【Poster Presentation (Regular Papers) May 25】

1A01 Medical Industry Cluster Promotion Unit Commerce, Industry & Labour Department Fukushima Prefectural Government

Introducing the Fukushima Medical Device Development Support Centre

* "Utsukushima (Beautiful Fukushima) Next-Generation Medical Industry Agglomeration Project" started in 2005 in Fukushima, with collaborative effort between industry, academia, and government as a foundation, to promote and clustering the medical device related field.
Clustering of medical related industry was designated as one of the bases for recovery from the 2011 Great East Japan Earthquake; as the core of this recovery effort, the Fukushima Medical Device Development Support Centre (the Centre) opened in November, 2016, to provide integral support from development to commercialization of medical device.
The Centre has four functions including preclinical safety evaluation, training, business matching, and consulting. Preclinical safety evaluation function includes conducting biocompatibility testing on laboratory swine, ensuring compliance with the GLP regulations. Up to 150 animals may be housed in the clean area of the animal facility. Initially, implantation testing (subcutaneous, muscle, bone, vascular) will be conducted at the Centre. However, chronic systemic toxicity testing and other tests evaluating performance of medical device may be included in the near future.
For training function, surgical training on swine is available for medical personnel. Procedures are performed in the dedicated mock operating room or in the mock angio-hybrid operating room. Availability of such training is limited to procedures involving the use of medical devices that are already on the market, and to procedures whose requirement for animal use is clearly indicated, such as those listed as requirement for the final stage of residency training, or for medical specialist certification processes.
Biocompatibility testing will be conducted in compliance with the GLP regulations and the ISO 10993 guidelines. Additionally, all experiments will be conducted, ensuring compliance with the ILAR Guide (the Guide for the Care and Use of Laboratory Animals), 8th ed, to obtain accreditation from AAALAC International in the near future. Only experiments whose protocols (for both GLP testing and training) approved by the Institutional Animal Care and Use Committee (IACUC) will be conducted. Biocompatibility testing will be conducted upon requests from manufactures, and will be limited to medical devices whose specification have already been finalized, and are intended for submission for approval within and outside of Japan. This type of training will not be available for medical devices that are still in development.
Animals used for testing and training described above will be limited to minipigs and farm pigs bred and raised for research purposes, and their specific pathogen free (SPF) status meet the standard set by the Centre. The SPF status was determined to provide clean and controlled environment required for medical device testing, prevent zoonosis, and improve animal welfare.
Currently, we are working towards the GLP certification and AAALAC accreditation of the Centre at the earliest possible date, and are in the process of preparing the facility and equipment, and establishing the personnel training program, IACUC operation, and the veterinary care program.
Opening of this Centre will contribute to the recovery of Fukushima prefecture from the earthquake, increased number of 'made-in-Japan' medical devices competitive in the international market, and improved safety in medical field.
*http://fuku-semi.jp/iryou-pj/English/

1A02 Planning and Assemblying of UT Medical Technology Evaluation Laboratory

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The development of medical technology has been the urgently needed national strategy. Developing and launching of medical devices especially require swiftness on that strategy. Although many research and development projects involving the university-derived technology are going on, there are still many problems to bring technologies to practical use. Solving the lack of communication between developer and user might help improve this problem.
Our new laboratory is located in the medical and engineering cooperation center, Molecular & Life Innovation Building. The surgical experiment room has enough space as wide as hybrid operation room for clinical use. The room is equipped with angiography system. A workshop, a central supply room and a biochemistry laboratory are located in peri-operative area. In addition, the facility can take in middle-sized animals such as swine, canine and rabbit. Also quarantine and habituation equipments for swine are located in this area. The traffic line is planned to prevent cross contamination between researchers, animals and devices. The IC card-based key system helps to restrict researchers' migration.
Since the laboratory has only 1 operation room and 2 animal pens, and is restricted to acute experiment, the system must be all-in & all-out way, avoiding the contamination between users or animals. To optimize the allocation of cost, usability and operation, we limit the number of monitoring-needed swine infectious disease to no practical impact on experiment.

1A03 Damage caused by the Kumamoto earthquake to rack devices for breeding genetically engineered mice

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We keep around thirty thousand genetically engineered mice (GEM) on the eighth, ninth and tenth floors of our new animal facility. Last year, Kumamoto was struck by two fierce earthquakes, known as the 2016 Kumamoto earthquakes. Although almost all of rack devices survived the earthquakes safely, two rack devices on the tenth floor containing GEM for breeding fell down. Some of mice kept in the rack devices died, and the remaining mice escaped into the mouse room. In this presentation, we would like to explain about our investigation into the cause of the damage and future countermeasures that we will take against the problem.

1A04 Creating a short-term stable environment for rabbits in a cargo van

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【Background】 We are investigating the ocular damage threshold by millimeter wave exposure using laboratory rabbits at the Research Center for Development of Far-Infrared Region, University of Fukui (FIR-UF) . There is no available animal facility for rabbits at FIR- UF and the nearest facility is 10 km away. Since rabbits are susceptible to transportation stress, we set up a short-term housing for rabbits in FIR- UF using a cargo van.

【Materials and Methods】 A cargo van was rented from the local car rental agency and was brought into the laboratory at FIR-UF. An air conditioner, humidifier, dehumidifier, and LED lamp were purchased from retailers. The Photocatalyst Deodorization Device (Renatech, Japan) was used to remove animal odors. Six male Dutch rabbits (10 to 11-weeks old and retired breeders) were housed individually in their cages for up to 6 days. Food and water were given daily. Pet pads were changed daily.

【Results and Conclusion】 We evaluated microbial contamination in the van by passive sampling method. After 70% ethanol sanitation, settle plates were exposed to air for 30 min and cultured. Average numbers of bacteria and fungi were 0.2/dish and 4.7/dish, respectively. This indicates that the van is as clean as non-barrier animal facility. Temperature, humidity, and CO_2 concentration were monitored. The temperature was stable (average 20.2°C) and ranges 17.1 to 22.6 °C. We managed the humidity by either dehumidifier or humidifier, resulted in an average 50.7% (33.1 to 70.8%) . Ammonia concentration was under detectable level (0.5 ppm) . CO_2 concentration, illumination, noise, and air speed were within appropriate levels. We conclude that a cargo van can be a choice for a short-term rabbit housing. The FIR-UF room with a cargo van is approved by Animal Research Committee, Univ. of Fukui.

Ultraviolet cured resin coat for floor of laboratory animal facility

1A05

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Laboratory animal facilities need especially cleanliness environment. Therefore the floor becomes the factor that is important to the maintenance of the cleanliness.

We considered ultraviolet cured resin coating to floor. Ultraviolet (UV) curable resin combines strongly when it receives UV dose. By this strong combination, UV coating shows a lot higher performance than a general floor wax.
UV cured resin is low odor and organic-solvent-free because of waterborne lacquer. Good re-coatability even after UV curing. Excellent scratch resistance, abrasion resistance and stain resistance

These results, useful thing was suggested to UV cured resin coating to floor of laboratory animal facility protecting flooring material from transformation by exposures, such as attrition by daily cleaning, crack, antiseptic solution, and reagents. Moreover, it improved cleaning antiseptic property by heightening the dust removal effectiveness, and it was suggested that it can carry out migration retention of the breeding environment where cleanliness factor is high at longterm.

The importance of vaporizing hydrogen peroxide in decontamination

1A06

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The purpose of this study was to investigate the effects of decontamination of laboratory animal rooms with the vapor-phase of hydrogen peroxide (VHP). Additionally, we examined material compatibilities with vaporized hydrogen peroxide. The laboratory animal room was exposed to VHP. The effects of decontamination with VHP were determined by biological indicators. 39 kinds of materials were exposed by 100 cycles to VHP in the test chamber. The materials tested contained plastics, metals, woods and plating or coating goods. According to the four-grade system, we evaluated the materials abilities to undergo exposure to vaporized hydrogen peroxide.

The exposure test to VHP provided complete sterilized effects at room humidity of not more than 75 %. Some damage was found in copper, brass, chromium plate and galvanized iron immediately following exposure to VHP. Repeated decontamination caused marked damage in stainless steel and urethane-, silicone- or epoxy-coating materials. Condensation of VHP posed severe damage for the surface the materials. We confirmed that VHP immediately sterilized microorganisms. The increasing concentrations of VHP with condensation caused severe changes in the materials during prolonged exposure. It was highly important to decontaminate with VHP.

1A07 Photocatalyst deodorization in a breeding environment using individual ventilation cage rack

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In recent years, the use of an individual ventilation cage system (IVC-System) has been increasing in many animal laboratories for housing mice and rats.
The IVC-System has a ventilation structure for each cage that reduces the microbiological cross-contamination risk between cages. The cage is of a high-density type in the IVC-System. Therefore, the number of accommodating cages is larger and less space than an open rack or laminar flow rack. However, the high-density housing apparatus has a disadvantage that odors (animal smell and ammonia) tend to be accumulated in the animal room. To solve this problem, updating the exhaust system of the existing animal room is effective but costly.
Therefore, we produced a photocatalytic deodorizing device dedicated to the IVC- System (Lab Products RAIR IsoSystem Super Mouse 1800), which is an indoor exhaust type for the animal room. When titanium oxide is irradiated with ultraviolet rays, the titanium oxide is activated and has a strong oxidizing activity. This photocatalytic technology is used to decompose organic matter that is a source of odor. In the breeding environment of experimental animals using the IVC-System, the deodorizing effect of the photocatalyst deodorizing device was evaluated for animal odor and ammonia levels.
We confirmed that both animal odor and ammonia levels were reduced significantly ($p < 0.05$). However, the reduction rate of ammonia was low at 21.9% which needs to be improved in the future.

1A08 Development of a New Air Filter Material for Vinyl Isolators That Has Satisfactory Dust Collection Efficienc

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The FG-50, which is a medium-efficiency glass wool mat produced by AAF, has been used since 1955 as an air filter material for vinyl isolators; however, airborne dust still manages to penetrate through the FG-50. Therefore, we have developed a new air filter material for vinyl isolators with the objective of improving the internal environment of the vinyl isolators while also improving the air filter itself. We created a prototype for the new air filter material (new HEPA) in which the HEPA was clamped between the Emirent; then, we compared this with the conventional FG-50. Upon measuring the airborne dust in a chamber that was mounted onto an isolator, in a sterilized can and in a transport can that has been negatively-pressured using an air blower that has been wrapped around the germ-free transport can, we found that the number of 0.3 μ m particles in 0.1CF was extremely small at 1 or less in all cases for the new HEPA. The new HEPA was also found to have the same dust collection efficiency as a dust collector when compared with the FG-50, and the HEPA could keep a significantly low degree of airborne dust from infiltrating inside the isolator. We also evaluated factors such as the number of air changes, wherein we felt that the HEPA would be useful as an air filter material for isolators.

1A09 Impact of environmental enrichment on mouse keeping

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【Objectives】 Recent international trends have led to the widespread improvement of animal welfare. In this study, the impact of environmental enrichment on animal welfare was evaluated based on wound rate due to fighting, while the impact on experimental results was assessed using a subcutaneous xenograft model in athymic mice.
【Methods】 Male BALB/c nude mice were group housed in a cage containing bedding material. In addition, nesting material was provided for the nesting material (N+) group, but not for no-nesting material (N-) group. 1) The number of wounded mice due to fighting was counted for 7 days. 2) To compare tumor growth between the two groups, human lung cancer cell lines NCI-H1975, HCC827 and A549 (sub strain) were implanted into right flanks of the mice. 3) To compare impact on the anti-tumor effects of an anti-cancer drug (cabozantinib) , tumor growth rates in the A549 (sub strain) xenograft model were evaluated in both groups.
【Results】 1) The rate of wounded animals significantly decreased in N+ group than in N- group (3.6 ± 0.8%, 32.6 ± 3.2%, $p<0.001$) . 2) No differences in tumor growth were observed between the two groups in any xenograft models. 3) Significant anti-tumor effects were detected both in N+ and N- group. Relative tumor volume ratio with cabozantinib treatment when compared to controls was 55.6 ± 2.1% ($p<0.001$) and 51.0 ± 1.9% ($p<0.001$) . No differences in anti-tumor effects were observed between the groups.
<Conclusion> We found that environmental enrichment is useful for reducing the aggression of BALB/c nude mice and refining living conditions without affecting tumor growth or the effects of anti-cancer drugs in the subcutaneous xenograft model.

1A10 Successful derivation/rearing of germfree pigs and a trial to rear a SCID pig

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Recently, we reported the generation of X-linked severe combined immunodeficiency (SCID) pigs that lack interleukin-2 receptor gamma chain gene. SCID pigs are vulnerable to infection and difficult to grow. The purpose of this study is to rear SCID pigs in germfree conditions. To this end, we first tried to rear wild-type pigs under germ-free conditions. Piglets were derived from wild-type pregnant pigs in a germfree isolator by hysterotomy or hysterectomy. The survival rate of the piglets was 20% (1 pregnant pig; 2/10) with hysterectomy while 60% with hysterotomy (1 pregnant pig; 3/5) . The survival rate with hysterectomy was significantly improved to 86% (3 pregnant pigs; 19/22) after we achieved to shorten the time required for the piglet derivation with a newly devised isolator. There was a strong negative correlation between the survival rate and the time required from uterus amputation to piglet derivation ($r = -0.97$, $P < 0.05$). Microbial cultures of the skin, oral mucosa and stool swabs from all of the piglets that were born alive have been negative for bacteria and fungi for up to 4 weeks ($n = 9$) . We then tried to rear a SCID pig. We successfully derived a SCID piglet from a SCID carrier pregnant pig. The SCID piglet has been no infections or other abnormal signs under germfree conditions for more than 10 weeks at the present. The SCID pigs would be a great model for the gene therapy with genome editing.

1A11 Study of an anesthetic mixture of medetomidine, midazolam, and butorphanol in mice –comparison of three different doses of medetomidine–

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【Objective】 An anesthetic mixture of medetomidine (MED): 0.3 mg/kg, midazolam (MID): 4 mg/kg, and butorphanol (BUT): 5 mg/kg has been usually used in mice. However, sometimes a dose of MED: 0.3 mg/kg has been reported not to be a sufficient dose for surgical anesthesia in mice. We then studied the anesthetic and physiological effects of 3 different anesthetic mixtures at doses of MED: 0.3, 0.5 and 0.75 mg/kg with MID: 4 mg/kg and BUT: 5 mg/kg in mice.
【Methods】 Male ICR mice (7 ～ 9 weeks) were used. Experimental groups were 1. MED: 0.3 mg/kg group (M0.3group), 2. MED 0.5 mg/kg group (M0.5group), and 3. MED 0.75 mg/kg group (M0.75group). After each anesthetic mixture was administered intraperitoneally, the anesthetic score was measured using 5 reflexes and a noxious stimulus using a forceps at 5 minutes intervals. The duration for which a mouse showed a score of 6 was considered sufficient for surgical anesthesia. We measured the time when righting reflex disappeared, surgical anesthesia started and ended. We also measured oxygen saturation (O_2-saturation), heart rate, respiratory rate, and blood pressure (BP) during anesthesia.
【Results and Discussion】 There were no significant differences in the disappeared time of righting reflex between the 3 groups. However, the M0.3group showed a later tendency when compared to the other groups. The M0.3group showed significant later starting and shorter ending time of surgical anesthesia compared to the other groups. There were no significant differences in heart rate and respiratory rate during anesthesia between the 3 groups. The M0.75group showed lower O_2-saturation compared to the other groups. The BP of the M0.75group was significantly lower than those of the other groups. These results indicated that an anesthetic mixture of MED: 0.5 mg/kg, MID: 4 mg/kg, and BUT: 5 mg/kg might be a suitable anesthesia in mice.

1A12 Gender and strain differences in experimental mice of a novel combination of alfaxalone combined with medetomidine and butorphanol for inducing anesthesia

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We have already reported the effects of alfaxalone combined with medetomidine and butorphanol to induce anesthesia in female ICR mice. In order to make that versatility to other mouse strains, this study was carried out to investigate the effects of various doses of alfaxalone combined with 0.3 mg/kg of medetomidine, 5.0 mg/kg of butorphanol (M/B/A) to induce anesthesia in both of male and female ICR, BALB/c and C57BL/6 mice, respectively. Intraperitoneal injection of any tested dose of M/B/A could not achieve surgical anesthesia in any tested mice. Subcutaneous administration of the combination of 0.3 mg/kg of medetomidine, 5.0 mg/kg of butorphanol and 40 mg/kg of alfaxalone (M/B/A40) achieved surgical anesthesia within 5-10 min of administration and anesthesia was maintained for 40-80 min in various female mouse strains. In male mice, one of 6 tested mice died in both of BALB/c and C57BL/6 mice, respectively. Furthermore, M/B/A30 (30 mg/kg of alfaxalone) administration to BALB/c male mice also caused deaths in a few cases. These results suggest that gender and strain differences exist in mice for response to alfaxalone combined with medetomidine and butorphanol.

Pharmacological properties of anesthetic protocols under surgical invasion in rat

1A13

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For the achievement of appropriate anesthesia in animal experimentation, it is necessary to understand the features of anesthetic protocols. Particularly, under the surgical procedure, anesthetic time and depth, and surgical invasion should be considered. In the present study, we investigated the pharmacological property of various anesthetic protocols under the surgical procedure of castration in rats. Eight-week Wistar rats were anesthetized with 6 anesthesia, including standard and high dose of ketamine / xylazine (K/X), medetomidine/midazolam/ butorphanol (M/M/B), isoflurane, and sevoflurane. Castration was performed in each anesthesia, and assessed the anesthetic depth and times. For the safety assessment, vital signs were evaluated.

K/X showed enough anesthetic depth with rapid induction and recovery. However, bradycardia was prominent in high dose K/X, compared with other anesthetic protocols, indicating that standard dose of K/X is recommended for the surgical anesthesia of castration. M/M/B had relatively strong cardiorespiratory depression, and some rats showed inadequate anesthetic depth or delayed recovery from anesthesia. It is reported that inhalant anesthesia such as isoflurane had strong respiratory depression in rodents. However, in the present study, the decrease of respiratory rate was mild in isoflurane and sevoflurane. Other vital signs were also stable in both inhalant anesthetic protocols. In summary, all protocols investigated in the present study were applicable to the surgical procedure of castration in rat. Inhalant anesthesia is superior to injectable anesthesia in terms of safety and anesthetic depth regulation.

Time-dependent Effects of Different Anesthesia Methods in Rats

1A14

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We previously reported literature about the effects of different anesthesia on hematological parameters in rats and mice. However, a time-dependent detailed examination of blood property and biological reaction in anesthesia has not been studied. In the present study, we examined the time-dependent effects of different anesthesia in the rats.

We divided Wistar male rats into five groups, namely 1) medetomidine (0.15mg/kg) + midazolam + butorphanol tartrate group (MMB), 2) medetomidine (0.375mg/kg) + midazolam + butorphanol tartrate group (MMB2.5), 3) isoflurane inhalation group (ISO), 4) pentobarbital + butorphanol tartrate group (PB), 5) alfaxalone + medetomidine + butorphanol tartrate group (AMB), 6)MMB + ISO and 7) AMB + ISO. Blood samples were collected via the cervical vein for complete blood count and analysis of serum chemistry with Vet Scan HM2, Vet Scan VS2 and Piccolo Xpress. Reflex and rectal temperature measurements were carried out on each individual rat to evaluate the depth and duration of anesthesia.

It was indicated relatively low value in rectal temperatures of groups except for PB. However, surgical anesthesia was not achieved in PB, because the areflexia did not reach anesthesia levels. On the other hand, Glu and BUN in groups except for PB were time-dependent high value. Rectal temperatures decrease and Glu increase were enhanced in MMB + ISO and AMB + ISO. Present study indicates that there are some differences of hematological parameters and biological reaction between each anesthesia methods, and these results suggest the necessity of considering selection of the anesthesia methods.

Development of an automatic monitoring system for the moving activity of a mouse as a potential indicator of physiological conditions

1A15

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【Introduction】Large variations of experimental results are serious problem in studies using animals such as mice and rats. To reduce these variations, breeding conditions should be maintained at a constant level. The appearance and weight of each animal are indicators so far used routinely. However there are intense needs for more specific indicators that can evaluate physiological states of animals in every cage in rapid, simple, and non-invasive manners. Candidate indicators are moving activity, amount of diet, properties of feces and urin. Then we have focused the moving activity of mice. Our hypothesis is that higher activity of the movement should cause less obesity and better healthy condition.

【Results】Test mice of the movement mode (1) and the control mice of the movement mode (2) showed a similar values of surface movement indicators, the number of frequencies that a mouse moved between 2 sensors. In contrast, the hanging time of the control mice were much longer than that of the test mice as expected. Therefore a novel experimental method for the comparative study of mouse moving activity has been established.

Analysis of Locomotor Activity in the Period for Convalescence and Acclimation Using "nano tag", a New-type Device

1A16

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Physical activity of experimental animals has been measured with some appliance such as infrared sensor, video tracking, and wheel running system. In many cases, these methods analyze the animals one by one: the individual measurement of activity in plurality breeding is not popular. Recently, "nano tag®" (KISSEI COMTEC), a new-type device to analyze the activity, was released. This device can measure locomotor activity for laboratory animals without limitation of the number of the animals in same cage. Any kind of cages can be used for the analysis anytime, anywhere.

In this study, we investigated locomotor activity of mice and rats after surgery and transportation with nano tag® to check appropriate period for convalescence and acclimation. Animals were operated for the device implantation subcutaneously at SLC (Shizuoka, Japan) and were transported to Shinshu University (Nagano, Japan) after the appropriate period. The data were analyzed after breeding for acclimation at Shinshu University. From the operation to the end, Animals were housed 2 to 5 per cage at constant rearing condition.

Significant decreased activity was observed until 8 days after the operation in mice. In contrast, locomotor activity of rats was not changed by the surgery. Significant changes of activity were observed until 1 day (mice) or 8 days (rats) after the transportation.

Generally, experimental animals were given a period of over 1 week for convalescence and/or acclimation. In this study, the animals calmed down around 1 to 8 days after the stress. These results suggest appropriation of an established theory.

1A17 Evaluation of the Autonomic Nervous System under with 'Next Generation' Digital Implantable Telemetry in Canine

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【Purpose】 Cynomolgus and miniature pigs and dogs have been widely used in basic medicine ·research on drug discovery and biological fields from old days to the present day.Many animal facilities that receive audits by AAALAC International and other third party organizations have also become larger.Also, Data Sciences International [DSI] released the Next Generation' Digital Implantable Telemetry that can Group housing.The group housing which could not be done on the system until now can be done.In this study, We compared the single housing and the group housing、and we analyzed the characteristics of autonomic nervous system by frequency analysis and time domain analysis.

【Materials & Method】 Using a beagle , place the transmitter in the flank in the animal under isoflurane anesthesia, submit the attached antenna subcutaneously blood pressure catheter inserted from the right femoral artery and in front of the left renal artery Fixed to be placed, the electrocardiogram was fixed to the cardiac capsule Ⅱ to lead. After the recovery period, blood pressure, electrocardiogram, body temperature and activity level were measured with PonemhVer.5.2 [DSI]. For the analysis, the obtained electrocardiogram was subjected to frequency analysis and time domain analysis in PonemahVer 6.3 [DSI].

【Results & Discussion】 Heart rate circadian rhythm showed clear diurnic characteristics of both single housing and group housing. In addition, the heart housing rate of the single housing was lower than that of the group housing.I believe that the range of activity is related to the breeding space.On the other hand, in the autonomic nervous system, the group housing was stable.

【Summary】 From the above results, using the PhysioTel Digital telemetry system enables unrestricted telemetry measurement under Group housing breeding environment, allowing for the design of new test designs, breeding in a socialized state It is now possible to improve animal welfare than before.

1A18 A novel portal vein cannulation technique

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The blood flowing into the liver has two courses that is portal vein and hepatic artery, and the ration of inflow is done with 70% and 30% each. The portal vein is important vessel to transport nutrition which is absorbed from gastrointestinal tract to liver. In the obese patients, it is suggested that increase of the free fatty acid in portal vein is related to glucose metabolism abnormality in the liver. Therefore, portal blood collection and the test are important for the study of glucose and lipid metabolism abnormalities.

Until now, some methods are reported that for the rabbit portal blood collection. Although, their methods are difficult to collect the portal blood stably in a long time. In this time, we devised a new cannulation method for the portal blood collection from the rabbit. And then, it was examined about the usefulness and the influence for the rabbit.

Postprandial hypertriglyceridemic (PHT) rabbits were used for the study. The rabbits were fasting 24 hours before surgical procedure. After anesthesia, rabbits were exposed the portal vein and inserted a catheter and fixed it. The catheter was filled up with heparin and periodically flushed. We proved that our newly devised method could collect blood continuously.

1A19 Establishment of disinfectant evaluation system using skin tissues excised from hairless miniature pigs

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To establish a new disinfectant efficacy evaluation system, usefulness of the excised and preserved skin tissues of hairless miniature pigs was examined.
Hairless miniature pigs were obtained from National Livestock Breeding Center Ibaraki Station. Bacteria were harvested by scrub cup method from the epidermis of skin of live animals, and skin samples immediately after removal, after storage at 4 °C for 24 hours, and after storage at -80 °C for 14-15 days. The number of viable bacteria was measured on neutralizing TSA plates after incubation at 35 °C for 48 hours. In the disinfectant efficacy evaluation test, bacteria were collected from skin samples 10 minutes and 6 hours after application of disinfectant, and viable bacterial count was measured in the same manner.
The number of bacteria isolated from the excised skin did not differ much from that from the skin of live animals regardless of storage conditions. A disinfectant efficacy evaluation test using a standard surgical disinfectant, 10% isodine solution was conducted by using the excised skins. The disinfectant efficacy index Log Reduction values (LR values: logarithmic values of baseline bacterial number – post disinfectant treatment bacterial number) were 10 min - LR value: 1.41 ± 0.49, 6 hr - LR value: 2.03 ± 0.48 on the skin stored at -80 °C. A 95% confidence interval of the LR value for the frozen storage skin was calculated as 1.00 to 1.82 for the 10 min - LR value, and 1.63 to 2.43 for the 6 hr - LR value. These results indicate that skin tissues excised from hairless miniature pigs retain the same number of viable bacteria as live animal skin even after frozen storage at -80 °C for 2 weeks, and that the disinfectant efficacy evaluation test using the frozen skin tissues is a feasible method.

1A20 Glutamine, dietary fiber, oligosaccharide and *Bifidobacterium lungum* exert symbiotic effects on wound healing in genetically diabetic mice

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【Objective】 Recently, some studies have shown that the intestinal microbiome might be an important contributor to the development of several diseases, such as obese, diabetes and allergic diseases. This study was carried out to evaluate the possibility of synergism between GFO®, which is a dietary supplementation product enriched with glutamine, dietary fiber, and oligosaccharide as a prebiotics, and *Bifidobacterium longum* BB536 as a probiotics on wound healing of genetically diabetic mice.
【Materials and Methods】 The cellulose-free AIN-93G was prepared by replacing equivalently cellulose with sucrose in the AIN-93G content. GFO® and *B. longum* BB536 were added together or separately in the cellulose-free AIN-93G, GFOB diet or GFO diet. Six-week-old, male C57BL/KsJ*db+/db+* mice were fed with AIN-93G or these test diets for 8 weeks. After that, a round full-thickness wound having a diameter of about 1.6mm was made with scissors on the clipped dorsal skin of an animal under anesthesia. The wound was covered with a transparent occlusive dressing. The dressing was changed every 2 days to measure the wound area. Cecal contents were analyzed by 16s rRNA gene sequencer.
【Results】 GFOB diet intake significantly accelerated the healing of a full-thickness wound on days 14 or 16. GFO diet and/or GFOB diet intake improve a reduced *Firmicutes* to *Bacteroidetes* ratio in cecal content.
【Conclusion】 These results provide a preclinical experimental basis for the synergistic effect of glutamine, dietary fiber, oligosaccharide and *Bifidobacterium longum* BB536 on an intractable wound via improving an intestinal dysbiosis.

Evaluation of aortic stiffness in the normal and KHC rabbits -Comparison of aortic PWV with aortic stiffness parameter beta-

1A21

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Aortic stiffness has been evaluated by pulse wave velocity (PWV) and cardio-ankle vascular index (CAVI) in clinical practice. PWV does not show particular aortic stiffness because PWV depends on arterial pressure at the time of measurement. In the present study, we calculated aortic beta (AoBeta) by applying the theory of stiffness parameter β to the aorta similarly to CAVI. We compared aortic PWV (AoPWV) and AoBeta when blood pressure (BP) was changed by the intravenous infusion of angiotensin II (Ang II) and sodium nitroprusside (NTP) in Japanese White normal and Kurosawa and Kusanagi- hypercholesterolemic (KHC) rabbits aged 10-12 months. Two chatheter-tip transducers were advanced to the ascending aorta and distal abdominal aorta via the left common carotid and iliac arteries, respectively under pentobarbital (30 mg/kg, i.v.) and butorphanol tartrate (0.2 mg/kg, i.m.) anesthesia. Changes in AoPWV and AoBeta in response to the infusion of Ang II and NTP were recorded at mean BP level of 60, 80, 100, 120 and 140 mmHg. AoBata was determined as $2\ \rho\ /\ \Delta\ P \times \ln SBP/DBP \times PWV^2$, where ρ, SBP, DBP, Δ P were blood density, systolic BP, diastolic BP and pulse pressure. AoBeta distributed relatively within narrow pressure range in the control group. AoBeta correlated weakly with SBP and DBP, whereas AoPWV did strongly in the two strains. Correlation coefficients of AoBeta to SBP and DBP were significantly smaller than those of AoPWV in the two strains. In conclusion, AoBeta is useful to estimate particular aortic stiffness.

