다. 국내에서는 최근 소비량 증가와 생산량이 감소함에 따라 일부 어종에 대해서는 자원관리를 위한 금어기 설정과 인공종자의 방류사업을 수행하고 있는 실정이다. 문어, 오징어와 같은 두족류는 짧은 생활사(short life span)와 빠른 성장(fast growth) 때문에 양식 대상종으로서 주목받아 왔다. 따라서 국내외적으로 양식을 위한 연구는 오래전부터 시작되어 왔으나, 대부분의 두족류는 초기생활사 단계에서 성체와는 다른 형태인 유생단계를 거치기 때문에 종자생산연구를 위해서는 이 단계를 이해하고 접근할 필요가 있었다. 국외에서의 대문어 종자생산연구는 1970년대부터 시작하여 1980년대 1건의 성공 보고가 있었으나, 이후 성공한 사례가 없을 정도로 난이도가 높다. 참문어는 1960년대부터 다양한 먹이 공급 시도와 사육방법을 통해 2000년대에 들어와서 어린 참문어 형태까지 사육할 수 있었으며, 최근에는 스페인, 일본 등에서 상품 출하 크기까지 성장시키는 시험양식에 성공하였으며, 상업적인 양식을 위한 준비를 하고 있다. 살오징어는 생태학적 특성으로 인해 초기생활사에 대한 접근이 어려웠으나, 1990년대에 인공수정 기술이 개발됨으로써 유생을 확보하여 생활사 초기단계의 연구가 시작되었으나, 초기 먹이를 밝혀내지 못해 유생 단계에 머무르고 있다.

한편, 국내에서의 두족류 종자생산연구는 2010년대부터 일부 연구자에 의해 수행되기 시작하였다. 대문어, 참문어의 경우, 성숙 암컷의 확보를 통한 산란과 부화를 통한 유생 생산까지는 가능하지만, 어린 문어의 형태까지는 사육하지 못하고 있는 실정이다. 또한, 살오징어는 어미의 실내 축양과 인공수정 기술은 확보하여 유생 생산은 가능하지만, 초기 먹이를 밝혀내지 못하고 있는 실정으로 발생생물학을 포함한 다양한 학문적 접근을 통한 생활사 초기과정의 이해가 필요하다고 생각된다.

Keywords: 문어류, 오징어류, 양식기술, 인공종자생산

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Strategies for mass cultivation of marine algae

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The cultivation of marine algae has been gaining attention due to its potential for carbon dioxide reduction and high economic value. Various strategies, such as nutrient regulation and environmental factor control, are being proposed for mass cultivation. Among them, we introduce increasing the microalgae and seaweed biomass using light-emitting diodes (LED) and microbiome.

To enhance the biomass of microalgae, we conducted a study using various wavelengths of LED. Three microalgae species, *Pavlova lutheri, Chlorella vulgaris,* and *Porphyridium cruentum*, were cultured under various single wavelengths. Biomass production by *P. lutheri, C. vulgaris,* and *P. cruentum* were the highest with blue, red, and green LED wavelength with 1.09 g dcw/L, 1.23 g dcw/L, and 1.28 g dcw/L on day 14, respectively. Biomass production was highest at the complementary LED wavelength to the color of microalgae. Lipid production by *P. lutheri, C. vulgaris,* and *P. cruentum* was the highest with yellow, green, and red LEDs wavelengths, respectively. Eicosapentaenoic acid production by *P. lutheri, C. vulgaris,* and *P. cruentum* was 10.35%, 10.14%, and 14.61%, and those of docosahexaenoic acid were 6.09%, 8.95%, and 11.29%, respectively.

For seaweed cultivation, the sample was collected from Sinyang Beach, Jeju, Korea where the green algae blooms have resulted in numerous ecological, environmental, and economic problems. The analysis identified the seaweed sample as *Ulva reticulate.* Additionally, the extracted genomes from the collected *U. reticulate* was subjected to metagenome analysis using next-generation sequencing. For the sustainable supply of seaweeds industrially, *U. reticulate* was cultured in the 5-ton tank. We discovered that a culture with a microbiome enhances the biomass compared to one without the microbiome. This study highlighted that understanding the functional roles of associated microbiomes in seaweed can mitigate the algae blooms in Jeju beach, and positively affect restoration. Furthermore, the suggested approach provides cost-effective technology for a sustainable supply of *U. reticulate.*

Keywords: microalgae, macroalgae, cultivation, light-emitting diodes (LED), microbiome

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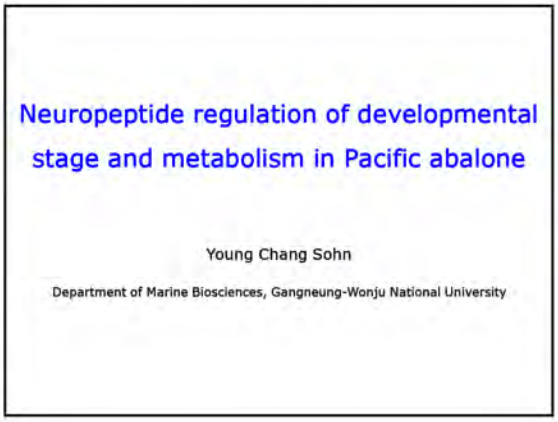
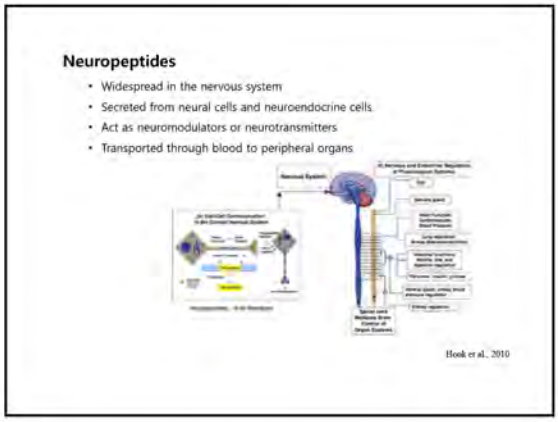
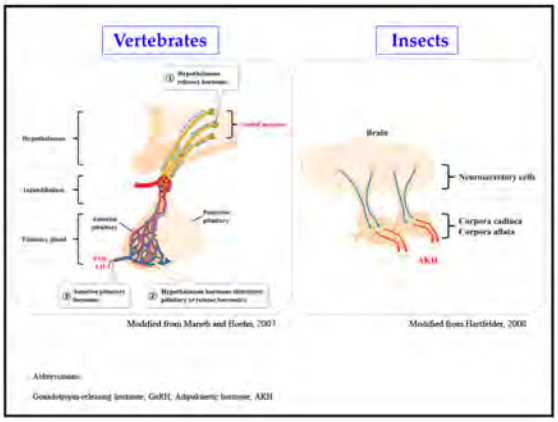
Neuropeptide regulation of developmental stage and metabolism in Pacific abalone

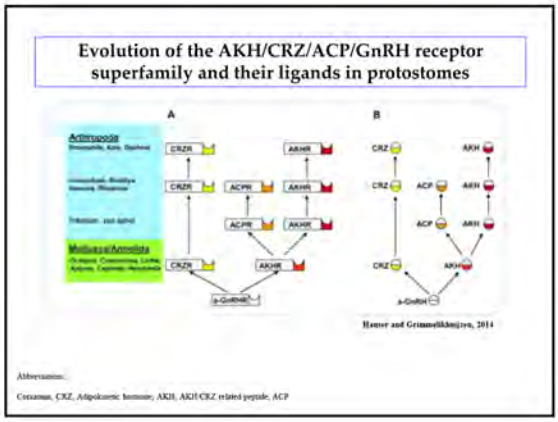
손영창

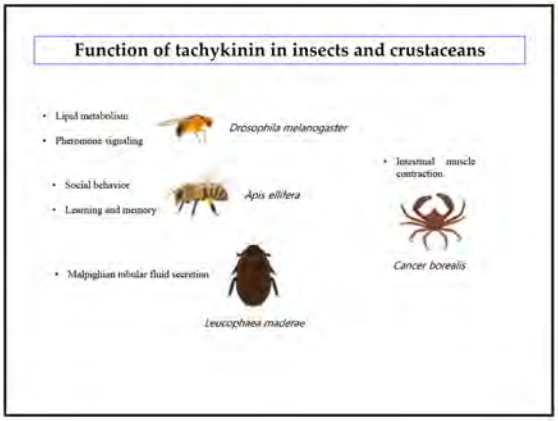
강릉원주대학교

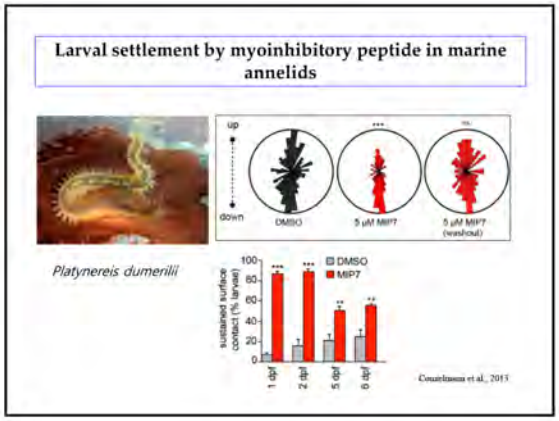


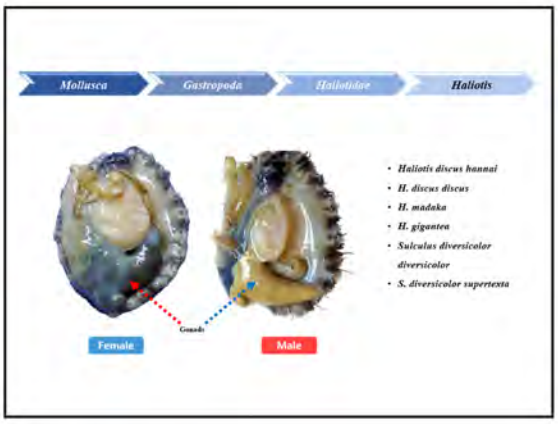


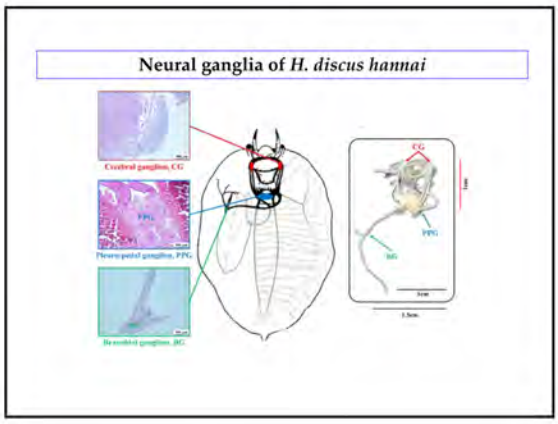


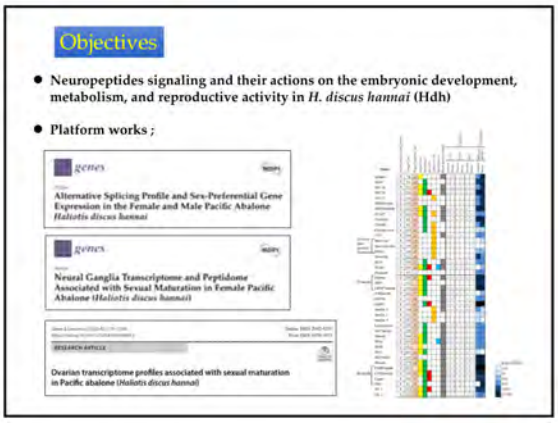


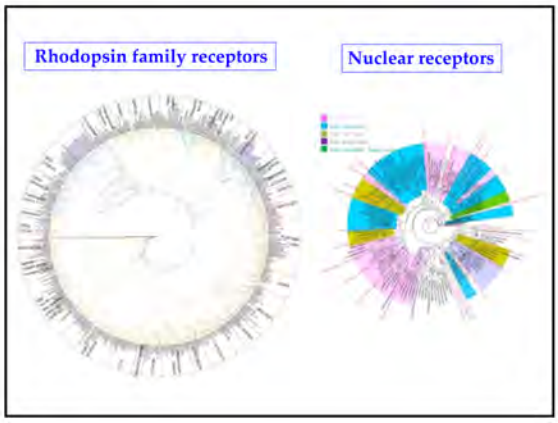


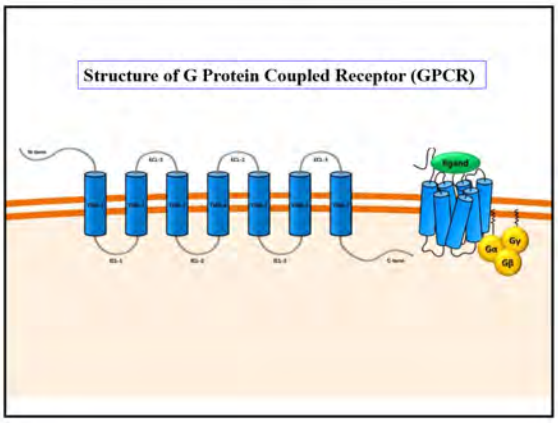


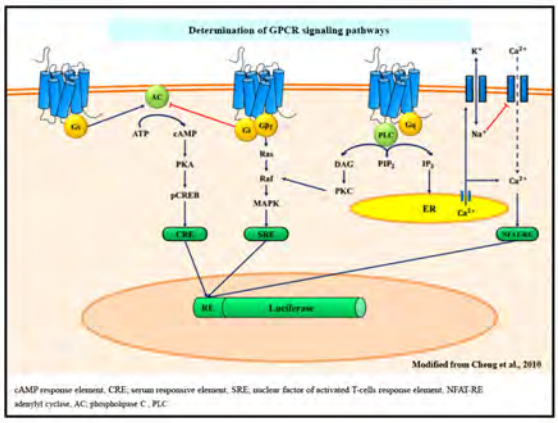

Mollusca
Gastropoda
Haliotidae
Haliotis
Female
Male
Haliotis discus hannai
H. discus discus
H. madaka
H. gigantea
Sulculus diversicolor diversicolor
S. diversicolor supertexta


Neural ganglia of H. discus hannai
Cerebral ganglion, CG
Pleuro-pedal ganglion, PPG
Branchial ganglion, BG