Effects of calcium concentration in medium on acrosome reaction and *in vitro* fertilization rate of rat spermatozoa

1B01

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We investigated the effects of the Ca concentration in the medium on acrosome reaction (AR) and *in vitro* fertilization (IVF) of rat spermatozoa. Spermatozoa were collected from the cauda epididymis of mature male Wistar-Imamichi (WI) rats and placed in capacitation media, Enriched Krebs-Ringer bicarbonate (EKRB, Ca^{2+}:1.00 mM), modified Krebs-Ringer bicarbonate (mKRB, Ca^{2+}:1.71 mM) and Human tubal fluid (HTF, Ca2+:5.14 mM). The spermatozoa were incubated at 37° C 5% CO_2, and the AR rate were investigated by Coomassie brilliant blue G (CBB) staining after 0.5, 2, 2.5, 3, 4 and 5h incubation. IVF were performed with superovulated eggs obtained from female WI rats in the media and investigated sperm penetration (SP) and pronuclei formation (PN). The AR rate in the EKRB was significantly lower than that in the HTF. When Ca^{2+} concentration of EKRB increased to 2.57 and 5.15 mM, the AR rate in Ca^{2+} rich EKRB was raised. In contrast, the AR rate reduced when Ca^{2+} concentration of HTF decreased to 2.57 and 1.29 mM. In addition, the AR rate in the mKRB was the same as that in the HTF although Ca^{2+} rich mKRB (5.14 mM Ca^{2+}) increased the AR rate rather than the HTF. On the other hand, the results of IVF showed lower SP and PN rate in all media except for the HTF. These results showed that the AR is caused efficiently when the Ca^{2+} concentration in the medium was about 5 mM, but it alone was insufficient for improving the fertilization efficiency. Because capacitated spermatozoa exhibit hyperactivation together with the AR, we will investigate about sperm motility in these media in the future.

Methyl-beta-cyclodextrin induces hyperactivation by altering the environment of membrane lipids in cold-stored mouse sperm

1B02

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The cold storage of the cauda epididymis, a male reproductive organ, has proved to be efficient in transporting genetically-modified mice as an alternative to live-animal shipment. The fertility of cold-stored sperm decreases in a time-dependent manner. However, the cause of the reduction in the fertility of sperm after cold storage remains unclear. It is known that cholesterol efflux from the sperm membrane is a trigger of capacitation. The dysfunction of cholesterol efflux due to changes in the state of the membrane at low temperatures may explain the reduced fertility. In this study, we examined the fertility (fertilization rate, acrosome reaction, and motility) and the amount of cholesterol in cold-stored mouse sperm after treatment with two cholesterol acceptors: bovine serum albumin (BSA) and methyl-beta-cyclodextrin (MBCD) . MBCD-treated mouse sperm exhibited the highest rate of fertilization. MBCD also displayed greater ability than BSA to remove cholesterol from the sperm membrane. The acrosome reaction occurred strongly in MBCD-treated mouse sperm. In a motility analysis, MBCD improved the lateral amplitude of head movement and beat frequency. These results suggest that MBCD-induced cholesterol efflux promotes acrosome reaction and hyperactivation, resulting in improved fertility of cold-stored mouse sperm.

The level of membrane cholesterol determines the cryotolerance of mouse sperm

1B03

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Sperm cryopreservation is a useful method to efficiently archive genetically engineered mice. Till date, we have improved the protocols of sperm cryopreservation and *in vitro* fertilization. The fertilization rate of frozen-thawed sperm reached to over 80% in the major strains of mice. However, an interesting question remains about the strain-dependent difference of cryotolerance among mice. In this study, we measured the amount of cholesterol on the sperm membrane and examined the motility, viability, and fertilizing ability of fresh and frozen-thawed sperm in major inbred and hybrid strains of C57BL/6, BALB/c, DBA/2, and B6D2F1 mice. The amount of cholesterol in DBA/2 and B6D2F1 mice was higher than that of C57BL/6 and BALB/c mice. After freezing and thawing, the sperms of DBA/2 and B6D2F1 mice indicated higher motility and viability than that of C57BL/6 mouse. *In vitro* fertilization using the frozen–thawed sperm showed that the fertilization rate of C57BL/6 mouse was the lowest among all strains. Removing membrane cholesterol from frozen–thawed sperm using methyl-beta-cyclodextrin elevated the rates of fertilization in all strains. In summary, membrane cholesterol is a crucial factor to determine the cryotolerance of sperm among mouse strains.

1B04 Effect of β-NMN supplemented with mTaM medium on gene expression of in vitro matured oocyte

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Declining of oocyte quality during *in vitro* maturation will affect embryonic development. We discovered that addition of β-Nicotinamide mononucleotide (β-NMN) suppresses the reactive oxygen species (ROS) production in ooplasm (Anzai *et al*., 2016). This study examined the effect of β-NMN on embryonic development and gene expression in *Sirt1* and *Foxo1*. The germinal vesicle oocytes collected from ovary of C57BL/6J mice. Then, those oocytes were cultured for 16hours in mTaM medium with β-NMN of each concentration (1,2,5mM). After fertilization, 2-cell embryo transferred into pseudopregnant mice. Next, we observed the amount of ROS in ooplasm using CM-H2DCFDA kit. Furthermore, each oocytes were performed RT-PCR to detect *Sirt1* and *Foxo1* gene expression. We suggested that the developmental rates of blastocyst stage from 2cell stage improve to compare addition of β-NMN (1mM:30%, 2mM:32%) with non-addition. In the results of embryo transfer, the production of offspring was improved. Then, we observed to increase *Sirt1* and to decrease *Foxo1* in β-NMN. These results suggested that β-NMN act as a regulation of *Sirt1* or *Foxo1* expression and inhibition of ROS generation in ooplasm.

1B05 Supplementation of L-carnitine into the mTaM medium improved *Cpt2* gene expression of subsequent *in vitro* matured oocytes

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L-carnitine is known essential cofactor of fatty acids metabolism. It is important oogenesis and embryogenesis. The interaction of L-carnitine and carnitine palmitoyl transferase (CPT) is necessary to import fatty acid into mitochondria (Dunning *et al*., 2010). L-carnitine contained in mTaM medium were improved embryonic development and inhibited overproduction of reactive oxygen species (Inoue *et al*., 2016). In this study, the expression of the *Cpt* family was observed in MII oocytes matured with mTaM medium containing L-carnitine. Immature oocytes were collected ICR mice. Then, isolated GV-stage oocytes were introduced to mTaM medium containing 0-5mM L-carnitine for 16hours. Next, maturated oocytes were fertilized using the zona drilling method (Nishimura *et al*., 2010). And, we transferred embryo to uterus of pseudopregnant mice. Moreover, GV and MII-stage oocytes were checked *Cpt2* expression. The rates of blastocyst development were 45% (2mM-add) and 31% (Non-add). While the group which 2mM L-carnitine was added into showed higher than that of non-added ($p<0.05$). Also, the result of IVF / embryo transfer was 22% (2mM-add) live pups. Moreover, expression of *Cpt2* mRNA was improved in the 2mM added L-carnitine in the MII-stage oocytes after in vitro maturation.

The effect of ultra-superovulation on the genetically engineered mice and spontaneous mutant mice

1B06

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The number of ovulated oocytes in the C57BL/6J is approximately 20 per female by conventional superovulation (pregnant mare serum gonadotropin: PMSG treatment). It was recently reported that ultra-superovulation technique using inhibin antiserum (IAS) was obtained ovaluated oocytes of about 100 per C57BL/6J female. In this study, we examined the effect of ultra-superovulation on the genetically engineered mice (5 lines) and spontaneous mutant mice (2 lines).

At 4 or 10 weeks of age, the mutant female mice were administrated PMSG or IAS, followed by injection with human chorionic gonadotropin (hCG) 48h later. At 17h after hCG injection, culmus-oocytes complexes were collected from the oviducts. Sperm of same strains were collected from cauda epididymides. At 24h after insemination, the number of 2-cell embryos, unfertilized oocytes and degenerated oocytes were counted.

In all mutant mice, IAS administration increased the number of ovulated oocytes and normal oocytes compared with PMSG administration. On the other hand, the number of ovulated oocytes of all mutant mice was lower than wild type. There were no significant differences in the fertilization rates between all mutant mice.

These data suggest that IAS treatment is useful for ultra-superovulation in various mutant mice.

Comparison of the superovulation effect on inhibin antiserum derived from individual female goats

1B07

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The administration of inhibin antiserum (IAS) promotes the secretion of follicle stimulating hormone (FSH), resulting in an increase in the number of ovulated oocytes in mice. Recently, we developed a new superovulation method via the coadministration of IAS and equine chorionic gonadotropin (eCG). Our new method increased the yield of oocytes in comparison to the administration of IAS or eCG alone. We produced IAS derived from blood which was collected from immunized goats administrated with inhibin peptide. We monitored the microbial quality of the IAS before administering it to female mice. We then accumulated data concerning quality control of IAS in our lab. In this presentation, we will introduce data for various lots of IAS derived from individual female goats.

1B08 Ultrasuperovulation improves the efficiency of embryos fertilized *in vivo* and animal production

○ Yumi Takeshita[1, 2], Kiyoko Yamashita[1, 2], Yukie Haruguchi[1, 2], Tomoko Kondo[1, 2], Yuko Nakamuta[1, 2], Tomoko Umeno[1, 2], Eri Ishida[1, 2], Mari Iwamoto[1], Fumi Takahashi[1], Wataru Sakamoto[1], Yoshiko Nakagawa[1], Kaori Sakaguchi[1], Shuuji Tsuchiyama[1], Ayumi Mukunoki[1], Toru Takeo[1], Naomi Nakagata[1]

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Recently, we developed a new superovulation method using inhibin antiserum (IAS) and equine chorionic gonadotropin (eCG), which we termed ultrasuperovulation. Our ultrasuperovulation technique yielded an increase in the number of ovulated oocytes compared with the conventional method using eCG or IAS only. Thus, ultrasuperovulation can be applied to improve the efficiency of reproductive technology. In this study, we evaluated the efficiency of embryos fertilized *in vivo* using ultrasuperovulation treatment in female mice of various ages (4, 6, 8, 10 and 12 weeks old). Two-cell embryos were produced via natural mating and then transferred into oviducts of pseudopregnant female mouse to confirm their developmental ability to live pups. We found that ultrasuperovulation increased the number of two-cell embryos in female mice of all ages compared with conventional superovulation using eCG only. In addition, the transferred embryos developed normally into live pups. These results suggest that ultrasuperovulation enhanced the efficiency of embryos fertilized *in vivo* and of animal production.

1B09 Improvement of *in vitro* fertilization using vitrified-warmed mouse oocytes derived from ultrasuperovulation

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Oocytes vitrification is a useful method for efficient preservation and production of genetically engineered mice. Mouse oocytes can be cryopreserved by simple vitrification method. However, to improve the technique of mouse-oocyte cryopreservation, we have to enhance the yield of cryopreserved oocytes and the efficiency of embryo production. In this study, we evaluated the effectiveness of ultrasuperovulation technique, coadministration of inhibin anti serum (IAS) and equine chorionic gonadotropin (eCG), abbreviated as IASe, and *in vitro* fertilization (IVF) using N-acetyl cysteine (NAC) in mouse oocyte cryopreservation, and 4-weeks-old oocytes were collected from the eCG- or IASe-treated female mice of C57BL/6J strain. Later, the oocytes were vitrified by a simple vitrification method. Treatment of IASe increased the number of vitrified-warmed oocytes. After vitrifying and warming, NAC -treatment of the oocytes improved the *in vitro* fertilization rate. NAC increased the level of thiol group in the zona pellucida and promoted its expansion. After IVF using NAC-treated oocytes, the two-cell embryos derived from IASe treatment normally developed into blastocysts and live pups by embryo culture and transfer. These results suggest that the ultrasuperovulation using IASe and IVF technique using NAC are useful in improving the technique of oocytes vitrification in mice.

1B10 Superovulation using inhibin antiserum in NOG female mice

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【Introduction】
The high number of fertilized egg collection is desired to maintain the mouse inbred strain cryopreservation. In this point of view, it's very important to improve superovulation. Recently, new superovulation protocol by coadministration of eCG and inhibin antiserum (IAS) was reported using C57BL/6J mice at 4 weeks old (Takeo andNakagata, PLoS ONE, 2015). In this study, we applied this protocol for immature and matured female NOG mice.
【Materials and methods】
Induction of superovulation was performed by injecting 0.1ml of eCG/IAS mixture (3.75IU eCG/0.05mL, inhibin antiserum 0.05 mL, i.p.) to 4 and 8-12 week age of female NOG mice and injecting of human chorionic gonadotropin (7.5IU hCG/0.2mL,i.p.) 48 hours later. After that, *in vitro* fertilization (IVF) was performed.
【Result】
Coadministration of IAS and eCG (IASe) showed to produce more than 50 oocytes from a single female young NOG mouse.The number of ovulation form the mature NOG mice significantly increased, compared with eCG, to 50 oocyte.The administration of IASe increased the number of ovulates oocytes from a 30week old mouse by approx. 9 times than the eCG alone.
IVF indicates high fertilization rates in all groups.
IASe decreased the Birth rates compared with eCG. It was found that superovulation by IASe is effective in NOG background as well.

1B11 The effect of superovulation using the combined administration of inhibin antiserum and pregnant mare serum gonadotropin on aged B6 female mice

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【Introduction】 Superovulation using the combined administration of inhibin antiserum (IAS) and pregnant mare serum gonadotropin (PMSG) were developed by Takeo & Nakagata (2015). Our facility has kept many genetically modified animals utilized for gerontological researches. It might be very useful if the method could also increase the number of ovulated oocytes especially in aged female transgenic mice. In the present study, we investigate the effects of superovulation with IAS and PMSG on aged C57BL/6N female mice, which is one of the main genetic background strains of genetically modified mice.
【Materials and Methods】 Female or male C57BL/6NCrSlc mice were used as oocyte donors at 17 and 19 months-old (M) (n=4-6/group) or sperm donors at 3M (n=2). Female mice were administered combined IAS and PMSG (HyperOva®, Kyudo Co.) (0.2ml) (HO group), or 7.5IU PMSG (0.2ml) (SO group). 48h after the injection of these reagents, 7.5IU hCG was administered to mice. In vitro fertilization was conducted according to the protocol established by CARD. Two-cell embryos were cultured in KSOM medium for 3 days. The fertilization rate and average number of ovulated oocyte were assessed as well as the rates of. Additionally histological sections of the isolated ovaries were made and stained with HE.
【Results and Discussion】 The average number of ovulated oocyte was very small in both HO and SO groups (2.0-5.5 oocytes/female) at 17 and 19M, while mice with no ovulate oocyte were more often observed in SO group. Additionally, there was no significant difference in the rate of fertilization and blastocyst development, and the ovarian morphology between two groups. Our results show superovulation might be usable in aged female mice, but the effects were very weak.

1B12 CARD HyperOva treatment for mouse is also effective in superovulation of BN rats

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Owing to the current development of genome editing, generation of gene-manipulated rats has become relatively easier, only if sufficient embryos are available. Conventionally, rat superovulation (SP) is induced by intraperitoneal injections of pregnant mare's serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG), but this method is not applicable in some strains such as BN rats. In order to establish a stable method of SP for every strain of rat, we examined if CARD HyperOva (HO) treatment, which is effective in C57BL/6 mouse line, can be used in rat SP using ovulated oocytes in BN rats. BN female rats were intraperitoneally administered 15IU PMSG (control group) or 200 μ L CARD HO (test group), and 48hr later given intraperitoneal injection of 15IU hCG, and crossbred. After confirming their crossbreeding in both groups the next day, the mating rate, the number of ovulated females, the number of ovulated oocytes and the number of two-cell embryos were checked. The ovulation rate was 66% in control group, and 100% in test group. The total number of ovulated oocytes was 40 in control group and 92 in test group, and the number of two-cell embryos was 5 in control, and 69 in test group. In addition, 2 cell embryos developed normally into pups. These results show the CARD HO treatment is useful for production of BN rats.

1B13 Effects of Blue LED on the Development of Mouse Embryos

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National Institutes of Biomedical Innovation, Health and Nutrition

The use of light-emitting diodes (LEDs) has increased in several biological and biomedical research areas. In this study, we examined the effects of blue-phase LED light (about 450 nm of wavelength, 500-600 Lx of illuminance) on the development of mouse embryos.

[Method] Two cell embryos were collected from a B6D2F1 (C57BL/6 × DBA2) mouse that had been treated with hormones to determine superovulation. The collected embryos were washed with culture medium and exposed to blue-phase LED light in an LED lighting box placed into an incubator under a humidified atmosphere of 5% CO_2 at 37 ° C. The embryos were exposed for different time periods (1 to 9 hours). After LED exposure, the embryos were cultured in a no-light incubator and we observed the development of the embryos. The embryos exposed to blue-phase LED were also transferred into ICR recipient mice. The experimental study was approved by the animal experiment committee of the National Institutes of Biomedical Innovation, Health and Nutrition, in Japan.

[Results and Discussion] The rate of blastocyst development in the one-hour LED light exposure group was 73.6%, whereas in the control group it was 93.4%. The development rate decreased with the extension of LED exposure time. Most of the embryos stopped growing after 9 hours of LED light exposure. Both implantation and delivery rates decreased in proportion to the extension of LED exposure. Rates for embryos subject to 4 hours of LED light exposure were significantly low. Blue LED light has a negative effect on embryo development and an extension of exposure time increased the risk of damage. Thus, blue LED light does cause damage to a preimplantation embryo.

1B14 Effect of buserelin (gonadotropin-releasing hormone) on *in vitro fertilization* rate of 129 strain knockout mice

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The 129 strain mice are well recognized for their low fertility and it is speculated that this low of fertility may be due to the oocyte.

In this study we investigated superovulation methods for the 129 knockout mice strain to improve the fertility rate of *in vitro fertilization* (IVF).

Female mice were divided into three groups based on hormone and timing of injection. Group 48hPH received pregnant mare serum gonadotropin (PMSG) and 48 h later human chorionic gonadotropin (hCG); using the same dose, group 55hPH received hCG 55 h post-PMSG. Group 55hBPH received buserelin (gonadotropin-releasing hormone agonist [GnRH]) followed 24 h later by PMSG and then hCG 55 h post-PMSG.

IVF was performed using Plk2-KO mice oocytes and sperm.

The IVF fertility rate was 11.4% (Group 48hPH), 19.9% (Group 55hPH) and 25.6% (Group 55hBPH). These results suggest that extending the interval time between PMSG and hCG and giving GnRH in addition to the standard PMSG and hCG treatments can improve IVF fertility rate of Plk2-KO mice.

On the other hand, the average number of 2-cell stage embryos and the fertilization rate of the TP group from which embryos were collected by oviduct perfusion were 19.8 and 52.1%, respectively. This result was about 2 times better than the 55hBPH group that showed good results in *in vitro fertilization*.

1B15 A simple vitrification method for mouse two-cell embryos in straws

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Embryo cryopreservation is a useful teachnique for the efficient storage of genetically engineered mice. There are mainly two protocols of embryo cryopreservation, based on slow freezing or vitrification respectively. At our facility, we use a simple vitrification protocol using 1M dimethyl sulfoxide (DMSO) and DAP213 (2M DMSO, 1M acetamide, 3M propylene glycol) as cryoprotectants. Using this method, we preserve mouse two-cell embryo in cryotubes. In Europe and the U.S., however, a slow freezing protocol using straws is widely used. Thus there is a potential need for institutes in these regions to adopt the vitrification method. Unfortunately, the use of cryotubes as containers for two-cell embryos is a technical barrier hindering the uptake of the vitrification method. In this study, we evaluated the adaptability of straws for mouse embryo vitrification. Results showed that the average recovery and survival rates of vitrified-warmed two-cell embryos preserved in straws were 97% and 92% respectively. These results suggest that straws are a viable alternative to cryotubes when using our simple vitrification protocol.

1B16 Study into the birth rate of the DBA/2FG-pcy mouse strain created by transplantation of refrigerated embryos

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Kyudo Co., Ltd.

【Introduction】 DBA/2FG-pcy mouse strain (pcy) is a mutant mouse strain that spontaneously develops polycystic kidney disease. We previously reported that a comparison of birth efficiency by natural mating and that by embryo transplantation (ET) showed that ET had higher production efficiency. On the other hand, in pcy, a comparison of ET using 2-cell embryos with or without freeze and thaw treatment (hereafter the latter embryos will be called 'intact embryos') showed that intact embryos had a higher birth rate. This result indicated that freeze and thaw treatment decreased the birth rate. Recently, a refrigerating method at 0-4 °C has been found to maintain the embryogenesis stage until the following day. Here, we compared the birth rate of refrigerated 2-cell embryos with that of frozen and thawed 2-cell embryos in pcy.
【Methods】 Twenty-four hours after *in vitro* fertilization, we obtained 2-cell embryos. We divided the embryos into three groups: an intact embryo group; a refrigerated embryo group that will be stored at 4 °C; and a cryopreserved embryo group that will be frozen and stored. Intact embryos were immediately transplanted into the ampulla of the uterine tubes of pseudopregnant female mice. Refrigerated embryos were preserved at 4 °C for twenty-four hours and transplanted into pseudopregnant female mice. Meanwhile, cryopreserved embryos were thawed the next day and transplanted as shown above.
【Results and Discussions】 In pcy, the birth rate of cryopreserved embryos was lower than that of intact embryos, while the birth rate of refrigerated embryos was higher than that of cryopreserved embryos. These results suggest that in pcy, the decrease of the birth rate caused by the freeze and thaw treatment can be improved using refrigerated embryos.

1B17 Scheduled production of pseudopregnant mice by progesterone injections

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Pseudopregnant mice are indispensable for reproductive and developmental engineering as recipients of embryo transfer. To produce a pseudopregnant mouse by standard method, it is necessary to maintain about 8 times (unpublished data: 1,873/15,399 mice) number of females to select proestrus stage-females by visual observation of the vagina. In this study, we examined the effectiveness of a scheduled production of pseudopregnant mice using progesterone (P4), which we reported to be effective for synchronization of estrous cycle in B6J mice (63th JALAS meeting and Biol Reprod (2016)). After P4 injections on Days 1, 2, 85% of ICR females were synchronized to metestrus on Day 3. When females with (P4 (+)) or without (P4 (-)) P4 treatment were paired with vasectomized males for 4 days, the highest rates of mating were found on Day 7 in both groups. The mating rate in P4 (+) group was similar to that of the standard method group (61%) and higher than that of the P4 (-) group (69% vs 25%). As same as above, in the embryo transfer test using vitrified-warmed C57B6/J embryos, there was no difference between the P4 (+) and standard method groups (52% vs 50%). Females of the P4 (+) group could be used as recipients of embryo transfer, because of their ability to produce normal offspring. These results indicated that our protocol for preparing pseudopregnant mice by progesterone injections can omit the procedure of visual observation for the proestrus stage and decrease the size of female colonies necessary for obtaining enough numbers of recipients; the efficiency of preparing usable recipient females per colony improved from 1/8 to 1/1.4 (69% plug-positive rate without selection).

1B18 Nutritive supplements to culture medium for rat embryos based on oviductal fluid

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The rat is widely used as an experimental animal for research, and genetically engineered rats are essential for the generation of animal models of several diseases. Although embryo manipulation techniques are indispensable to produce them, the technology for culturing rat preimplantation embryos is not as advanced as it is for mouse embryos. Therefore, we developed a new culture system for rat preimplantation embryos focusing on nutritive supplements to potassium simplex optimized medium (KSOM), which is a versatile medium used in the culture of mouse embryos. In a previous study, we analyzed the amino acid and taurine profiles in the oviductal fluid of female Wistar rats (Nakamura K et al., 2016). Glycine, glutamate, alanine and taurine were abundant in the oviductal fluid. We then assessed the effect of addition of these amino acids and taurine to modified KSOM on rat zygote development. The rates of zygote development were increased by the three amino acids and taurine in concentration-dependent manners (0.2–1mM) ($p<0.05$), and we confirmed that blastocysts cultured in our modified KSOM could develop to full term after implantation in pseudopregnant rats. However, this positive effect could not be achieved without refreshing the medium once every 24 hrs. It is known that supplementation of culture medium with amino acids leads to the accumulation of ammonium, which is detrimental to embryo development. Indeed, the ammonium levels in KSOM were increased by the addition of amino acids and taurine in concentration-dependent manners ($p<0.001$). These results suggest that not only the addition of nutrients, but also removal of waste metabolites such as ammonium should be considered for the development of more effective culture systems for rat embryos.

1B19 The effect of the Kumamoto earthquakes on the quality of cryopreserved mouse embryos and sperm

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Mouse banks play an important role in the collection, production, preservation and supply of genetically modified mice. Our center was the first mouse bank in Japan, and we have archived more than a million cryopreserved embryos and thirty thousand straws containing cryopreserved sperm taken from genetically engineered mice. Last year, Kumamoto suffered two massive earthquakes, known as the 2016 Kumamoto earthquakes, and thereafter experienced more than four thousand aftershocks. Before the earthquakes, we had separately preserved the cryopreserved samples as a countermeasure against natural disasters. Fortunately, we succeeded in protecting the cryopreserved samples without any damage. However, there have been no reports on the effect of the earthquakes on the quality of the cryopreserved embryos and sperm. In this presentation, we will report the results of the fertilization ability of cryopreserved sperm and the viability and developmental ability of cryopreserved embryos pre- and post-earthquakes.

1B20 CARD-IP mouse sperm and embryo cryopreservation course at Institut Pasteur

○ Yuko Nakamuta[1, 2], Kiyoko Yamashita[1, 2], Yukie Haruguchi[1, 2], Tomoko Kondo[1, 2], Yumi Takeshita[1, 2], Tomoko Umeno[1, 2], Eri Ishida[1, 2], Mari Iwamoto[1], Fumi Takahashi[1], Shuuji Tsuchiyama[1], Ayumi Mukunoki[1], Hidetaka Yoshimoto[1], Toru Takeo[1], Kristy Williams[3], Jorge Sztein[4], Lluis Montoliu[5], Jean Jaubert[6], Franck Bourgade[6], Naomi Nakagata[1].

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Genetically engineered (GE) mice are frequently used for research in the field of life science. We have developed mouse reproductive technologies to improve the systems of our mouse bank, which plays an important role in efficiently producing, archiving and distributing GE mice. Since 2000, we have held 54 training workshops to share our knowledge and ability pertaining to mouse reproductive techniques with researchers and technician worldwide. Indeed, more than 500 researchers and technicians have participated in our workshops. In 2016, CARD and Institut Pasteur, a research institute which is authorized for infectious disease and microbiology research, entered into a departmental agreement on academic and personnel exchanges. As the first project of the agreement, we held the CARD-IP Mouse Sperm and Embryo Cryopreservation Course at Pasteur Laboratories in France. In this presentation, we will introduce the outline and the findings of our workshop.

1C01 Comparison of DBA/2-*mdx* mice with the background strains on body and organ weight

○ Yui Kaneko[1], Yuyo Ka[1], Masahiko Yasuda[1], Mika Yagoto[1], Chie Shimomura[2], Takuma Mizusawa[1], Nao Yoneda[1], Takayuki Goto[2], Tomoyuki Ogura[1], Kenji Kawai[1], Riichi Takahashi[1]

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【Introduction】

CIEA has provided DBA/2N-*mdx* (D2-*mdx*) mice to researchers. This strain, established by backcrossing C57BL/10Sc-*mdx* (B10-*mdx*) to DBA/2N (D2), shows significant muscle weight decrease and weakness. In this study, we focused on body and organ weight of D2-*mdx* at 10 weeks old.

【Materials and methods】

We used D2-*mdx* and D2 mice (10 weeks of age, male and female, 20 heads/group). We measured weights of body and organs (brain, heart, lung, liver, kidney, spleen, and testis/ovary), calculated organ-to-body ratio, and analyzed the data with those of B10-*mdx* and C57BL/10ScN (B10) mice, previously reported at JALAS in 2016 (Yoneda, *et al.* P-30).

【Result】

Comparison between D2-*mdx* and D2 showed significantly lower body weight and higher organ (brain and liver) weight of D2-*mdx*.

And comparison between B10-*mdx* and B10 showed significantly higher body weight of female B10-*mdx*, and lower organ (brain and kidney) weight of B10-*mdx*.

The comparisons above also indicated that both of D2-*mdx* and B10-*mdx* were higher than D2 and B10 in the weight of liver, however, in the weight of brain, B10-mdx was lower than B10, contrary to D2-*mdx*.

These results seem to attribute to characteristic of DBA/2-*mdx* mice.

In the future study, we are planning further analysis to find the cause of these differences.

Comparison between DBA/2N-*mdx* and DBA/2N on Wire Hanging Test at 5 and 10 weeks old

1C02

○ Yuyo Ka[1], Masahiko Yasuda[1], Tomoyuki Ogura[1], Takayuki Goto[2], Takuma Mizusawa[1], Yui Kaneko[1], Mika Yagoto[1], Motohito Goto[1], Kenji Kawai[1], Riichi Takahashi[1]

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【Introduction】
CIEA has been providing muscular dystrophy *mdx* mice, backcrossed to the parental DBA/2N strain (DBA/2N-*mdx*), to the researchers. This strain showing significant muscle weight reduction and muscle weakness. In this study, we focused on the muscle strength of D2-*mdx* and DBA/2N (D2) at 5 and 10 weeks old.
【Materials and methods】
We used D2-*mdx* and D2 (5 and 10 weeks of age, male and female, 20 heads/group) for weight measurement and muscle strength test. In the method, mice were hanged from a wire mesh and the hanging time was measured.
【Result】
Body weight of D2-*mdx* was significantly lower than D2. In the muscle strength test, hanging time of D2-*mdx* was significantly shorter than D2. In muscle strength comparison between 5 weeks old and 10 weeks old age, there was no difference in males of D2-*mdx*. In muscle strength comparison between sexes, time of male at 10 weeks old D2-*mdx* was shorter than female.
【Conclusion】
D2-*mdx*'s muscle strength showed lower already at young age (5 weeks of age), and male didn't show increase in muscle strength with growth and development, and it was increased found that the difference from D2.

Analysis of background data in the DBA/2N-*mdx* mice

1C03

○ Masahiko Yasuda[1], Yuyo Ka[1], Takayuki Goto[2], Mika Yagoto[1], Yoko Kamai[1], Chie Shimomura[2], Takuma Mizusawa[1], Motohito Goto[1], Tomoyuki Ogura[1], Riichi Takahashi[1], Kenji Kawai[1]

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CIEA maintains a variety of model mouse strains of muscular dystrophy and has supplied them to several research institutions from the 1960s. The *mdx* mouse is the most common mouse model of Duchenne muscular dystrophy. In this study, to contribute to muscular dystrophy research, we analyzed body weight, blood biochemical (CPK, LDH, AST, ALT, Cr, Glucose, TP, ALP, T-Cho, TG, BUN, Na, K, and Cl), and historical background data from DBA/2N (D2)-*mdx* mice and compared these to data from NOD/Shi-*scid*, IL-2R γ KO (NOG)-*mdx*, C57BL/10Sc (B10)-*mdx*, D2, NOG, and B10 mice (10 weeks of age, male, 10 animals/group). Compared to serum/plasma levels in respective wild mice, levels of CPK, LDH, AST, and ALT in all *mdx* mice significantly increased, reflecting skeletal and cardiac muscle damages. In particular, elevated CPK levels were remarkable. Quantification of CPK serum level is used as a diagnostic biomarker for the detection of muscular dystrophies. Histopathology of skeletal muscle in D2-*mdx* mice revealed dystrophic changes with remarkable fibrosis. Therefore, the D2-*mdx* mouse is an extremely useful and new animal model for Duchenne muscular dystrophy.

1C04 Behavioral impairment in brain-specific heterochromatin protein 1 (HP1)-deficient mice

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Epigenetics is defined as heritable changes in gene activity and expression that occur without alteration in DNA sequence. It is widely described that breakdown of epigenetic regulation is a candidate risk factor for mental disorder like autism, bipolar disorder, depression etc.

We have focused on heterochromatin protein 1 (HP1) as an epigenetic factor that recognizes methylated 9th lysine (H3K9me2/3) of histone H3. Mice lacking this gene systemically on hybrid strain are infertile (Naruse et al., 2007), impaired germ cell meiosis (Takada, Naruse et al., 2011) and proliferation of primordial germ cells (Abe et al., 2011). Although we generated mice deficient in this gene on C57BL/6 inbred strain for functional analysis in the central nervous system, we could not obtain homozygous adult mutant mice because the animals died immediately after birth.

In this presentation, we report the results of behavioral analysis of the test battery especially for conditional knockout (cKO) mice in which HP1 deficit only in the nervous system.

The cKO mice obtained by mating with Nestin-Cre mice were born with desirable Mendelian ratio and some growth retardation (low body weight) was observed, but they developed to adulthood.

Ten paradigms of behavioral tests were comprehensively carried out for male mice of approximately 3 months of age in order to analyze activity, anxiety/depression, learning/memory, attention, social interaction and motor function. As a result, cKO mice exhibited remarkable low activity in novel situation, as well as an increase in immobility time in forced swimming situation. These results suggest that our cKO mice could be a candidate animal model for some kinds of mental disorder.

1C05 Epileptogenesis genes in NER

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NER (Noda Epileptic Rat) exhibits generalized tonic–clonic seizure (GTCS). We performed a genetic linkage study using (F344×NER) F1× NER backcross progeny and produced congenic lines. Two genomic regions, *Ner1* on Chr1 and *Ner3* on Chr5, were associated with spontaneous GTCS. The wild-type *Ner1* allele partially suppressed GTCS incidence and the wild-type *Ner3* allele postponed GTCS onset in single-locus congenic lines. Both loci suppressed GTCS when they were combined in double-locus congenic lines. To identify candidate genes within the GTCS-associated loci, global expression analysis and *de novo* BAC sequencing were performed. Five genes for the *Ner1* locus and one gene for *Ner3* locus were significantly downregulated. We detected an insertion of an endogenous retrovirus sequences in the *Ner3* gene. Interactions among these genes would be crucial for epileptogenesis in NER.

Mechanism of impaired incretin-induced insulin secretion in a model of obese type 2 diabetes: the ZFDM rat

1C06

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We recently established a novel animal model of obese type 2 diabetes, the Zucker fatty diabetes mellitus (ZFDM) rat strain harboring the fatty mutation (*fa*) in the leptin receptor gene. Here we tried to clarify the mechanism of impaired incretin-induced insulin secretion in the ZFDM rats. In *fa/fa* male rats, the number of large islets (diameter > 300 μm) increased from 7 to 11 weeks of age. At 11 weeks of age, gene expression levels of incretin receptors were decreased in both large and normal islets of *fa/fa* male rats, while a defect in incretin-induced insulin secretion was obvious in the large islets. RNA-seq analysis revealed a higher expression of glycolysis-related genes and a lower expression of the TCA cycle- and the malate-aspartate shuttle-related genes in islets of *fa/fa* male rats as compared with *fa/+* male rats. Metabolome analysis also clarified an acceleration of glycolysis and an increased production of lactate, while a defect in glutamate production from glucose accompanied by suppression of the TCA cycle and the malate-aspartate shuttle. In addition to the downregulation of incretin receptors, an age-dependent increase of large islets exhibiting impaired glucose metabolism and defective glutamate production may cause impaired incretin-induced insulin secretion.

Analysis of dynamic behavior of NKT cells in pre-diabetes model mice

1C07

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Pre-diabetic patients have a high risk of developing diabetes as well as other associated diseases. From the viewpoint of risk assessment and to assist the development of protective therapies, we focused on the functional role of natural killer T (NKT) cells in pre-diabetes. We found that the activity of NKT cells, as estimated by the expression level of specific gene, *Vα14-Jα18*, was significantly lower in specific tissues/organs such as adipose tissue and pancreas in non-obese pre-diabetes model mice than in their normal littermates. Subsequently, in the pre-diabetes model mice, *Vα14-Jα18* was activated with α-galactosylceramide (α-GalCer) and its effect on glucose tolerance was estimated. The simultaneous injection of α-GalCer and lymphocytes improved glucose tolerance with its maximum effect on the 3rd day. An analysis of circulating cytokine levels revealed that interferon-γ, which is a pro-inflammatory cytokine, was secreted only 0 the 1st day after treatment with α-GalCer and that interleukin (IL) -4, which is an anti-inflammatory cytokine, was secreted from the 1st to 4th days. The prolonged secretion of IL-4 was thought to substantially contribute to the improvement of glucose tolerance. Based on these results, the functional role of NKT cells in pre-diabetes is to improve metabolic dysfunctions.

1C08 Relationship between occlusal surface wear of molar teeth and salivary gland dysfunction in type-2 diabetic db/db mice

○ Soma Shirakashi, Kiyokazu Ozaki, Tetsuro Matsuura

Laboratory of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University

【Background】 We showed that dental caries associated with persistent hyperglycemia was frequently observed in type-2 diabetic db/db mice. Meanwhile, occlusal wear of molar teeth other than development of dental caries reportedly occurs due to salivary gland dysfunction in alloxan-induced type-1 diabetic rat. However, there is no report analyzing the relationship between occlusal wear of molar teeth and salivary gland function in type-2 diabetic animal model. Thus, we investigated whether these similar changes are induced in db/db mice.
【Methods】 Male db/db mice aged 20, 30 and 40 weeks along with age-matched db/+ mice were used. The saliva volume was measured by stimulation of pilocarpine. Immediately after salivary functional test, animal was autopsied and the salivary glands were removed. After observing molar surface under a stereoscopy, the cusp heights of first (M1), 2nd (M2) and 3rd (M3) molars were measured. All molars and salivary glands were histologically examined.
【Results】 The average saliva volume in db/db mice has been already significantly decreased at 20 weeks of age compared to db/+ mice, although the incidence and severity of molar caries in db/db strain were much higher than in db/+ strain after 30 weeks of age. The cusp heights of mandibular molars (M1 and M2) in db/db mice were significantly shorter than those in db/+ mice at any weeks of age, however there was little difference in cusp heights of all maxillary molars and mandibular M3 between 2 strains. Histopathol -ogically, tertiary dentin formation was formed at the dentin just below the site of occlusal wear in all mice.
Conclusion: In type-2 diabetic db/db mice, occlusal wear of molar teeth progresses, and the lesion may be involved in dysfunction of salivary glands due to persistent hyperglycemia.

1C09 Long-term hyperglycemia naturally induces dental caries but not periodontal disease in type-2 diabetic db/db mouse

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Periodontal disease (PD) in diabetic patients is described as the 6th complication of diabetes. Several recent studies have reported that long-term hyperglycemia can cause naturally occurring PD in diabetic rats. Moreover, we have previously shown that diabetes increases dental caries, and periodontitis and gingivitis might be a secondary change resulting from dental caries in spontaneous and chemically induced diabetic rodent models. However, the possibility that hyperglycemia may induce PD in diabetic animals could not be completely eliminated because we could not definitively distinguish PD-derived inflammation from carious inflammation around the dental root. The goal of this study was to confirm the presence of PD in type-2 diabetic animal models by preventing carious inflammation with fluoride administration.
【Methods】 Male diabetic *db/db* and nondiabetic *db/+* mice were used and examined at 40 weeks of age. Mice were also given tap water or tap water containing fluoride (10-100 ppm) from 10 weeks of age onward.
【Results】 A cariostatic effect of fluoride was apparent in the diabetic db/db mice, as expected, and the progression of dental caries in the diabetic animals was markedly suppressed by fluoride treatment. Fluoride treatment drastically attenuated the incidence and severity of periodontitis and gingivitis along with prevention of dental caries, whereas the lesions were notably enhanced in the diabetic condition. Furthermore, with fluoride treatment, periodontitis was notably nonexistent in the periodontal tissue surrounding noncarious molars, whereas the caries-forming process was clearly observed in the teeth that were enveloped with persistent periodontitis, thus suggesting that enhanced periodontal inflammation might have been derived not from PD but from dental caries in the diabetic mice. In conclusion, long-term hyperglycemia naturally induces dental caries but not periodontal disease in type-2 diabetic mouse.

1C10 Antihypertensive effect of gamma-aminobutyric acid (GABA)-rich brown rice in spontaneously hypertensive rats (SHR)

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【Objectives】Gamma-aminobutyric acid (GABA), a non-protein amino acid, is an inhibitory neurotransmitter known for its antihypertensive effect. In this study, the antihypertensive effect of GABA was investigated in spontaneously hypertensive rats (SHR) by the long-term dietary administration of GABA-rich brown rice.
【Methods】Seven-week-old male and female SHR were given free access to either a regular diet supplemented with 5% corn starch or a GABA-diet supplemented with 5% GABA-rich brown rice (40.7 mg/100 g GABA) for 12 weeks. Blood chemistry was performed, and the body weight, blood pressure, and anti-oxidative stress were measured.
【Results】In both control and GABA groups, male and female SHR showed steady weight gain with no signs of distress. In male SHR, there was a small but significant difference in blood pressure in the 6th week of treatment (192 and 174 mmHg for control and GABA groups, respectively). This trend persisted for 9 and 12 weeks, indicating a significant antihypertensive effect of GABA. In female SHR, there was a significant difference in blood pressure in the 9th week of treatment (170 and 153 mmHg for control and GABA groups, respectively); however, the difference was not significant in the other weeks. SHR were given free access to the GABA-rich diet, and the daily GABA intake was approximately 1.8 mg/kg BW in males and 1.2 mg/kg BW in females.
【Conclusions】The study demonstrated the antihypertensive effect of GABA. The effect persisted in males, but was transient in females. The difference in GABA intake may have contributed to the difference in the observed antihypertensive effect between males and females.

1C11 Increased salt intakes have an insignificant effect on amelioration in renal cystic disease progression in PCK rats with high water intakes

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【Background】Polycystic kidney disease (PKD) is one of genetic disorder and is characterized by an aberrant proliferation of tubule cell in the kidney. We reported that high water intakes (HWI) reduced the kidney/body weight ratio (KB%) in PCK rats, an orthologous model of human autosomal recessive PKD. However, HWI in patients with autosomal dominant PKD resulted in higher total kidney volume, urine sodium and urine volume. These suggest increased salt intakes deteriorate effect of treatment by high water intakes on PKD. In the current study, we investigated the combination effect of high salt and high water on PKD in PCK rats.
【Methods】Male PCK rats were assigned randomly to CONT group (distilled water (DW)), HWI group (5% glucose in DW) or HWS group (5% glucose and 0.45% NaCl in DW) and treated from 4 to 20 weeks of age.
【Results】Total water intakes were significantly increased in HWI and HWS compared with CONT, whereas total food intakes were not different among all groups. NaCl intake in HWS was higher than CONT or HWI. Systolic blood pressure (SBP) was increased in HWS compared with HWI from 5 to 20 weeks of age. KB% in HWI and HWS were lower than CONT, whereas there is no difference in HWI and HWS.
【Conclusions】High NaCl intakes induced high blood pressure. However, high NaCl intakes dose not deteriorate renal cystic disease progression in high water loaded PCK rats.

The development of atherosclerotic lesions in various arteries of WHHLMI rabbits

1C12

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【Purpose】In this study, we analyzed the development of atherosclerotic lesions in various arteries of WHHLMI rabbits.

【Methods】We examined arteries the aorta, basilar artery, carotid artery, pulmonary artery, renal artery, and femoral artery of WHHLMI rabbits aged 6, 10, 20, and 30. Macrophotographs of the intima surface was taken, and the lesion area was measured with Image_J. Pathological sections were prepared at the lesion site, and the intimal thickening and cross-sectional narrowing were measured with Image_J.

【Results】In the aorta, the progression of lesions was prominent in the aortic arch, the proximal areas of the thoracic and abdominal aorta. In the pulmonary artery, lesions were observed frequently. The lesion in the right pulmonary artery was more extent than those in the left pulmonary artery. In the carotid artery, advanced lesions were observed at the bifurcation. The lesion was progressing in the right carotid artery compared to the left carotid artery. In the renal artery lesion, a mild lesion was observed at the branch from the aorta and the branch on the peripheral side, and the lesion was progressing in the right renal artery as compared with the left renal artery. In the femoral artery, mild lesions were found only in the branch of the iliac artery, the branch of the femoral artery, and the branch of the popliteal artery and popliteal artery after 10 months of age. In the basilar artery, mild lesions were found in the branch from 6 months of age, but the lesions were all mild.

【Conclusion】This study revealed that there is a big difference in lesion occurrence / extension in the type and site of the artery.

Therapeutic role of *Asparagus cochinchinensis* extract as a NGF stimulator and anti-oxidant in the Tg2576 model for Alzheimer's disease

1C13

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Use of multifunctional drugs with neurotrophic supporting and oxidative stress suppressing activity may be considered a therapeutic strategy to protect or repair cellular damage caused during the progression of Alzheimer's disease (AD). In this study, we investigated the therapeutic effects of aqueous extract of *A. cochinchinesis* root (AEAC), particularly its role as a nerve growth factor (NGF) stimulator and anti-oxidant in Tg2576 mice showing AD phenotypes of human. AEAC containing flavonoids, phenols, saponins and protodioscin induced enhancement of NGF secretion and decreased intracellular ROS in the neuronal and microglial cell line. These effects as well as enhanced SOD levels were also detected in AEAC treated Tg2576 mice. The expression of p-Akt among downstream effectors of the high affinity NGF receptor was dramatically recovered in AEAC treated Tg2576 mice, while the expression of p75NTR was slightly recovered in the same group. Significant recovery on the level of A β-42 peptides and the expression of β-secretase members including PS-2, APH-1 and NCT were detected in AEAC treated Tg2576 mice. Furthermore, AEAC treated Tg2576 mice showed decreased numbers of dead cells and suppressed acetyl choline esterase (AChE) activity. These results suggest that AEAC contribute to improving the deposition of A β-42 and neuronal cell injuries during the pathological progression stage of AD in the brain of Tg2576 mice through increased NGF secretion and suppressed oxidative stress.

1C14 Combined (-)-Epigallocatechin-3-Gallate and Ferulic Acid Effectively Modify Alzheimer-like Pathology

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"Nutraceuticals" with therapeutic potential, are promising for Alzheimer disease (AD) therapy. Among these, we found two anti-amyloidogenic nutraceuticals: (-)-epigallocatechin-3-gallate (EGCG), an promotor of α-secretase activity, and ferulic acid (FA), a β-secretase modulator, and attempted to examine whether combination therapy further ameliorates AD-like pathology than each single treatment. At 12 months of age, single or double EGCG and/or FA (all at 30 mg/kg) as well as vehicle was received orally to PSAPP amyloidosis model mice once daily for 3 months. At 15 months of age, combined treatment effectively reversed most behavioral outcome measures. Moreover, EGCG plus FA doubly-treated PSAPP mice had additionally ameliorated brain parenchymal and cerebral vascular β-amyloid deposits, and decrease in abundance of amyloid β-protein (Aβ) species when compared with EGCG or FA treatment alone. Of note, combination therapy upregulated nonamyloidogenic soluble amyloid β-protein precursor (APP)-α and a disintegrin and metalloproteinase domain-containing protein 10 expressions, while this treatment downregulated amyloidogenic β-carboxyl-terminal APP fragment and β-site APP cleaving enzyme 1 expressions. Supporting to this, the ratio of β-carboxyl-terminal APP fragment to α-carboxyl-terminal APP fragment was also decreased. Together, these beneficial effects were attributed to orchestration of α-secretase promotion and β-secretase inhibition, polarizing APP cleavage towards nonamyloidogenic direction. Our observation provides that combination therapy by α- and β-secretase modulators holds greater promise for AD remedy.

1C15 Alzheimer's Disease Model in latent infection with herpesvirus using APP × PS2 double transgenic mice

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【purpose】We are researching in latent infection model of swine herpes virus (Aujeszky's disease virus) to confirm the relationship between Alzheimer's Disease and infection with herpes simplex virus. However, keeping latent infection after six months of the infection was instability and we need the strain in which β-amyloid accumulate early. Then we established the Alzheimer's Disease Model in latent infection with herpesvirus using APPxPS2 double transgenic mice observed early accumulation of β-amyloid after two to four months of birth, we considered the kinetics of β-amyloid in brain.

【method】We mated a female PS2 mouse with a male Tg2576 hemi mouse and selected double transgenic mice. We administered a serum of pigs anti Aujeszky's disease virus to mice of 5 weeks old and challenged with 100$LD_{50}$of YS-81 after 30 minutes. We regarded mice survived over two months as latent infection mice. We conducted virus reactivating test after 2 months of the infection and detected virus DNA by PCR from nasal swabs. As the control group, we set not-infection group. After virus reactivating test, we harvested brain samples from all mice and detected β-amyloid by ELISA.

【results】We obtained 14 double transgenic male mice. Mice stood virus challenge after priming. The virus excretion in reactivation test and viral DNA in trigeminal ganglia were found. We confirmed the kinetics of β-amyloid in brain after two months of infection, Aβ rose and higher in latent infection group than not-infection group.

【discussion】We confirmed that APPxPS2 double transgenic mice were also latent infection with Aujeszky's disease virus. In addition, β-amyloid in brain rose after two months of infection and rose highly by reactivating. Therefore, we conclude that this model is useful.

A catalog of patient-derived xenograft model mice

1C16

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We introduce a catalog of the patient-derived xenograft (PDX) model mice we have established.
The male NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug}/Jic (NOG) mice and female C.B-17/*Icr-scid/scid*Jcl (SCID) mice were maintained under SPF condition, temperature (22–23 °C), relative humidity (40–60 %), ventilation (20 cph) and light (7:00-19:00), in accordance with the guidelines of the facility.
All procedures involved in the transplantation were undertaken in the safety cabinet. The solid tumor tissues, excised from the patients, were cut into small pieces (approximately 1 mm) and injected subcutaneously to the back. The samples from leukemia patients were transplanted by a single intravenous injection of tumor cells (1×10^6 cells) via tail vein. Then, the mice were monitored for body weight, solid tumor size, and cell fraction of peripheral blood and tibial bone marrow cells by flow cytometry. The derived tumors were assessed by histopathology and comprehensive gene expression analysis.
On the catalog published in September 2016, 21 lines of solid tumor PDX models and 9 lines of leukemia models were listed. These PDX model mice will be useful for investigating pathophysiological mechanisms of tumorigenesis and developing new therapeutic methods.

Differential expression pattern of epithelial-type galectins in unique mouse model of gastric cancer

1C17

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A4gnt KO mice, a spontaneous model of gastric cancer, were utilized to determine the expression pattern of epithelial-type galectins. Stomach samples from 10-, 35- and 50-week old KO mice and age-matched controls were analyzed by histological and molecular biological approaches. Immunohistochemical detection showed moderate to strong cytoplasmic and nuclear galectin 4-expression in 5- and 35-week old KO mice and a characteristic reduction from diffusely cytosolic to a restricted nuclear pattern in 50-week old KO mice. However, other galectins (2, 3 and 7) showed markedly reduced to no immunoreaction in relation to galectin-4 and WT control. Real-time PCR analysis, on the other hand, revealed highly increased expression with significant levels in 35- and 50-week old KO mice relative to WT controls. In contrast, levels of galectins-1, 2, 3, 7 and 8 were either insignificant or downregulated with respect to WT mice across all age group. The present findings suggest the promising potential of galectin-4 as a biological marker in a subset of gastric cancer.

1C18 Effects of enriched environment on suppression of cancer progression in mice

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【Objective】 We have examined the effects of living in a simplified enriched environment (EE) on mice compared to general housing. We previously reported that EE leads to progression of motor function and learning ability, and suppression of tumor growth in mouse models of carcinoma. In this study, to further explore anti-tumor effects of living in EE, it was tested in a model of spontaneous metastasis.

【Methods】 C57BL/6 mice were used for the experiments. Mice in simplified EE and control environment were breed and housed in normal space cages supplemented with or without Mouse Igloo & Fast-trac (Animec) which provide a nesting shelter with running wheel. A mouse model of spontaneous metastasis to lung tissue was induced by intravenous injection of Lewis lung carcinoma cells.

【Results and Discussion】 The number of pulmonary nodules in the lungs of mice under EE was significantly decreased compared to control mice. Interestingly, the averaged body temperature of mice in EE was 0.5 ℃ higher than control group mice. In this time, difference of body temperature between light/dark cycles in EE mice was bigger than that in control mice. Furthermore, we observed that the mass of brown fat tissue in EE mice was significantly decreased at the light-to-dark switching compared to control mice. Now we are trying to clarify the mechanism of underlying cancer inhibiting effects of housing in EE, which includes control of body temperature and changes of energy metabolism.

1D01 Case Report: Feeding Program for Excessive Weight Loss after Phalange-injured Common Marmoset (*Callithrix jacchus*)

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It is important to manage continuous and excessive weight loss of animals by reduction of feed intake associated with loss of appetite, especially for small animal such as common marmoset (*Callithrix jacchus*). However, it is very difficult to gain weight with only feeding of ordinary diet. In our case, a right 2nd proximal phalange of one female marmoset (295 g of body weight and 13 month-old) was severed in an accident during the quarantine (day 0). After this accident, her body weight was constantly decreased to 191 g at day 52 accompanied with decreases in water/diet intake, urination and defecation. We checked body weight, food intake amount and defecation once a day for 4 months. In addition to common pellet diet, we additionally supplied 2 eggs of a quill, 2 g of chicken breast, 2 g of biscuit and 2 g of sponge cake daily as preferable items for aggressive diet program. Also, we performed forced feeding with liquid diet (the canned high protein-nourishing liquid food (68%), a yogurt (15%) and 5% dextrose fluid (17%) until body weight reached above 260 g. Volume and number of dose of liquid diet were regulated according to body weights as follows: 4 mL three to four times daily for below 200 g; 2 mL three times daily for 200-230 g; 2 mL twice daily for above 230 g. The body weight was increased steadily during force-feeding and even after the end of force-feeding. Finally, the body weight at the top was 358 g at day 117 after force-feeding and maintained without any problems. In conclusion, our feeding program might be helpful for common marmosets with excessive weight loss and contribute to animal welfare.

Key words: Feeding program, Excessive weight loss, Common marmoset, Liquid diet

1D02 Case of right heart failure cause of congenital in Cynomolgus monkey

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Experimental animal that more closely related in human is necessary to clarify the pathology and pathophysiology. Macaque is most closely experimental animal to human in gene, anatomy and physiology, and there is important for human medical science. Especially heart disease occupies the top cause of human death, and experimental animal model is highly desired for clarification of disease condition and examination of treatment method. VSD which is review in this report is most common abnormality in cardiovascular congenital deformity.

This report indicates congenital abnormality; pulmonary artery stenosis (PS) , ventricular septal defect (VSD) in newborn cynomolgus monkey that breed in Tukuba primate research center, NIBIOHN. Congenital abnormality is important disease in human medical, and especially VSD is most common human congenital abnormally. This case monkey is male, one month after birth, and this animal presented growth delay, exercise intolerance, cyanosis at eating and drinking. We try some exam that is echocardiography, chest radiography, calculate blood-gas, CBC, blood-chemistry cardiac hormone (ANP and BNP) and ECG, because animal show severe cyanosis and respiratory failure, and their show severe right cardiovascular dilatation and VSD.

In addition, there are severe dehydration, malnutrition, and increase cardiac hormone in blood test. In pathologic anatomy, there are PS and VSD, and there is fibrosis around stenosis in histological search.

From these things, this case may be affected severe right cardiovascular failure from congenital abnormality that is PS and VSD and secondary respiratory failure. This case report would give much helpful information for human medical and experimental animal management in future.

1D03 Especially in heart disease cynomolgus monkey's hair with ICP-AES

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Hair coat is important excretion pathway that one way for nocuous chemical substance and metallic materials. Known elongation rate in hair coat is so leisurely that 0.3 - 0.5 mm/day, around 1cm/month. Study using hair coat have started from 1960s, but report is not much. Genus macaca are most important experimental animals because there are most related to human. Diagnosis using hair coat microelement is less stress than other examination and it is easy than other examination. Hair coat is especially useful for diagnosis of clonal disease because it is reflect long-term metabolic effect. However, almost not report spotted microelement of hair coat in macaque. Recently cynomolgus monkeys are in use many scenes (cardiovascular disease, nervous disease…). These many clonal diseases assume effect of blood mineral dynamic and accumulate abnormal protein at central nervous systems. There are store in their hair coat microelement. Accordingly, there hair coat microelement analysis may be a marker of human, nonhuman primates and other animal's clonal disease. Heart disease is known clonal cardiovascular partial in any animals. Circulatory failure may come clonal deposition in their hair coat like already reported. This study used 42 healthy cynomolgus monkey, and there are breed in same environment. We pick up heart disease monkeys from this group by physical examination, echocardiography and x-ray. At result, no significance between control and diseases, and no correlate in age, in addition to some items show significance in the heart disease group. This study analysis and desire reference value and contrast the normal one with the heart disease one in hair coat microelement of cynomolgus monkeys. This data is a helpful thing in cynomolgus monkeys and other experimental animals and will be useful data in human bedside science.

1D04 Safety evaluation of fecal microbiota transplantation materials for *Clostridium difficile* infection in common marmosets

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Clostridium difficile (CD) is a species of commensal bacteria in the gut of mammals including humans. An imbalance of gut microbiota may result in the abnormal increase of CD numbers; the CD toxin can cause chronic diarrhea. Recently, researchers have begun to study the therapeutic effects of fecal microbiota transplantation (FMT) – the transfer of healthy donor stool into the gut of patients with various diseases, including CD infection. Pseudomembranous enteritis and chronic diarrhea caused by CD have also been observed in common marmosets, and examinations of treatment methods are required. A preliminary study of FMT therapy using healthy marmoset feces has been initiated, but whether the feces contain unknown pathogens has not been established. Therefore, we designed a safety evaluation study of the FMT materials using germ-free mice. Frozen feces derived from five healthy marmosets were administrated to germ-free mice (two mice per each feces, IQI, male, nine weeks of age) in orally and/or rectally; these mice were defined as the marmoset microbiota-associated (MMA) mice. We collected feces from the MMA mice every week during breeding and collected cecum and colon contents after four weeks. Composition of fecal microbiota was predicted by analyzing DNA extracts using a next generation sequencer.

One of 10 MMA mice died at the fourth week of the study, but no remarkable abnormal findings were observed. An abscess-like form was confirmed under the kidney of a second mouse. An anaerobic culture test detected *Bacteroides fragilis, Enterococcus faecalis,* and *E. coli*. The fecal microbiota composition of MMA mice showed a decrease in the order *Bifidobacteriales* and an increase in the order *Fusobacteriales* compared to donor marmosets.

1D05 Curative effects of tranexamic acid with supportive measures on wasting marmoset syndrome

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Wasting marmoset syndrome (WMS) has a high incidence and death rate and is one of the most important problems in captive common marmoset (*Callithrix jacchus*) colonies. Although there are several reports on WMS, little or no reports exist concerning its reliable treatment. We previously reported that marmosets with WMS had high serum matrix metalloproteinase 9 (MMP-9) levels. MMP-9 is thought to be a key enzyme in the pathogenesis of inflammatory bowel disease (IBD), the main disease state of WMS, and is activated by plasmin, a fibrinolytic factor. In mice, it was reported that inhibition of plasmin using an antibody prevented the progression of IBD. Thus, this study aimed to examine the efficacy of tranexamic acid, a commonly used plasmin inhibitor, for the treatment of WMS, with supportive measures including amino acid and iron formulations. Six marmosets in our colony were diagnosed with WMS and received tranexamic acid therapy with supportive measures for 8 weeks. The body weight, hematocrit values, and serum albumin levels of the six marmosets in this study significantly increased, while serum MMP-9 levels significantly decreased after tranexamic acid therapy with supportive measures. Thus, tranexamic acid therapy may be a new and useful treatment for WMS.

1D06 Treatment of obesity in cynomolgus monkeys by reducing feed, and changes in the effect of ketamine administration on their behavior

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Obesity in cynomolgus monkeys was successfully treated by reducing feed, and the dosage of ketamine required to immobilize the monkeys decreased as treatment progressed. Two obese cynomolgus monkeys (female, 5-6 years old) with a Body Condition Score (BCS) of 4.0 or more and abdominal body fat (BF) of 30% or more were used. The resting energy requirement was calculated from the average body weight of an individual with standard physique that had the same trunk length as the obese individual, and was used as a measure of the daily energy requirement during treatment. During treatment, the monkeys received soybeans and radishes as a supplement. Body weight and BF were measured once a week, and BCS, waist girth, trunk length under ketamine anesthesia, and blood biochemistry were measured every 3 weeks. The treatment endpoint was achieved when all conditions of (1) BCS less than 4.0, (2) BF less than 30%, and (3) waist girth to trunk length ratio less than 1.10 were met, and this endpoint was met by one animal at 18 weeks and by the other at 24 weeks. Blood biochemical test values were all normal during treatment, which suggests that obesity can be controlled healthily by decreasing the feed based on the resting energy requirement calculated from the average body weight of an individual with standard physique. During treatment, behavior was observed for 10 minutes after intramuscular administration of ketamine, and the time to asymptomatic wobble and weakness was measured. As treatment progressed, the animals succumbed to ketamine doses that were not effective when they were obese, which suggests that the pharmacokinetics of drugs like ketamine with high fat solubility may differ as body fat levels change.