Objectives
Neuropeptides signaling and their actions on the embryonic development, metabolism, and reproductive activity in H. discus hannai (Hdh)
Platform works ;
genes
Alternative Splicing Profile and Sex-Preferential Gene Expression in the Female and Male Pacific Abalone Haliotis discus hannai
genes
Neural Ganglia Transcriptome and Peptidome Associated with Sexual Maturation in Female Pacific Abalone (Haliotis discus hannai)
Ovarian transcriptome profiles associated with sexual maturation in Pacific abalone (Haliotis discus hannai)

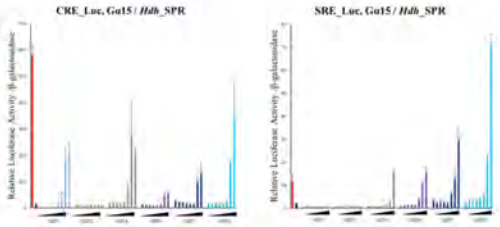

Rhodopsin family receptors
Nuclear receptors

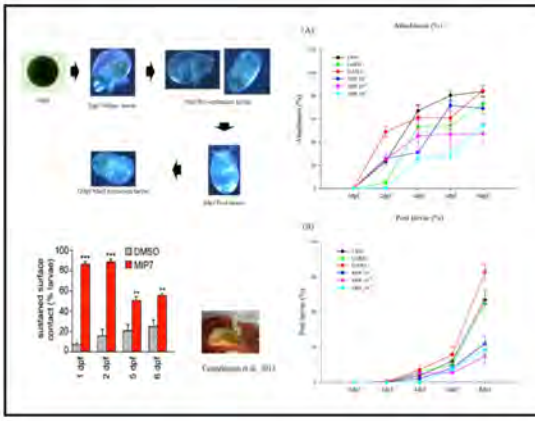

Structure of G Protein Coupled Receptor (GPCR)
Ligand
Gα
Gγ
Gβ


Determination of GPCR signaling pathways
AC
ATP
cAMP
PKA
pCREB
CRE
Ras
Raf
MAPK
SRE
PLC
DAG
PIP2
IP3
PKC
ER
RE
Luciferase
Modified from Cheng et al., 2010
cAMP response element, CRE, serum responsive element, SRE, nuclear factor of activated T-cells response element, NFAT-RE
adenylyl cyclase, AC; phospholipase C , PLC

Myoinhibitory peptide (MIP) signaling system

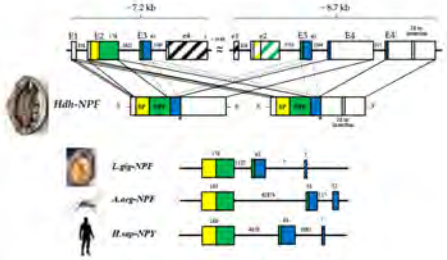
Functional characterization of Hdh-SPR





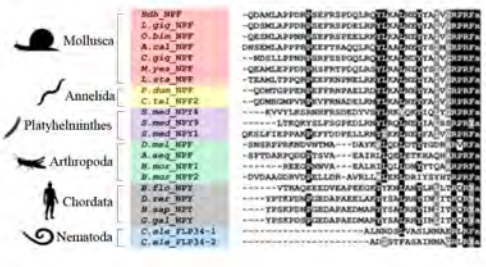
Abalone NPF (*H. discus hannai* NPF, Hdh-NPF)

- NPF precursor; signal peptide + proNPF (NPF peptide+G+R)
- Conserved in NPF/Y gene organization



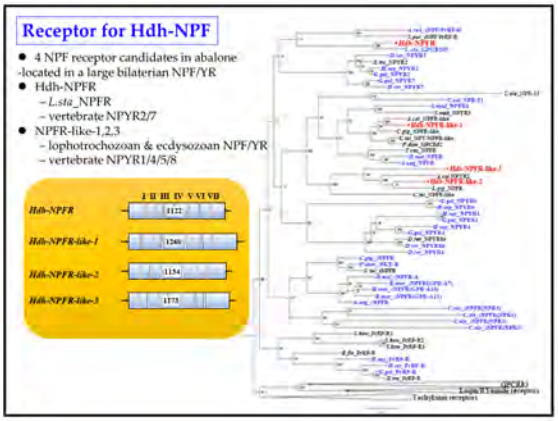
Abalone NPF (*Haliotis discus hannai* NPF, Hdh-NPF)

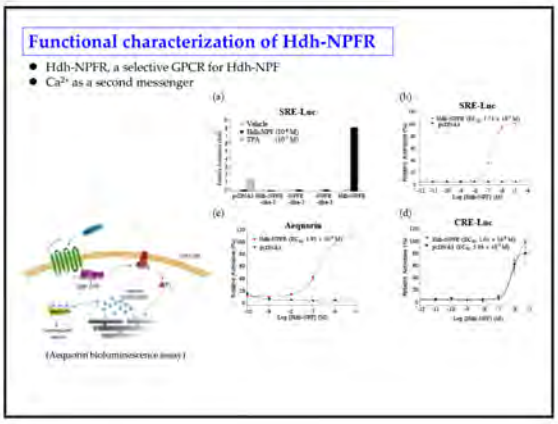
- Well conserved aa, RPRFa in C-terminal and P8, P11
- Hdh-NPF as ortholog of chordate NPY
- Water soluble; signal transducton by binding with membrane receptor

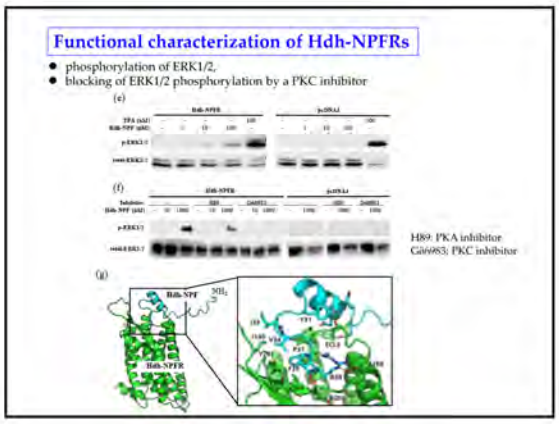


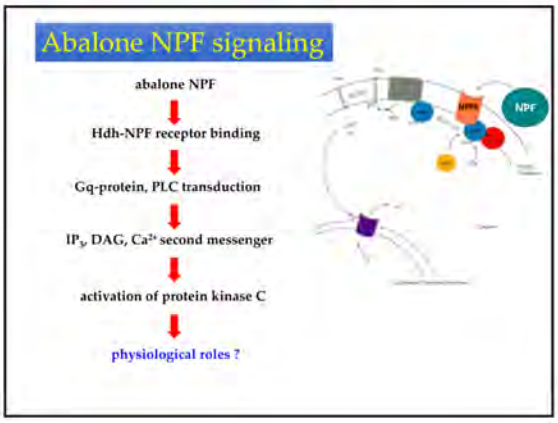
Receptor for Hdh-NPF

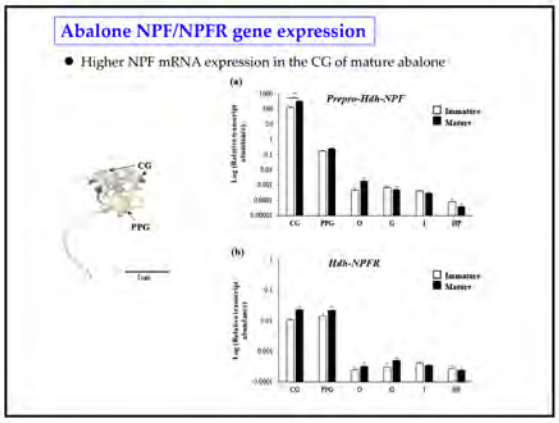
- 4 NPF receptor candidates in abalone
  -located in a large bilaterian NPF/YR
- Hdh-NPFR
  - *L.sta*_NPFR
  - vertebrate NPYR2/7
- NPFR-like-1,2,3
  - lophotrochozoan & ecdysozoan NPF/YR
  - vertebrate NPYR1/4/5/8

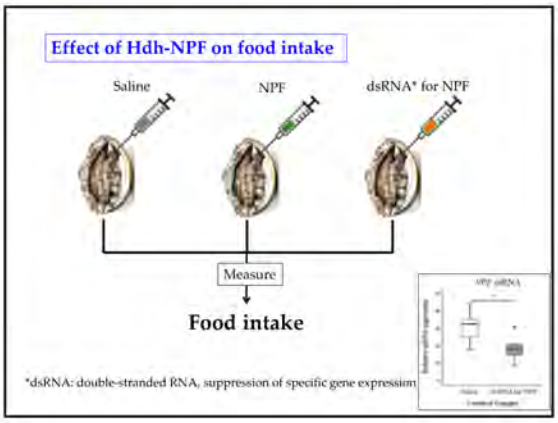


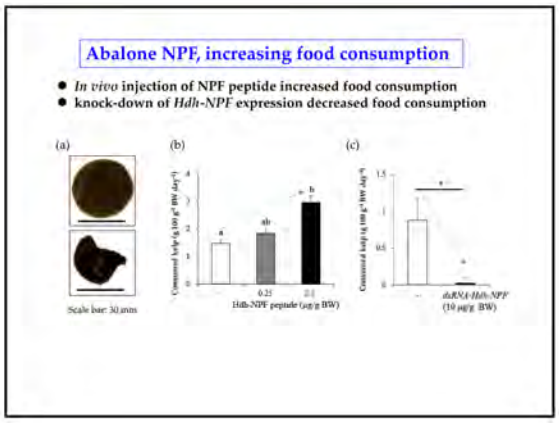

Functional characterization of Hdh-NPFR
Hdh-NPFR, a selective GPCR for Hdh-NPF
Ca^{2+} as a second messenger
SRE-Luc
Aequorin
CRE-Luc
(Aequorin bioluminescence assay)

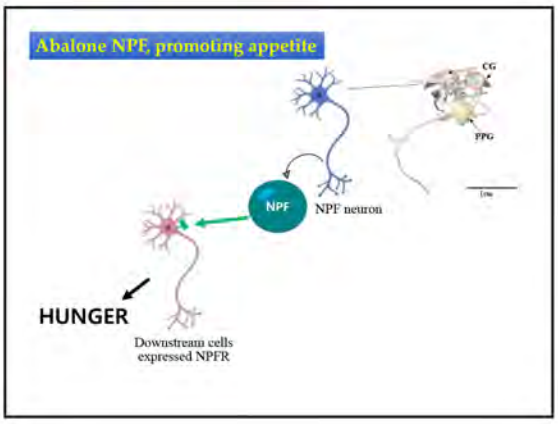

Functional characterization of Hdh-NPFRs
phosphorylation of ERK1/2,
blocking of ERK1/2 phosphorylation by a PKC inhibitor
H89: PKA inhibitor
Gö6983: PKC inhibitor
Hdh-NPF
Hdh-NPFR

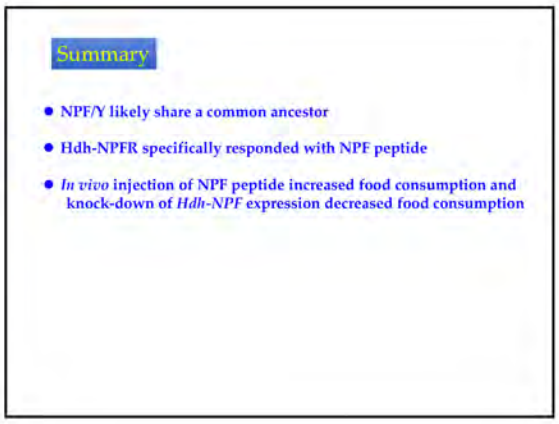

Abalone NPF signaling
abalone NPF
Hdh-NPF receptor binding
Gq-protein, PLC transduction
IP_3, DAG, Ca^{2+} second messenger
activation of protein kinase C
physiological roles ?
NPF

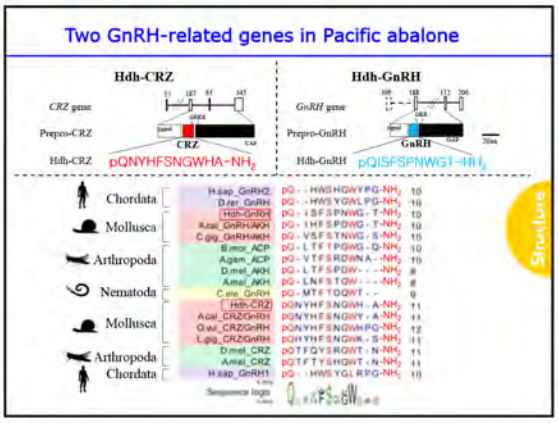

Abalone NPF/NPFR gene expression
Higher NPF mRNA expression in the CG of mature abalone
CG
PPG
Prepro-Hdh-NPF
Hdh-NPFR
Immature
Mature

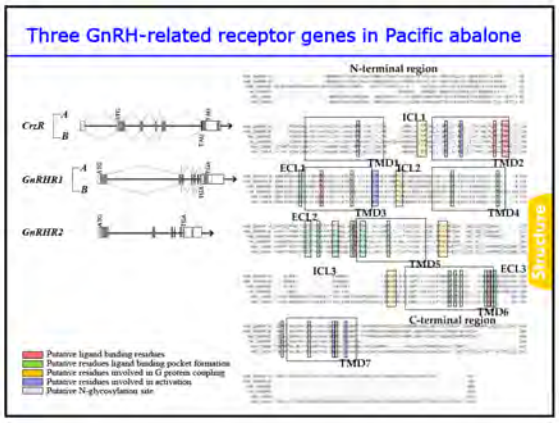

Effect of Hdh-NPF on food intake
Saline
NPF
dsRNA* for NPF
Measure
Food intake
*dsRNA: double-stranded RNA, suppression of specific gene expression

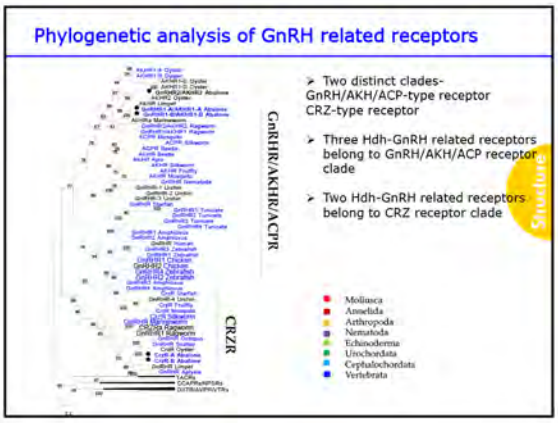

Abalone NPF, increasing food consumption
In vivo injection of NPF peptide increased food consumption
knock-down of Hdh-NPF expression decreased food consumption
Scale bar: 30 mm
Hdh-NPF peptide (pg/g BW)
dsRNA-Hdh-NPF
(10 μg/g BW)

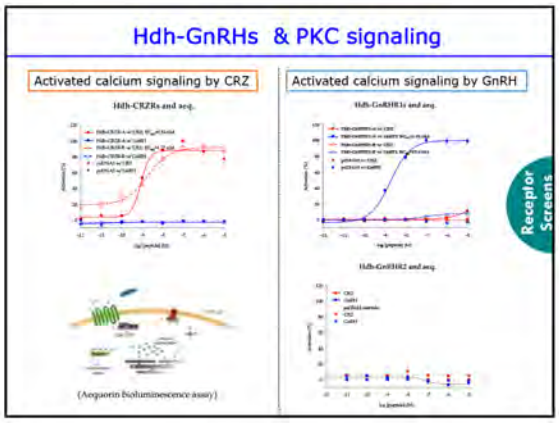

Abalone NPF, promoting appetite
CG
PPG
NPF
NPF neuron
HUNGER
Downstream cells
expressed NPFR

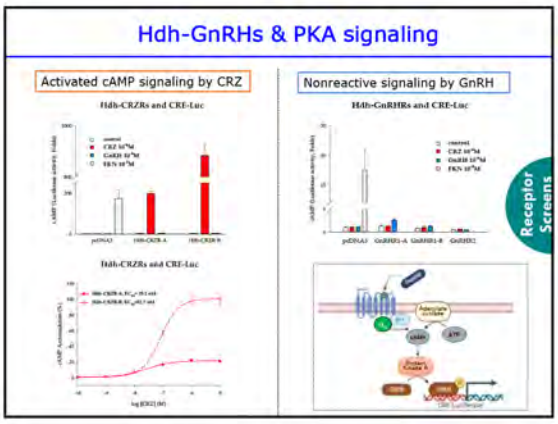

Summary
NPF/Y likely share a common ancestor
Hdh-NPFR specifically responded with NPF peptide
In vivo injection of NPF peptide increased food consumption and knock-down of Hdh-NPF expression decreased food consumption

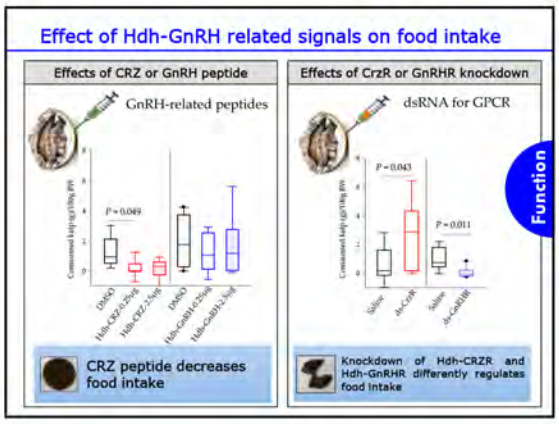

Two GnRH-related genes in Pacific abalone
Hdh-CRZ
Hdh-GnRH
CRZ gene
GnRH gene
Prepro-CRZ
Prepro-GnRH
CRZ
GnRH
Hdh-CRZ
pQNYHFSNGWHA-NH2
Hdh-GnRH
pQISFSPNWGT-NH2
Chordata
Mollusca
Arthropoda
Nematoda
Mollusca
Arthropoda
Chordata
Structure

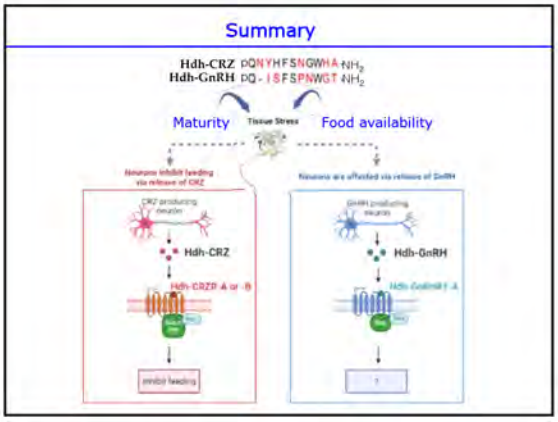

Three GnRH-related receptor genes in Pacific abalone
N-terminal region
CrzR
GnRHR1
GnRHR2
ICL1
ECL1
TMD1
ICL2
TMD2
ECL2
TMD3
TMD4
ICL3
TMD5
ECL3
TMD6
C-terminal region
TMD7
Structure

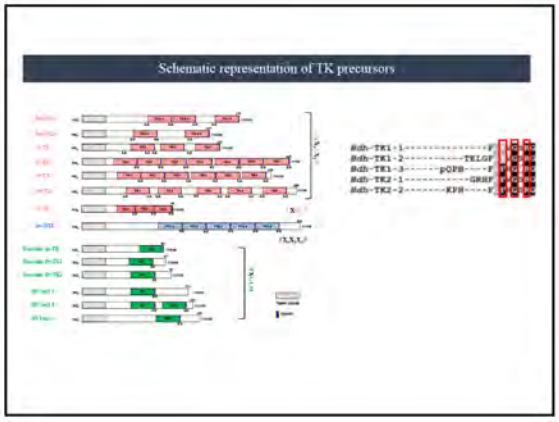

Phylogenetic analysis of GnRH related receptors
Two distinct clades- GnRH/AKH/ACP-type receptor CRZ-type receptor
Three Hdh-GnRH related receptors belong to GnRH/AKH/ACP receptor clade
Two Hdh-GnRH related receptors belong to CRZ receptor clade
GnRHR/AKHR/ACPR
CRZR
Mollusca
Annelida
Arthropoda
Nematoda
Echinoderma
Urochordata
Cephalochordata
Vertebrata
Structure

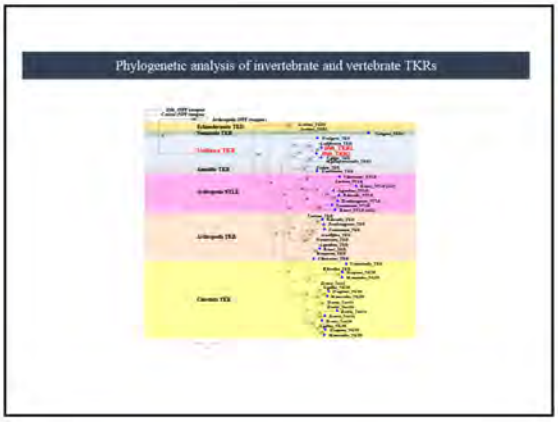

Hdh-GnRHs & PKC signaling
Activated calcium signaling by CRZ
Activated calcium signaling by GnRH
Hdh-CRZRs and seq.
(Aequorin bioluminescence assay)
Receptor Screens

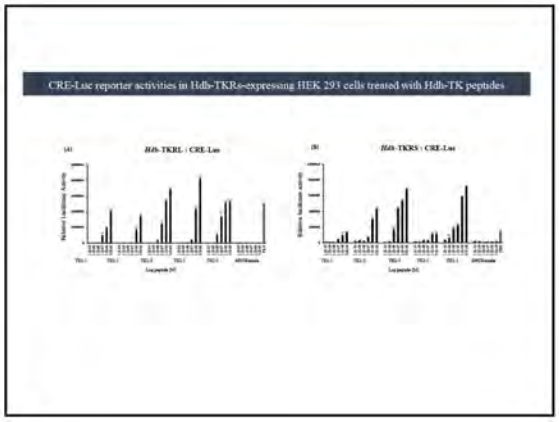

Hdh-GnRHs & PKA signaling
Activated cAMP signaling by CRZ
Nonreactive signaling by GnRH
Receptor Screens


Effect of Hdh-GnRH related signals on food intake
Effects of CRZ or GnRH peptide
Effects of CrzR or GnRHR knockdown
GnRH-related peptides
dsRNA for GPCR
Function
CRZ peptide decreases food intake
Knockdown of Hdh-CRZR and Hdh-GnRHR differently regulates food intake

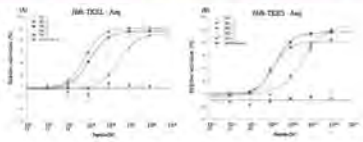

Summary
Maturity
Food availability
Hdh-CRZ
Hdh-GnRH


Schematic representation of TK precursors

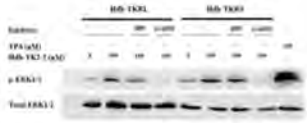

Phylogenetic analysis of invertebrate and vertebrate TKRs


CRE-Luc reporter activities in Hdh-TKRs-expressing HEK 293 cells treated with Hdh-TK peptides

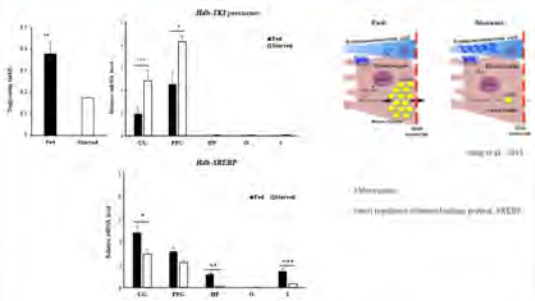
Dose-response curves for intracellular Ca^{2+} in Hdh-TKRs- and aequorin- transfected CHO-K1cells



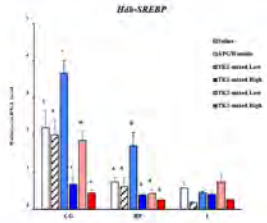
Effects of the PKA inhibitor H89 and the PKC inhibitor Gö6983 on Hdh-TKR-mediated ERK1/2 phosphorylation



Gene expression of Hdh-TK precursors, Hdh-TKR and Hdh-SREBP, and triglyceride level differences in fed and starved abalone



Effect of Hdh-TK peptides on *Hdh-SREBP* transcript in abalone



Suggestion:

Possible roles of TK peptides in regulating lipid metabolism in the neural and peripheral tissues

References

- Kim MA et al., Alternative Splicing Profile and Sex-Preferential Gene Expression in the Female and Male Pacific Abalone *Haliotis discus hannai*. Genes (Basel). 2017 Mar 9;8(3):99.
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Recent studies on anorexia and tissue wasting induced by cancer cachexia

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Cancer cachexia-anorexia is a multi-organ metabolic syndrome characterized by anorexia and weight loss. Generally, such symptoms are a serious problem in cancer patients, adversely affecting chemotherapy success and survival rate. Cachexia has been reported to accompany up to 80% of gastrointestinal cancers, such as pancreatic, lung, and colon cancer, though it is relatively rare in lymphoma or breast cancer patients. It is also known that cancer-induced anorexia occurs independently of chemotherapy, although decreased appetite due to chemotherapy is well reported. Since the mechanism of cancer cachexia is not yet fully understood, there are currently no therapeutic agents or diagnostic markers to treat it. A recently published study identified a substance secreted from cancer cells that induces cancer anorexia, and the molecular mechanism causing the eating disorder was discovered. An increase in the expression of this substance has been shown to be statistically correlated with the symptoms of cachexia in cancer patients, and it is therefore expected to be applicable in the diagnosis and development of therapeutic agents for cancer cachexia.

Keywords: cancer anorexia, cachexia, cytokine, tumor-host interaction

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Developmental myogenesis and basal proliferation of satellite cells in craniofacial muscles

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Craniofacial muscles are essential skeletal muscles and critical to the most basic functions of life including breathing, speaking, feeding, facial expression and eye movements. These muscles, composed of approximately 60 muscles, have a number of properties that distinguish them from the all other skeletal muscles. Although craniofacial muscles have significantly different embryonic origin, known as pharyngeal mesoderm, most current skeletal muscle differentiation protocols using human induced pluripotent stem cells (iPSCs) are based on somite-derived limb and trunk muscle developmental pathways. We have developed an optimized specific protocol to generate craniofacial myogenic precursor cells (cMPCs) from human iPSCs by mimicking key signaling pathways during craniofacial embryonic developmental myogenesis. By performing RNA sequencing, we identified iPSC-derived cMPCs compared with human primary myoblasts from craniofacial muscles. We also determined how cell-intrinsic and niche factors influence to the basal proliferation of craniofacial skeletal muscle stem cells, known as satellite cells (SCs) versus limb SCs. We found that fibroadipogenic progenitors (FAPs) in the pharyngeal muscles are a major cell type providing hepatocyte growth factor (HGF) to activate pharyngeal SCs. These study give new insights to explain the distinctive SC characteristics of craniofacial muscles, which helps to understand unique physiology of craniofacial muscles.

Keywords: craniofacial muscle, induced pluripotent stem cells, satellite cells, fibroadipogenic progenitors

Acknowledgements: This research was supported in part by grants from National Institutes of Health (NIH) NIAMS (R01 AR071397, HC) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2021R1C1C2007132, EK). We appreciate Emory Body Donor Program to arrange autopsy, Xuewen Wu (Xiangya Hospital of Central South University) to dissect human muscles by autopsy, and Jill Wards (Emory University) to share an autopsied human TA muscle sample. CT and LM were financed by INSERM, Sorbonne Universite, Association Française contre les Myopathies (AFM-Téléthon) and the Fondation Recherche Médicale (EQUIPE FRM EQU201903007784).