1D07 Preparation of streptozotocin-induced type 1 diabetes model in cynomolgus monkeys and its maintenance

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HAMRI Co., Ltd.

【Objective】 Type 1 diabetes model was prepared by administration of streptozotocin (STZ) to cynomolgus monkeys according to the method reported by Dufrane et al. (Transplantation 2006; 81: 36-45), and conditions for keeping the animals for an appropriate period were examined.
【Materials and Methods】 Two groups of 3 male cynomolgus monkeys were injected iv with a single dose of saline (vehicle) or STZ at 50 mg/kg. Clinical observation, measurement of body weight and fasting blood glucose (FBG) and GTT including measurement of plasma C-peptide were performed at appropriate intervals. After diabetic condition was developed, a daily sc dosing of insulin was done at appropriate doses. Animals were sacrificed after 63 days and pancreas was subjected to histopathological examination.
【Results】 In STZ-treated animals, elevated FBG, reduced glucose tolerance and lack of plasma C-peptide increase in GTT were noted, suggesting a type 1 diabetic condition was developed. Insulin was dosed from 8, 15 or 19 days after STZ administration to the end at dose ranges of 5-7 units/body/day based on daily individual clinical signs and FBG. As a result, FGB were controlled at around 200 mg/dL throughout the observation period. Histological examination revealed a marked decrease of β cells in the Langerhans islets in the pancreas of STZ-treated animals.
【Conclusion】 Type 1 diabetes model was prepared by STZ treatment in cynomolgus monkeys. It was possible to keep the diabetic animals for a significant period of time by appropriate treatment with insulin. This animal model was considered useful for developing a novel medical remedy, especially a regenerative medicine-related remedy, for type 1 diabetes.

Development and Evaluation of the Cynomolgus Monkey (*Macaca fascicularis*) Model for Induced EndometriosisTitle

1D08

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Endometriosis is a significant gynecological disease that influences female quality of life. The pathogenesis of endometriosis is complicated and not clear. There are also no fundamental treatment methods. Animal models for endometriosis research are needed to elucidate the disease mechanism. In our study, we showed that spontaneous endometriosis in the cynomolgus monkey is similar to that of humans (Hum Reprod, 2016) . We sought to establish an experimental cynomolgus monkey model for the study of endometriosis and to analyze clinical conditions.

We examined 16 female cynomolgus monkeys with normal menstrual cycles. Nodular and superficial endometriosis was induced in the subjects by grafting autologous uterine endometrium onto the peritoneum and by scattering minced endometrium in the peritoneal cavity. After surgery, the lesions were evaluated macroscopically by laparoscopy and were measured by an ultrasonic diagnostic system and MRI. Induced lesions were identified by histological evaluation and immunohistochemical analysis. The experimental study was approved by the animal experiment committee of the National Institutes of Biomedical Innovation, Health and Nutrition in Japan.

One month after transplantation, we found the endometrium completely fixed to the abdominal wall in all 16 females. Superficial endometriosis was observed laparoscopically in some females. Nodular lesions included chocolate cystic components. Laparoscopic observation was very useful in this experiment, but ultrasonic diagnostics and image analysis by MRI were difficult. Microscopic lesions induced after endometrium grafting showed endometriosis, characterized by scattered glands. Lesions also revealed angiogenesis and thickened interstitium by fibrosis.

This model may be of value for the elucidation of the mechanisms underlying the development of this disease, and enable further studies and the development of new strategies for endometriosis treatment.

Thioacetamide-induced hepatic fibrosis in the common marmoset

1D09

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The common marmoset (*Callithrix jacchus*) is a non-human primate that is useful in preclinical research on stem cell transplantation therapies, because of its similarity to human beings and its small size enable to test with few cells. In this study, we developed a marmoset hepatic fibrosis modelfor regenerative medicine research on liver cirrhosis. Five female marmosets (4 ～ 6 years old) were administered thioacetamide (TAA) at a dose of 2.5 ～ 40 mg/kg two or three times a week. The blood biochemistry and weight of the animals were monitored once a week. Hepatic fibrosis was assessed by liver biopsy under anesthesia when liver damage was detected based on the blood chemistry, and the serum levels of type IV collagen 7S was also measured at that time. Marmosets administered with TAA increasesd in total bile acid, aspartate aminotransferase, alkaline phosphatase, and total bilirubin and decreased in serum levels of albumin. After administering TAA for at least 120 days, histological samples showed markedly hepatic fibrosis in all animals. The fibrosis was detected untill90 days after the final dosing of TAA. The levels of type IV collagen 7S in animals with hepatic fibrosis were significantly higher than those of normal animals. Hepatic fibrosis was successfully induced with TAA treatment in marmosets. Serum levels of type IV collagen 7S may be a useful marker of hepatic fibrosis in marmosets, as in humans. Now, we plan to use this model on novel stem cell therapies.

1D10 Ultrasonographic evaluation of arterial resistivity in old common marmosets

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The common marmoset (*Callithrix jacchus*) is a small non-human primate with a shorter life span than other non-human primates and has high potential as a model of human aging. In this study, we measured the resistivity index of the ophthalmic artery in old marmosets using ultrasonography to evaluate the vascular resistance related to glaucoma and age-related arteriosclerosis. Eleven old marmosets (average age 12.5 ± 1.9 years) and seven young marmosets (average age 2.9 ± 0.7 years) were examined under anesthesia with ketamine and xylazine. Glaucoma was diagnosed based on the morphology of the optic disc cupping by ophthalmoscopy. The arterial resistivity index (RI= [peak systolic velocity (PSV) – end diastolic velocity (EDV)]/PSV) of the ophthalmic artery or central retinal artery was measured using color Doppler ultrasonography (Hitachi Aloka ProSound Alpha7). Glaucoma was detected in 3 of the 11 old marmosets and no young marmosets. The average RI of the ophthalmic artery was 0.729 ± 0.040 in normal old animals, 0.805 ± 0.034 in glaucoma animals, and 0.609 ± 0.095 in young animals. The RI of the glaucoma or old animals was significantly higher than that of young animals. Spontaneous glaucoma with high ophthalmic arterial resistivity detected in old marmosets indicates that the marmoset is a useful animal model of age-related diseases. A high arterial RI measured in old marmosets suggests that age-related arteriosclerosis occurs in marmosets and can be evaluated with a non-invasive ultrasonographic examination.

1D11 Application of Tarsorrhaphy Technique for Cynomolgus Monkey

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【Introduction】 In the field of ophthalmologic clinical practice, there is a technique called lap suturing. This prodedure is done to make the eyelid closure by suturing so as not to physically touch the eyes, to protect the corneal epithelium in corneal ulcer and to promote healing. It is a procedure that is usually done in clinical practice.In this report, we observed what behavior of cynomolgus monkey when applying lidplate suturing to protect the corneal epithelium of cynomolgus monkey in corneal epithelial stem cell exhaustion model. We examined the effects of enrichment and tranquilizer.

【Materials and Methods】 With regard to three cynomolgus monkeys, 24-hour video recording of the behavior of 1 to 3 days after laparoplasty was performed, visually confirmed what action to take. In addition, we applied enrichment and tranquilizer during application of lid board suturing technique, and we checked what action to take 24 hours video recording and confirmed visually.

【Results】 With respect to three cynomolgus monkeys, the action of touching the eyes which is not normally observed was confirmed. Among them, the action rubbing the eyelid on the treatment side was confirmed 54%, the action of pulling and pulling the suture 15% was confirmed. For 1 cynomolgus monkey, the action of touching the eye on the treatment side after enrichment was reduced from 104 times to 40 times. In addition, regarding one cynomolgus monkey, the action of touching the eye on the treated side after applying the tranquilizer decreased from 16 to 7. There was no sudden change in food consumption, activity level, body weight.

【Discussion】 Although cynomolgus monkeys who performed lid plate suture surgery increased their behavior of touching eyes that are not normally seen, enrichment and tranquilizer are useful for reducing such behavior It was suggested.

1D12 Effect of Two Vaginal Short-Chain Fatty Acids (Butyric Acids) on Fertility in the Long-Tailed Macaque (*Macaca fascicularis*)

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In many mammals, vaginal scent and chemical signals play an important role in mating behavior; that is, it involves an active, pheromone-like substance. In both primates and humans, some lower fatty acids can be detected in a female's vaginal scent. However, the influence on behavior due to these lower fatty acids is not yet clear, as some primates don't have the vomeronasal/Jacobson's organ for detecting pheromones. In our study, we detected vaginal fatty acids by gas chromatographic/mass spectrometric (GCMS) analysis and analyzed the relevance between the amount of fatty acid and female fertility in terms of age and number of past pregnancies.

【Method】 All of the long-tailed macaque were born and raised in the Tsukuba Primate Research Center, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN). Vaginal fluid was collected from the females under full anesthesia 7-10 days from last menstruation (time of ovulation) using a swab. This study was approved by the ethics committee of NIBION. The samples were analyzed using GCMS and the relationship between the amount of fatty acid and each female's age/past gravidity were investigated using a statistical correlation analysis.

【Results and Discussion】 The GCMS analysis detected lower fatty acids in the vaginal fluid. We focused on three: acetic acid, butyric acid, and propionic acid. The statistical analysis showed that these three acids correlated with a female's age. In addition, butyric acid affect female fertility. In this study, specific short-chain fatty acids were found to be related to female characteristics. Our results are a first step toward showing that lower fatty acids play an important role in primate mating behaviors.

1D13 Development of techniques for shortening the generation cycle using immature spermatogenic cells in marmosets

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The present study was undertaken to see the possibility of shortening the generation turnover in marmosets by sperm (or spermatid) injection. To establish marmoset microinsemination system using first-wave male germ cells, we examined marmoset male germ cells retrieved from immature testes at different stages. Early spermatids appeared at 10-11 months and elongated spermatids and spermatozoa appeared at 12 months. Then, we assessed the oocyte-activating capacity of adult marmoset male germ cells by microinjecting them into mouse oocytes. Early round spermatids inefficiently activated oocytes (7/46), but late round spermatids and testicular spermatozoa activated most oocytes (44/58 and 24/27, respectively). When marmoset testicular spermatozoa and elongated spermatids were injected into marmoset oocytes, fertilized oocytes developed to the blastocyst and 8-cell stages, respectively. Next, we attempted to determine whether marmoset male germ cells could be cryopreserved for effective use of valuable genetic resources. The best result was obtained when the cells were cryopreserved in 1:1 mixture of the basic medium for mouse testicular cell freezing (7.5% glycerol + 7.5% serum in PBS; Ogura et al., 1996) supplemented with 0.25M sucrose and CELLBANKER. About 70-80% of male germ cells survived freezing and thawing. The frozen-thawed male germ cells showed the oocyte-activating capacities similar to those of fresh cells. The present study demonstrated that marmoset male germ cells acquire the oocyte-activating capacity at the late spermatid stage. We also succeeded in cryopreservation of male germ cells. By using marmoset first-wave male germ cells (about 12 months-old), we may shorten the generation turnover to about 1 year shorter.

Ovarian stimulation with a GnRH antagonist in cynomolgus monkeys

1D14

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In this study, mature cynomolgus monkeys, which were bred and maintained at the Tsukuba Primate Research Center, were subjected to an ovarian stimulation method using a GnRH antagonist (GnRH-ant) . In the conventional ovarian stimulation method, a GnRH agonist (GnRH-ago) is administered on the first or second day of menstruation and followed by recombinant FSH (rFSH) administration once daily for 9 days. We used the following protocol with GnRH antagonist: several days after menstruation, rFSH was administered once daily for 8 days, and several days before hCG administration, GnRH-ant was administered once daily. There were no significant differences ($P > 0.05$) between the GnRH-ant and GnRH-ago groups with respect to the percentage of the collected MII (18% vs. 19%) and MI (44% vs. 47%) stage oocytes. However, at oocyte collection, the body weights of the monkeys in the GnRH-ago group decreased significantly than that of the GnRH-ant group (-302.9 ± 78.9 g vs. -37.5 ± 19.3 g, $P < 0.05$) . Considering the short schedule of ovarian stimulation and effect on the body condition, the administration of a GnRH antagonist was considered to be a more suitable ovarian stimulation method than GnRH agonist administration.

Effect of Blue LED Light on the Growth of a Somatic Cell

1D15

○ Mohammad Abu Jafor Bapary, Aya Ito, Jun-ichiro Takano, Shogo Soma, Tadashi Sankai

National Institutes of Biomedical Innovation, Health and Nutrition

【Introduction】 In recent years, the use of light-emitting diodes (LEDs) has increased in biological and biomedical studies. In our study, we examined the growth of and DNA damage to a somatic cell under blue LED light (about 450 nm) to assess its use in embryological studies and animal experiments.

【Materials and Methods】 The COS-7 cells were cultured in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin, and maintained under a humidified atmosphere of 5% CO_2 at 37 °C. Blue LED light was exposed to the cells immediately after seeding (0-hour cultured, non attached cells) or after attachment (24-hour cultured cells) at different exposure times.

【Results and Discussion】 Growth suppression was higher in the 0-hour cultured cells than in the 24-hour cultured cells, demonstrating that cell damage declines with the progression of cell growth. A higher suppression of cell growth was observed under a longer period of blue LED light exposure. Cell viability also changed in accordance with light exposure time and duration. The percentage of apoptotic cells (53% for 1 hour, 79% for 3 hours and 92% for 6 hours) increased with increased light exposure time. Blue LED light exposure for 1 hour, 3 hours and 6 hours induced 1.28, 1.58 and 1.98-fold reactive oxygen species (ROS) generation, respectively. A comparison to dark-maintained cells revealed that blue LED light generated intracellular ROS plays a significant role in causing cellular dysfunction in DNA in a time dependent manner.

【Conclusion】 This study will contribute to the understanding of the basic mechanism of somatic cell death under visible light conditions in the beneficial use of LEDs.

Search for definitive senescence biomarkers in rats

1D16

○ Kohei Tomita[1], Shinya Tamura[1], Shunichi Tanii[1], Satomi Takano[2], Kaori, Muguruma[2], Noboru Ogiso[2]

[1]KAC Corporation, Laboratory of Experimental animals, [2]National Center for Geriatrics and Gerontology

【Introduction】Various kinds of animal experiments have been conducted to clarify the mechanisms of senescence. However definitive biomarker which indicates individual senescence has not been discovered in animal models. Our facility has kept many naturally-aged animals used for gerontology and geriatrics researches. In the present study, we evaluate several candidate biomarkers of senescence in those aged animals.

【Materials and Methods】Sixteen to 35 male rats (F344/NSlc, one month old) were obtained from Japan SLC every three months. Rats were allowed free access to a commercial standard diet (Labo MR Stock; Nosan Co., Yokohama, Japan) and Reverse Osmosis (RO) water with ≤ 2.0ppm chlorine. As candidate biomarkers, body weight, daily food and water consumption were measured in addition to systolic blood pressure (SBP) (using a non-preheating MK-2000ST, Muromachi Kikai Co., Tokyo, Japan) and serum biochemistry parameters (AST, ALT, LDH and CK were determined by an auto-analyzer Hitachi 7180, Hitachi, Japan).

【Results and Discussion】Body weight peaked at 13 months-old (M) and then start to decline at 19 M. There was no significant change in food or water intake with aging. Systolic blood pressure was highest at 4M (164 ± 14.6 mmHg) and lowest at 19 M (128 ± 16.5 mmHg). No significant difference in serum biochemistry parameters was found among the age groups, while some rats showed more than twice higher levels of LDH and CK than others. As stated above, senescence biomarkers of human, such as SBP, show low correlation with aging in our rats. We will continue to search for novel biomarkers and analyze their age-related changes.

Age-related changes found in naturally-aged mice

1D17

○ Noboru Ogiso[1], Kaori, Muguruma[1], Satomi Takano[1], Kohei Tomita[2], Kazumichi Yamaguchi[2], Naomi Matsui[1], Mitsuo Maruyama[1]

[1]National Center for Geriatrics and Gerontology, [2]KAC Corporation

【Introduction】Our facility has kept many naturally-aged animals (mice and rats) used for gerontology and geriatric researches. We have examined various characteristics of these animals to evaluate them as animal models of aging research. In the present study, we will provide basic information especially about age-related changes found in the naturally-aged mice.

【Materials and Methods】Four-weeks old mice were obtained from Japan SLC every three months, and were kept over their lifetime. Physiological (measurement of body weight, food, water consumption and survival rates, and analysis of intestinal flora), behavioral (rotarod and grip strength test), morphological (autopsy, MRI and histological examination) analysis were performed. Some mice were also checked with a blood test.

【Results and Discussion】Body weight of male mice peaked at 18-19 months-old (M) and decreased rapidly at around 25M, while that of female mice peaked at 14-16M and there was no significant changes up to 27M. Survival rates started to decline gradually at 20M in both sexes. Some alteration of the intestinal flora might occur at around 12M. Both rotarod performance and grip strength tended to decline with age-related weight gain. Enlarged seminal vesicles in male mice or splentic tumors in both sexes were often found at autopsy. Blood test showed the percentage of each type of white blood cells (WBC) tended to change with aging, and elderly mice (33M) had relatively high proportion of large-sized WBCs (Neut) with low number of platelets. Various age-related changes found in our study can be candidate senescence biomarkers at individual level. We will analyze these parameters in detail and continue to search for novel biomarkers.

Establishment of an evaluation system for muscle tissue-derived fibroblasts collected in Kindai University

1D18

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Currently, improvement of various for eating habits and living spaces in exhibition of zoo animals. And the management of sustainable zoo have progressing (Kodama, 2016). We are also considering to establish primary cultures cells of animal tissues, genetic resources preservation and their evaluation system based on developmental engineering technology which has been established in experimental animal science in collaboration with zoo. In this study, we attempted to examine cell markers using fibroblast derived from aged animals. The establishment of fibroblast cell line were 11.5 days fetal mice, and skin tissue for 48 months of aged mice. Also, for establishment of fibroblast from zoo animals were used both muscle tissue of *Aliurus fulgens* (*i.e.*, postnatal of 65 and 246 months) from Asa zoological park. Each established fibroblast confirmed the influence on the early and late passages using karyotype analysis. Next, the senescent cells were measured the activity of β-galactosidase using Senescence Detection Kit. These results that more than 60% of mice fibroblast indicated the normal karyotype (n=40). And the karyotype analysis of *Aliurus fulgens* fibroblast were retained more than 65% of the normally (n=36). Furthermore, mice fibroblast aged for 48 months showed a positive tendency. Presently, we are comparing with fibroblast derived from *Aliurus fulgens*.

Comparison of age-dependent hearing phenotypes among C57BL/6J-*Cdh23*$^{c.753A/A}$, -*Cdh23*$^{c.753A/G}$, and -*Cdh23*$^{c.753G/G}$ mice

1D19

○ Sari Suzuki[1], Yuki Miyasaka[1,2], Yuta Seki[1], Shumpei P Yasuda[1], Kunie Matsuoka[1], Hiroshi Shitara[1], Yoshiaki Kikkawa[1]

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The C57BL/6J (B6J) mice have long been studied as a model of age-related hearing loss (AHL). Previous studies strongly suggested that homozygosity of the c.753A (*Cdh23*$^{c.753A}$) allele of the cadherin 23 gene is responsible for AHL in B6J mice. In this study, we performed age-dependent phenotypic analysis of hearing in B6J mice with the *Cdh23*$^{c.753A/A}$, *Cdh23*$^{c.753A/G}$, and *Cdh23*$^{c.753G/G}$ genotypes to investigate this mutation effect. *Cdh23*$^{c.753A>G}$ knock-in mice were produced using CRISPR/Cas9-mediated genome editing. In B6J mice with the three genotypes, we measured the ABR to sound stimuli at 4, 8, 16, and 32 kHz. *Cdh23*$^{c.753A/A}$ mice developed AHL following stimulus at all frequencies at least 10 months of age. In contrast, the development of AHL was restrained in *Cdh23*$^{c.753A/G}$ and *Cdh23*$^{c.753G/G}$ mice, which showed normal ABR at all time points. However, the *Cdh23*$^{c.753A/G}$ and *Cdh23*$^{c.753G/G}$ mice gradually developed AHL after 12 months of age. The hearing levels of these mice were also assessed by measuring DPOAE. The AHL of B6J mice is caused by progressive functional loss of the outer hair cells (OHC). DPOAE provides information related to OHC function. Reductions in the DPOAEs in mice with the three genotypes showed similar patterns with those of ABRs, suggesting that functional loss of OHCs was restrained, while the functions were not completely recovered by *Cdh23*$^{c.753A>G}$ knock-in. These results suggest that other genetic factor (s) affect hearing in B6J, although *Cdh23*$^{c.753A/A}$ is a strong risk factor for AHL.

【Poster Presentation (Regular Papers) May 26】

2A01 Functional analysis of chromosome specific clustered trap region (CSCT)

○ Masatake Araki, Iyo Takeda, Toshinori Ohga, Satoshi Eto, Mai Nakahara, Kumiko Yoshinobu, Kimi Araki

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We developed the Database for the Exchangeable Gene Trap Clones (EGTC) (http://egtc.jp) . During the annotation of trap clones, we found new genome element CSCT (Chromosome Specific Clustered Trap region) . There were 39 EGTC clones mapped in the CSCT region, they were distinguished in CSCT2, CSCT4, CSCT12 or CSCT13. Using CRISPR/Cas9 system, CSCT13 region (1.6 Mbp) was deleted in the mouse ES cells (CSCT KO) . We could establish CSCT KO mouse line. Mating between heterozygotes gave apparently normal homozygotes. However, mating between homozygotes gave relatively small number of pups. Moreover, CSCT13 KO mice showed comparatively low rate of homologous recombination during meiosis for the region corresponding to CSCT13. On the other hand, outside of this region showed up-regulation of homologous recombination during meiosis. This study suggest that CSCT13 might be related with the early embryogenesis and homologous recombination during meiosis.

2A02 Functional analysis of HP1 in neural stem cells

○ Chie Naruse[1], Toru Yoshihara[1], Kanae Abe[2], Tomoaki Kato[3], Takumi Nishiuchi[3], Masahide Asano[1]

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Heterochromatin Protein 1 (HP1) family members are components of constitutive and facultative heterochromatin in eukaryotic cells. HP1 protein binds to di- and tri-methylated histone H3 lysine 9 (H3K9me2/3) and recruits heterochromatin-forming factors such as DNA methyltransferases, histone methyltransferases and histone deacethylases to form heterochromatin. We revealed functions of HP1 in gametogenesis and immune systems, however, functions of HP1 in neural cells are still unclear despite of its strong expression in these cells.

We analyzed HP1 deficient embryonic brains and neurospheres, and found HP1 deficient neurospheres became to have a tendency toward differentiation. The proliferation ratio of the HP1 deficient neurospheres derived from embryonic day 14.5 embryos was comparable to that of wild-type neurospheres. Expression array and quantitative reverse transcription polymerase chain reaction (qRT-PCR) analyses revealed several genes that are normally expressed in differentiated neural cells were upregulated in HP1 deficient neurospheres compared to wild-type neurospheres. Levels of tri-methylated histones were reduced around the transcription start sites of these genes in HP1 deficient neurospheres compared to wild-type neurospheres. The levels of histone methyltransferase were decreased at the promoter sites of these genes in HP1 deficient neurospheres. suggesting that HP1 in murine neurospheres was required for recruitment of histone methyltransferases to promoter sites of differentiated cell-specific genes to repress expression of these genes in the neural precursor cells.

2A03 Genomic imprinting reconstituted with an artificial imprinting control region

○ Daichi Kuramochi[1], Hitomi Matsuzaki[2,3], Aki Ushiki[1], Keiji Tanimoto[2,3]

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Genomic imprinting in mammals is a mono-allelic gene expression mechanism that is controlled by allele-specific DNA methylation of gametic differentially methylated region (gDMR) in the gene locus. It is accepted that this imprinted methylation pattern is established during gametogenesis and is maintained after fertilization, yet underlying molecular mechanism of which remains to be fully elucidated.

H19-ICR is a classical example of gDMR that is located within *Igf2/H19* gene locus. Imprinted expression of the locus is controlled by paternal allele-specific DNA methylation of the *H19*-ICR. We previously revealed by using transgenic mouse that *H19*-ICR carried sufficient information to establish methylation imprinting, and therefore, we have been focusing on and analyzing *cis*-regulatory sequences within the *H19*-ICR.

We have disclosed indispensable roles for CTCF and Sox-Oct binding motifs in maintaining maternal *H19*-ICR hypomethylation and 118bp sequence in paternal *H19*-ICR hypermethylation in transgenic mouse. Furthermore, we found that λ -DNA fragment supplemented with above-mentioned *cis*-regulatory sequences (termed "LCb118") was capable of generating imprinted methylation status in transgenic mouse.

In this study, endogenous mouse *H19*-ICR was replaced by the LCb118 sequence and its effect on genomic imprinting was tested. Based on our results, possible molecular mechanism of genomic imprinting establishment/maintenance at the *Igf2/H19* locus would be discussed.

2A04 Cytoplasmic region of IZUMO1 is not essential for male fertility in mice

○ Haruhiko Miyata[1], Samantha A. M. Young[1,2], Yuhkoh Satouh[1], Masanaga Muto[1], Martin R. Larsen[3], R. John Aitken[2], Mark A. Baker[2], Masahito Ikawa[1]

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IZUMO1, a single transmembrane protein in the sperm head, has been identified as essential for sperm–egg fusion. Its binding partner in the oocyte has been discovered (JUNO, a GPI-anchored protein) ; however, the roles of several domains within IZUMO1 remain unexplored. For fusion to take place, IZUMO1 needs to translocate from the acrosome to the equatorial segment during the exocytotic event called acrosome reaction. It has been reported that the cytoplasmic region of IZUMO1 may be involved in this translocation. Therefore, to understand the role of the cytoplasmic region of IZUMO1, we utilized the gene manipulation system of CRISPR/Cas9 to generate a point mutation resulting in a premature stop codon, producing mice with truncated IZUMO1. We injected pX330 plasmid expressing Cas9 and sgRNA, and single-stranded oligonucleotide that has about 65 bp homologous arms into 88 oocytes. 7 pups were obtained and 3 of those mice possessed the desired point mutation. Mice without the cytoplasmic region of IZUMO1 showed normal translocation and fertility but decreased amount of protein, indicating that while this region is important for the expression level of IZUMO1, it is dispensable for fertilization in mice.

2A05 Generation of point mutant mouse model responsible for human globozoospermia by CRISPR/Cas9 system

○ Yoshitaka Fujihara, Asami Oji, Kanako Kita, Masahito Ikawa

Research Institute for Microbial Diseases, Osaka University

Globozoospermia is a human infertility disorder caused by defects manifested during spermatogenesis. The characteristic feature of globozoospermia is the malformation or loss of the acrosome accompanied by an abnormal nuclear shape and a disarrangement of the sperm mitochondria. In the gene knockout (KO) mouse experiments, various genes were found to be associated with globozoospermia. We have reported that sperm membrane protein SPACA1 disrupted mice are sterile with abnormally shaped sperm heads reminiscent of globozoospermia (Fujihara et al. Development. 2012) . However, almost all of these genes (10 out of 13 genes) are ubiquitously expressed and therefore are suitable for mouse models of human globozoospermia. Recent studies of human patients have identified spermatogenesis associated 16 (SPATA16) and dpy-19-like 2 (DPY19L2) as autosomal recessive causes of globozoospermia. In this study, we generated Spata16 KO and point mutant mice by the ES cell-mediated CRISPR/Cas9 system and analyzed the reproductive phenotype. Evaluating the phenotypes of these mutant mice will help elucidate the molecular mechanism underlying globozoospermia.

2A06 The role of rat acrosin in fertilization

○ Ayako Isotani[1,2], Takafumi Matsumura[1], Masaki Ogawa[1], Takahiro Tanaka[1], Kazuo Yamagata[1,3], Masahito Ikawa[1], Masaru Okabe[1]

[1] Osaka University, [2]Nara Institute of Science and Technology, [3]KINDAI University

Acrosin is a trypsin-like serine protease in the sperm acrosome, and its involvement in fertilization has been suggested in various species such as rats, bulls, and pigs. Therefore, it was believed to be a key enzyme required in fertilization. However, gene disruption experiments in mice have shown that acrosin-deficient male mice are fertile. Thereafter, acrosin was not considered to be an essential enzyme in fertilization. On the other hand, it has been reported that the serine protease activity, including acrosin activity, is significantly weaker in mice than in other species including rats. We generated and analyzed acrosin-deficient rats; the acrosin-deficient male rats were fertile. However, their litter size was smaller than that of heterozygous mutant rats. Acrosin-deficient spermatozoa had no abnormalities in sperm formation, sperm motility, acrosome reaction, sperm–zona pellucida binding, and sperm–egg
fusion.

In *in vitro* fertilization, an equal ability to penetrate the zona pellucida was possessed by the acrosin-deficient and wild-type spermatozoa; however, cumulus cell layer dispersal was delayed in the acrosin-deficient spermatozoa compared to the wild-type and heterozygous spermatozoa. These results suggest that rat acrosin plays a role in the sperm penetration of the cumulus cell layer, which was speculated to be the cause of the small litter size of the acrosin-deficient male rats.

Screening for extracellular matrix factors that may concern in male fertility

2A07

○ Daiji Kiyozumi, Tomohiro Tobita, Ryo Yamaguchi, Masaru Okabe, Masahito Ikawa

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Extracellular matrix (ECM) regulates cellular functions in various physiological processes. In mammalian reproduction, the roles of extracellular matrices have been elucidated in female reproductive organs, whereas those in male have remained elusive. Here we screened ECM factors that may concern in male fertility. To this end, we utilized expression profile datasets representing gene expression in male reproductive organs deposited in NCBI databases. ECM-related genes were selected from the database based on the following criteria: (1) their expression is reproductive organ-specific, or (2) their expression is up-regulated along spermatogenesis. Finally we isolated several genes that fulfilled the above criteria. Knockout mice for the selected ECM-related genes were generated by CRISPR/CAS9-mediated genome editing and their fertility is now being investigated.

RNA-seq analysis to reveal genetic basses of tameness in mice

2A08

○ Yuki Matsumoto[1,2], Hirofumi Nakaoka[1,3], Hiromichi Nagayama[1,2], Tsuyoshi Koide[1,2]

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Tame behavior is a major behavioral factor for the domestication of animals. To reveal genetic bases of the behavior might be contributed to establish a method for efficient selective breeding. Previously, to identify genes associated with tame behavior in mice, we established the combination method using selective breeding for tame behavior and following genomic analysis using the Wild-derived heterogeneous stock (WHS) . The stock has high levels of genetic variation because the stock derived from 8 wild strains (MSM, HMI, BLG2, PGN2, KJR, CHD, NJL, and BFM/2) originated from various geographic regions. As a result of the analysis, we identified the partial genomic region on Chromosome 11 originated from MSM. Further, to elucidate gene (s) or gene network in the expression level related to tame behavior we conducted RNA-seq analysis. Total RNA was extracted from the hippocampus of 10 selected and 10 control mice and the RNA were used for the analysis. Here we are going to discuss about the candidate genomic region on Chromosome 11 and gene expression potentially affecting tame behavior in mice.

2A09 Forward genetic approach-based investigation of novel modifier gene on mouse chromosome 12 for age-related hearing loss

○ Shumpei P. Yasuda[1], Yo Obara[1], Sari Suzuki[1], Toyoyuki Takada[2], Toshihiko Shiroishi[2], Yoshiaki Kikkawa[1]

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C57BL/6J (B6J) mice exhibit age-related hearing loss (AHL) due to the effect of a *Cdh23*$^{c.753A}$ allele of the cadherin 23 gene in chromosome (Chr) 10. In contrast, MSM/Ms (MSM) mice maintain good hearing until at least 20 months of age. Several B6J-MSM consomic strains, in which a part of the chromosome of B6J mice has been replaced with the chromosome derived from MSM mice, also exhibit delayed AHL onset despite bearing the *Cdh23*$^{c.753A}$ allele. This result suggests that there are several modifier genes in the genetic backgrounds of B6J and/or MSM mice. To identify these genes, we performed a forward genetic analysis of a B6J-Chr12C^{MSM} subconsomic strain, in which an approximately 71-Mb region of MSM-derived Chr 12 is placed into the B6J genome, that showed delayed AHL onset. We focused on the hearing ability for 32 kHz sound stimulus of B6J-Chr12C^{MSM} mice because the phenotypic analysis can quickly perform due to B6J develop early-onset AHL. We performed audiometry for the F_1 and F_2 offspring of B6J-Chr12C^{MSM} and B6J mice. F1 mice showed hearing ability of an intermediate level between that of B6J and B6J-Chr12C^{MSM} mice, and hearing thresholds of F_2 mice showed bimodal distribution. Next, using a genetic mapping approach in F_2 mice, we identi¬fied the hearing phenotype was significantly linked to a region approximately 0-9-Mb. There are 36 protein-encoding genes and 12 non-coding RNAs reported so far. We performed *in silico* analysis for the candidate genes and found that 10 carry deleterious mutations in B6J mice. This suggests that AHL in B6J-Chr12C^{MSM} mice is delayed both by the genetic substitutions from the MSM mouse genomic region on Chr 12 and by the mutations already present in the genes of B6J mice.

2A10 Genome-wide mutation screening of genes associated with eye defects in NAK/Nokh rat

○ K. Wada, H. Munakata, H. Uchiyama, S. Okubo, M. Watanabe, A. Abe, Y. Kikkawa

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The NAK/Nokh (NAK) is an eye deficient mutant rat strain which was spontaneously isolated from SD colony. NAK mutation leads to the various degree of eye defects among offspring generated by backcrossing with Brown Norway or Wister strains. We previously identified a causative genetic locus for NAK phenotype on chromosome 16 by genome-wide linkage analysis. However, the complete association between genotype on chromosome 16 and eye phenotype could not be confirmed. These results suggested that NAK phenotype is caused by multiple gene mutations. We performed linkage analyses using phenotypes which were distinguished between right and left side eyes. The genetic loci with highest LOD score associated with the weight of left and right side eyes were detected on chromosome 16 and 2, respectively. Mutation analysis based on the RNA-seq and WGS identified a large deletion of genomic region encoding *Cyp4v3* gene in NAK rat. We confirmed that this deletion mutation causes partial loss of 3' -terminal region on *Cyp4v3* transcript by RT-PCR analysis. Based on this mutation, we genotyped *Cyp4v3* in [NAK x (BN x NAK) F_1]N_2 individuals, and investigated the correlation between eye phenotype and genotypes. The complete lack of left side eye were observed in 63.3% of *Cyp4v3*$^{nak/nak}$ individuals. Meanwhile, the association between the absence of right side eye and the *Cyp4v3*$^{nak/nak}$ was weak compared to that of left side eyes (48%). Moreover, the left side eye weight of *Cyp4v3*$^{nak/nak}$ showed significantly reduced compared with the right side eye in *Cyp4v3*$^{nak/+}$ ($P < 0.01$). Therefore, we suggested that *Cyp4v3*^{nak} mutation affects to NAK phenotype, especially in left side eye development.

Functional analysis of *Dnase1l2* gene using the gene knockout mouse

2A11

○ Masaru Tamura, Yasuyo Kozawa, Mao Ozaki, Shigeharu Wakana

RIKEN BRC, Technology and Development Team for Mouse Phenotype Analysis

DNASE is an enzyme that has a digestive activity of the deoxyribonucleic acid (DNA) , and it is roughly divided into two groups, endonuclease and an exonuclease. *DNASE1* family is composed of 4 genes, including the *DNASE1* and *DNASE1L2*, all of which have exonuclease activity. It was reported that the mutation of *DNASE1* causes the autoimmune disease, systemic lupus erythematosus, and that haploinsufficiency of *DNASE1* is involved in 16p13.3 partial deletion syndrome. *DNASE1L2* is most resemble to the *DNASE1* among the *DNASE1* gene family at the amino acid sequence level, and is located on human chromosome 16, 16p13.3, near side of *DNASE1*. However, function of *DNASE1L2* and correlation of *DNASE1L2* mutation and human syndrome remains unclear.

Previously, we have generated *Dnase1l2* KO mouse as a part of IMPC project, and reported its phenotypes, such as joint fusion and low body weight. These phenotypes were similar to the symptoms of 16p13.3 micro-deletion syndrome that lack extremely short genome fragment including *DNASE1L2*. On the other hand, up to 16 weeks of age of *Dnase1l2* KO mice did not show autoimmune disease phenotype, and FACS analysis also showed no abnormality in the immune system of *Dnase1l2* KO mice.

In this study, we carried out late onset analysis of *Dnase112* KO mice at over one year after birth, whether *Dnase112* is involved in autoimmune disease related to the aging. In addition, we also conducted an embryonic analysis of *Dnase112* KO mice in order to clarify when joint fusion occurs. We will report on these results, and discuss the relationship between *DNASE1L2* and human chromosomal disorder.

Monitoring of genetically engineered mouse strains using high-throughput genetic profiling system in Japan Mouse Clinic

2A12

○ Ikuo Miura, Akiko Shinogi, Daiki Usuda, Tomohiro Suzuki, Hideki Kaneda, Kimio Kobayashi, Masaru Tamura, Shigeharu Wakana

RIKEN BioResource Center

In performing phenotype analysis for genetically engineered mouse lines, the genetic background should be uniform by enough backcrossing to elucidate effects of the modified gene on the targeted genes. Hence, in order to monitor the genetic background, whole genome scanning with the high-throughput genetic profiling system has performed in the Japan Mouse Clinic. This system was possible to get information such as the ratio of replacement to backcrossing strain, the paternal or maternal origin by SNPs markers and the existence of transgenes as gene modification and, moreover, was able to closely distinguish each C57BL/6 (B6) substrains and production breeders of B6/J and B6/N by SNPs markers. The whole genome scanning was performed to 137 strains analyzing in the JMC. As the results, the strains of the non-uniform genetic background led by inappropriate cross were often recognized. The lack of backcross and maintenance with intercross were 35.0%. The confusion by having used plural B6 strains while generating congenic strain was 35.0%. Non-replacement of the Y Chr. by backcrossing using only male carrier as backcross partner was 22.6%. The unexpected remaining of transgenes as genetic modification was 3.7%. In this study, we propose guidelines on accurate development of congenic mouse strain to uniform the genetic background.

2A13 Genetic profiles of inbred mice with the SNP analysis

○ Masafumi Yamamoto, Nobuhito Hayashimoto, Riichi Takahashi, Hiroshi Suemizu

Central Institute for Experimental Animals

Genetic monitoring is essential for quality control when breeding inbred and transgenic animals.
There are several methods used to genetic monitoring including physical characteristics, biochemical and immunological techniques and DNA-based techniques. Recently genetic monitoring testing has become mainstream to analyze the SNP markers. At the previous meeting, we also reported that there was no difference in testing quality between the conventional method and SNP analysis by 8 years test using three inbred stains.
CIEA SNP marker panel of inbred mice consist of 32 markers. It can distinguish the commercially available major inbred mice strain (129, BALB/c, C3H/He, C57BL/6, DBA/2, NOD) in Japan, and each marker located on autosome respectively. In this study, we revealed the genetic profiles of 44 mouse strains (35 inbred and 9 hybrid) that commercially available laboratory animal in Japan. As the result, in the following four strains C57BL/6, BALB/c, C3H/He and NOD/Shi (including NOG) , there were no differences in genetic profile between substrains respectively. On the other hand, few numbers of differences was observed in the substrains 129 and CBA. Genetic profiles of hybrid strain (F1) indicated heterozygote between the inbred strains.
We construct the database of these genetic profiles on the web as CIEA Mouse Strain Check Program. This program is available viewing the genetic profiles of inbred mice and comparing an SNP profile with database.

2A14 Identification of a quantitative trait gene for kidney weight in mice by quantitative complementation tests

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Previous QTL analysis using backcross mice of the C57BL/6JJcl (B6JJcl) inbred strain and wild mice captured in Philippines revealed several QTLs affecting growth related traits on a 44-Mb region of chromosome 2. A subcongenic strain (SR24) previously created has a 3-Mb target region derived from the wild mouse. *Gcg*, *Ly75*, and *Fap* are located on the region as candidate genes for some of the QTLs. This region may include a QTL affecting organ weight which was mapped previously. In this study, we confirmed whether this QTL is present in the subcongenic region by phenotypic analysis and we narrowed candidate genes for the QTL by gene expression analysis. By quantitative complementation tests, we identified a causal gene for the organ weight QTL. Phenotypic analysis showed that body weight was not significantly different between knockout (KO) mice and its background C57BL/6J (B6J) strain and between SR24 and B6JJcl. However, KO showed significantly higher kidney weight than B6J, and SR24 showed significantly higher kidney and heart weight than B6JJcl. On the other hand, SR24 showed significantly lower lungs weight than B6JJcl. *Gcg* expression in jejunum was not significantly different between B6JJcl and SR24. However, SR24 showed significantly higher expression of *Ly75* and *Fap* in liver than B6JJcl. Quantitative complementation tests revealed significant interaction effects between KO genotypes and QTL genotypes for kidney, heart and lungs weights. The results suggested that at least the gene knockouted is a causal gene for the kidney weight QTL.

2A15 *K-rasG12V*-mediated lung tumor models identified new quantitative trait loci modifying events post-*K-ras* mutation

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A high incidence of oncogenic *K-ras* mutations is observed in lung adenocarcinoma of human cases and carcinogen-induced animal models. The process of oncogenic *K-ras*-mediated lung adenocarcinogenesis can be dissected into two parts: pre- and post-*K-ras* mutation. Adoption of transgenic lines containing a *flox-K-rasG12V* transgene eliminates the use of chemical carcinogens and enables us to study directly crucial events post-*K-ras* mutation without considering the cellular events involved with oncogenic *K-ras* mutation, e.g., distribution and metabolism of chemical carcinogens, DNA repair, and somatic recombination by host factors. We generated two mouse strains C57BL/6J-*Ryr2*^{tm1Nobs} and A/J-*Ryr2*^{tm1Nobs} in which *K-rasG12V* can be transcribed from the cytomegalovirus early enhancer/chicken beta actin promoter in virtually any tissue. Upon *K-rasG12V* induction in lung epithelial cells by an adenovirus expressing the Cre recombinase, the number of tumors in the C57BL/6J-*Ryr2*$^{tm1Nobs/+}$ mouse line was 12.5 times that in the A/J-*Ryr2*$^{tm1Nobs/+}$ mouse line. Quantitative trait locus (QTL) analysis revealed that new 13 modifier loci were involved in the differential susceptibility between the two line.

2A16 Wapl develop oncogenic activity with HPV E6/E7

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Human papillomavirus (HPV) are the primary causal agents responsible for the development of cervical cancer, and expression of two viral agents E6 and E7 is considered to develop of cervical cancer. Approximately 90-100% of women infected with HPV during their lives. Chromosomal instability is observed in most cancers that it develop into malignancy. Wapl deficiency causes chromatin compaction by stabilizing cohesin on DNA. The compaction state of interphase chromatin, therefore, depends on the stability of cohesion and DNA interactions, which is controlled by Wapl. Thus, Wapl is an essential regulator of chromatin structure and chromosomal stability. Moreover, High-level expression of hWAPL (the human homologue of wapl) was observed in cervical cancers; it is necessary to reveal about the regulation of development of cervical cancer by E6/E7 and Wapl. Recently, we have demonstrated that transduction of Wapl with E6/E7 is sufficient for the development of canceration in epithelium cells. Here we show that transduction of Wapl with E6/E7 expression causes increase not the only number of colonies but also the formation of colonies. Furthermore, E6/E7 and Wapl-overexpressing epithelium cells developed into tumors on injection into a severe combined immunodeficient mice. These results suggest that Wapl could be developed not only oncogenic activity in epithelium cells with E6/E7 but also canceration in epithelium cells.

2A17 Analysis of *cis*-DNA element of mouse *renin* gene invoved in its regulation in hypertensive environment

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Renin is a late-limiting enzyme of the renin-angiotensin system that plays a pivotal role in blood pressure homeostasis. *Renin* gene expression is controlled by a feedback regulation of blood pressure change and suppressed in hypertensive-environment, molecular mechanism of which remains to be elucidated.

Previously, we analyzed a couple of transgenic mouse lines each carrying a distinct portion of mouse *renin* gene locus, and found that a *cis*-DNA element responsible for its transcriptional attenuation in hypertensive environment was located away from its protein-coding region. To roughly locate this putative regulatory element, we generated mutant mice with their 5′or 3′flanking regions of the endogenous *renin* gene deleted by CRISPR/Cas9 genome editing. Expression analysis of these mutant alleles in the hypertensive-environment revealed that *cis*-responsive element of the gene was located within its 5' -flanking region. Next, we further analyzed the 5' -flanking region in renin-producing As4.1 cells, and identified a novel enhancer element by using a luciferase assay and DNaseI hypersensitive site mapping.

Finally, we generated a mutant mouse with the enhancer deletion in the endogenous *renin* gene locus. Based on the observations in this mutant mouse, we would discuss the molecular mechanism of *renin* gene attenuation in hypertensive-environment.

2A18 Functional validation of each domain of tensin2 by CRISPR/Cas9-mediated genome editing

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Chronic kidney disease (CKD) is a public-health problem characterized by either kidney damage or a long-term decline in kidney function regardless of disease type or cause, and is becoming increasingly common in both developed and developing countries across the globe. Proteinuria is intimately involved in the dysfunction of glomerular podocytes or slit diaphragms, and glomerulosclerosis sequentially starts with a decreased podocyte count. Glomerulosclerosis (GS) is one of the most common histopathological findings of CKD, and mutations in a number of podocyte-specific genes responsible for glomerulosclerosis have been identified in human. Therefore, podocyte injury is a common determining factor for progression toward many types of kidney disease that result in CKD. The ICGN mouse is a good model of glomerular dysfunction that shows gross morphologic changes in the podocyte foot process accompanying proteinuria. Previously, we demonstrated that proteinuria in ICGN mice might be caused by the null mutation in the tensin2 ($Tns2^{nph}$) gene. Tns2 is a multidomain protein composed of a PTPase domain at the N-terminus followed by a Src homology 2 (SH2) /phosphotyrosine binding (PTB) domain at the C-terminus. However, little is known about the function of these domains *in vivo*. To test whether Tns2 mutation might cause the GS phenotype and to clarify the biological role of these domains, we created the diminished PTPase activity mutant mice ($Tns2^{C231S}$) and SH2/PTB domain deficiency mice ($Tns2^{\Delta C}$) via CRISPR/Cas9-mediated mutagenesis. Herein we report the phenotypic differences among these mutant mice.

Generation of hairless mice for *in vivo* imaging of embryo in the uterus

2A19

○ Yoshikazu Hoshino[1,2], Kanako Kato[1], Saori Mizuno[1], Yoko Tanimoto[1], Miyuki Ishida[1], Tomoki Sakasai[1], Yoshihiro Miwa[1], Satoru Takahashi[1], Ken-ichi Yagami[1], Seiya Mizuno[1], Fumihiro Sugiyama[1]

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At present, *in vivo* imaging of mouse makes it possible to analyze disease progresses non-invasively through reporter gene expression. Because the removal of hair improve accuracy of *in vivo* imaging, gene-modified mice with reporter gene are often crossed to the Hos:HR-1 mutant mice which carried the *hr* gene mutation and exhibit the hair loss phenotype. However, it is time consuming to produce the mice carried both reporter gene and mutated *hr* gene by mating. In addition, there is the risk that genetic background of the gene-modified mouse would be changed by mating. To solve these problems, we try to establish a simple method to generate hairless mice keeping its genetic background by CRISPR technology. Firstly, we construct *pX330* vector which targets exon 3 of the *hr*. Then, this DNA vector (5 ng/μL) was microinjected into pronuclear of C57BL/6J mice. Accordingly, induced *hr* gene mutations were found in many founders (76.1%) and these mutations were inherited. Next, we tried *in vivo* imaging by using these gene-modified hairless mice. As expected, the luminant objects in their body were detected by *in vivo* imaging. This our study clearly showed that hairless mice could be simply generated by CRISPR/Cas9 system, and this method might be useful for *in vivo* imaging studies with various gene-modified mice.

Exploring and characterization of new gene integration site 'FN-Locus'

2B01

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A knock-in mouse is an essential tool for biological research, but the stability of expression of an integrated gene strongly depends on where it is integrated in the mouse genome. The genome loci suitable for gene knock-in, such as the Rosa26 locus, are wholly scarce. In this study, we developed an efficient strategy for identifying the genome locus suitable for gene knock-in and characterized Fam168a neighborhood locus (FN-Locus) that we identified. We successfully identified some candidates of genome loci suitable for gene integration utilizing gene-trapping strategy and evaluation of chimera embryos. We focused on one of the identified loci (FN-Locus) and generated KI mouse that fluorescent protein expression cassette is integrated into the locus. The KI mice exhibited ubiquitous expression of integrated gene without any notable abnormality. Remarkably, the FN-Locus KI mice exhibited more ubiquitous expression of the integrated gene than Rosa26 KI mice in some tissues. This indicated that FN-locus possessed high potential as gene integration site for ubiquitous expression.

2B02 Examination of specificity in novel site-specific recombination systems, VCre/VloxP and SCre/SloxP, in mice

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【Introduction】 Cre/loxP, a site-specific recombination system, is a powerful tool for development of transgenic animals including conditional knockout mice. Cre recombinase recognizes two loxP sites, each of which consists of 34-bp DNA, recombines two DNA fragments containing a loxP site respectively. Suzuki (2011) developed new site-specific recombination systems similar to Cre/loxP, VCre/VloxP and SCre/SloxP. These systems provide unique opportunities to express multiple transgenes. The specificity and efficiency of them was previously revealed in *E.coli* or Medaka, but not in mouse. In this study, we constructed 4 novel knock-in mice, to examine whether VCre/VloxP and SCre/SloxP systems work in mice with their specificity.

【Methods】 We constructed two reporter ROSA26-KI mice, VloxP-EGFP or SloxP-tdTomato KI mice, in which floxed-stop codon is inserted between the CAG promoter and the reporter gene. Thus, GFP in VloxP-EGFP or tdTomato in SloxP-tdTomato KI mice can be expressed in case of exclusion the floxed-stop cassette with VCre or SCre recombinases, respectively. We also produced two deleter ROSA26-KI mice, VCre and SCre KI mice, in which the recombinase is ubiquitously expressed by the CAG promoter. We crossed VloxP-EGFP KI and SloxP-tdTomato KI mice with three delete mice; VCre KI, SCre KI, or Cre Tg mice to confirm the specificity of each system.

【Results and discussion】 GFP expression was observed in F1 mice, only when VloxP-EGFP KI was crossed with VCre KI mice, but not with other deleters. The expression of tdTomato was also observed, when SloxP-tdTomato KI and SCre KI mice, but not other deleter strains, were crossed. These results suggest the specificity of the VCre/VloxP and SCre/SloxP systems in mice. We will discuss the usefulness of these systems for generation of transgenic mice.

2B03 Efficient gene-targeted disruption using CRISPR/Cas9 system for gene editing in mice

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Recently, CRISPR/Cas9 system have been used to generate knockout mice by generating DSB and followed by NHEJ-mediated repair, resulting in mutant mice carrying indel mutation. In this study, we show generation efficiency of mutant mice with targeted mutation in seven target genes by CRISPR/Cas9 system, respectively. Cas9 mRNA and sgRNA for five target genes (A ～ E) were injected into the cytoplasm of oocytes at the pronuclei stage, respectively. Cas9 mRNA and sgRNA for target gene F and donor vector (ssODN) were also injected. Expression vector, containing sgRNA and Cas9 mRNA, and donor vector for target gene G were co-injected into oocytes with spermatozoa. The 2-cell stage embryos developed from injected oocytes were transferred into oviducts of pseudopregnant mice at 0.5 dpc. After birth, genomic DNA was extracted from the tail tips of weaned pups and subjected to PCR. The purified PCR products were analyzed by T7E1 assay and TA cloning/ sequencing for genome modification. We found that 7 (70%), 4 (100%), 14 (100%), 9 (75%) and 6 (86%) pups were mutated for A, B, C, D and E target genes, and in except for C target gene were generated homozygous mutant. On the other hand, F and G target genes, 1(8%) and 1(7%) pups were inserted of donor genes, respectively. These results indicate that the CRISPR/ Cas9-mediated genome editing makes possible highly effective method for the generation of knockout mice and the one-step generation of homozygous mutants for indel mutation. However, the efficiency of HDR-mediated gene editing using CRISPR/Cas9 system for generating knockin mice was less than that of NHEJ -mediated.

2B04 Genetation of FLAG-tag knock-in mice using CRISPR/Cas system

○ Emi Tsuru[1], Masaaki Mizobuchi[1,2], Kiyohito Hisakawa[1,2], Atsuya Nobumoto[1,2], Hiroki Mogawa[1,2], Masayuki Tsuda[1]

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In general, gene targeting by homologous recombination in embryonic stem cells has been used to generate knock-out or knock-in mice. Recently, genome editing technologies, such as CRISPR/Cas system which enables site-specific mutagenesis, become widespread and allow to quickly and easily generate mutant mice. On the other hand, CRISPR/Cas system has been reported to have low knock-in efficiency *in vivo* genome editing. Here we report the CRISPR/Cas9 mediated generation of FLAG-tag knock-in mice by microinjection of all-in-one CRISPR/Cas9 plasmid vector and single- stranded oligonucleotide (ssODN, donor oligos) into zygotes.

To test the knock-in efficiency, we designed some guide RNAs (gRNA). We demonstrated higher knock-in efficiency using gRNA located more close to the insertion site, whereas some mice harbored incorrect FLAG-tag gene recombinations. We now further analyze germline transmission of knock-in allele.

To examine the difference of the efficiency of all-in-one CRISPR/Cas9 vector system in mouse strain, we used zygotes from inbred C57BL/6N and hybrid B6D2F1 mice. We found that the birth rate was very low using C57BL/6N zygotes, compared with B6D2F1. In microinjection of plasmid DNA, it showed that zygote survival was mouse strain dependent and influenced by transgene quality. Further improvements of CRISPR/Cas9 vector system are needed to increase the efficiency in inbred mouse strains.

2B05 Generation of knock-in mice at the GT-repeat region with long single strand DNA

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Mouse Genomics Resource Laboratory, National Institute of Genetics

Genome editing technologies such as CRISPR/Cas9 enabled us to generate genetically modified animals. We have established several types of KI rats using single-stranded oligonucleotides, such as SNP substitution and small DNA fragment insertion (Yoshimi K et al., Nat Commun 2014). Furthermore, we also demonstrated efficient KI in rodents by combining CRISPR/Cas9 with long single strand DNAs (lssDNAs) purified by nicking endonucleases. (Yoshimi K et al., Nat Commun 2016).

In this study, we tried to replace the GT-repeat region of C57BL/6 mouse strain with that of MSM/Ms strain. Previous study indicated that a length of the GT-repeat region at *Adcyap1* locus can affect anxiety-like behaviors in wild-derived strains.

About 0.8kb lssDNA was synthesized by nicking endonucleases from the plasmid including long GT-repeat region from MSM/Ms. Co-injection of the lssDNA with guide RNA and Cas9 protein could generate GT-repeat KI at the *Adcyap1* locus in C57BL/6 mice. Any off-target effect was detected in all KI founders.

These results suggested that lssDNA-mediated KI can apply to any target site including repeat regions, thus simplifying genome engineering in living organisms with a better cost/benefit ratio.

Production of Gdf9-BiCre Knock-in mice with CRISPR/Cas9

2B06

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The Cre/loxP system is most often used in conditional knock-out mouse experiments. We have produced bicistronic *Cre* knock-in (*biCre*-KI) mouse strains for precise cKO experiments. In our *biCre*-KI mice, the 2A sequence connected *Cre* gene fragments are inserted (Knock-in) just before stop codon of some endogenous genes which are expressed tissues/time specific. Here, we introduce a novel oocyte-specific Cre direver, *Gdf9-biCre*-KI mice.

The *Gdf9* gene expression in oocyte is found in primary follicle and it continues until ovulation. However, this genes expression immediately stopped after fertilization. To establish the oocyte-specific *biCre*-KI driver mice, we tried to insert the 2A connected *Cre* gene into *Gdf9* gene locus by embryo-based genome editing using with CRISPR/Cas9. Firstly, CRISPR targeted sequence, which overlapped stop codon of *Gdf9* gene, was selected. The 20-nt DNA coding the gRNA target sequence was introduced in pX330 plasmid (Addgene#42230). We also constructed the donor plasmid DNA vector in which 5' and 3' homology arms (1.4 kb each) and 2A-*Cre* gene fragment (1.7 kb) were included. These two vectors were co-microinjected into pronuclear of 147 C57BL/6 mice zygotes and 40 newborn mice were obtained. The *Gdf9-biCre*-KI allele were found in 3 funders. To confirm the *Cre* recombination activity, KI founder was mated with the *Cre*-reporter mice. In F1 from this mating, recombination happed in oocyte, as expected. However, recombined cells were also found in cerebellum. We then checked *Gdf9* expression in wild type cerebellum and it was detected. It means that *Cre* expression in cerebellum is due to endogenous Gdf9 promoter activity.

In conclusion, the *Gdf9-biCre*-KI mouse strain have enough *Cre* recombination activity in oocytes but we must mind the recombination in cerebellum.

The culture time of zygotes before microinjection affects the production efficiency of CRISPR-Cas9-mediated knock-in mice

2B07

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Reproductive engineering techniques are required for the quick and efficient production of genetically modified mice. We have previously reported on the efficient production of genome-edited mice using reproductive engineering techniques, such as the ultra-superovulation method, *in vitro* fertilization (IVF) and vitrification/warming of zygotes. We usually use vitrified/warmed fertilized oocytes created via IVF for microinjection for reasons of work efficiency and flexible scheduling. Here, we investigated whether the short-time culture of zygotes before microinjection influences the efficiency of the production of knock-in mice. Knock-in mice were created using the clustered regularly interspaced short palindromic repeats (CRISPR) -CRISPR-associated protein 9 (Cas9) system and the single-stranded oligodeoxynucleotide (ssODN) or PITCh (Precise Integration into Target Chromosome) system, the method of integrating a donor vector assisted by microhomology-mediated end-joining (MMEJ). The cryopreserved fertilized oocytes were warmed, cultured for several hours, and microinjected at different timings. The microinjection was carried out using Cas9 protein, guide RNA (s) , and ssODN or PITCh donor plasmid for the ssODN knock-in and the PITCh knock-in respectively. The different production efficiencies of knock-in mice were confirmed by changing the timing of the microinjection. Our study provides useful information for the CRISPR-Cas9-based generation of knock-in mice.

Genome editing of embryos by electroporation

2B08

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The CRISPR/Cas9 system is a powerful tool that can directly edit genomic DNA in fertilized eggs.
For an effective genome editing, however, it is necessary to inject Cas9 mRNA and sgRNA into the cytoplasm of fertilized eggs by microinjection. This microinjection operation requires a skill.
Here we report a highly efficient genome editing using a simple and easy electroporation method.

Generation of genome-edited mice using the electroporation

2B09

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【Purpose】 It has been taken about 2-3 years to generate transgenic mouse using ordinary method such as homologous recombination with ES cells, however, new method with electroporation (EP) enables us to generate genome-edited mouse, quickly and easily as reported (Hashimoto et al. Sci Rep 2015). Thus, we have just begun to establish this experimental system to generate knockout/in mice such as single nucleotide mutation or mice harboring loxP sequence using C57BL/6 embryos in our facility. In this study, we report the results of the optimization of the electroporation conditions.
【Material and Methods】 Embryos were obtained from naturally mated females or *in vitro* fertilization (IVF). First, we compared EP condition by +5 repeat, ± 6 repeat and no pulse using Genome Editor™ (BEX). Next, we compared normal developmental rate and genome editing rate between embryos obtained from natural mating and IVF. Under appropriate condition determined by these results, we performed knock-in mutagenesis by homologous recombination using ssODN.
【Results】 As a result from pulse number optimization, +5 repeat condition showed the highest developmental rate into 2 cell stage. Normal developmental rate and genome editing rate is 80% and 89% in natural mating, 62% and 44% in IVF, respectively. ssODN mediated knock-in mutagenesis was successfully performed with 13% mutation rate.
【Conclusion】 Based on these results, we determined to use embryos obtained from IVF because of its possible reduction of the number of animals, with pulses of +5 repeat. Under these condition, we confirmed integration of ssODN mediated sequences in edited mouse genome. Now, we are trying to generate flox or Tag mice using this strategy.