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Application of sonic hedgehog signaling-induced *in vitro* oocyte maturation to *klotho*-knockout pig production

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Genetically modified pigs produced by somatic cell nuclear transfer (SCNT) have been widely used as animal models for studying human disease. However, the developmental competence of porcine oocytes matured *in vitro*, the most important requirement for SCNT, still remains low. Therefore, this research was conducted to investigate the applicability of sonic hedgehog (SHH) signaling-induced *in vitro* oocyte maturation system to *klotho*-knockout pig production. In part I, the relationship between oocyte quality and SHH signaling was investigated. High-quality oocytes have a greater potential to expand their surrounding cumulus cells with active SHH signaling and a lower apoptosis, providing cumulus-oocyte complexes with a proper environment for maturation. Next, resveratrol and/or melatonin were used to activate SHH signaling during oocyte maturation and to subsequently improve embryo development. Based on the results, it was demonstrated that their combination has synergistic effects on oocyte maturation and finally improves cloning efficiency. In part II, this improved IVM system was applied to *klotho*-knockout pig production. The *klotho* gene is considered to be one of the aging-suppressor genes that control life span. *Klotho* monoallelic knockout fetal cell lines were established by recovery of fetuses cloned from porcine fibroblasts transfected with Cas9-sgRNA ribonucleoproteins. Using these *klotho*-knockout cell lines as nuclear donors, *klotho*-knockout cloned embryos were generated and transferred to 11 recipients. Seven from 11 recipients (63.6%) became pregnant. However, none of the pregnancies was maintained to term. After protein and gene expression analysis using placentas, it was speculated that the reason why *klotho* monoallelic knockout fetuses were not maintained to term might be due to decreased klotho protein expression and negatively changed aging- and apoptosis-related genes in placentas. In conclusion, although SHH signaling-induced IVM system is demonstrated to be applicable in generating genetically modified pigs, recipients carrying *klotho* monoallelic knockout fetuses failed to maintain full-term pregnancies.

Keywords: sonic hedgehog signaling, *in vitro* oocyte maturation, somatic cell nuclear transfer, *klotho*, pig

포스터 초록

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A-01

Single cell transcriptomic profiling reveals dysregulation of oligodendrocytes in Alzheimer's disease

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Alzheimer's disease (AD) is a disease in which neurons degenerate progressively as amyloid-beta (Aβ) or tau proteins accumulate in the brain. Further, non-neuronal glial cells were recently reported to play an important role in the development of AD. However, the role of oligodendrocytes in AD pathogenesis remains unclear. Here, we elucidated a previously undiscovered disease-associated subpopulation of oligodendrocytes during AD progression in AD mouse models and patients using single-cell RNA sequencing analysis. Aberrant Erk1/2 signaling was found to be associated with the activation of disease-associated oligodendrocytes (DAOs) in the AD brain. Notably, inhibition of Erk1/2 signaling in DAO rescued impaired axonal myelination and ameliorated Aβ-associated pathologies and cognitive decline in an AD mouse model. Therefore, DAO molecular profiling is a promising new therapeutic strategy for the treatment of AD. Acknowledgement: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No.2021R1C1C1006551).

Keywords: Alzheimer's disease, snRNA-seq, oligodendrocytes, Erk1/2, DAO

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A-02

GJA1 depletion causes ciliary defects by affecting Rab11 trafficking to the ciliary base

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The gap junction complex functions as a transport channel across the membrane. Among gap junction subunits, gap junction protein α1 (GJA1) is the most commonly expressed subunit. A recent study showed that GJA1 is necessary for the maintenance of motile cilia; however, the molecular mechanism and function of GJA1 in ciliogenesis remain unknown. Here, we examined the functions of GJA1 during ciliogenesis in human retinal pigment epithelium-1 and *Xenopus laevis* embryonic multiciliated-cells. GJA1 localizes to the motile ciliary axonemes or pericentriolar regions beneath the primary cilium. GJA1 depletion caused malformation of both the primary cilium and motile cilia. Further study revealed that GJA1 depletion affected several ciliary proteins such as BBS4, CP110, and Rab11 in the pericentriolar region and basal body. Interestingly, CP110 removal from the mother centriole was significantly reduced by GJA1 depletion. Importantly, Rab11, a key regulator during ciliogenesis, was immunoprecipitated with GJA1 and GJA1 knockdown caused the mislocalization of Rab11. These findings suggest that GJA1 regulates ciliogenesis by interacting with the Rab11-Rab8 ciliary trafficking pathway.

Keywords: GJA1, ciliogenesis, Rab11, CP110, *Xenopus laevis*

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A-03

Perfluorooctanoic acid diminishes sperm functions during capacitation

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Perfluorooctanoic acid (PFOA) is a manmade chemical substance that has been applied in numerous industrial goods such as fire-fighting products, non-stick kitchen appliances, medicine, paints, and cosmetics for over 70 years. Although exposure to PFOA affects cancer development, people are constantly exposed to it. PFOA is known to be bioaccumulated in testicles, causing health risks. However, the effects of PFOA on male fertility are unclear. Therefore, the objectives of this study are to investigate reproductive effects of PFOA on sperm functions with different concentrations of PFOA (0.1, 1, 10, and 100 μM). Sperm functions (sperm motility and motion kinematic parameters, capacitation status), cell viability, intracellular ATP, and western blot were analyzed after capacitation. Sperm motility and numerous motion kinematic parameters showed significant dose-dependent decreases. As a result of capacitation status, capacitated spermatozoa were substantially decreased after PFOA treatment. Moreover, intracellular ATP was remarkably diminished after PFOA exposure, and expression levels of phospho-PKA and tyrosine-phosphorylated proteins were significantly decreased. Altogether, PFOA may suppress sperm functions, causing male infertility. Therefore, the use of PFOA requires the caution for reproductive toxicity.

Keywords: perfluorooctanoic acid, boar spermatozoa, reproductive toxicity, sperm functions, capacitation

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A-04

Effects of 2-naphthol and 2-hydroxy fluorene on development in *Xenopus* embryo

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2-Naphthol and 2-hydroxy fluorene are part of the polycyclic aromatic hydrocarbon metabolites (PAHs). Polycyclic aromatic hydrocarbons (PAHs) are organic compounds consisting of two or more benzene rings in a linearly angled or closely packed structure and are formed from the incomplete combustion of coal, oil and gas, garbage, or organic materials. Emissions from these sources can enter the soil or vegetation through the process of wet deposition, or they can be released into the air and reach the surface. In particular, coal combustion emissions, motor vehicle fuels and exhaust, and tobacco smoke can contaminate the environment, leading to human exposure through fish and produce, and PAHs can also be present in cooked and unprocessed foods. Polycyclic aromatic hydrocarbons (PAHs) have multiple benzene rings and exist as mixtures rather than as single compounds, so evidence of human carcinogenicity is only reported for mixtures. As such, there is no specific cancer type at increased risk, but prolonged exposure may increase the risk of respiratory, skin, and bladder cancers. To date, there is a paucity of studies on direct eye contact between 2-naphthol and 2-hydroxy fluorene. In this study, the Frog Embryo Teratogenesis Assay-Xenopus (FETAX) was used to investigate the adverse effects of 2-naphthol and 2-hydroxy fluorene on organs, including the eye. Among the various 2-naphthol concentrations, embryos exposed to 50 μM developed malformations such as reduced length, edema, small eyes, and tail flexure. To compare the gene expression locations of 2-naphthol-exposed embryos, eye marker genes such as rx1, six3, prox1, and cryba1 were analyzed by whole-mount *in situ* hybridization (WISH). The results showed that the expression regions of rx1 and six3 were reduced in the 2-naphthol-exposed embryos. In the same experiment with 2-hydroxy fluorene, we found that embryos exposed to a concentration of 15 μM developed malformations such as edema, lens dilation, tail flexure, pigment loss, and misfolded gut, and that the expression regions of cryba1 and prox1 were increased when lens specific marker genes were analyzed by in situ hybridization (WISH). In conclusion, our study shows that 2-naphthol, 2-hydroxy fluorene affects gene expression during *Xenopus* embryonic development, resulting in developmental toxicity and teratogenicity.

Keywords: 2-naphthol, 2-hydroxy fluorene, FETAX, toxicity, teratogenicity

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A-05

Teratogenic effects of butylparaben and oxybenzone in *Xenopus* embryos

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Butylparaben and oxybenzone are Endocrine Disrupting Chemicals (EDCs) that have raised significant concern due to their extensive use in various consumer products and their potential impacts on ecosystems. In this study, we conducted a comprehensive investigation using the Frog Embryo Teratogenesis Assay Xenopus (FETAX) to evaluate the teratogenic effects of these EDCs. Our findings indicate that butylparaben and oxybenzone possess the potential to act as teratogens and growth inhibitors. Moreover, we observed severe damage to eye development induced by these chemicals, which was corroborated by the downregulated mRNA expression levels of eye and lens specific markers, including *pax6*, *rx1*, *six3*, *prox1*, and *cryba1*, as analyzed using qPCR. Additionally, through *in-situ* hybridization, we could observe the spatial developmental changes in the early stages of *Xenopus* embryos using one of the previously mentioned eye and lens specific markers. Furthermore, we investigated malformations previously observed in FETAX such as immature gut, growth retardation and bent notochord. We then evaluated if there were any changes in the expression of specific markers linked to these malformations through the application of in-situ hybridization. However, we did not detect any significant changes, suggesting that further studies are required to elucidate these observations fully. Results from this study suggest that butylparaben and oxybenzone are a developmental toxicant and teratogen, particularly on eye development and emphasizes the importance of continued research to understand and mitigate the risks associated with these EDCs in consumer products and environmental settings.

Keywords: butylparaben, oxybenzone, eye development, FETAX, EDCs

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A-06

Regulation of FAM3D in mouse uterus by estrogen during the estrous cycle

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The uterus is a female reproductive organ regulated by sex hormones, estrogen and progesterone. Due to dynamic hormonal changes, the mouse uterus undergoes various physiological changes, estrous cycle. Among these changes, proestrus and estrus stages are strongly influenced by estrogen. The family with sequence similarity 3 (Fam3) family is a cytokine-like gene family with four members Fam3a, Fam3b, Fam3c and Fam3d. However, the regulation of Fam3 family in mouse uterine physiology remains largely unknown. Through analysis of mouse uterine RNA-seq data, we observed that the expression of Fam3b, Fam3c, and Fam3d was upregulated during the proestrus and estrus stages. Notably, Fam3d expression showed dynamic regulation during the estrous cycle, with high expression levels at proestrus and estrus stages. To investigate whether Fam3d expression is regulated by estrogen, we administered E_2 at different time points to ovariectomized mice. The results showed that Fam3d expression was highest 24 hours after E_2 injection, suggesting that estrogen plays a significant role in regulating Fam3d expression. Furthermore, we conducted inhibition experiments using the ER alpha antagonist, ICI, which revealed that estrogen regulates Fam3d expression through the ERα-mediated pathway. Interestingly, immunofluorescence staining demonstrated that FAM3D was exclusively expressed in the luminal epithelium and glandular epithelium, but not in the stroma. Additionally, FAM3D was found to be localized predominantly in the cytoplasm and not in the nucleus, with a predominant localization in the apical region. These findings provide valuable insights into the potential role of FAM3D within the uterus, laying the groundwork for future research on its function and significance in uterine physiology.

Keywords: uterus, estrous cycle, estrogen, estrogen receptor, FAM3D

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A-07

Functional modulation of lysophosphatidic acid type 2 G-protein coupled receptor facilitates alveolar bone formation

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Lipid biosynthesis is recently studied its functions in a range of cellular physiology including differentiation and regeneration. However it still remains to be elucidated in its precise function. To reveal this, we evaluated the roles of lysophosphatidic acid (LPA) signaling in alveolar bone formation using the LPA type 2 receptor (LPAR2) antagonist AMC35 (Amgen Compound 35) using tooth loss without periodontal disease model which would be caused by trauma and usually requires a dental implant to restore masticatory function. In this study, *In vitro* cell culture experiments in osteoblasts and periodontal ligament fibroblasts revealed cell type-specific responses, with AMC35 modulating osteogenic differentiation in osteoblasts *in vitro*. To confirm the *in vivo* results, we employed a mouse model of tooth loss without periodontal disease. Five to ten days after tooth extraction, AMC35 facilitated bone formation in the tooth root socket as measured by immunohistochemistry for differentiation markers KI67, Osteocalcin, Periostin, RUNX2, TGF-β1 and SMAD2/3. The increased expression and the localization of these proteins suggest that AMC35 elicits osteoblast differentiation through TGF-β1 and SMAD2/3 signaling. These results indicate that LPAR2/TGF-β1/SMAD2/3 represents a new signaling pathway in alveolar bone formation and that local application of AMC35 in traumatic tooth loss can be used to facilitate bone regeneration and healing for further clinical treatment.

Keywords: lysophosphatidic acid receptors, G-protein-coupled receptors, intracellular signaling proteins, transforming growth factor beta1, SMAD2/3 proteins, osteoblasts, alveolar bone regeneration

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A-08

MTX2 is crucial for on the craniofacial and eye development in *Xenopus leavis*

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Mitochondrial Outer Membrane Import Complex Protein 2 (*mtx2*) is associated with the cytosolic face of the outer mitochondrial membrane and involved in protein import into the mitochondrion. Since the developmental function of *mtx2* has not been thoroughly studied, we examined the physiological role of *mtx2* during embryonic development using *Xenopus laevis* as an animal model. In temporal and spatial expression patterns of *mtx2* transcripts during *Xenopus* embryogenesis, *mtx2* gradually increased in the late tailbud stage and mainly expressed in the branchial arch and eyes. Knockdown of *mtx2* using morpholino oligonucleotides (mo) reduced the size head and eyes of embryos. Alcian blue staining of the cartilage indicated that *mtx2* knockdown interfered with branchial cartilage formation, causing small gill arch. We elucidated that these results were due to reduced cell division in the overall craniofacial area using Phosphor H3 staining. We also examined the effect of mtx2 knockdown on cartilage and eye formation in molecular level using whole-mount *in situ* hybridization (WISH) and Real-Time qPCR. WISH data showed that *mtx2* morphants had not only down-regulated cartilage-related factors such as *col2a1, sox9* at late tailbud stage but also eye-related factors including *six3, rx1, cryba1*, and *prox1*. Real-time qPCR analysis indicated that *mtx2* morphants decreased levels of *bmp2, sox9*, the master regulator of collagen formation, *col2a1*, a major component of cartilage, and *acan*, a major element of proteoglycans, while increased level of *mmp13*, a cartilage-degrading protein, suggesting that *mtx2* knockdown inhibited cartilage formation by affecting cartilage-related gene expression. In addition, we also found that *mtx2* knockdown reduced expression of eye marker genes including *six3, pax6, rx1* and *otx2*, and lens marker genes, *cryba1* and *prox1*. Rescue experiments with *mtx2* domains indicated that the GST C domain of MTX2 was required for cartilage development and the GST N domain was required for eye development. Therefore, this study suggests that *mtx2* is required for cartilage and eye development by affecting gene expression and cell proliferation.