2B10 Genome editing mice generated by CRISPR / Cas9 system using cryopreserved embryos and electroporation

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The CRISPR/Cas9 system is one of the simple genome editing tools. Microinjection of gRNA and Cas9 mRNA into the pronuclear-stage embryos is a common method to generate genome editing animals. Recently, we have established zygote electroporation, which provides extremely quick and easy transfer of gRNA/Cas9 into a dozen of fertilized eggs (Kaneko et al. *Sci Rep* 2014) . In this study, we combined the zygote electroporation with the cryopreserved mouse embryos that were prepared with high-quality and easy thawing.

We designed gRNA using "CRISPR design tool (http://crispr.mit.edu/)". Long single-stranded DNA (lssDNA) was manufactured using nicking endonucleases from double-stranded plasmid vectors. We introduced gRNA, Cas9 mRNA, with or without the lssDNA, into fertilized eggs by electroporation using NEPA21 electroporator (NEPA GENE) , and cultured them overnight. Then, we selected 2-cell stage embryos, and transferred them to the pseudopregnant recipients.

We optimized several conditions to transfer RNA into the cryopreserved embryos and we could generate genome editing mice with high efficiency. We also succeeded to produce flox-mice from cryopreserved embryos by zygote electroporation with gRNA, Cas9 mRNA, and lssDNA including two LoxP sequences.

The easy protocol for zygote electroporation with cryopreserved embryos we established here will facilitate more production of genome editing mice.

2B11 Efficient generation of knock-in rats using CRISPR/Cas9

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【Background】

CRISPR/Cas9, the valuable genome editing technology, has made it possible to genetically modify a wide range of experimental animals such as drosophila, zebrafish, mouse, and rat. We have already established CRISPR-based technologies to produce genetically modified rats such as knockout and knock-in alleles. Especially, the generation of knockout animals via nonhomologous end joining (NHEJ) is now routine experiments, and the time required for animal production has been greatly shortened. On the other hand, preparation of large cassette knock-in animals or conditional knockout animals inserting loxP sequences is still a challenging task even its high demand. In this study we report efficient and simple protocols to produce large cassette-knockin or conditional knockout rats.

【Methods】

Rat pronuclear-stage embryos were cultured in mKRB medium for several hours. OPTI-MEM medium containing 400 ng/ μ l Cas9 mRNA, 200 ng/ μ l gRNA, and 40 ng/ μ l long single stranded DNA (lssDNA) (Yoshimi et al, *Nature Commun*, 2016) were used for zygote electroporation. On the day after the gene transfer, fertilized eggs were transplanted into pseudopregnant females.

【Conclusion】

By using this method, knock-in animals can be routinely produced with a success rate of about 10%. This protocol will provide the easy and efficient production of knock-in animals, such as large cassette knock-ins or conditional knockouts.

Electroporation method for generation of genome editing animals

2B12

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Electroporation method (TAKE method) has been developed for generation of genome editing animals by Kaneko et al. In this study, we examined the development of embryos introduced Cas9 protein by TAKE method. Cas9 protein and gRNA targeted Tyrosinase gene was introduced into rat embryos. Embryos were then transferred to pseudopregnant females. The development to offspring of embryos introduced Cas9 protein and gRNA by TAKE method were 68%. And, all offspring showed modification of targeted gene. This study demonstrated that the TAKE method was easy and high efficiency method for generation of genome editing animals.

FGF2 contribute to the cell fate determination of undifferentiated spermatogonia in mice

2B13

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Self-renewal of undifferentiated spermatogonia including spermatogonial stem cells (SSCs) are supported by GDNF. Our previous report demonstrated that FGF2 also possess these functions *in vitro*. In the present study, we investigated the function of FGF2 on mouse undifferentiated spermatogonia in vivo.

By using gelatin microgels as FGF2 delivery carrier, we could successfully stimulate undifferentiated spermatogonia *in vivo*. After stimulation, we found that $GFRA1^+$ undifferentiated spermatogonia exhibited hyperproliferation to form unphysiologically large colonies, suggesting that FGF2 act as self-renewal factor even *in vivo*. However, morphology and phenotype of these cells are different from undifferentiated spermatogonia stimulated with GDNF. GDNF-stimulated undifferentiated spermatogonia formed dome-like 3D colonies with $RARG^-$ phenotype, whereas FGF2-stimulated cells formed 2D colonies containing $GFRA1^+RARG^+$ population. Given that retinoic acid (RA) induce spermatogonial differentiation and RARG is a RA receptor exclusively expressed in spermatogonia, FGF2 is considered as an inducer of $RARG^+$ population in undifferentiated spermatogonia, thereby facilitating the differentiation of undifferentiated spermatogonia. We also found that FGF2 also act to enhance RA action in germline niche by downregulating RA metabolizing enzyme. Taken together, our finding suggested that FGF2 show ambivalent behavior in germline niche. Considering that FGF2 regulate RA action, FGF2 might also contribute to determine the species specific-period of seminiferous epithelial cycle in combination with RA action.

2B14 Transplantation of cryopreserved ovarian cells in *Xenopus laevis*

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【Objective】 Although numerous endangered wild populations of amphibians exist, the only method currently available for preserving the resources is to rear live individuals, as protocols for cryopreservation of amphibians eggs or embryos have not yet been successfully established due to their large size. To develop the system for preservation of amphibians, we aimed to cryopreserve germline stem cells (oogonia) which would differentiate into both sperm and eggs. At first, we tried to transplant cryopreserved ovarian germ cells into surrogate recipients.

【Methods】 We vitrified whole ovaries of *Xenopus laevis* (albino) including oogonia, and the vitrified ovarian cells were then transplanted into surrogate recipients (*Xenopus tropicalis*) after staining with PKH26. Since recipients producing gametes derived only from transplanted donor germ cells are desirable, infertile triploids were used as the recipients.

【Results】 When viability of ovarian germ cells was assessed with trypan blue staining, 52.5% of ovarian germ cells were survived after vitrification. After staining of cryopreserved ovarian germ cells with PKH26, they were transplanted into hatching larvae. After 2 weeks, PKH-fluorescence of transplanted cells was observed in gonads of recipients. Cryopreserved ovarian germ cells may be incorporated in gonads of recipients. Moreover, it was confirmed that surrogate recipients were triploids by ploidy analysis. Next, we are going to investigate whether transplanted ovarian germ cells can be differentiated into oocytes.

2B15 Effect of histone deacetylase inhibitor on development of interspecies nuclear transfer embryos

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Mammalian oocytes can initialize somatic cells to totipotency state. However, major of reconstructed oocytes are low production efficiency. Then the cause and the mechanism is still unknown. Matoba *et al*., reported that arrest of embryonic development following somatic cell nuclear transfer (SCNT) caused by persisting histone methylation. In this study, we investigated that histone deacetylase inhibitor effected on development of interspecies nuclear transfer embryos. First, fibroblast cells were established from tail tissues of the large Japanese field mouse (*Apodemus speciosus*). Second, it put to produce the somatic cell nuclear transfer embryos. After mice oocytes (*Mus musculus*) were enucleated, the fusion of cell-cytoplasm used HVJ Envelope Cell Fusion kit. Activation of the reconstructed oocytes treated for 8, 10 and 24 hours with CZB Ca^{+} free medium supplemented 50nM Trichostatin A (TSA) and 10mM $SrCl_2$. In these results, the rate of cell fusion in interspecies SCNT oocytes was 96% (218/226). After activation, reconstructed pronuclear oocytes were formed (78% (170/218)), the oocytes treated with 50nM TSA 8h, 10h and 24h. Also, there is no difference in the development of the 2 cell stage embryos at various TSA treatment times (8hpi: 88% (60/68), 10hpi: 83 (43/52), 10hpi: 86% (43/50)). At present, there have been further validated trimethylation levels of H3K9 and assayed regulation of gene expression via retrotransposon in the 2cell embryo.

ES cell derivation is affected by the genetic background

2B16

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Embryonic stem cells (ESCs) are derived from inner cell mass (ICM) of blastocysts in mice. ESCs are self-renewing in the presence of cytokine LIF in culture. The robustness of ES cells' self-renewal in standard serum containing culture (FCSLIF) is highly dependent on their genetic background. ES cell derivation experiments in FCSLIF have revealed that the 129 strain is permissive while the NOD (Non-Obese Diabetic) strain, a model of Type I diabetes (T1D), is not (ref 1). However, it is still unclear that how ICM cells of NOD strain behave when establishing ESC lines directly from blastocyst under FCSLIF culture condition.

Here we report that the FCSLIF culture condition that gave rise to ESCs efficiently from the 129 strain blastocysts as reported elsewhere resulted in capturing the primitive endodermal (PrE) like cells instead of ESCs from the NOD strain. These NOD derived cell lines express *Gata6* and *Dab2* (PrE markers) and stably self-renewed for at least a month. Further gene expression analysis in these cell lines indicates a striking similarity to that of extra-embryonic endoderm stem (XEN) cells.

However, we still do not know molecular mechanism of the standard ESCs culture condition (FCSLIF) captures PrE cells from NOD ICM cells. We are investigating the mechanisms by which the same culture condition gives rise to different cell fates (either ESCs or the PrE-like) depending on the genetic background of the parental mouse strain.

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Attempt to establish feeder-free ES cells in a mouse model of cardiomyopathy (4C30)

2B17

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【Aim】 We established a mouse model (4C30) for dilated cardiomyopathy, which is a transgenic mouse line over-expressing ST3GalII transgenes with the C57BL/6NCr background. For further utilization of the strain, we tried to establish feeder-free embryonic stem (ES) cells. [Methods] In vitro produced 4C30 blastocysts were cultured in serum-free medium with inhibitors for GSK β and ERK/MEK (ESGRO-2i Medium, Merck Millipore). First, the blastocysts were cultured in 100-μL droplets of the medium for approximately 10 days, then transferred to gelatin-treated 4-well plates (NUNC), and then to gelatin-coated 6-well plates (IWAKI). The establishment of ES cells was judged by the appearance.

【Results and discussion】 We obtained four and twelve ES cell lines from 20 and 40 blastocysts, respectively, in two trials. Compared with our experience for ES-cell establishment with feeder cells and serum-containing media, inner cell masses were easily isolated because trophoblast proliferation was highly inhibited in the 2i medium. In the early phase in culture, blastocyst-derived cells were difficult to be attached to the gelatin-coated surface of culture plates and, therefore, embryoid-body-like masses floated in wells. In the later phase, the masses gradually attached to and spread on the gelatin-coated surfaces of the culture dish and formed an appearance like ES cells as reported. Attachments to well surfaces and proliferations of the feeder-free ES cells differed among six sources of commercially available 6-well plates even though the same gelatin treatment was employed, suggesting that lot checks of culture plates are recommended. Since feeder-free ES cells are easy to be maintained and pure without any feeder cells, we believe that the cells obtained in this study are useful for studying cardiomyopathy at a cellular level.

Kidney generation from mouse ES cells in homozygous *Sall1*-KO rats by xenogeneic blastocyst complementation approach

2B18

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Regeneration of human kidneys in animal model would provide enough number of functional donor kidneys in transplantation therapy. This study was designed to generate mouse ES cell-derived kidney in homozygous *Sall1*-KO rats by xenogeneic blastocyst complementation approach. *Sall1* gene of Wistar rats was knocked-out (replaced) with tdTomato gene by conventional homologous recombination of rat ES cells and chimeric rat generation, to generate the kidney-deficient model. Day-4 blastocysts were collected from uteri of the *Sall1*-tdTomato heterozygous KO females that had been mated with the heterozygous KO males. Seven GFP-positive mouse ES cells were injected into each blastocyst with piezo-manipulation system, and the injected blastocysts were transferred into Day-3 pseudopregnant rat uteri. Transfer of 168 injected blastocysts resulted in the harvest of 111 live fetuses, including 64 rat/mouse chimerae, on Day-17.5 to 21.5. Genotyping showed that these rat/mouse chimeric fetuses were composed from 29 Sall1-wildtype, 25 heterozygous *Sall1*-KO, and 10 homozygous *Sall1*-KO fetuses. Kidneys in 6 of the 10 homozygous *Sall1*-KO fetuses were observed with strong GFP fluorescence and no tdTomato-positive signals, while those in heterozygous *Sall1*-KO fetuses had both GFP fluorescence and tdTomato-positive signals. No kidney complementation occurred in the other 4 homozygous *Sall1*-KO fetuses. Flowcytometric quantification showed that cell proportions of mouse lymphocytes in homozygous *Sall1*-KO, heterozygous *Sall1*-KO, and *Sall1*-wildtype xenogeneic chimera spleen were 0-8.7%, 0-7.0% and 0-12.1%, respectively. These data suggest that mouse kidneys were successfully generated from ES cells in the developmental niches for kidneys of homozygous *Sall1*-KO rats.

Porcine derived induced pluripotent stem cell with the expression of six reprogramming factors

2B19

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We established porcine-induced pluripotent stem (iPS) cells with the expression of six reprogramming factors (Oct3/4, Klf4, Sox2, c-Myc, Lin28, and Nanog) . The resulting cells showed growth dependent on LIF (leukemia inhibitory factor) and expression of multiple stem cell markers. Furthermore, the iPS cells caused teratoma formation with three layers of differentiation and had both active X chromosomes (XaXa) . Our iPS cells satisfied the both of important characteristics of stem cells: teratoma formation and activation of both X chromosomes. Injection of these iPS cells into morula stage embryos showed that these cells participate in the early stage of porcine embryogenesis. Furthermore, the RNA-Seq analysis detected that expression levels of endogenous pluripotent related genes, NANOG, SOX2, ZFP42, OCT3/4, ESRRB, and ERAS were much higher in iPS with six factors than that with four reprogramming factors.

2B20 Triple-target CRISPR enabled almost perfect whole-body bi-allelic knockouts at first generation

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The efficient production of biallelic KO mice can be facilitated by recently developed genome editing techniques, including the CRISPR/Cas nuclease system. This method accelerates the generation of KO animals via the co-injection of RNA encoding the Cas9 protein and target-locus-specific guide RNAs into embryos. However, two problems remain: (1) first-generation mice often contain a mosaic of wild-type and KO cells, and (2) the rate of complete biallelic mutant mice generated is relatively low (usually ～ 60%–80% at best) . In order to achieve almost complete biallelic knockout at first generation, we improved CRISPR/Cas system by developing a new method to design gRNAs with high efficiency and low off-targets, and utilizing three gRNAs within a target gene to obtain maximum knockout efficiency. As a result, the triple-target CRISPR method elicited almost perfect (～ 96%–100%) whole-body KO of the Tyrosinase (Tyr) gene, which is functionally evaluated by animal coat color. This KO efficiency was confirmed using three independent sets of gRNAs. The highly efficient production of whole-body KO by the triple-target CRISPR method also enabled us to obtain clock mutant phenotypes reliably, not only of Bmal1 single-KO mice but also of Cry1/Cry2 or Per1/Per2 double-KO mice. By using this system to comprehensively analyze all of the N-methyl-D-aspartate (NMDA) receptor family members, we found Nr3a as a short-sleeper gene, which is verified by an independent set of triple-target CRISPR. These results demonstrate that the triple-target CRISPR method enabled highly efficient gene KO phenotype screening with lesser efforts and fewer animals.

2C01 Analysis for factors affecting the tapeworm (*Hymenolepis diminuta*) biomass in infected rat

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Hymenolepis diminuta is a parasite of the rat small intestine and is easily maintained in the laboratory using rats (definitive host) and flour beetles (intermediate host) . This tapeworm is regarded as a valuable model for the analysis of cestode-host interactions. In this study, we report the tapeworm biomass in three rat strains with compromised immune systems: X-linked severe combined immunodeficient (XSCID) rats lacking T, B, and NK cells; nude rats that are T cell deficient; and mast cell deficient rats. Rats were infected with five cysticercoids and worm biomass in the small intestine was determined after three weeks.

Total worm weight in XSCID (F344-xscid) rats was greater than controls (F344) indicating that the permissible capacity of worm biomass in the rat small intestine is controlled by host immunity. Total worm weight in nude rats (F344-rnu) was intermediate between XSCID and control rats suggesting that both T cells and other immune cells (B and NK cells) are involved in determining the permissible capacity of worm biomass. Total worm weight was not much different between mast cell deficient (WsRC-Ws/Ws) and control (WsRC-+/+) rats. Thus, mast cells are not major effector cells for the control of permissible capacity of worm biomass.

Effects of antibiotics with different mechanisms of action on chronic hyperplastic candidiasis in diabetic mice

2C02

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【Introduction】
The chronic hyperplastic candidiasis (CHC) caused by *Candida albicans* (*C. albicans*) infection is characterized by proliferation of mucosal squamous epithelium with chronic inflammation in the oral cavity and esophagus, and the lesion progresses to cancer in some patients. Oral administration of *C. albicans* to diabetic mice induces squamous proliferative lesions with *C. albicans* in the forestomach. Thus, we established a murine model for CHC. Meanwhile, CHC is inevitably accompanied by bacterial infections as well as *C. albicans* infection. In this study, we evaluated whether antibiotics with different mechanisms of action vary *C. albicans* infection, inflammation and proliferative lesions.
【Methods】
Diabetes was induced by alloxan treatment at 6 weeks of age. Female ICR mice were divided into 5 groups. Control diabetic mice were given chlorinated water (AL), and 4 groups were treated with different antibiotics, tetracycline (AL+TC), penicillin (AL+PCN), streptomycin (AL+STR), and penicillin and streptomycin (AL+PS), respectively, from 11 weeks of age. They were sacrificed at the 41 weeks of age.
【Results】
Squamous hyperplasia of the forestomach was induced in almost all mice, and the degree of this lesion was the strongest in the AL+TC group. Yeast-like and hyphal form- *C. albicans* and bacteria were infected in the cornified layer of mucosal surface in all groups. The shape of *C. albicans* varied between groups, and yeast-like and hyphal form-fungi predominated in the AL+PCN and AL+PS groups, and the AL+TC group, respectively. In addition, the number of viable *C. albicans* and bacteria cultured from the mucosa was the largest in the AL +PCN group followed by the AL+PS group.
【Conclusion】
Antibiotics with different mechanisms of action could change the number of bacteria as well as the number and shape of *C. albicans*. However, these changes were not correlated with the severity of the mucosal proliferative lesion.

Murine vasculitis model mimicking Kawasaki disease by administration of *Lactobacillus casei* cell wall extract

2C03

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【Background】 Kawasaki disease (KD) is a systemic vasculitis of unknown etiology that affects young children. About 2.6% of KD patients have coronary artery lesions (CALs) as complications, with the resultant aneurysmal rupture occasionally being lethal. However, since the mortality rate is quite low, the mechanisms of onset of CALs in KD have not been elucidated. After single peritoneal injection of *Lactobacillus casei* cell wall extract (LCWE), mice develop coronary arteritis that resembles the CALs found in human KD.
【Aim】 To evaluate the characteristics and time course of coronary vasculitis induced by LCWE.
【Methods】 Four-week-old male C57BL/6J mice were intraperitoneally injected with 300 μg of LCWE. Serial sections of their aortic roots including bilateral coronary arteries, myocardium, and epicardium were extracted at pre-injection and 3, 5, 7, 14, and 28 days after injection (n=5–15 in each group) and stained with hematoxylin and eosin, Elastica–Masson (elastin), and CD169 (macrophages). For flow cytometry analysis and serum cytokine measurement, blood samples were collected at 2, 6, and 24 h and 14 and 28 days after LCWE or phosphate buffered saline injection.
【Results】 Inflammatory cells were observed around coronary arteries 3 days after LCWE injection. On the seventh day after injection, aortitis and coronary perivasculitis peaked in severity. Additionally, the incidences of pericarditis and myocarditis in LCWE-treated mice were 67% (10/15) and 80% (12/15), respectively. Moreover, arterial wall destruction including elastin breaks and intramural hemorrhage were observed 28 days after LCWE injection.
【Conclusion】 We present here a murine vasculitis model with pathological features similar to KD-related CALs in humans. This mouse model should thus help in developing novel therapeutic drugs and elucidating the pathology of CAL formation.

2C04 Deficiency of secretory-type non-specific ribonuclease in mice causes immune abnormality

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Ribonuclease (RNase) is thought to play an important role in maintenance of homeostasis by rigorously controlled RNA degradation. However, it is reported that a wide variety of molecules has RNase activity, and the roles of RNase in immune homeostasis had not been completely elucidated. In this study, we focused secretory-type non- specific (sns) RNase and analyzed the function of snsRNase *in vivo* by generation of knock-out (KO) mice.

In mice, snsRNase are encoded by duplicated genes with strong homology at a distance of 1 Mb in the same chromosome, so we tried to generate KO mice by CRISPR/Cas9 method. To reduce off-target risk, off-set nicking strategy was adopted in this study (Fujii W *et al*., BBRC 2014) . Mutation was introduced into snsRNase genes with high efficiency, and *cis*-targeted lines were successfully established in C57BL/6N back- ground. All snsRNase KO mice spontaneously developed splenomegaly and abnormal accumulation of immune cells in lung and liver at 8 weeks old.

These data suggested that deficiency of sns- RNase caused immune abnormality under dysregulation of non-specific RNA degradation. snsRNase KO mice are thought as a novel, unique disease model animal.

2C05 Generation of mice cloned from antigen-specific CD4$^+$ T cells

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Although somatic cell nuclear transfer (SCNT) is a sole reproductive technique that enables us to produce individuals from somatic cells, types of somatic cells which can be used as nuclear donors have been still limited. T cells possess antigen-specific T cell receptors and play significant roles in various immune system. However, SCNT from T cells has never been successful with one-step procedure. In this study, we attempted to produce mice cloned from CD4$^+$ T cells immunized by specific antigen to understand detailed functions of CD4$^+$ T cells. We used (BALB/c x DBA/2) F1 (CDF1) mice as nuclear donors. CDF1 male mice were immunized with mite, cedar pollen or ovoalbmin antigens. Isolated CD4$^+$ T cells were cultured *in vitro* and used for SCNT donors. SCNT was carried out with the Honolulu method. Reconstructed embryos were treated with Latrunculin A and trichostatin A. As a preliminary experiment, we attempted to produce mice cloned from peripheral lymphocytes collected from a non-immunized CDF1 mouse. Although we successfully obtained one cloned mouse for 46 transferred embryos (2.2%) , the 2-cell ratio was low because almost half of embryos were fragmented before the 2-cell stage (2-cell ratio: 46.9%, 46/98) . With sensitized T cells, we generated 1,817 transferrable T cell cloned embryos and obtained 33 fetuses. Among them, 20 mice grew into adults. When we examined reactivity to the immunized antigen using peripheral lymphocytes, nine mice showed antigen specific reactivity. In this study, we demonstrated that CD4$^+$ T cell can be utilized as nuclear donor for SCNT. As CD4$^+$ T cells have been demonstrated to be involved in immunological diseases, our CD4$^+$ cloned mice may be applicable for analysis of these diseases.

Rapid development of allergic airway inflammation in cloned mice of antigen-specific CD4+ T cells

2C06

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$CD4^+$ T cells are essential for the development of allergic and immunological diseases. Topical application of antigens to target tissues of animals previously immunized by systemic injection of corresponding antigens induces tissue-specific allergic inflammation. By employing cloned mice generated from antigen-specific $CD4^+$ T cells, new murine models in which upper and lower airway inflammation could be induced simply by several antigen applications were developed. Cloned mice generated from $CD4^+$ T cells of *Dermatophagoides* mite-immunized mice were repeatedly challenged by intranasal injection of the mite antigen with 3 to 4-day interval. At 72 h after the last antigen challenge, bronchial asthma -like airway inflammation was evaluated by bronchoalveolar lavage and the assessment of bronchial hyperresponsiveness (BHR). Following only 4-time antigen challenge, the lung infiltration of inflammatory cells and BHR were induced in cloned mice expressing both rearranged TCR α and β from donor $CD4^+$ T cells but not in wild-type mice. The existence of either rearranged TCR α or β was sufficient to induce the airway inflammation. Allergic rhinitis-like nasal inflammation represented by immediate nasal response and nasal hyperresponsiveness was induced in the cloned mice from $CD4^+$ T cells of ovalbumin (OVA) -immunized mice by only 3 to 5-time antigen challenge. The nasal inflammation was also induced in mice expressing either rearranged TCR α or β.

The pathogenesis of asthma and allergic rhinitis could easily be investigated by using cloned mice from antigen-specific $CD4^+$ T cells.

DNCB-induced dermatitis in KFRS4/Kyo rats

2C07

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【Background】

We have recently established female KFRS4/Kyo rat as a model of atopic dermatitis (AD). The KFRS4 rats spontaneously develop dermatitis that accompanied an elevation of IgE and scratching behavior. In the skin lesions, mast cells and eosinophils are observed. Although the dermatitis in the KFRS4 rats closely resembles that seen in human AD, it takes almost 6 moths that the dermatitis develops. It was reported that hapten can induce stable clinical AD-like skin diseases in NC/Nga mice. Here, we examined the hapten-induced dermatitis in the KFRS4 rats.

【Materials and Methods】

We used 2,4-dinitrochlorobenzene (DNCB) to induce the dermatitis. For sensitization, 1.5 % DNCB was applied on right ears of KFRS4 and PVG rats at day1 and day3. At days 7, 9, 11, 16, and 18, 1.5 % DNBC was challenged on right ears. At 6 hrs after each challenge ear thickness was measured. At the end of experiments, blood samples were collected for IgE measurements and ears were harvested for histopathological examination.

【Results and Discussion】

We observed swelling of ears at day11 in PVG and at day16 in KFRS4 rats, respectively. Although numbers of mast cells significantly increased in both strains, no infiltration of eosinophils was observed in both strains. Serum IgE level was elevated in PVG but not in KFRS4 rats. These findings indicated that the DNCB could induce dermatitis in KFRS4 rats in almost 2 weeks and suggested that the KFRS4 rats exhibited more resistant to the DNCB-induced dermatitis.

Generation of inner ear cells from iPS cells targeting hereditary deafness

2C08

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GJB2 encodes connexin (Cx) 26, a component in cochlear gap junction. We recently demonstrated that the drastic disruption of gap junction plaque (GJP) macromolecular complex composed of Cx26 and Cx30 are critical pathogenesis starting before hearing onset (Kamiya, Journal of Clinical Investigation, 2014;124 (4) :1598–1607) . To develop the effective therapy for GJB2 associated hearing loss, restoration of gap junction plaque (GJP) macromolecular complex using virus vectors or multipotent stem cells such as induced pluripotent stem (iPS) cells are expected to rescue the hearing function of GJB2 related hearing loss. Mouse induced pluripotent stem cells (iPS) were used for generation of Cx26-expressing cells with proper gap junction plaque between the cells. Adeno associate virus (AAV) were used for the GJB2 gene transfer and restoration of GJP (Human Molecular Genetics. 2015, 24 (13) :3651-61) . By differentiation of iPS cells, we generated the Cx26-expressiong cells with large gap junction plaque as cochlear cells. Furthermore, these cells from CX26-deficient mice recapitulated the drastic disruption of GJPs, the primary pathology of GJB2-related hearing loss (Fukunaga, Stem Cell Reports, 2016, 7 (6) , 1023–1036) . These in vitro models should be useful for establishing inner-ear cell therapies and drug screening that target GJB2-related hearing loss.

Oncomodulin plays essential roles in maintaining the organ of Corti of cochlear outer hair cells

2C09

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The oncomodulin gene (*Ocm*) encodes an EF-hand Ca^{2+}-binding protein specific expressed in cochlear outer hair cells (OHCs) . We established Ocm-deficient ($Ocm^{-/-}$) mice and analyzed the hearing phenotypes to study the roles of OCM in the auditory system. The ABR measurements confirmed that $Ocm^{-/-}$ mice exhibit early-onset progressive hearing loss to sound stimuli at 4–32 kHz. DPOAEs of the cochleae in $Ocm^{-/-}$ mice were also drastically decreased across the 4–32 kHz frequency range. In morphological phenotypes, there were no remarkable differences in spiral ganglion, spiral ligament, and stria vascularis between WT and $Ocm^{-/-}$ mice. However, the outer tunnel in the organ of Corti (oC) was significantly narrow in $Ocm^{-/-}$ mice. SEM imaging revealed that $Ocm^{-/-}$ mice exhibited progressive degeneration of stereocilia (SC) . Typically, SC form the normal staircase shape at 3 weeks of age. However, we found that the SC of $Ocm^{-/-}$ mice are slightly degenerated. The morphology of SC showed age-related degeneration. Several SC on the OHCs of $Ocm^{-/-}$ mice were lost, shortened, and disrupted at 3 and 5 months of age. Although there are no significant difference in the number of OHCs between $Ocm^{-/-}$ and WT mice at 1 months of age, losses of approximately 15% and 67% were observed at the 3 and 11 months of age, respectively, in $Ocm^{-/-}$ mice. Thus, our data suggests that OCM is involved in the maintenance of the oC.

2C10 Establishment and analysis of mitochondrial disease model mice with pathogenic mtDNA mutation

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Mitochondria are organelle producing ATP and containing hundreds to thousands copies of mitochondrial DNA (mtDNA) per cell. Accumulation of pathogenic mtDNA mutations results in respiratory dysfunction and induces mitochondrial diseases. The expression mechanisms of disease phenotypes are unclear, and no therapeutic protocol has been developed. To elucidate these problems, establishment and analysis of disease model mice is required. However, genetic manipulation of mtDNA is not easy contrasting to nuclear DNA, thus establishment of mitochondrial disease model mice is also very difficult despite causal mutations have been identified.