Keywords: *Xenopus*, *mtx2*, branchial arch, gill arch, eye development

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A-09

Comparative analysis of the morphology and nervous system in two species of leeches (*Hirudo nipponia*, *Glossiphonia complanata*) from different behaviors in South Korea

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The purpose of this study is to investigate the differences of mophology and nervous system among two species of leeches (*Hirudo nipponia*, *Glossiphonia complanata*) from different behaviors such as crawling and swimming. It has been known that species adapted to different morphology and dietary requirements will develop different neuronal networks and morphological traits. Firstly, phylogenetic analysis based on mitochondrial COI gene sequences in two species of leeches was performed. For morphological and anatomical analysis, nerve cords were dissected out from two species of leeches, and immunohistochemistry was performed to examine the nervous system including neural connectivity and ganglion. This research is expected to contribute to our understanding of the differences in ecological adaptability and neurogenesis between two species leeches.

Keywords: leech, neurogenesis, ganglion, morphology, nervous system

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A-10

The leech *Helobdella* as a system for characterizing the expression and function of PIEZO, a putative mechanotransduction protein

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Mechanosensory transduction is the process by which mechanical stimuli are transformed into electrical signals that the brain can interpret. A reasonable assumption would be that mechanosensory transduction would involve proteins in the plasma membrane of the cell. Experiments conducted on mice and Drosophila suggest that mechanosensory transduction involves a highly conserved multi-pass transmembrane protein called PIEZO (also referred to as FAM38). We want to investigate the links between these two apparently divergent functions of PIEZO proteins. *piezo* genes have been found in a variety of species including those lacking nervous systems. We have found that the leech *Helobdella austinensis* has three *piezo* genes, *Hau-piezo1*, *Hau-piezo2* and *Hau-piezo3*. Moreover, leeches feature identified mechanosensory cells in the adult nervous system and large accessible embryonic blastomeres in which changes in cell adhesion associated with mitosis can readily be observed. Thus, the leech provides a useful system in which to explore *Hau-piezo1*.

Keywords: mechanosensory, leech, PIEZO, *Helobdella austinensis*, nervous systems

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A-11

Pubertal reproductive dysfunction in propylthiouracil-induced hypothyroid male rats and recovery by levothyroxine

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Propylthiouracil (PTU) is an anti-thyroid drug that acts on thyroid hormone and inhibits thyroid peroxidase, thereby suppressing thyroid hormone production. PTU-induced hypothyroidism was exposed at various times from the fetal period to adulthood, and it mainly progressed in fetal and lactating periods and in adults. The purpose of this study was to investigate the effect of propylthiouracil (PTU)-induced hypothyroidism on the development of the reproductive system from immediately after weaning to puberty in male rats and the recovery effect of levothyroxine (LT4) administration.

Male rats were randomly divided into three groups immediately after weaning at 21 days of age. The CON group received regular drinking water and oral saline. In the PTU group, hypothyroidism was induced by oral administration of PTU-diluted water (0.025%) and saline. The P+LT4 group received oral administration of thyroxine at 20 mg/kg body weight daily with PTU dilution water (0.025%).

Body weight and AGD measured during the experimental period decreased in the PTU group compared to the CON group from 35 days after birth and were normalized again in the P+LT4 group ($p<0.001$). Hypothyroidism was confirmed through changes in thyroid microstructure ($p<0.05$), a decrease in serum thyroxine level ($p<0.001$), an increase in thyroid weight ($p<0.001$), and normalization of serum thyroxine level confirmed whether the LT4 dose was appropriate. The PTU group showed a decrease in testis weight ($p<0.01$), epididymal weight ($p<0.001$), prostate weight ($p<0.001$), seminal vesicle weight ($p<0.05$), and levator ani-bulbocavernosus (LABC) weight ($p<0.001$). Cowper's glands weight ($p<0.01$), preputial glands weight ($p<0.001$), mesenteric white adipose tissue (mWAT) weight ($p<0.001$), and epididymal white adipose tissue (eWAT) weight ($p<0.001$) compared to the CON group and normalized in the P+LT4 group. A decrease in serum testosterone level ($p<0.05$) and decreased expression of StAR ($p<0.05$) and Hsd17b3 ($p<0.05$) genes were also observed in the PTU group. Histological analysis of the testis showed that hypothyroidism increased the luminal sloughing of spermatocytes attached to the walls of the seminiferous tubules ($p<0.01$). Also, sperm count ($p<0.01$) and penis length ($p<0.05$) were decreased in the PTU group. In contrast, the P+LT4 group recovered most of the defective pubertal features previously observed in the PTU group.

Through PTU-induced hypothyroidism, this study confirmed that thyroxine deficiency during infancy decreased reproductive tissue development, testosterone secretion, and transcription of StAR and Hsd17b3 genes and increased abnormalities in the testis. These results indicate that the thyroid may

play an important role in pre-pubertal reproductive tissue development. Furthermore, it suggests the possibility of early diagnosis and recovery through thyroxine supplementation of some epigenetic reproductive system development disorders caused by infancy hypothyroidism.

Keywords: rat, puberty, propylthiouracil, levothyroxine, hypothyroid

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B-01

Establishment of an upper motor neuron-induced neurotoxicity model using iPSC

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Research of brain organoid using human pluripotent stem cells is a promising tool for screening new drugs and cytotoxicity tests. In this present study, we established an optimal model for forebrain-like cortical organoid (FLCO) with upper motor neuron such as glutamatergic neuron and medium spiny neuron, which were derived from human iPSCs. Especially, we focused on the formation of high-purity organoid for studying the ability motor cortex to regulate neurotoxicity-induced motility. Our 3D FLCO iPSC-derived model showed that a similar gene expression pattern (vGLUT1, 2, ARPP21, DARPP32), compared with human primary motor cortex tissue. Furthermore, the result of neuronal characteristics between 2D and 3D FLCO indicated that more similar to that of human forebrain neurons, compared with human neural cell line model (2D and 3D). Additionally, 3D FLCO iPSC-derived profoundly enhanced the neuronal survival (ATG5 and Caspase 3) and regeneration (GAP43). Finally, there was a difference of neuronal toxicity results between 3D iPSC-derived organoid and 3D neuronal cell line model. Therefore, this study suggests that 3D FLCO iPSC-derived combined with neurotoxicity test could be a powerful tool in new drug development toxicity evaluation system.

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B-02

Estrogen receptor alpha-dependent regulation of IL36α expression in mouse uterus

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The uterus, a vital female reproductive organ, undergoes intricate and cyclical transformations driven by the ovarian sex hormones, estrogen and progesterone. These cyclic alterations, known as the estrous cycle in mice, encompass four distinct phases: proestrus, estrus, metestrus, and diestrus. Throughout this cycle, the uterine endometrium engages in processes like proliferation and apoptosis, orchestrated by hormonal influences. According to microarray data, we confirmed that the expression of IL36 (Interleukin) subfamily is specifically regulated in epithelial cells of the mouse uterus through estrogen receptor alpha-dependent singling pathway. Comprising Il36α, Il36β, and Il36γ, this subfamily belongs to the IL1 gene family. As IL1 gene family, the IL36 subfamily serves as pro-inflammatory cytokines, actively involved in immune modulation, cellular apoptosis, and proliferation. While numerous studies have suggested links between the IL36 subfamily and pathological conditions like cancer or psoriasis, the precise expression patterns of IL36 subfamily within the uterus remain unknown. In this study, we verified that the transcription of IL36 subfamily was highly induced during the proestrus and estrus stages in the uterus. Furthermore, the mRNA expression of IL36 subfamily was notably increased in the uterus of ovariectomized mouse upon administration of 17β-estradiol. Consistent with the microarray data, derivation of IL36 subfamily expression was inhibited by ICI, a specific antagonist of estrogen receptor alpha. Among the IL36 subfamily members, IL36α exhibits the most conspicuous dynamics in protein expression. Same as the mRNA level, protein expression of IL36α was significantly high at the stage of proestrus and estrus. In ovariectomized mouse uterus, IL36α protein expression reached its peak 24 hours after treatment of 17β-estradiol. Immunofluorescence staining results reveal the exclusive presence of IL36α in the luminal epithelial cells of the mouse endometrium. Based on the above *in vivo* experiment results, we validated that the expression of IL36α is controlled by estrogen receptor alpha-dependent signaling. Following isolation and culture of uterine primary cells *in vitro*, we unveiled distinct transcription pattern of IL36α based on specific cell types. The expression of IL36α mRNA was only confirmed in the epithelial cells after estrogen treatment not in the stromal cells. These results support the bioinformatic data analysis, IL36α was primarily localized in the luminal epithelial cells of the uterus. Our study revealed that estrogen can trigger an increase in IL36α mRNA and protein within uterine epithelial cells. It is recognized that IL36α also plays the role in immune modulation and cellular apoptosis. This implies the potential for IL36α to have a significant role in regulating the dynamic changes in the uterus driven by estrogen.

Keywords: uterus, IL36α, estrogen, estrogen receptor alpha

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C-01

Ethylene oxide has detrimental effects on male reproduction

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Ethylene oxide (E.O) is currently one of the widely used materials in various industries. E.O is generally used as an intermediate material for sterilizing industrial products that cannot be sterilized at high temperatures or for making ethylene compounds. Despite the versatility of E.O, several studies have reported the dangers of exposure to E.O. Representatively, it has been reported that exposure to E.O causes cancer and has toxic effects on several cells. However, despite these reports recognizing the risks, sufficient studies of exposure to E.O in male reproductive cells have not been performed. Therefore, this study was designed to investigate the toxic effects of E.O exposure on male reproductive cells. This study treated duroc spermatozoa with various concentrations of E.O (0, 0.1, 1, 10, and 100 μM) during capacitation. Then, sperm functions (sperm motility, motion kinematics, capacitation status, ATP level, and cell viability), PKA activity, and tyrosine phosphorylated protein expression levels were evaluated. As a result, the motility and several motion kinematics were significantly decreased in the highest concentration group (100 μM). The capacitation status was also significantly increased in the highest concentration group (100 μM). The ATP level was significantly decreased in a dose-dependent manner. The tyrosine phosphorylated protein levels were significantly decreased in a concentration-dependent manner. In addition, abnormal PKA substrate levels were observed in treatment groups. Comprehensively, the results demonstrated that E.O exposure may have detrimental effects on sperm functions. Therefore, the risk of reproductive toxicity should be considered when using E.O in various industries.

Keywords: ethylene oxide, sperm function, capacitation, reproductive toxicity

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C-02

Effect of Rho-associated kinase inhibitor Y-27632 on the proliferation of cynomolgus monkey induced pluripotent stem cells

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Rho-associated Kinase (ROCK) has been identified as an important regulator of proliferation and cell cycle progression in a number of cell types. Y-27632 markedly diminishes human embryonic stem cell and induced pluripotent stem cell (iPSC) dissociation-induced apoptosis and increases cloning efficiency in a feeder-free culture system. In the present study, our aim was to investigate the effect of ROCK inhibition by Y-27632 on the proliferation of Cynomolgus monkey-iPSC (miPSC).

iPSCs (cmKF-iPS-C5) were provided by the National Primate Research Center (NPRC), Korea Research Institute of Bioscience and Biotechnology (KRIBB) and used in the experiment (Stem Cell Res. 2022;64:102887). miPSCs were maintained in TeSR-E8 medium on a Matrigel, diluted 1:30 in KO-DMEM)-coated plate in a 37℃ incubator with 5% CO_2. Individual miPSC-colonies were passaged using ReLeSR at a ratio of 1:6 every 5 days. According to the experimental design, miPSCs were treated with 10 μM Y-27632 for 24 hours after thawing and subculture.

As a result of the experiment, miPSC treated with Y-27632 showed a significantly lower cell number than the control group (5×10^6 cell/mL Vs. 1.3×10^7 cell/mL) when the cell number was examined after 168 hours of culture. Alkaline phosphatase (AP) staining showed that miPSC lines in all experimental groups had AP activity in colonies. It was confirmed through qRT-PCR analysis that the undifferentiated pluripotent cell markers *POU5F1*, *NANOG*, and *KLF4* were expressed in the miPSCs derived from control and Y-27632 groups. In addition, the expression of the *BCL2* gene was significantly ($p<0.05$) higher in the Y-27632 treatment group compared to that of the control group.

In conclusion, treatment with Y-27632 inhibited the proliferation of miPSC, but had the effect of preventing apoptosis. Additionally, it is necessary to investigate the effects of various concentrations and treatment times of Y-27632.

Keywords: induced pluripotent stem cells, Rho-associated kinase inhibitor, Y-27632, proliferation, cynomolgus monkey

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C-03

Enhanced survival and *in vitro* fertilization capacity of cryopreserved boar sperm by nobiletin (NOB)

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Nobiletin (NOB) is a bioflavonoid compound isolated from citrus fruit peels. The present study aimed to elucidate whether NOB facilitates the survival and *in vitro*-fertilization (IVF) capacity of boar sperm after freezing-thawing. To this end, spermatozoa were diluted and cryopreserved in a freezing extender supplemented with 0 (control), 50, 100, 150, and 200 μM Nobiletin. Frozen-thawed (FT) sperm kinematics were assessed (CASA software) at 30 min and post-incubation for 90 minutes after thawing. Viability, acrosome integrity, and mitochondrial membrane potential (MMP) were measured 30 min after thawing using SYBR-14/PI, PSA/FITC, and R123/PI respectively. Lipid peroxidation was determined using MDA assay after incubation for 90 min. The addition of 100 μM and 150 μM NOB to the extender significantly improved sperm progressive motility, and acrosome integrity compared to the control group ($p<0.05$). The proportion of viable spermatozoa was significantly higher in the 150 μM NOB group. MDA levels were less in 50 μM and 150 μM NOB treated groups compared to the control. In addition, pre-treatment with 150 μM NOB before cryopreservation increased the cleavage and blastocyst formation rates compared to the control group. Furthermore, the relative expression of POU5F1 and AMPK, genes related to pluripotency and cell differentiation were significantly upregulated in embryos resulting from NOB-treated sperm compared to the control group. These results suggest that Nobiletin is a functionally novel phytochemical to mitigate oxidative stress during the freezing-thawing of porcine spermatozoa as reflected by improved FT sperm quality and IVF outcome.