MELAS, a mitochondrial disease with the highest morbidity, is mainly caused by point mutations on $tRNA^{Leu\ (UUR)}$ gene encoded by mtDNA. Establishment of MELAS model mice is effective for understanding of MELAS expression mechanisms and development of therapies. We are now challenging to this important and difficult task, and in this presentation, current progress will be reported. First, mtDNA-encoded $tRNA^{Leu\ (UUR)}$ region of mutagen-treated mouse cell line was sequenced clonally, and the A2748G mutation was identified. This mutation corresponds to the human A3302G mutation reported as one of causal mutations for MELAS. Next, we established the mouse cell line with high-rate of the A2748G mutation, and confirmed induced respiratory dysfunction. Then the A2748G mutation was introduced into mouse ES cells by fusion of ES cells and enucleated these cells. Currently, a chimera mouse was obtained from this ES cell line, however, the A2748G mutation was not detected from of the offspring of the chimera mouse. We are now evaluating mitochondrial function of each tissue obtained from this chimera mouse.

2C11 Development of Model for Recessively Transmitted Male Genital Diseases in Mouse

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In human, male genital diseases are general term for several diseases including prostate disease, erectile dysfunction and testicular tumor. In particular, male genital diseases are associated with symptoms of functional and/or morphological aberration in male genital system. Mouse models are expected to become the key to the treatment of intractable illnesses such as male genital diseases. In our project, we have been aimed to develop mouse mutants by genome-wide screening for various phenotypes observed in ENU-mutagenized mice to provide resources for studying the functions of genes, and to establish animal models for human diseases. In the recessive screening, we found out that the mutant family with symptoms identical to human male genital diseases, priapism. The phenotype state with onset usually around the 5 weeks, remain in maintenance of erection. Furthermore, this mutant showed body size, gait abnormality, but survival curve within the normal range. We begin more elaborate studies about histological analysis for male genitals, reproduction-related and lipid metabolic analysis. At the same moment, to gene identification, we have been next-generation sequencing. As a result, we found out a missense mutation of a candidate gene. We report here on recent progresses concerning the association between phenotypes and a candidate gene.

2C12 Prmt5 in mouse primordial follicle oocytes is essential for oogenesis

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Oogenesis in mammals is a complex process involving bi-directional communication between the oocyte and surrounding soma. In the mouse ovary, primordial follicles containing the dormant oocyte are surrounded by flatten soma, which are formed shortly after birth. The primordial follicles are stored in the ovaries and provide growing follicles, which act as the primary, secondary, and antral follicles during the entire reproductive lifespan. For long female reproductivity, it is essential to retain the dormant oocytes in the proper state, such as by preserving genome integrity. Here, we report that the arginine-methyltransferase Prmt5 is preferentially expressed in mouse oocytes of primordial and primary follicles and that it interacted with Sohlh1, which is an essential transcription factor for oocyte maturation. We are currently analyzing an oocyte-specific *Prmt5* conditional knockout mouse; in these mice, females showed sterility after approximately 10 weeks of age due to the upregulation of retrotransposons and the disorganization of organella. In the meeting, we would like to discuss the molecular function of *Prmt5* in oogenesis.

2C13 Expression changes in uterine epithelial genes in implantation-defective mice due to *Sox17* haploinsufficiency

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Implantation is a complicated phenomenon with drastic morphogenetic changes at the cellular and tissue levels. We have revealed that *Sox17* heterozygous mutant females are subfertile due to impaired implantation. However, the role of *Sox17* in implantation remains largely unknown. Here, we report expression profiles of group-F Sox genes *Sox17* and *Sox7* and possible *Sox17* downstream genes in the uterine epithelium. In quantitative PCR analysis, intense *Sox17* expression in the uterine epithelium started from day 3 of pregnancy, reached at the maximal level at day 4, at which the uterus is receptive for embryo implantation, and slightly decreased in day 5. Distinct from *Sox17*, *Sox7* expression was maximal at day 1 and decreased by 50% at day 4, implicating different role of *Sox7* in pregnancy. Microarray analysis was performed to compare gene expression profiles between wild-type and *Sox17* heterozygous mutant uterine epithelium at day 3. Expression of *Sox17* in *Sox17* heterozygous mutant was 0.6-fold lower than that of wild-type. Among other *Sox genes*, *Sox7*, *Sox5*, and *Sox9* showed 2-, 0.5-, and 2.4-fold changes in *Sox17* heterozygous mutant. Gene ontology analysis showed that decreased and increased genes were predominantly categorized in extracellular- and cell death-related functions, respectively. Currently we are investigating which gene (s) are the most responsible for the implantation failure. *Sox17* is also expressed in human uterine epithelium. Therefore, it is expected that clarification of the role of uterine *Sox17* in mice may contribute to understand pathology of implantation failure in human and to improve the success rate in IVF treatment.

Analysis of Placenta of *Chst14* Gene-deleted Mice as the Model of DDEDS, a New Type of Ehlers-Danlos Syndrome

2C14

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Recently, a new type of Ehlers-Danlos syndrome (EDS) called dermatan 4-O-sulfotransferase-1 (D4ST1) -deficient EDS (DDEDS) was reported. Mutation of *CHST14* gene induces loss-of-function in D4ST1, and is the cause of DDEDS. Ablation of D4ST1 induces impaired assembly of collagen fibrils and can be cause of embrittlement connective tissues. DDEDS is characterized by multiple congenital malformations and progressive fragility related manifestations. Especially, the large hematomas are one of the most serious complications accompanied by decreased QOL and potential lethality. However, the mechanisms of the symptoms are unclear.

In this study, we evaluated *Chst14* gene-deleted mice (*Chst14-/-*) as the model animal of DDEDS. Most *Chst14-/-* mice died in utero and only limited number of adult mice were available. In this study, we investigated embryo and placenta of *Chst14-/-*. The embryo showed short stature, but body weight and appearance were not changed in E18.5. The placenta of *Chst14-/-* fetal mice showed reduced weight, alterations in vascular structure, and ischemic changes. D4ST1 was not detected in the placental villus of *Chst14-/-* fetal mice. Electron microscopy demonstrated abnormal structure of vascular basal laminae on the villus. Gene expressions of collagens were not changed. These results suggested that structural change of extracellular matrix influences the condition of DDEDS rather than expression of collagens.

These findings suggest that *Chst14* gene would essential for placental vascular development in mice. Furthermore, placenta of *Chst14-/-* could be a useful model for the symptoms with vascular manifestations such as large subcutaneous hematomas.

Function analyses of novel gene mutations causing small eye, omphalocele, and respiratory failure in mice

2C15

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RIKEN BioResource Center

In the ENU mutagenesis project of RIKEN GSC we found two mutant mice showing the optic nerve coloboma caused by an autosomal dominant mutant gene. As the results of fine mapping of the causable gene, different nonsense mutations were detected in a same locus. It is novel gene that has not been reported about human patients of hereditary disorder and mouse gene targeting by this gene.

For understanding of the gene function in mammals, the homozygous mice were produced. As the results, microphthalmia, spinal curve, and omphalocele (about 20% in homozygous mice) were observed in both mutants and the newborn pups died by respiratory failure with cyanosis. In addition, lung morphological abnormality, bone morphological abnormality, failure of optic fissure closure, loss of retinal pigment, nuchal edema, and hydronephrosis were observed in the both mutant mice. These results indicate the importance of the gene for the ontogeny of mammals and survival after birth.

In this study, we will identify the details of characters of the each phenotype by molecular biological and histological analyses.

2C16 Melanocyte differentiation in a novel pink-eyed dilution mouse showing age-related pigmentation in the eyes and coat hair

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Oca2$^{p\text{-}cas}$ (oculocutaneous albinism II; pink-eyed dilution castaneus) is a new coat color mutant gene on mouse chromosome 7 that arose spontaneously in Indonesian wild *Mus musculus castaneus*. Mice homozygous for *Oca2*$^{p\text{-}cas}$ usually have pink eyes and gray coat on a nonagouti C57BL/6JJcl (B6) background. Recently, a novel spontaneous mutation occurred in a mixed strain carrying this gene on the B6 background (Exp. Anim. 64: 207, 2015) . The eyes and coat hair of this novel mutant progressively become dark with aging. In this study, we clarified differences in melanocyte proliferation and differentiation between the ordinary pink-eyed and novel black-eyed mutants using a serum-free primary culture system of melanocyte-proliferation medium. The proliferation of melanoblasts did not differ between the two mutant mice. However, when L-tyrosine was added in the medium, black-eyed melanocytes greatly differentiated in a concentration-dependent manner, compared to pink-eyed melanocytes. Immunocytochemistry demonstrated that the protein expression of TYR and TYRP1 was increased both in pink-eyed and in black-eyed melanocytes whereas MITF expression was increased only in black-eyed melanocytes. Real-time PCR analysis of the eyes revealed upregulation of *Mitf* mRNA expression in black-eyed melanocytes. The results suggest that the age-related darkening in the black-eyed mutant may be caused by the increased ability of melanocyte differentiation dependent on L-tyrosine through the upregulation of TYR, TYRP1 and MITF.

2C17 Preparation of partial spinal intervertebral disc defect model in the sheep and its maintenance

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HAMRI CO.,LTD.

【Introduction】 Recently sheep are increasingly used in studies on the spinal diseases, while the usage of this species as an experimental animal is lower than other species in Japan. The contract of such studies has been initiated in our company in 2016. In this study, preparation and maintenance of a partial spinal intervertebral disc defect model and its maintenance in the Suffolk strain was examined.

【Materials and methods】 Two male Suffolk strain sheep were used. These animals were housed in a pen at room temperature and fed with solid diet. In addition, grass hay and mineral salt was adequately given and water freely to prevent urinary calculus formation. The pen was cleaned twice a day to prevent from infectious diseases fur staining with animal excreta.

A partial spinal intervertebral disc (SID) defect model was prepared by removing a central part (0.1g) of nucleus pulposus from SID of lumbar vertebra at L1/L2 and L5/L6. In order to prevent a reflux of gastric contents, animals were fasted for 4-5 days before operation and were held in a recumbent position and a respirator was used during operation. Disinfectant, antibiotic and analgesic agent were adequately administered after operation. After 5 weeks of observation period the tissue specimen were prepared and examined histopathologically.

【Results】 No abnormalities were seen throughout the observation period. Nucleus pulposus defect was histopathologically noted in a central part of each SID examined.

【Conclusion】 A partial SID defect model was successfully prepared and maintained for a significant period of time under animal care and operation conditions applied in this study. This model was considered useful for the development of a new therapeutic agents against a partial SID defect or for some related studies.

2C18 Cathepsin S over-expressed transgenic mice manifests excessive autoimmune responses and renal disorders

○ Jinhee Lee, Zae Young Ryoo

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Systemic lupus erythematosus (SLE) is an autoimmune disorder which affect systemic multi-organ. Despite the various investigations recently, the mechanisms of lupus are not completely understood. We injected pristane to Cathepsin S (CTSS) over-expressed transgenic (Tg) mice. CTSS over-expressed mice demonstrated a predominant increase in IL-17, TNF-alpha, IFN-alpha and IFN-gamma cytokine production which are known as cytokines associated with lupus. And autoantibody levels of anti-dsDNA and ANA were both significantly increased. The glomerulonephritis are also severe that confirmed deposition of immunoglomulin and complement and albumin level of uria. These phenotypes are much more excessive when treat pristane on Tg mice than Tg non-treated mice, but symptoms of Tg mice without pristane are severe than pristane treated WT mice. These results demonstrate that CTSS are required for aggravate lupus-like autoimmunity and end-organ damage induced by pristane. We think this is due to the inherent characteristics of CTSS which involve to increase the expression of MHC class II in macrophage and granulocyte. The CTSS over-expressed Tg mice are good for experimental lupus disease model.

2C19 The expression of Lin28a protects streptozotocin-induced beta-cell destroy, preventing type 1 diabetes in mice

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Lin28a is highly a conserved RNA-binding protein and represses miRNA, let-7. Lin28a has an effect on early embryogenesis timing. In previously studies, Lin28a regulates glucose metabolism and insulin sensitivity and promotes cancer cell proliferation. The over-expression of Lin28a was enhanced cell proliferation in pancreatic beta cells, Min6 cells, and the ability of glucose transport. Next, we treated streptozotocin (stz), killing pancreatic beta cells. Lin28a over-expressed pancreatic beta cells more survived compared to mock cells. Lin28a was increased proliferation and decreased apoptosis in stz-treated cells. Also, glucose transport was enhanced in Lin28a over-expressed cells. Lin28a inhibited let-7 expression and up-regulated PI3K/Akt signaling pathway. Furthermore, We generated Lin28a over-expressed mice. We performed stz-treated experiments like type 1 diabetes in vivo. Lin28a over-expressed mice were decreased blood glucose levels and survived pancreatic beta cells. Their pancreas secreted more insulin than WT's pancreas in stz-treated mice. In conclusion, lin28a protected stz-induced pancreatic beta cell destruction and promoted cell proliferation in pancreatic beta cells.

2C20 JAZF1 can regulate the expression of lipid metabolic genes and required for adipogenesis

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Jazf1 (Juxtaposed with another zinc finger protein 1) is a 27 kDa nuclear protein containing three putative zinc finger motifs that is associated with diabetes mellitus and prostate cancer; however, little is known about the role that this gene plays in regulation of metabolism. The differentiation of committed preadipocytes to adipocytes is controlled by PPARgamma and several other transcription factors, but the molecular basis for preadipocyte determination is not understood in adipogenesis. To elucidate Jazf1's role in adipocyte differentiation, we fed a 60% fat diet for up to 8 weeks in Jazf1 +/- mice. We determined that weight gain was found to be markedly impaired in Jazf1 +/- mice. Furthermore, in the molecular basis analysis, we found that Jazf1 can regulates PPARgamma expression. These results suggest that Jazf1 plays a required for adipogenesis. Finally, Jazf1 may provide a new therapeutic target in the management of obesity and diabetes.

2C21 hMAGEA2 promotes progression of breast cancer by regulating Akt and Erk1/2 pathways

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Breast cancer is the most abundant cancer worldwide and a severe problem for women. Notably, breast cancer has a high mortality rate, mainly because of tumor progression and metastasis. Triple-negative breast cancer (TNBC) is highly progressive and lacks the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Therefore, there are no established therapeutic targets against TNBC. In this study, we investigated whether the expression of human melanoma-associated antigen A2 (hMAGEA2) is associated with TNBC. We found that hMAGEA2 is significantly overexpressed in human TNBC tissues; we also observed oncogenic properties using TNBC cell lines (MDA-MB-231 and MDA-MB-468). The MDA-MB-231 cell line, in which hMAGEA2 was overexpressed, showed dramatically increased cellular proliferation, colony formation, invasion, and xenograft tumor formation and growth. Conversely, knockdown of hMAEGA2 in MDA-MB-468 cell line suppressed cellular proliferation, colony formation, and xenograft tumor formation. Additionally, we showed that hMAGEA2 regulated the activation of Akt and Erk1/2 signaling pathways. These data indicate that hMAGEA2 is important for progression of TNBC and may serve as a novel molecular therapeutic target.

Humanized mice as a small animal model for measles virus infection

2D01

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Humans are the only natural hosts of measles virus (MV). To elucidate the pathogenic mechanisms upon MV infection, various animal models have been established. In particular, nonhuman primate models utilizing monkeys are susceptible to MV infection and develop symptoms that are similar to those of human patients; efficient infection to lymphocytes, leukopenia, and rushes. However, experiments in monkeys are expensive and cumbersome. Recently, mouse lines expressing MV receptor human SLAM were reported, but limited MV replication in lymphoid tissues was supported only when crossed with mice lacking IFN-a/b receptor.

In this study, we aimed to establish a small animal model for MV infection by utilizing humanized mice, which were produced by transplantation of human CD34-positive cord blood cells into NSG immunodeficient mice. When we challenged the humanized mice with a recombinant wild-type MV expressing EGFP, a strong EGFP fluorescence was observed in spleen, lymph nodes, and bone marrow. The fluorescence was confirmed to associate with MV infection of human T and B cells. Leukopenia and subsequent recovery of human lymphocyte was also observed. Moreover, green fluorescent spots similar to skin rashes were observed on the abdominal skin. The spots were found to be due to accumulation of human lymphocytes around the hair follicles. These results indicate that our small animal model resembles the nonhuman primate model and would be useful for elucidating the mechanism of MV infection and its pathogenesis.

Humanized mouse as a model of human pregnant immunity

2D02

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【Background】The mother constructs a specific immune system during pregnancy to accept its allogenic fetus. We hypothesized that this pregnant immunity system might correlate with cancer immunity. Beause, the study of human pregnant immunity has limits, we tried to establish the humanized pregnant NOG mouse system as a research model of human pregnant immunity and to analyze the mobility of engrafted T cells.

【Materials and Methods】Mated female immunodeficient NOG mouse was transplanted with 5×10^6 human PBMC, which we call humanized pregnant NOG mouse (pPB-NOG). Fourteen days later, bone marrow, peripheral blood, spleen, and placenta were removed from the mouse and cell suspension of each tissue was prepared. These cells were stained with fluorochrome-labeled antibodies against human antigen, and analyzed using the Flow cytometry (FCM). Cell apoptosis was analyzed by TUNEL method, and the localization of the transplanted human leukocyte was monitored using immunohistochemical staining with anti-CD45 antibody.

【Results】The structure of pPB-NOG mouse placenta was similar to that of normal mouse, and the number of fetus was not significantly decreased. The human leukocyte cells were localized in the spongiotrophoblast of fetus origin in the placenta. However the number of the apoptotic cell was in the normal range and only in the decidua of mother's origin, and apoptosis was not enhanced. The percentage of killer T cell was significantly higher than that of helper T cell in pPB-NOG placenta. While, helper T cells were observed more frequently than killer T cells in the spleens. According to our results, the immunity of humanized mouse tend to be suppressed by pregnancy, especially cytotoxic, while the killer T cell were dominant in the placenta.Body.

Immune response induced by allergens sensitization in mice: Comparative analysis between dog allergen Can f 1 and ovalbumin

2D03

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【Objective】
Many studies on induced mechanisms of food allergies have been performed using mice sensitized by ovalbumin (OVA). However, there have been few reports about induced mechanisms of animal allergies. In this study, to reveal whether animal allergen sensitization induced the immune response similar to OVA sensitization, we analyzed the immune response induced by dog allergen Can f 1 compared with OVA sensitization in mice.
【Materials and Methods】
Purified Can f 1 protein (INDOOR) and OVA (Sigma) was used as antigen of animal allergies and food allergies, respectively. Female C57BL/6 (B6) and BALB/c mice were sensitized s.c. in the neck with 5 μg antigens or PBS (negative control) only or adsorbed 1 mg aluminium hydroxide (Alum) on day 0, 14, 28 and the mice were euthanized on day 35. Serum levels of IgE and cytokine content, IL-13 and IFN-g, in splenocyte stimulated by the 5 μg antigens in vitro culture supernatants were analyzed by ELISA.
【Results and Discussion】
The serum levels of IgE were significantly increased in BALB/c than C57Bl/6 sensitized by OVA only. In contrast, no between-strain differences were observed in the mice sensitized by Can f 1 only. The production of IL-13 was significantly increased in the supernatants from the splenocyte of the mice sensitized by Can f 1 only than Can f 1 with Alum in both strains. On the other hand, no differences between OVA only and OVA with Alum were observed in the mice of both strains. These results suggest that Can f 1 sensitization induced different immune responses from OVA sensitization.

Development of antibody production method using goldfish

2D04

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Antibodies are an essential molecular and biological tools for widely utilizing not only basic biology, antibody drugs, inspection diagnostics, and analytical reagents. However, since host animals among well-conserved to each other have never produced the antibodies against structurally complicated proteins such as GPCR and post-translated proteins by sugars and lipids that have already been existed in their cells, it is very difficult to obtain specific and high titer antibodies against them. In this study, we have focused on the lymph fluids of goldfish, Bubble Eye as a host animal having both innate and acquired immunity that are located at upstream among vertebrates and have developed antibody production method using them. The anti-gIgM antibody was prepared from rabbits for detection of antibodies in the lymph fluids of Bubbly Eye. The antigen was used for the region of heavy chains in gIgM which was cloned expressed in Escherichia *coli* and specific anti-gIgM polyclonal antibody was obtained by injected into the rabbits. Next, after immunization of EGFP as an antigen to the lymph fluids of goldfish several times, the lymph fluids were taken from them. Then, the lymph fluids including gIgM were used to the method of ELISA as detection against EGFP as a target protein. By using anti-gIgM polyclonal antibody against rabbits, gIgM in the lymph fluids was detected and quantitatively estimated. Furthermore, specific anti-gIgM antibody of goldfish that EGFP as an antigen was injected was produced and detected. In future works, while a gene library of specific anti-gIgM antibodies from immunized goldfish is constructed and screened, highly specific and affinity ScFv (single chain antibody) against a target antigen would be obtained and characterized towards drug discovery for antibody drugs.

Survey of Murine astrovirus in mouse experimental facilities in Japan

2D05

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【Purpose】
Astrovirus is one of the viruses isolated from many mammals and is known to cause viral enteritis. Recently mouse astrovirus (Murine astrovirus) has also been detected from mice in laboratory animal facilities. This Murine astrovirus (MuAstV) is thought to be nonpathogenic to mice, but its distribution and pathogenicity are unknown. The objective of the study is to reveal the prevalence of MuAstV in mouse experimental facilities in Japan.
【Method】
The survey was performed for three months (from October 2016 to December 2016). We examined 1212 mice (immunodeficient mice: 12 samples, immunocompetent mice: 1200 samples) from 226 facilities. Necropsy was performed on the all of mice, and nucleic acids from the cecum samples were extracted. All samples were tested by PCR for MuAstV and analyzed these sequences.
【Results and Conclusions】
As a result, 424 MuAstV infected mice were found in 1212 out of 226 facilities tested in mouse experimental facilities, and the facility positive rate was 50.4%, mice positive rate was 35.0%. Gross lesions in intestinal associated with MuAstV were not observed for both immunocompetent mice and immunodeficient mice. This survey provided evidence on the prevalence of MuAstV in mouse experimental facilities in Japan. Furthermore, it is necessary to confirm pathogenicity by infection experiment.

Development of immunochromatographic test for serological diagnosis of major infections in laboratory mouse

2D06

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【Objective】
We have reported that immunochromatographic (ICG) test is a rapid and simple test for serological monitoring of infectious diseases among laboratory rat (The 63rd JALAS). In this study we have aimed to apply ICG test for detection of antibody to major infectious diseases in mouse.
【Materials and Methods】
Following three major infectious diseases are selected: MHV (Mouse hepatitis virus), HVJ (Sendai virus), and Tyzzer (*Clostridium piliforme*). Antigens used in MONILIZA were used in ICG tests. Colloidal Gold WRGH2 (Wine red Chemicals) and Protein A (Nakarai) were used.
【Results and Discussion】
ICG tests were carried out with various concentrations of positive serum (1:50, 100, 200) and antigens (1:1, 10, 100). ICG test with antigens with following concentrations, MHV (0.5mg/ml), HVJ (0.5mg/ml), Tyzzer (0.6mg/ml), able to detect antibody at serum dilution of 1:200. Under the conditions, no nonspecific reaction was observed in normal serum. These results indicate that the ICG test will be applicable for mouse serum as a rapid, simple and safe diagnosis. Further studies for determine sensitivity and specificity will be necessary.

Effect of cultured cells on antibody test of mouse hepatitis virus (MHV)

2D07

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There are many nonspecific reactions when examining antibody test of mouse hepatitis virus (MHV). One of the causes is considered to be reaction with cultured cells used for antigen production. We compared nonspecific reactions using MONILISA, MHV-S strain antigen, MHV-Nu strain antigen and DBT cells antigen.

【Material and Methods】 ELISA was used for screening tests and IFA was used for conformation tests. Mouse and rat antisera were used to compare the differences in antigen reactivity. Furthermore, in total, 733 serum samples were tested.

【Results】 In experiments using 1000-fold diluted antisera, MONILISA, MHV-S strain antigen, MHV-Nu strain antigen was positive reaction (OD value 0.3 or higher).

DBT cells antigen were negative reactions (OD value of 0.01 or less).

Nonspecific reactions were compared using 733 serum samples from mice and rats.

The proportion of positive reactions (OD value 0.3 or higher) was 16.4% for MONILISA, 16.9% for MHV-S strain antigen, 16.8% for MHV-Nu strain antigen and 9.4% for DBT cell antigen. Only 6 samples were positive in IFA.

【Discussion】 Half of the samples showing nonspecific reactions were reactive to DBT cells antigen. From these results, we think that DBT cells antigen may cause nonspecific reactions.

Investigation of non - specific reaction caused by the ELISA

2D08

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Antibody screening test by ELISA often shows non-specific reaction to high detection sensitivity. In this study, we investigated non-specific reaction of ELISA that samples had been serological test at ICLAS Monitoring Canter in 2016. The test items are Sendai virus (HVJ), Mouse hepatitis virus (MHV), *Mycoplasma pulmonis* (Myco), *Clostridium piliforme* (Tyzzer), Hantavirus (Hanta) and Ectromelia virus (Ectro). This study was carried out by following two method, ① First screening was performed using ELISA (cut-off value was set to OD 0.3). Positive samples were additionally tested by IFA. The number of samples was 25,380. ② Positive samples that self tested by user using the ELISA kit (MONILIZA, Wakamoto pharmaceutical Co., Ltd.) were tested by IFA. The number of samples was 2,480.

The results are shown in below. ① [Number of samples ; IFA positive / ELISA positive] HVJ [20,297 ; 3/370], MHV [20,378 ; 43/2630], Myco [20,344 ; 7/477], Tyzzer [20,660;47/ 518], Hanta [4,503 ; 0/1374] and Ectro [21,777, 0/540]. ② [IFA positive/ELISA (Moniliza) positive] HVJ [0/362], MHV [31/1,108 (2.8%)], Myco [0/252], Tyzzer[74/384 (19.3%)] and Hanta [0/781]. Theses result indicate that frequency of non-specific reaction of MHV and Hanta are higher than other test items.

Comparison of fecal DNA extraction kits for analysis of murine gut microbiota

2D09

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The success of microbiota analysis using molecular techniquies requires effective cell lysis of various bacteria in the materials. Since the physical disruption using bead-beating is known as a method for efficiently lysing bacterial cells, we perform bead-beating before DNA extraction by phenol/chloroform method. Currently, several companies sell fecal DNA extraction kits including bead-beating step. These kits can easily and rapidly recover high quality, inhibitor-free DNA from the materials. In this study, we compared the relative efficacy of two commercial DNA extraction kits in extracting bacterial genomic DNA from murine fecal samples.

Feces samples were collected from 15 mice and rats, respectively. Each feces was thoroughly mixed and divided into three equal parts. Fecal DNA was extracted by conventional method or two commercially fecal DNA extraction kits. Fecal microbiota analysis was performed by terminal restriction fragment length polymorphism (T-RFLP) method. The T-RFs were analyzed by electrophoresis on an ABI PRISM 310 Genetic Analyzer in GeneScan mode.

As a result of T-RFLP analysis, the proportion of Lactobacillus was significantly lower in fecal DNA samples extracted by one kit than in fecal DNA samples extracted by other methods. This result suggested that one kit has low extraction efficiency of gram positive bacteria. Choice of DNA extraction kit significantly influences the results of downstream microbial community analysis and thus should be taken into consideration for gut microbiota analysis.

Detection of trichomonads of mouse by PCR and rRNA analysis

2D10

○ Ayako Kajita, Chiimi Ogawa, Hiromi Sakata, Naoki Hirano, Akemi Yasui, Noriko Hiraiwa, Atsushi Yoshiki, Fumio Ike

Experimental Animal Division, RIKEN BRC

Tritrichomonas muris is parasitic flagellate in intestines of mice. Although *T. muris* may shows no pathogenicity in mice, many mouse colonies were infested with *T. muris*. We regularly perform *T. muris* monitoring on mice by microscopy at RIKEN BioResource Center. In this study, we tried PCR detection of trichomonads among mice by using previously reported and newly designed primers. We also estimated rRNA partial sequence of *T. muris*.

【Materials and methods】 Cecal samples from mice housed in conventional facility were checked by microscopy. DNA was extracted from cecum using FastDNA kit (MP Biomedicals).

【Results and discussion】 Protozoa showing diagnostic undulating membrane in cecal contents from mice under microscopy were identified as *T. muris*. DNA extracted from cecum contents was applied to PCR. PCR products were used for DNA sequencing. One of obtained sequences showed 95% homology to 18S rRNA (Acc. No. AY886846) of *T. muris* infested in *Apodemus flavicollis*. It showed 85% homology with ITS1, 5.8S rRNA, ITS2 and 23S rRNA region. Another sequence showed 99% homology with 18S rRNA of *T. musculis* (KX000921). ITS1 and 5.8S rRNA region of this was conformed with those of *T. musculis* (KX000922) completely. At present, a limited number of trichomonad genes of mice are available on database. A textbook said morphologically identified several trichomonads inhabited in mouse intestines. Our results also indicate that genetically distinct trichomonads inhabits indeed in mouse intestines. Comprehensive genetic analysis is further necessary to detect trichomonads by PCR.

2D11 Application of Automated Hematology Analyzer XN-30 for an experimental rodent malaria model

○ Takahiro Tougan[1], Sawako Itagaki[1], Yuhgi Suzuki[2], Munehisa Izuka[2], Kei Aono[2], Tomonori Okazaki[2], Kinya Uchihashi[2], Toshihiro Horii[1]

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Malaria is one of three major infectious diseases. The malaria parasite is a unicellular eukaryote that infects humans via mosquitoes. The blood stage in humans causes symptoms such as fever, anemia, and splenomegaly, and is clinically important. Therefore, experimental rodent malaria model is used for malaria immunology, malaria biology, as well as for antimalarial drug development.

We have previously reported that the Automated Hematology Analyzer XN-30 (manufactured by Sysmex corp.) can rapidly calculate parasitemia and developmental stage ratio by detecting DNA content and morphological changes through the application of flow cytometry. Here, we report that XN-30 can measure parasitemia in mice by reanalyzing the data. Furthermore, XN-30 can also comprehensively recognize a change in the relationship between parasitemia and erythrocyte concentration in mouse blood after infection by simultaneously measuring the number of hematocytes.

In this presentation, we demonstrate that XN-30 can monitor changes in blood condition as well as precise parasitemia, and thus recommend the application of XN-30 for malaria biology and antimalarial drug treatment.