Keywords: flavonoid, boar, cryopreservation, cryoinjury, *in vitro* fertilization, artificial insemination

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C-04

Assessing the compatibility of customized fluids for intravenous therapy in physiologically intact swine

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Administering commercially available fluids intended for human use to pigs undergoing intravenous fluid therapy is a widely adopted practice. Prior to developing a fluid formulation for porcine use, a comprehensive examination was conducted to analyze the composition of bodily fluids in pigs during various reproductive cycles. Our investigation indicates the presence of a negative association between the age of sows and piglets and their respective glucose concentrations. Furthermore, female pigs encountered a reduction in hematocrit (Hct) and hemoglobin (Hgb) concentrations because of bleeding after giving birth. The primary objective of this study was to investigate the potential negative outcomes associated with the administration of personalized fluids in a population of healthy pigs. The ions of Hartmann's solution (H/S) were modified and subsequently classified into a comprehensive set of five categories. In Group 1, the quantity of sodium lactate was augmented; in Group 2, sodium chloride; in Group 3, potassium chloride; and in Group 4, a combination of sodium lactate and potassium chloride was increased, aiming to closely resemble the composition of pig body fluid. Following the administration of anesthesia, all fluids were introduced through an auricular vein. Blood samples were collected from the external jugular vein before and after fluid injections, and subsequently analyzed using an EPOC® blood analysis system. The findings derived from the pre- and post-fluid delivery blood tests demonstrated a lack of statistically significant disparities in most of the results. Furthermore, the physical examination did not yield any clinical observations. After the administration of fluid injection, it was observed that only Group 2 displayed a reduction in lactate levels, whereas the control group exhibited an elevation. Further research will perform a comparative analysis between a recently developed fluid and a H/S solution which focus is to assess the degree of improvement in dehydrated patients after receiving these fluids.

Keywords: fluid therapy, pig, bodily fluid composition, customized fluid

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C-05

Supplementing boar semen with myo-inositol before freezing enhances the quality of thawed semen and increases fertility

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Myo-inositol (MI) is classified as vitamin B_8, a member of the B-vitamin family. In the reproductive system, it enhances sperm acrosome integrity, induces sperm maturation, and regulates reproductive hormones such as FSH, LH, and inhibin B secretion. In this study, we investigate the effect of myo-inositol on boar semen.

The semen was cryopreserved in a BF5 extender containing varying concentrations of myo-inositol, (0, 0.5, 1, 1.5, and 2 mg/mL). After thawing, the semen was assessed for motility, viability, acrosome integrity, lipid peroxidation, gene expression, fertilization ability, ROS, and apoptosis levels. The results reveal that a concentration of 0.5 mg/mL of MI enhances total and progressive motility, acrosome integrity, decreases expression of BAX and ROMO1, increased the expression of SMCP gene, and improves cleavage and blastocyst rates in *in vitro* fertilization results compared to the control groups. Also, significant enhancements in viability were observed in the groups that received 0.5 and 1 mg/mL MI. Moreover, all groups that received MI showed less apoptosis and ROS levels compared to the control group, with the 1 mg/mL group showing a significant decrease.

In conclusion, we recommend to using myo-inositol at a concentration of 0.5 mg/mL as a pre-freezing supplement to improve cryopreserved boar semen parameters and embryo production rate.

Keywords: myo-inositol, antioxidant, boar semen, cryopreservation, fertilization.

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C-06

Generation of artificial cardiovascular spheroids with biomimicking microfluidic concave system for heart failure therapy

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In order to develop cardiomyocytes without proliferative capacity as cell therapy products, increasing the survival rate of transplanted cardiomyocytes is an important issue. Recently, the dual stem cell therapy method using endothelial cells has been proposed as a strategy to improve the survival and engraftment of injected cardiomyocytes. Despite progress in this direction, a problem remains the advancement of cardiomyocyte and endothelial cell co-transplantation techniques. Accordingly, we designed a microfluidic concave platform with an *in silico* simulation to form cardiovascular spheroids. Indeed, we successfully generated cardiovascular spheroids by encapsulating endothelial cells within cardiomyocytes and confirmed the long-term culturing possibility of spheroids. Remarkably, our cardiovascular spheroids exhibited several advantages: 1) improved the survival rate of cardiomyocytes under a 3% hypoxic environment, 2) could be stored for emergency patient through cryopreservation, 3) showed therapeutic efficacy compared to single cardiomyocytes in animal models of myocardial infarction. These findings emphasize the enhanced functionality of cardiovascular spheroids. Based on our results, we suggest that the technology for forming cardiovascular spheroids utilizing microfluidic concave systems holds promise as the advanced cell therapy for heart failure. This work was supported by the Bio&Medical Technology Development Program of the National Research Foundation (NRF) (No. RS-2023-00220207) and (No. 2022R1A2C1006622) of Korea grant funded (MSIT, Republic of Korea).

Keyword: *in silico*, microfluidic concave, cardiovascular spheroids, heart failure

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C-07

Establishment of simplified serum-free media for porcine embryonic stem cells through inhibition of WNT and SRC pathways

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Stable porcine embryonic stem cells (pESCs) derived from in vitro blastocysts provide powerful tools for beneficial applications in animal breeding as well as evaluating the safety and efficacy of stem cell-based therapies. Most pESCs require complex media compositions and feeder layers. We derived pESCs using simplified culture media (F12-FIW) consisting of FGF2, canonical WNT inhibitor (IWR-1), and SRC inhibitor (WH-4-023) in serum-free medium. The established pESCs-FIW capable of single cell passage with short cell doubling time (about 12 hours). These cells exhibited alkaline phosphatase (AP) activity and expressed pluripotency markers, OCT4, SOX2, and NANOG, as well as cell surface markers, SSEA1, SSEA4, and TRA-1-60. Established pESC showed formative and primed characteristics based on the negative expression for the naive and primed marker, whereas strong expression for the formative marker with high levels of related genes. Transcriptome analysis showed that pESC-FIW had similar cellular identities to reported pESC maintained in complex media formulations and had characteristics of gastrulating epiblast cells. The pESCs maintained until late passages (above p50) showed similar proliferation rates and single cell clonal efficiencies with dome shape morphologies as those of early passages (below p30). They also could be maintained on Matrigel, fibronectin, LN-521, and VTN-N using mTeSR™, a feeder-free culture medium for human pluripotent stem cells. Expression of pluripotency marker genes in pESCs maintained on each of the four matrices showed similar levels to those in pESCs under the feeder condition. These results indicate that inhibition of the canonical WNT pathway and SRC pathway is sufficient to establish pESCs with properties similar to those established in complex media formulations and is applicable to feeder-free expansion. Easy to maintain-pESCs can be used to develop complex genetic modifications useful for agriculture and biomedicine, and manufacture cell-derived meat and other products.

Keywords: porcine embryonic stem cells, serum-free media, canonical WNT inhibition, SRC inhibition, feeder-free adaptation

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C-08

Myoinositol improves developmental competence in porcine embryos and reduces oxidative stress via the *NRF2-HO-1* signaling pathway

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Myoinositol (MI) is an antioxidant and is expected to have a positive effect on early embryonic development in mammals. However, there is a paucity of studies about the effect of MI on porcine embryonic development. Therefore, we evaluated the effect of MI supplementation on porcine embryos and its antioxidant effect. We cultured porcine parthenogenetic-derived embryos in a porcine zygotic medium (PZM3) with various concentrations of MI (0, 5, 10, and 20 mM) for 7 d. MI at 20 mM significantly increased the blastocysts formation rate compared to the control group; however, cleavage rate and total cell number did not increase significantly compared to the control group. MI also improved significantly antioxidant-related genes such as *NRF2*, *HMOX1*, and *GCLC* in the 20 mM group on day 7 compared to the control group blastocysts. Moreover, 20 mM MI significantly increased the mitochondrial quantity, mitochondrial membrane potential and reduced ROS in mitochondria at day 7 blastocysts compared to the control group. These results showed that MI improved the developmental competence of porcine preimplantation embryos by activating *NRF2/HO-1* pathway against oxidative stress.

Keywords: myoinositol, embryos, oxidative stress, mitochondria, genes

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C-09

Improving cryopreservation efficiency and pregnancy rate through superstimulation with FSH in Korean Hanwoo cows via ovum pick up

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Given the global demand for high-quality cattle, it is necessary to develop effective techniques for producing oocytes from high-quality cows. Ovum pick-up (OPU) methods play a significant role in oocyte collection worldwide and are the most efficient means of enhancing genetic advancement through maternal lines in cattle. This study aimed to establish an efficient OPU-derived transferable embryo production system. Oocytes were collected from 20 control and 15 follicle-stimulating hormone (FSH)-treated female Hanwoo. A combination of decreasing doses of FSH (36, 36, 24, and 24 mg, 12 h apart), progesterone, estrogen, and prostaglandin was administered to synchronize and mildly stimulate the animals. *In vitro* blastocysts were generated by *in vitro* maturation, fertilization, and culture. The FSH-treated group (1,125 oocytes) and the control group (1,022 oocytes) exhibited a higher proportion of Grade A and B oocytes (88.2%) than other grades ($p<0.05$), with the majority of them in the germinal vesicle 2 stage (64.0%). Moreover, the FSH group had a significantly higher blastocyst rate (44.7%) than the control group (31.1%) ($p<0.01$). After vitrification and *in vitro* culture warming, the embryos of the FSH group exhibited higher re-expansion rates (Grade 1: 86.9% and Grades 2 and 3: 57.9%) than the control group ($p<0.01$). FSH treatment also reduces working hours, making it an efficient method for embryo production, freezing, and preservation.

Keywords: Hanwoo cow, ovum pick-up, cryopreservation, embryo development, embryo transfer

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C-10

Effect of metal components in PM2.5 derived from porcine farm exposure on sperm function in mice

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Particulate matter (PM) is emerging as a global environmental problem and a significant burden on public health. However, the contribution of PM generated in agriculture or livestock farms has received little attention thus far. Understanding the relationship between livestock production rates and the occurrence of PM is crucial, especially considering its impact on reproductive functions. Furthermore, the specific effects and underlying mechanisms of metal components present in PM2.5 derived from porcine farms on reproductive toxicity remain poorly understood. Therefore, the objective of this study is to investigate the potential impact of major metal components on the reproductive system, sperm function, and embryo development. Male mice were exposed to varying levels of metal components present in PM2.5, including Ca, Fe, Al, Zn, Pb, and a mixture of all these metals. Our results indicate that metal exposure influenced the levels of inflammatory cytokines in the testis, as well as oxidative stress-induced cell death and apoptosis. Moreover, the exposure to metal components in PM2.5 was found to affect sperm deformation, capacitation status, testosterone levels, and testosterone biosynthesis. Additionally, the embryo development crucial for sperm fertility was also influenced by metal exposure in PM2.5. Overall, this study suggests that major metal components, when administered through intratracheal instillation, induce adverse effects on the male reproductive system. These findings highlight the need for further research into the reproductive toxicity associated with PM2.5, particularly its metal constituents.

Keywords: particulate matter (PM), sperm, testosterone, embryo development

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D-01

Effects of extruded pellet on the growth and digestive physiology of olive flounder (*Paralichthys olivaceus*) in Jeju local farm

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Local fish farmers who culture the olive flounder (*Paralichthys olivaceus*) prefer moist pellet (MP) over extruded pellet (EP) due to better growth enhancement. MP had high feed conversion ratios, however, discouraged due to its significant negative impacts on water quality deterioration and increase incidence of fish disease. The feeding trial was conducted for six months, and the experimental diets were MP which composed of mackerel and cutlass fish and EP is commercial diet. Weight growth rate was not significantly affected by MP and EP ($p>0.05$), although EP-fed fish had a better somatic yield ($p<0.05$). Plasma activity of growth hormone and insulin-like growth factor 1 (IGF-1) was comparably similar between diets, while the EP-fed olive flounder had a higher hepatic IGF-1 mRNA expression ($p<0.05$). The intestinal digestive enzyme activity was not significantly influenced by diets ($p>0.05$), whereas the EP- fed fish had a longer intestinal villi length and increased number of goblet cells in the pyloric caeca ($p<0.05$). This study shows that the EP diet may be suitable feed based on somatic growth and some aspects of the digestive physiology of the olive flounder aquaculture.

Keywords: flatfish aquaculture, feeding, somatic yield, somatic growth, digestive physiology

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D-02

배합사료와 생사료를 급이한 넙치(*Paralichthys olivaceus*) 친어로부터 생산된 수정란의 난질분석

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본 연구는 배합사료(EP)와 생사료(MP)를 각각 급이한 넙치 친어에서 생산된 수정란의 난질분석을 통해 종자생산 시 배합사료의 효율성과 적용 가능성을 제시하기 위해 진행되었다. 각 사료 급이구별 산란량 및 생산된 알의 수정률, 부화율, 기형율, 부화자어 먹이 무공급 생존지수(survival activity index, SAI) 및 난 발생 단계별 소요 시간과 발생 단계에 따른 GH(growth hormone)와 IGF-1(insulin like growth factor-1) 함량을 비교하였다. 발생 단계는 수정란, 8세포기, 128세포기, 상실기, 포배기, 낭배기, 배체형성기, 쿠퍼소낭(Kupffer's vesicle)기, 안포형성 및 근절발달기, 심장박동기, 부화직전, 부화직후를 선정했으며, 각 발생 단계에 도달한 수정란의 비율이 전체의 80% 이상을 차지한 시점을 기준으로 발생시간을 비교하였다. 산란량은 EP 사료구에서 많았으나, 수정률, 부화율, 기형율은 사료구별 큰 차이를 나타내지 않았고, SAI는 EP 사료구에서 높은 수치를 나타내었다. 수온 20℃에서 난 발생 단계별 소요시간은 MP 사료구에서 생산한 수정란이 수정 후 44시간 뒤에 부화했고, EP 사료구에서 생산한 수정란이 수정 후 43시간 뒤에 부화하여 1시간 감소한 것으로 나타났다. GH와 IGF-1의 함량은 모든 구간에서 EP 사료구의 수정란(GH: 평균 440.84±24.7 pg/mL, IGF-1: 평균 37.62±3.71 pg/mL)이 MP 사료구의 수정란(GH: 평균 347.01±46.97 pg/mL, IGF-1: 평균 23.89±2.41 pg/mL)보다 높은 함량을 나타냈다. 결과적으로 종자생산을 위한 양질의 수정란 생산 시, 본 연구에서 사용된 EP 사료가 긍정적인 영향을 미치는 것으로 판단되지만, MP의 사료원에 따라 다르게 나타날 수도 있을 것으로 사료된다.

Keywords: egg quality, egg development stages, GH, IGF-1, olive flounder

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D-03

Morphological assessment of egg quality for second generation of cultured broodstock small yellow croaker, *Larimichthys polyactis*

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The egg quality is a representative limiting factor in developing culture techniques for certain fish species. It is a known predictor of subsequent larval viability, quality, and stress resistance related to aquaculture productivity. Here we tracked egg quality in a second generation of broodstock small yellow croacker, *Larimichthys polyactis*. Cultured 2nd generation broodstock (3 years old, 600 fishes) was reared in indoor tank (14 tons). We induced natural spawning with heated water temperature (11.5 ~22.0℃) and regulation of photoperiod (9L:15D). We used spawning events from spawning period and monitored basic morphometrics such as: egg viability, egg diameter, oil droplet diameter and oil droplet volume. Natural spawning of the broodstock was maintained for 23 days. During this study we demonstrated that egg diameters and oil droplet diameters for *L. polyactis* decreased as the spawning season progressed and water temperature increased. We showed that smaller eggs lead to higher quality with viability, and that using eggs later in the spawning season would lead to better production. In addition, the volume of oil droplet was the strongest factors for prediction of egg viability for small yellow croaker.

Keywords: small yellow croaker, egg quality, morphometrics, egg diameter, oil droplet volume

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D-04

참다랑어 산란 유도를 위한 호르몬 임플란트 제작

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국내 참다랑어 양식 연구는 인공종자 양성, 어미화 등의 주요 핵심 요소 기술 개발에 박차를 가하고 있으나, 어미로부터 수정란 생산에 어려움을 겪고 있다. 이에 따라 국내 연안 환경에 따른 참다랑어 성성숙 특성을 구명하였고, 안정적, 계획적인 수정란 생산을 위한 추가적인 연구가 필요하였다. 현재 유럽을 비롯한 여러 나라에서 참다랑어의 인공적인 성성숙 유도를 위해 호르몬 임플란트 투여와 관련된 연구가 활발히 진행되어 왔다. 유럽연합 연구개발 프로그램 보고에 따르면, 호르몬 임플란트를 참다랑어에 투여시 정자량 증가 및 난소 발달에 효과적임을 보고하였다. 국내에서는 해외 수입 호르몬 임플란트 투여로 참다랑어의 수정란 생산에 성공한 바 있으나, 수입 호르몬 임플란트의 높은 단가 및 적기 물량 확보에 어려움이 있는 실정이다. 따라서, 본 연구진들은 어체중에 따라 농도 조절이 가능한 산란유도 호르몬 임플란트의 자체 제조기술 확보를 통해 비용 절감 및 기술의 국산화를 목적으로 호르몬 임플란트를 자체 제작하고자 하였다.

본 임플란트의 제작은 시약 ethylene vinyl acetate copolymer(EVAc)와 함께 혼합된 호르몬 혼합물이 EVAc의 미세공을 통해 어체 내에서 방출되는 원리를 이용하였다. 호르몬 임플란트의 제작은 목적 호르몬인 LHRA 또는 GnRH를 방출속도 조절에 관여하는 bovine serum albumin(BSA)와 inulin과 함께 증류수에 용해시켜 동결건조 후, 동결건조 부산물을 잘게 균질화 하여 10～15% EVAc 용액과 혼합시킨 후, 알루미늄 틀에 –20℃에서 24시간 보관하여 유기용매를 모두 증발시켰다. 이후 진공 데시케이터 상에서 24～36시간 보관하여 굳어진 호르몬 플레이트의 수분을 제거해 주었다. 마지막으로 수분까지 모두 제거된 호르몬 플레이트를 사용하고자 하는 농도에 맞게 재단하여 삽입용 촉, 모노필라멘트, 태그 등과 함께 삽입용 호르몬 임플란트의 형태로 제작하였다. 자체 제작 호르몬 임플란트는 작살용 촉과 결합 후, 호르몬 투여용 인젝터에 연결하여 활용하였다. 인젝터 활용 호르몬 투여시, 스토퍼에 의해 참다랑어 근육 내 호르몬 임플란트만 남게 되는 구조로 제작하였다. 호르몬 펠렛은 어체중에 따라 호르몬 펠렛 크기를 조절하여 용출되는 호르몬 농도의 조절이 가능하였다. 상기 결과로부터 어체 크기・체중에 맞춤형 호르몬 임플란트 제작이 가능하였으며, 참다랑어와 같은 핸들링 스트레스에 영향을 받은 어종을 대상으로 효과적으로 투여가 가능할 것으로 판단된다.

Keywords: 참다랑어, 성성숙 유도, 호르몬 임플란트

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D-05

Progesterone enhances the mRNA expression of FSHβ and LHβ genes in the pituitary cells of immature eels (*Anguilla japonica*)

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Fish reproduction is regulated by neurohormones secreted from the brain and gonadotropins from the pituitary. Progesterone (P4), one of the gonadal steroids, stimulates oocyte maturation and ovulation in females, and sperm maturation and motility in males. Many studies have reported the effect of P4 on animal reproduction and the importance of E2 priming for the action of this hormone. Thus, it is highly probable that P4 has similar effects on eel maturation and reproduction.

In this study, the pituitaries of immature eels were primary cultured and treated with P4 and E2 at different concentrations and experimental conditions in the presence of gonadotropin-releasing hormone (GnRH). Then, the mRNA expression of FSHβ (follicle stimulating hormone β), LHβ (luteinizing hormone β), GH (growth hormone), SL (somatolactin), pgr (progesterone receptor) genes were analyzed from pituitary cells treated with P4, E2 and E2+P4.

P4 enhanced the mRNA expression of FSHβ and LHβ in immature eel pituitary cells, but not the mRNA expression of GH, SL, and pgr. E2 and E2+P4 enhanced the mRNA expression of pgr but not the mRNA expression of FSHβ, LHβ, GH, and SL. These results suggest that P4 may play an important role in the early sexual maturation of eels by enhancing FSHβ and LHβ mRNA expression.

Keywords: *Anguilla japonica*, reproduction, progesterone (P4), 17β-estradiol (E2)

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D-06

참문어(*Octopus vulgaris*)의 번식생리학적 연구

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참문어(*Octopus vulgaris*)는 전세계 온대와 열대 사이에서 주로 분포하고 있으며, 조간대 모래, 자갈, 바위틈에서 서식하고 있고, 주로 밤에 포식활동을 한다. 수명은 대략 1～2년으로 알려져 있으나 교미 여부에 따라 달라지며, 대부분 교미 및 산란 후 죽는 것으로 알려져 있다. 한편, 참문어는 상업적으로 매우 중요한 생물종이기에 여러 나라에서 인공종자 생산 기술에 박차를 가하고 있다. Shigeki Dan et al.(2018)은 사육수조에서 상향식 수류시스템을 적용한 것과 갑각류 조에아 유생을 먹이원으로 공급 시 생존율이 우수하다고 보고하는 등 다양한 연구가 활발히 진행 중에 있으며, 국내에서는 자원조성, 성숙과 산란(Song et al., 2020; Yang et al., 2021; and) 등 연구가 국한되고 부진한 실정이다. 따라서 본 연구에서는 참문어의 산란기질 선호도, 산란량, 난발생 및 유생사육 등 번식생리학적 기초자료를 얻고자 수행하였다.

어미 참문어의 산란용 기질에 따른 선호도를 조사한 결과, 기질별 잠입 횟수는 나무 62회, 옹기 48회, 돌 46회, 고무 27회, PVC 20회로 나무에 대한 선호도가 특히 높게 나타났다. 기질별 산란개체 수는 나무 4개체 및 옹기 1개체였으며, 돌, 고무, PVC에서는 산란을 하지 않았다. 어미 크기에 따른 산란량을 조사한 결과, 어미의 체중은 946 g, 1,216 g, 2,313 g이었으며, 총 산란량은 각각 110,258개, 156,142개 및 182,606개로 어미 크기가 클수록 산란량이 많았다. Song et al.(2020)은 어미 1 g당 상대 포란수가 64～108개의 범위에서 평균 84개로 체중이 증가함에 따라 포란수가 증가하는 경향을 보고하였는데, 본 연구에서도 어미 크기가 클수록 산란량이 증가하는 것으로 나타났다. 참문어의 수온별 난발생 과정, 부화 소요일 및 부화율을 조사한 결과, 난발생 과정은 피포 형성, 안점 생성, 색소포 출현, 부화 순으로 나타났다. 수정률은 95% 이상이었으며, 수온별 피포가 생성되는 소요일은 수온 25℃에서 4일, 20℃에서는 7일 및 15℃는 11일이 소요되었고, 안점생성 시기는 수온 15, 20 및 25℃에서 각각 23일, 15일 및 11일이 소요되었다. 부화는 15, 20 및 25℃에서 각각 52일, 38일 및 28일이 소요되었고, 부화율은 수온 15, 20 및 25℃에서 각각 83.8±2.5, 92.0±1.6, 87.2±2.7%였으며, 수온 10, 30℃는 발생이 진행되지 않았다. 참문어 유생 사육은 수온 21℃ 내외에서 유수식으로 관리하였으며, 먹이생물로 알테미아(노플리우스 30마리/유생, 2～3 mm 배양개체 50마리/유생), 게류(깨다시꽃게, 꽃게) 조에아 유생(15마리/유생)을 공급하였고, 민들조개 다짐육, 냉동 곤쟁이 및 냉동 알테미아를 충분히 공급한 결과, 2022년도는 30일령에 7.3 mm까지 사육하였고 최종 40일령까지 사육하였다.

Keywords: cephalopoda, common octopus, paralarva, *Octopus vulgaris*, embryonic development.

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D-07

멍게 유생의 뇌에서 좌우비대칭적으로 발현하는 유전자

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피낭류로도 불리는 멍게(미삭동물)는 창고기(두삭동물), 척추동물과 함께 척삭동물문을 구성한다. 척추동물의 복잡한 발생과정을 이해하는 데 있어 멍게는 유용한 실험재료이다. 멍게의 유생은 복잡한 척추동물 체제(body plan)의 기본적인 특성을 보여주며, 척추동물과 멍게의 발생과정을 조절하는 핵심적인 유전자 중에서 공통적인 특성을 나타내는 것이 다수 존재한다. 멍게와 척추동물은 모두 몸의 등쪽에서 앞뒤로 뻗은 모양으로 형성되는 신경관으로부터 중추신경계가 유래하며, 신경관을 유도하는 신호는 신경관의 배쪽 부위에 위치하는 척삭으로부터 나온다. FGF 신호의 활성화에 의한 신경분화와 FGF 신호전달과정의 보존성은 대표적인 공통점이다. 21세기에 들어서 척추동물의 뇌 발생에 대한 이해가 비약적인 진보를 하였지만, 아직도 많은 영역이 미지의 상태로 남아 있다. 이는 척추동물 뇌 구조의 복잡성이 가장 큰 이유이다. 수백만에서 수십억 개 이상의 뉴런의 네트워크 형성과 이에 의한 복잡한 행동조절 메커니즘을 밝히는 것은 극도로 난해한 작업이다. 멍게의 유생은 1차 신경관 형성 방식으로 신경관이 닫히며, 뇌는 신경관의 앞쪽 부위에서 발달하는데, 약 400개의 세포로 구성되며, 이중에서 뉴런은 약 180개로 추정된다. 이와 같은 단순한 구성 때문에 멍게를 활용한 신경계와 뇌의 발생에 관한 연구는 많은 이점을 갖는다. Synaptic connectome, 유생 행동의 정량화 등을 이용하여 멍게 뇌 발생 연구로부터 척추동물의 뇌 발생을 이해하려는 연구는 최근 크게 주목받고 있다. 척추동물과 멍게의 몸은 뇌를 포함하여 좌우비대칭으로 형성되는 구조가 다수 존재한다. 멍게 유생의 경우, 안점(ocellus)과 광수용체 세포체는 오른쪽 뇌포(brain vesicle)에 존재하지만, coronet 세포와 antenna 세포는 왼쪽 뇌포에 위치한다. 이러한 차이를 만드는 과정에 척추동물처럼 Nodal 신호가 관여하는 것이 알려졌지만, 세부적인 내용에는 미지의 부분이 많이 남아 있다. 본 발표에서는 멍게 중추신경계에서 발현하는 유전자를 검색하는 과정에서 찾은 뇌의 오른쪽에서 특이적으로 발현하는 유전자를 보고한다. 이 유전자는 발현량이 매우 적어 검출을 위해 *in situ* 실험에서 오랜 시간 발색반응이 필요하였다. 수정란, 초기 난할기 및 신경배에서는 발현하지 않았고, 후기 tailbud 시기에 뇌의 오른쪽 부위에서 발현이 시작되었다. 앞으로 이 유전자를 이용하여 멍게 뇌 발생과 좌우비대칭 메커니즘을 연구할 예정이다.

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D-08

흰다리새우에서 수온, 염분 및 pH 변화에 의한 ferritin 유전자의 발현 연구

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해양생태계에서 수온, 염분 및 pH 등의 환경요인은 해양생물의 생식, 생리, 대사, 성장 및 삼투압 조절 등 해양생물의 생리적 변화 및 서식처에 영향을 미친다고 알려져 있다. 해양생물은 수온 변화에 매우 민감하게 반응하는 생물로서, 수온은 생물의 생식, 성장, 생리, 대사, 에너지 균형 및 면역 등에 영향을 미치는 중요한 요인으로 알려져 있다. 양식대상 생물종에서 수온의 조건은 종자생산, 양성 및 초기 발생에 있어서 매우 중요하다. 수온과 더불어 염분은 해양생물에게 중요한 환경요인으로 염분의 변화에 따른 해양생물의 스트레스에 의한 항상성의 불균형을 회복하기 위하여 체내 삼투 조절, 체액 농도 조절 및 산소 소비와 같은 대사활동을 통해 항상성을 유지하고자 한다. pH는 각종 용존 물질과 생물의 동화작용 및 호흡작용에 의하여 변화한다. 용존산소는 최적 양식 수용밀도와 생산량을 결정하는 중요한 환경요인이다. 용존산소량은 pH와도 관계가 있어 수중의 동일 산소량 존재에 pH의 산성화 및 알칼리화에 따라 생물에게 미치는 영향은 다르게 나타난다고 알려져 있다. Ferritin은 동물, 식물, 미생물 등의 대부분의 세포에 존재하는 단백질로서, 주요 기능은 세포 내 철분의 과잉에 따른 손상에 대비하며, 또한 철분 부족 시 capturing을 통한 철분의 보충 등 세포 내 철분의 항상성 조절을 한다. 환경적 요인에 의한 스트레스는 세포 내 활성산소종(ROS)을 생성하여 산화적 스트레스를 유도함으로서 면역 및 대사에 영향을 미치는데, ferritin은 산화적 스트레스를 조절하는 기능이 있다. 흰다리새우(*Litopenaeus vannamei*)는 광염성 종으로, 1～40 psu의 염분 범위에서 생존이 가능하여 지역의 제한을 받지 않고 양식이 가능한 생물이다. 본 연구의 목적은 수온, 염분 및 pH 등의 환경요인 변화가 흰다리새우에 미치는 스트레스 정도를 생체지표유전자의 발현 조사를 통해 파악하기 위한 연구이다. 연구결과, 수온, 염분 및 pH 등의 환경요인 변화는 ferritin 유전자의 차별적 발현 양상을 보였다. 따라서 ferritin 유전자는 흰다리새우의 스트레스 정도를 판단하는 생체지표유전자로 활용이 가능하다 생각된다.

Keywords: 흰다리새우, ferritin, 수온, 염분, pH

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D-09

Response of astaxanthin feed on body colour change and carotenoid-related gene expression in juvenile red spotted grouper, *Epinephelus akaara*

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Studies on aquaculture technology are being conducted, various studies of fish coloration are being conducted. Depending on the types of carotenoids, fish body coloration can take on diverse forms and recent studies has been conducted on improving fish body colour using astaxanthin. We investigated to changes of body growth, body colour development, chromaticity analysis and mRNA expression of carotenoid-related factors (*bco2*, *scarb1* mRNA) in the skin were subsumed in the red spotted grouper. The experiment was divided into a control group and two astaxanthin treatment groups (1,000 ppm and 2,000 ppm), and astaxanthin was adsorbed to the feed for five weeks on the 35 days after hatching red spotted grouper. As a result of the body growth, there was no difference between groups at the end of the experiment, but the weight and body length of the 1,000 ppm group tended to be higher than that of other groups. The results of the body colour development investigation indicated differences in body colour development between the treatment groups and the control group, starting from the third weeks of the experimental period. The treatment groups exhibited a tendency for faster colour development compared to the control group. The results of chromaticity analysis confirmed that the red chroma in the skin of the treatment group was higher than that of the control group. Measuring the size of the yellow spots that develop on the bodies of red spotted grouper, the showed that the control group 0.7±0.02 μm, the 1,000 ppm treatment group 0.88±0.04 μm, and the 2,000 ppm treatment group 0.93±0.04 μm. The spot size in the 1,000 ppm and 2,000 ppm treatment groups was significantly larger compared to the control group (p<0.05). Expression of *bco2* mRNA in the skin did not show significant differences among the groups, but the 1,000 ppm treatment group exhibited the lowest expression levels. On the other hand, scarb1 mRNA expression was significantly higher in the 2,000 ppm treatment group (p<0.05). Based on our research, astaxanthin supplements are thought to promote the timing of spots on the body of red spotted groups and improve spot size during the spot formation stage. Further studies are needed to determine the optimal concentration of astaxanthin in the future.

Keywords: astaxanthin, carotenoid, chromatophore, body colour, *Epinephelus akaara*

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D-10

The effect of water temperature on stress response-related gene expression in *Epinephelus akaara* and *Paralichthys olivaceus*

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Water temperature is an important part of aquaculture because it is a key factor in controlling metabolism in aquatic animals. Fish are more sensitive to changes in temperature conditions than other animals, and various physiological and environmental factors can cause stress. Heat shock protein (*HSP*) plays an important role in temperature adaptation and is an effective molecular chaperone that protects cells that are denatured by stress. *HSP70* is a protein triggered by a mechanism that protects cell from changes caused by oxidative stress, and *HSP90* is a protein that acts in the signaling pathway to steroid receptors. In this study, we investigate the expression of stress response-related factors; corticotropin-releasing hormone (*CRH*), *HSP90*, and *HSP70* mRNA for changes in the water temperature of *Epinephelus akaara* and *Paralichthys olivaceus*. The *E. akaara* and *P. olivaceus* were raised from February to November in a flow-through tank system (150×150×150 cm) at Jeju National University's Marine Science Research Institute. Sampling was conducted four times in February, May, August, and November. RT-qPCR was performed to analyze the expression of *CRH*, *HSP90*, and *HSP70* mRNA. In the case of *E. akaara*, *HSP90* mRNA in the liver had a lower expression level in November, and *HSP70* mRNA showed a decrease expression level from February to November. *CRH* mRNA expression in the brain was observed to increase from February to November. In the case of *P. olivaceus*, *HSP90* and *HSP70* in the liver were observed significantly high expression level in August. In the brain, *CRH* observed relatively low expression level in August. As a result of our study, it is assumed that *E. akaara* and *P. olivaceus* has difference of water temperature stress response according to seasonal changes. We believe that this study might be available as basic information on the hypothalamic-pituitary-interrenal (HPI) axis in response to stress caused by changes in the water temperature of the two fish in the future.

Keywords: *Epinephelus akaara*, *Paralichthys olivaceus*, heat shock protein, corticotropin-releasing-hormone, stress impact

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A novel method to establish porcine trophoblast stem-like cells from parthenogenetic activated embryos

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Trophoblasts play an important role in the embryo implantation and formation of the maternal-fetal interface. Neurotrophins are associated with implantation, maintenance of pregnancy, and regulation of placental angiogenesis. Here, we established trophoblast stem-like cells (TSCLCs) from porcine parthenogenetic activation (PA)-derived embryos treated with neurotrophin-4 (NT-4) during *in vitro* culture (IVC). On day 7 of IVC, PA-derived blastocysts were seeded on feeder cells in modified porcine TSCLCs medium to obtain TSCLCs. Porcine TSCLCs derived from embryos not treated with NT-4 during IVC were termed 'control TSCLCs', whereas the TSCLCs derived from embryos treated with NT-4 during IVC were termed 'NT-4-treated TSCLCs'. Both the established porcine TSCLC lines showed significantly decreased mRNA transcript levels of pluripotency markers (*SOX2* and *Nanog*) and increased mRNA transcript level of a trophoblast marker (*KRT18*). In particular, the mRNA level of the *CDX2* transcript was significantly increased in the NT-4-treated TSCLC line. Through immunostaining, we also confirmed that the trophoblast stem cell markers (TEAD4, YAP1, and CDH1) were expressed in all established porcine TSCLC lines. Although further studies will be needed, our study could serve as a useful model for studying porcine trophoblast and placental development *in vitro*.

Keywords: *In vitro* culture, trophoblast stem cells, parthenogenetic activation

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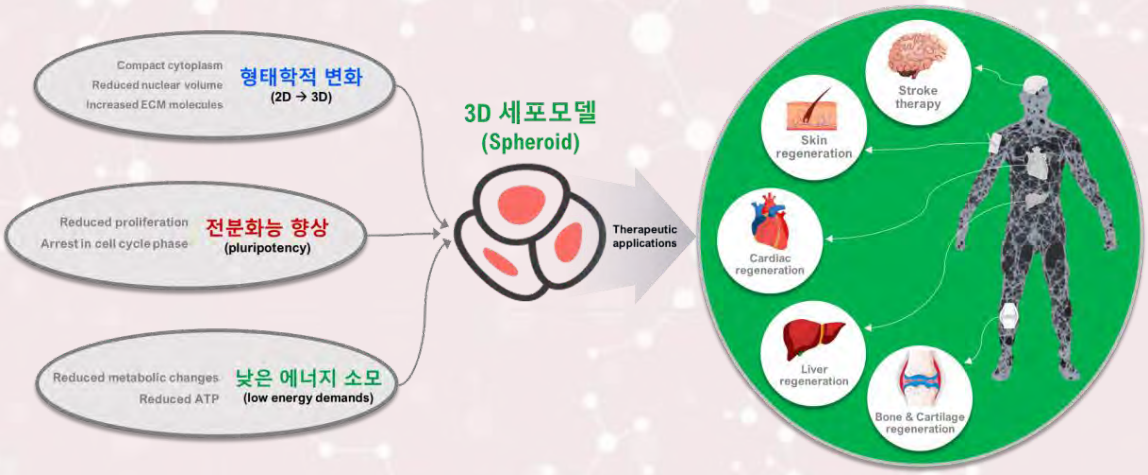

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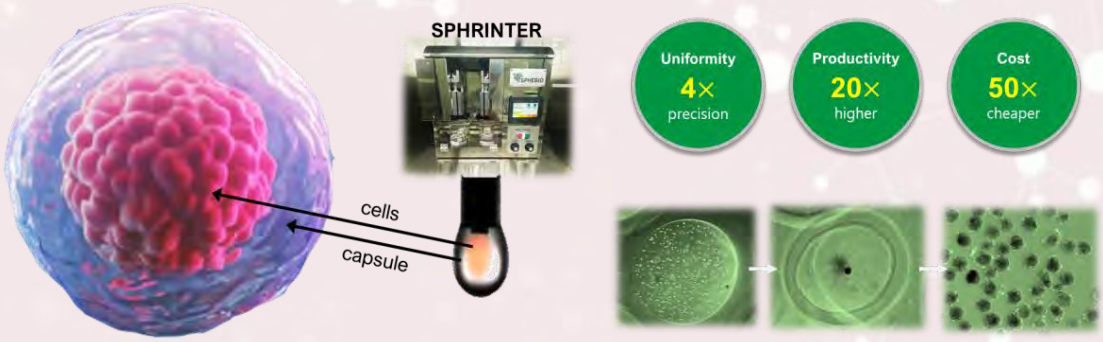

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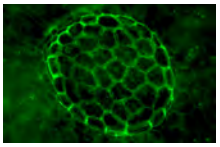
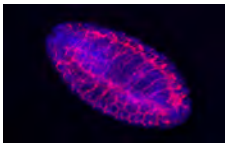
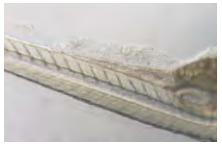


Identify and document weakly autofluorescent gland tissue of Nepenthes, Isolate glands of interest



Sort and evaluate Drosophila embryos, Acquire multidimensional images



Zebrafish Larva

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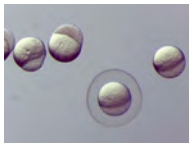
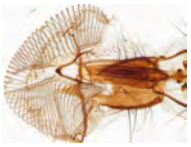


Zebra fish embryos, four hours after fertilization, obliquely illuminated in transmitted light brightfield, magnification: 25(as seen in the eyepiece)

Transmitted light with VisiLED HCT contrast stage. Lateral darkfield



Transmitted light with VisiLED HCT contrast stage. Brightfield

5:1 & 8:1 줌을 갖춘 컴팩트 실체 현미경
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Green lacewing acquired with reflected light, brightfield.

Butterfly wing acquired with vertical illumination, brightfield.

Zebreafish embryo

Royal fern - sori and sporangia acquired with reflected light, darkfield

Powdery mildew on Norway maple acquired with reflected light, darkfield

Zebreafish Larva

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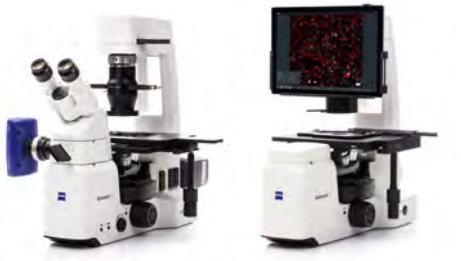

- 간단하고 스마트한 조작으로 높은 품질의 이미지 획득
- 최대 6개까지 장착 가능한 확장된 대물렌즈 및 리플렉터 슬롯
- 스마트 컨트롤 박스 내장으로 샘플 촬영부터 분석까지 일원화된 시스템

Flexible tablet concept - future proof

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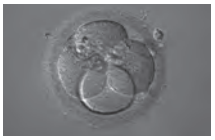
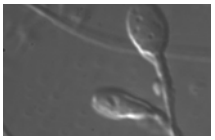
Smart control box compatible with many Axiocams

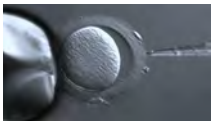
Only snap button - no other controls



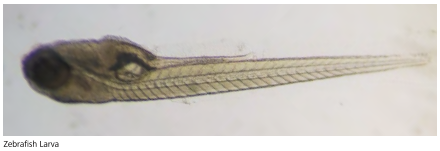
Embryo: Nucleus with nucleoli visible in right cell, iHMC



IMSI: Visualization of vacuoles in sperm cells, DIC



ICSI: Visualization of oocyte with Zona pellucida, PlasDIC



Zebrafish Larva

Auricularia, Dr. Moon Sunju, National Institute of Fisheries Science.
Research; Effects of Water temperature, Salinity and Stocking Density on Larva Survival and Growth of Sea Cucumber, Apostichopus japonicus

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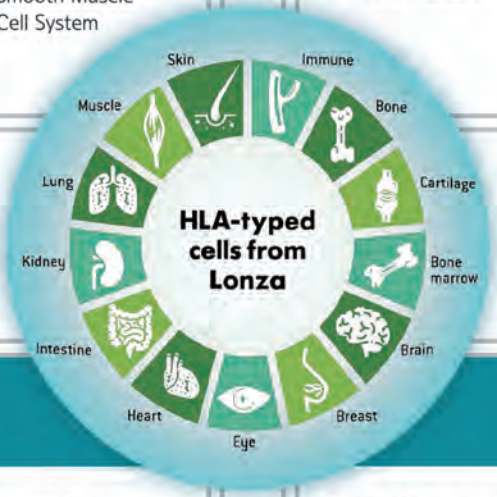

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