2D12 Survey of methicillin-resistant *Staphylococcus aureus* infection in experimental animal mice

○ Sayaka Ishiyama, Hanako Morita, Nobuhito Hayashimoto

ICLAS monitoring Center Central Institute for Experimental Animals

【Background and purpose】 *Staphylococcus aureus* common bacteria of immunocompetent mice and rats in Japan. Although drug resistance of *S.aureus* is problematized in human clinical medicine, the information of those of *S.aureus* derived from laboratory rodents is scarce. To reveal a current situation of prevalence of MRSA in laboratory mice, we surveyed mice from animal facilities of universities and institutes in Japan.

【Materials and Methods】 In total 1,737 mice were tested for six months (from July to December 2016). These mice were derived from 72 facilities of universities and institutes in Japan. Detection and identification test of *S.aureus* were carried out according to SOP in our center. To detect MRSA, commercially available selection ager of MRSA (chromoID™MRSA) was used, and PCR and following sequencing was used for identification of mecA gene.

【Results and discussion】 Of the 1737 mice, 179 mice were positive for *S.aureus* (positive rate : 10.3%). Among 179 isolates, 27 isolates were MRSA (total positive rate : 1.55%). From given results in this study, the prevalence of MRSA was shown in laboratory mice in laboratory animal facilities of university and institutes, although the rate was relatively low. The workers in animal facilities should re-recognized the risk of mutual infection between animal and humans.

2D13 Detection and clearance of *Helicobacter hepaticus* infection in mice at the Experimental Animals Facility

○ Toshiaki Kokubo[1], Kaori Tateno [2], Mizuki Iina [2], Takashi Ohkubo [2], Seiji Kito[1]

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The Animal Research Building conducts barrier management according to the guidelines of the specific pathogen–free (SPF) Experimental Animals Facility. Furthermore, the building permits the transport of animals to other facilities for radiation exposure experiments. In October 2016, mice were introduced from the rearing facility to the fourth floor mouse room A of our facility, where we discovered *Helicobacter hepaticus* (*H. hepaticus*) in the feces of the mice during the intake examination. We then investigated the presence of Helicobacter in other animal facilities and detected Helicobacter in mice in the third floor mouse room B of our facility and mouse room C of another rearing facility. All three rooms had received mice directly from the rearing facility or had a history of rearing mice received from the facility. The *H. hepaticus* infection was relatively contained, suggesting that the following four measures were performed appropriately: 1) strict work flows; 2) separation of rearing areas with consideration for the animal experiments being performed; 3) strengthened hygiene management of the cleaning rooms for equipment; and 4) re-education and training of animal researchers and handling staff. Measures for clearance of *H. hepaticus* include moving mice involved in experiments that cannot be immediately terminated to rearing rooms that can be depressurized, and where the breeding equipment can be autoclaved. In mice for which this is not possible, preventative measures against dust scattering can be implemented. In either case, it is important to ensure that the rearing rooms are disinfected within a specified time period. Additionally, needed mouse lines are bred from *in vitro* fertilization and embryo transfer.

2D14 Lethal infectious disease in highly immunodeficient X-SCID rats

○ Kazunori Ozawa[1,2], Yuko Yamamoto[1], Miho Sekiguchi[1,2], Kiyoaki Katahira[1]

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To develop patient-derived xenograft (PDX) model with rats, X-SCID rats were introduced from the National BioResource Project–Rat, Kyoto University, and bred to establish a colony. X-SCID rats were kept in Vent Rack (Allentown Inc.) or Clean Rack (CLEA Japan) in a SPF condition (22 ± 2 °C, 55 ± 5 %, lighting 7:00-19:00) with bedding (ALPHA-dri) and free access to foods and water. During breeding, aged rats of both sex exhibited dyspnea, wasting and then died. In histopathological analysis of the lung, inflammation (septic thickening, cell infiltration, foamy exudate) and dark-stained cysts by Grocott staining were observed, and *Pneumocystis carinii* was detected by microbiological examination. Then, *P. carinii* was eradicated by hysterectomy, but chromodacryorrhea, dyspnea and death were still observed. Through further examination, inclusion bodies were found in the lung, the salivary glands and the prostate, suggesting viral infection. Recently, a novel rat polyomavirus was found in immunodeficient rats in U.S. The situation was very similar to our case, so we implemented PCR test and investigated further towards establishment of a viral-free colony.

Immunoresponses in large intestinal mucosa of mice infected with *Helicobacter japonicum*

2D15

○ Hitoki Yamanaka[1], Hiroki Yamane[1], Ritsuko Masuyama[1], Toshikazu Takagi[1], Makiko Ohsawa[1], Noriaki Kubo[1], Naoto Yamamoto[1], Takahira Takemoto[1], Kazutaka Ohsawa[1]

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"*Helicobacter japonicum*" (MIT 01-6451) which predominantly colonizes in large intestinal tract of mice has frequently been detected among *Helicobacter* species in laboratory mice kept in Japan. In this study, to clarify the pathogenicities in immunocompetent mice of *H. japonicum* Mu-ngs1 (*Hj*) isolated from mice in our laboratory, the immunoresponses in the large intestinal mucosa were investigated using BALB/c mice.

At 3 weeks post infection (wpi), the expression of IFNγ, IL-4, IL-17A, and IL-10 mRNA in lamina propria cells of cecum and colon were significantly higher than those of uninfected mice. No differences in the cytokine mRNA expressions were detected between infected and uninfected mice at 6 months (m) pi. However, the mild inflammations including infiltration of granulocytes were observed in the cecal mucosa and the lymphoid follicles in the mucosa of colon was significantly increased comparing with uninfected mice while any inflammations were not observed in the colon of infected mice at 6 mpi. The IgA and IgG titers against *Hj* in sera and feces of infected mice were significantly higher than those of uninfected mice and these antibody titers in mice at 6 mpi were significantly higher than those at 3 wpi.

From the results obtained in this study, the *Hj* infection induced inflammatory responses including helper T cell 1, 2, and 17 responses in large intestinal mucosa. However, the severe responses might be inhibited through the production of IL-10 by regulatory cells, and then the stimulation of mucosal immune systems with *Hj* infection was regulated by the development of mucosal barrier systems including IgA secretion. Therefore, *Hj* may have potentials to induce typhlocolitis in immunocompetent mice.

Pilot study of *Filobacterium rodentium* culture on solid medium

2D16

○ Fumio Ike[1], Ayako Kajita[1], Atsushi Yoshiki[1], Toshiaki Kokubo[2]

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Filobacterium rodentium (formerly known as "CAR bacillus") has been reported to be non-culturable on classic agar plate. Liquid culture system by using conditioned medium is generally used to cultivate this organism in vitro. However it would be easy to pickup mutants of *F. rodentium* if this organism could grow on solid medium. In this study we attempted to resolve this issue by using gellan gum for solidification of medium instead of agar. [Materials and methods] *F. rodentium* (SMR-C^T, JCM 19453^T) grown by liquid culture was used. The conditioned medium was prepared by collecting culture supernatants of Vero E6 cells grown in IMDM medium (Gibco) supplemented with 10% fetal bovine serum (FBS). All cultures were carried out at 37 °C in 5% CO_2/95% air humidified chamber. Gellan gum was purchased from Wako pure chemical. [Results and discussion] Gellan gum is naturally derived highly purified polysaccharide and has a selfgelling property. Gellan gum in water was easily solubilized by autoclaving. Then, solated gellan gum was chilled to around 45°C and mixed with nutrient medium. Gellan gum containing medium was solidified at room temperature. We prepared 0.4% gellan gum in IMDM and 4% FBS (final) in culture dish (FALCON 1029). When *F. rodentium* grown in liquid culture was spread over these plates, colony formation was observed macroscopically after 3-4 weeks of inoculation. It is said that traditional agar might have unknown materials to prevent growth of many bacteria. In this study, we found gellan gum-base solid medium actually support *F. rodentium* growth. This improvement of culture method would have a potential to promote biology of *F. rodentium*.

2D17 Hepatic serum amyloid A upregulates IL-17 in gammadelta T cells through Toll-like receptor 2 and is associated with psoriatic symptoms in transgenic mice

○ Minjee Choi, Zae Young Ryoo

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Serum amyloid A (SAA) is an acute phase protein with pro-inflammatory cytokine-like properties. Recent studies have revealed that SAA promotes interleukin-17 (IL-17) production by gammadelta T cells, as well as IL-17 secretion in the T helper 17 (Th17) cells.

In this study, we established hepatic SAA1-overexpressing transgenic (TG) mice. In these mice, IL-17 was significantly increased in gammadelta T cells without any stimulation. We found that SAA-mediated IL-17 production by gammadelta T cells was dependent on Toll-like receptor 2 (TLR2). We also observed a thickened epidermis and found that cytokines associated with psoriasis symptoms were increased in the dorsal skin of TG mice. The deterioration of psoriasis is known to be associated with an increase in IL-17. Therefore, it seems that these symptoms were induced by gammadelta T cells increased by SAA.

These data indicate that SAA is a potent endogenous protein that promotes inflammatory responses associated with IL-17 production by gammadelta T cells via TLR2.

2D18 Triplex PCR for the simultaneous detection of pathogenic bacteria in respiratory system of rodents

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High quality laboratory animal is most important in the experiment for get reliability and reproducibility data. Bordetella bronchiseptica, Pasteurella multocida and Pasteurella pneumotropica infection were reported in respiratory system of laboratory animals. Microbiological control in laboratory animals is essential for experimental result because of laboratory animals are not treated the disease, prevention at periodic monitoring. B. bronchiseptica, P. multocida, and P. pneumotropica are similar colony morphology on 5% sheep blood agar. Morphology confirmed is subjective and not precision. However, it is not easy to identify bacterial and we determined method of rapid, objective and accurate diagnose bacteria. In this study, conventional PCR was used for rapid identification of B. bronchiseptica, P. multocida, and P. pneumotropica. On this basis, specific identification primers were designed and easy multiplex-PCR was established and optimized. All three bacteria were successfully each or simultaneous identified. B. bronchiseptica, P. multocida, and P. pneumotropica are often infected in many animals such as mice, rats, rabbits, guinea pigs, dogs, cats, pigs and primates. Thus, multiplex-PCR is useful and convenient assay in identification of bacterial pathogens in laboratory, pet and economic animals. Our multiplex- PCR method will be used to improve quality control in laboratory animals and laboratory animal facilities.

Key words: Bordetella bronchiseptica, Pasteurella multocida, Pasteurella pneumotropica, multiplex-PCR, Respiratory

【Poster Presentation (Regular Papers) May 27】

Maternal effects of gene mutation in the OCM related genes on behavioral phenotypes of progenies

3A01

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In the field of metabolic diseases, "Developmental Origins of Health and Disease" (DOHaD) is the concept that in utero experiences reprogram susceptibility to adult metabolic diseases and it is of interest to test if this concept is applicable to behavioral phenotypes. In our study, we are aiming to validate the DOHaD hypothesis in the behavioral phenotypes using mouse models. In the previous study, we conducted *in vitro* fertilization and embryo transfer in mice, and allowed protein restricted (PR) diet and folate supplemented PR diet to affect only fetal environments (Ref. 1) . Comprehensive phenotyping of PR and control-diet progenies showed moderate differences in fear/anxiety-like, novelty-seeking, and prosocial behaviors, irrespective of folate supplementation (Ref. 1) . Changes were also detected in gene expression and genomic methylation in the brain (Ref. 1) . Therefore, we focused on the one carbon metabolism" (OCM) in the present study. The OCM is consisted of methionine cycle and folate cycle. The folate cycle is involved in DNA synthesis and methionine cycle is involved in DNA methylation by donating methyl group. We produced wild type progeny from mutant females of key genes of the OCM such as *Mat2a* (methionine adenosyltransferase II, alpha) and *Tyms* (thymidylate synthase) by using *in vitro* fertilization technique and phenotyped the progenies. The progenies obtained from heterozygote of *Mat2a* and *Tyms* KO mice exhibited increased spontaneous locomotor activity in the behavioral phenotyping. We will perform epigenomic and gene-expression analyses in several brain regions.

Reference
1) Furuse et al. *Genes & Nutrition*. 2017; 12:1

Behavioral analysis of fear in PACAP over-expression mice

3A02

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【Aim】
PACAP is a peptidergic neurotransmitter that regulates stress response pathway in hypothalamus. The association between blood PACAP concentration and post-traumatic stress disorder was reported. However, the effects of high PACAP expression on fear memory is still unclear. Here we report the results of behavioral and neuroscientific analysis of fear responses using PACAP over-expression mice.
【Methods】
PACAP over-expression mouse was developed previously in our laboratory. This mouse is a congenic mouse that has a PACAP gene derived from Japanese wild mouse MSM/Ms on C57BL/6 genetic background. We analyzed freezing behavior in fear-conditioning test followed by fear conditioning and extinction using the adult male mice.
【Results and Discussion】
We observed freezing behaviors in the PACAP over-expression mice at the similar level with control mice in conditioning and extinction sessions. However, in test session, freezing behaviors were significantly higher in the PACAP over-expression mice than control mice. We speculated that the difference was caused by either the changes of PACAP expression in brain, the secondary effects of stress hormones or both. We will also report the results of the tests in female mice and the gene expression analyses such as c-fos and CREB genes.

Study for behavioral and neural basis of tameness

3A03

○ Hiromichi Nagayama[1,2], Yuki Matsumoto[1,2], Tsuyoshi Koide[1,2]

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An experimental mouse such as C57BL/6 mouse is easy to handle for human in experiment and breeding situations. This behavioral characteristics is known as "tameness". Through domestication for experiments, these animals have been selected for tameness by human. It has been reported that tameness has two potential components, reluctance to avoid humans (passive tameness) and motivation to approach humans (active tameness). However, behavioral and neural basis, particularly about active tameness, remain unclear. Therefore, the aim of this study is clarification of the tameness in behavioral neurology.

We have conducted selective breeding using wild derived heterogeneous stock mouse based on active tameness to establish mouse groups which exhibit higher active tameness. Until now, the selective breeding has been conducted for 18 generations, and established selected groups (tame groups) have a significant higher active tameness than control groups (non-tame groups) maintained randomly. There groups are outbred derived from 8 wild strains and have various traits. Thus, the resource is useful for comprehensive investigation about traits associated with active tameness.

In this study, we compared anxiety, curiosity for object and sociability using tame and non-tame groups to find common behavioral traits associated with active tameness. As results of open field, light-dark box, novel object, three chamber and social interaction tests, tame groups exhibited significant longer social activity than non-tame groups in social interaction test. This result indicates relations between active tameness and sociability.

In addition, to identify the brain regions associated with active tameness, c-Fos analysis was conducted by immunohistochemistry. As a result, more c-Fos positive cells were observed in basolateral amygdala (BLA) and hippocampal dentate gyrus (DG) of non-tame group with stimuli facing human hand. On the other hand, there were no changes in BLA and DG of tame group.

Evaluation of motor function of *Hcn1*-knockout rats

3A04

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HCN1 (hyperpolarization activated cyclic nucleotited1) channel is activated by action potential, permeates cations, and produces current called I_h. I_h is thought to be involved in transmission of rhythmic membrane potential, synaptic transmission, working memory and, motor learning. *Hcn1*-knockout (KO) mice exhibited defects in motor learning and memory. Here, we produced *Hcn1*-KO rats by TALEN and evaluated motor function of them. Two *Hcn1*-KO rat lines were established. They lacked 7-bp (c.1049_1055del) and 24-bp (c.1041_1064del) of the exon 4, respectively. Both lines exhibited low scores compared with the control F344/NSlc rats in the grip strength test, incline place test, balance beam test, and rotor rod test. In the foot print test, both KO lines exhibited wider step width of the hind limbs than the controls. These findings indicated that the *Hcn1*-KO rats exhibited muscle weakness, defects in motor coordination, and abnormal gaits. Thus, we concluded that the *Hcn1*-KO rats had impaired motor function.

Effects of the initial size and strain on the repair process after ischemic stroke

3A05

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Several models of ischemic stroke in human have been established. However, most of them shows large variation on size and region of lesions among strains, genetic backgrounds, and individuality. We, therefore, have established a new model in which the variation of on size and location of lesions was few, by using photochemical reaction.

By this model, we evaluated the effect of initial lesion size on the repair process. It was found that both larger and smaller lesions showed time-dependent shrinking, but it was faster in larger lesion. Furthermore, relating process including microglial accumulation and astrocyte activation were more remarkable in larger lesion. These findings indicate that ischemic stroke models that show larger variation on initial lesion size are not suitable for the analysis of the repair process after ischemic stroke. Then, we studied the repair process among C57BL/6, Balb/c and 129SV by using this model. It was found that the reduction of region size was more remarkable in Balb/c than others although microglial accumulation was comparable. These finding suggested that the repair process was different among different strains, which should be considered on the experimental design.

Strain difference in histology of cardiac muscle tissue in normal mice

3A06

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Heart pumps blood through the body. Cardiac myocytes drive the blood pump. In cardiomyopathy, which refers to a chronic disease of heart muscle marked by cardiac myocyte degeneration, cardiac function and output can decrease. On the other hand, it is said that some people who have cardiomyopathy show no symptoms and need no treatment throughout their life. In this study, we focus on "granular" cardiac fibers, which can be often observed in normal mice, so that they seemingly look like artifacts. To investigate whether or not strain difference is present in this histology of cardiac muscle, we collected heart samples from seven mouse strains (C57BL/6J, BALB/c, FVB/N, DBA/2, C3H/He, Jcl:ICR, Slc:ICR), and compared their differences. As a result, "granular" cardiac fibers were most commonly seen in ICR and C3H/He mice and in the papillary muscle and the endocardium side of the left ventricle. Currently we performed further analyses to investigate whether this strain difference is attributed to anatomical and embryological difference or differential cell responsiveness of cardiac myocytes.

3A07 Reduction of Myosin VI in mice leads to stereociliary fusion caused by disruption of actin networks in the apical region of cochlear hair cells

○ Yuta Seki[1], Yuki Miyasaka[1, 2], Sari Suzuki[1], Yoshiaki Kikkawa[1]

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An unconventional myosin, the myosin VI gene (MYO6), contributes to hearing loss in humans. *Myo6*^{ksv} mice harbor spontaneous mutations, and homozygous mutants exhibit congenital defects in balance and hearing caused by fusion of the stereocilia. We identified a *Myo6*$^{c.1381G>A}$ mutation that was shown to be a p.E461K mutation leading to alternative splicing errors in *Myo6* mRNA in *Myo6*^{ksv} mutants. An analysis of the mRNA and protein expression in animals harboring this mutation suggests that most of the abnormal alternatively spliced isoforms of MYO6 are degraded in *Myo6*^{ksv} mice. In the hair cells of *Myo6*$^{ksv/ksv}$ homozygotes, the MYO6 protein levels were significantly reduced in the cytoplasm, including the cuticular plates. MYO6 and stereociliary taper-specific proteins were mislocalized along the entire length of the stereocilia of *Myo6*$^{ksv/ksv}$ mice, suggesting that MYO6 was attaching to taper-specific proteins at the stereocilia base. Histological analysis of the cochlear hair cells showed that the stereocilia fusion in the *Myo6*$^{ksv/ksv}$ mutants developed through the fusion among stereociliary bundles, rising of cuticular plate membranes in the cochlear hair cells, and the outer hair cell-specific incorporation of the bundles into the sheaths of the cuticular plates. Interestingly, the expression of the stereociliary rootlet-specific TRIOBP was altered in *ksv/ksv* mice. The abnormal expression of TRIOBP suggested that the rootlets in the hair cells of *Myo6*$^{ksv/ksv}$ mice were excessively grown. Hence, these data indicate that reduced MYO6 levels in *Myo6*$^{ksv/ksv}$ mutants disrupt actin networks in the apical region of hair cells to maintain the normal structure of the cuticular plates and rootlets.

3A08 Deficiency of heat shock transcription factor 1 in non-CNS organs is critical for the life span shortening of Huntington's mice

○ Naoki Hayashida, Masami Momonaka, Yasuko Tokunaga

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Huntington's disease is a one of well-known neurodegenerative diseases and also polyglutamine (polyQ) diseases. PolyQ protein forms toxic insoluble oligomers and aggregates/inclusion especially in neuron and glial cells of CNS and other cells of non-CNS organs.

We discovered the Heat Shock Transcription Factor 1 (HSF1) has suppressive roles in Huntington's disease onset and progression (Hayashida et al., EMBO J 2010). We examine the brains of WT- and HSF1KO-Huntington's (HT) mice in detail in this study, but did not investigate other organs nevertheless polyQ protein expressed and often forms aggregates/inclusion in non-CNS organs. Here, we analyzed the tissues of non-CNS organs and report the novel findings.

We examined gene expression in the tissues of 10 organs, brain, heart, muscle, lung, liver, kidney, pancreas, spleen, stomach, and testis, of WT- and HSF1-KO HT mice at eight weeks old mainly by semi-quantitative RT-PCR. We examined Nfatc2, Pdzk3, Cryab, Csrp2, and Prame. The proteins encoded by these genes can suppress the intracellular aggregations/inclusion (Hayashida et al., EMBO J 2010). Unexpectedly, the expression levels of these five genes in most tissues including brain were not affected by HSF1 deficiency.

However, the expression of these five genes were prominently decreased in heart, muscle, and spleen. Especially, Nfatc2, Pdzk3, and Prame were decreased in heart, and all five genes were also prominently decreased in spleen.

Moreover, we selected 15 small chemical drugs and examined whether these drugs suppress the aggregates/inclusion by polyQ proteins. Finally, we discovered seven drugs suppress these formation. These small chemical drugs may become the therapeutics for Huntington's disease.

3A09 Identification and functional analysis of CABS1 protein in porcine testis

○ Hisashi Oishi[2,3], Hossam Shawki[1,2], Takumi Kigoshi[1], Yuki Katoh[1,4], Manabu Matsuda[1], Satoru Takahashi[2], Akihiro Kawashima[1]

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Previously, we have identified a calcium-binding protein, CABS1 that is specifically expressed in spermatids and localized to the flagella of the mature sperm in mouse. Here, we aimed to localize and clarify the role of CABS1 in porcine (pCABS1). We determined the full nucleotides sequence of pCABS1 mRNA. pCABS1 protein was detected on SDS-PAGE gel as two bands at 75 kDa and 70 kDa in adult porcine testis, whereas one band at 70 kDa in epididymal sperm. pCABS1 immunoreactivity in seminiferous tubules was detected in the elongated spermatids, and that in the epididymal sperm was found in the acrosome as well as flagellum. The immunoreactivity of pCABS1 in the acrosomai region disappeared during acrosome reaction. We also identified that pCABS1 has a transmembrane domain using computational prediction of the amino acids sequence. The treatment of porcine capacitated sperm with anti-pCABS1 antiserum significantly decreased acrosome reactions. These results suggest that pCABS1 plays an important role in controlling calcium ion signaling during the acrosome reaction.

3A10 Serum amyloid A1 is involved in amyloid plaque aggregation and memory decline in amyloid beta abundant condition

○ Soyoung Jang, Zae Young Ryoo

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Alzheimer's disease is a neurodegenerative disorder, characterized by cognitive impairment, progressive neurodegeneration, and amyloid-beta lesion. Inflammation is known to play an important role in neuronal death and disease progression. A previous study on serum amyloid A 1(SAA1) showed that the liver-derived SAA1 accumulated in the brain by passing through the brain blood barrier without damaging it. Since SAA1 triggers immune responses in other diseases, a double transgenic mouse was generated using amyloid precursor protein (APP)-c105 mice and SAA1-overexpression mice to examine the function of SAA1 in amyloid beta accumulated condition. Comparison between APP and APP/SAA1 transgenic mice showed that SAA1 exacerbated amyloid aggregation and inflammation in amyloid beta abundant condition in the brain. Behavior tests also supported this result; APP/SAA1 transgenic mice had greater memory decline compared to APP mice, which only expresses amyloid beta 1-42.

Tet1 overexpression induce anxiety-like behavior and enhanced fear memories in mice

3A11

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Ten-eleven translocation methylcytosine dioxygenase 1 (Tet1) initiates DNA demethylation by converting 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) at CpG-rich regions of genes and it has been revealed to have functions in adult neurogenesis, cognition, and memory extinction. However it is still unclear whether Tet1 overexpression is beneficial for the neuronal networks. In the present study, we found that Tet1-transgenic (Tet1-TG) mice display abnormal behaviors involving elevated anxiety and enhanced fear memories. Tet1 overexpression induced activation of intracellular calcium signals in prefrontal and hippocampal neurons, followed by the augmented expression of immediate early genes (IEGs), such as Egr1, c-fos, Arc, and Bdnf. The expression of gamma-aminobutyric acid (GABA) receptor subunits (Gabra2 and Gabra4) fluctuated in the prefrontal cortex (PFC) and hippocampus. In addition, we identified that Tet1 overexpression not only affect adult neurogenesis but also promote oligodendrocyte differentiation in the hippocampus of Tet1-TG mice. We evaluated the effects of Tet1 overexpression on intracellular calcium-dependent cascades with the activation of Egr1 promoter in vitro. Tet1 enhanced Egr1 expression, which affected alterations in Gabra2 and Gabra4 expression in neurons. Taken together, we suggest that chronic Tet1 overexpression in brain may induce harmful effects on maintaining the proper excitatory-inhibitory balance in neural networks.

Establishment of a supply system of Germfree Mice Jcl:MCH (ICR)[Gf] (1) Production efficiency

3B01

○ Takayuki Goto[1], Masahiko Yasuda[2], Chie Shimomura[1], Miho Ito[1], Toshihiko Tanaka[1], Kaori Takahashi[1], Yuki Narabe[1], Mika Yagoto[2], Yuyo Ka[2], Tomoyuki Ogura[2], Riichi Takahashi[2], Kenji Kawai[2], Kyoji Hioki[2], Hideki Shinohara[1]

[1]CLEA Japan Inc., [2]Central Institute for Experimental Animals (CIEA), [3] JAC

Jcl:MCH (ICR) mice makes 4 systems of inbred line mouse which makes the Jcl:ICR mice which is a closed colony an origin (IQI, IAI, IPI and ICT) an ancestor, and is uniform and is the mice from which stable reproducibility is obtained hereditarily.

Germfree mice of this system was made, data about (MCH [Gf]), the propagation and growth was collected and a comparative analysis with a SPF mice (MCH [SPF]) was put into effect this time.

My elder brother younger sister did living crossing of a pair of IQI female, IAI male, IPI female and ICT male, and my elder brother younger sister did living crossing of produced F1 propagation colony QAF1 female and PCF male, and a manufacturing system made Jcl:MCH (ICR).

MCH [Gf] was a vinyl isolater, and MCH [SPF] was a barrier system, and the culture environment calculated the production index from the natality at each culture environment bottom, newborn and the weaned rate and measured the weight in 3-8 week age.

Reproductive performance and the weight rate of increase were also with SPF by VI culture by the system to which the thing a germfree Jcl:MCH (ICR) mice produces in Four Muti Cross Hybrid gives hereditary system and reproducibility, and the difference wasn't admitted. When in vivo studies Flora in the gut, a germfree Jcl:MCH (ICR) mice can say the germfree mice system of the valuable role.

Establishment of a supply system of Germ free mice Jcl:MCH(ICR)[Gf] (2) Organ weight data analysis

3B02

○ Miho Ito[1], Masahiko Yasuda[2], Takayuki Goto[1], Chie Shimomura[1], Toshihiko Tanaka[1], Kaori Takahashi[1], Yuki Narabe[3], Mika Yagoto[2], Yuyo Ka[2], Tomoyuki Ogura[2], Riichi Takahashi[2], Kenji Kawai[2], Kyoji Hioki[2], Hideki Shinohara[1]

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The stable production supply of a germfree mice is important in a study of intestinal bacteria. We made germfree mice of Jcl:MCH (ICR) what have high level hereditary system and reproducibility .

This time, we put organ weighting and data collection of the enteron form of MCH[Gf] into effect, and did a comparative analysis.

We used 3-8 weeks age of MCH [Gf] and MCH [SPF], and 6 for each of a male and female-10.

The organ with which the weight was gauged are brain, thymus gland, lung, heart, liver, spleen, kidneys, stomach, small intestine, appendix, large intestines and genitals, in addition we measured the length of enteron and weight of enteron contents.

The weight of small intestine, appendix, appendix contents and the length of appendix indicated the statistical significant difference, these numerical values of MCH[Gf] were higher than the values of MCH[SPF] in both male and female.

The relative weight to the body weight average (g/100gBW) at 7weeks were MCH[Gf]7.9 and MCH[SPF]5.1 in the small intestine, MCH[Gf]5.7 and MCH[SPF]1.0 in the appendix, MCH[Gf]4.8 and MCH[SPF]0.6 in the appendix contents.

And the appendix length average of MCH[Gf] was 4.2cm and MCH[SPF]was 2.4cm, its difference was about 2times.

The difference between MCH [Gf] and MCH [SPF] showed conspicuously in the enteron.

These results showed that the influence by which intestinal bacteria give it to the form of enteron is big.

Establishment of a supply system of Germ free mice Jcl:MCH(ICR)[Gf] (3) Blood data analysis

3B03

○ Chie Shimomura[1], Takayuki Goto[1], Masahiko Yasuda[2], Miho Ito[1], Toshihiko Tanaka[1], Kaori Takahashi[1], Yuki Narabe[3], Mika Yagoto[2], Yuyo Ka[2], Tomoyuki Ogura[2], Riichi Takahashi[2], Kenji Kawai[2], Kyoji Hioki[2], Hideki Shinohara[1]

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Germ free is expected to be utilized for reseach of intestinal bacteria and the other micro-biome. Its background data is necessary for these reseach and is interesting.

In this study, we measured the Blood cell count (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT) and blood biochemical (CPK, LDH, AST, ALT, ALP、GLU, ChE, T-CHO, TG, Ca, IP, T-BIL, BUN, CRE, TP, Na, K, Cl) of 3-8weeks mice and compared and analyzed MCH[Gf] and MCH[SPF].

In the Blood cell count, there is no difference between MCH[Gf] and MCH[SPF] in RBC value.

HGB,HCT,MCV and MCH values of MCH[Gf] are always lower than the values of MCH[SPF],

both values increased gently, indicated a peak at 5weeks.A significant difference was admitted by female in particular.

In the Blood biochemical, TG value of MCH[Gf] was significantly lower than the value of MCH[SPF].

The value of both of MCH[Gf] and MCH[SPF] increased at 3-4weeks, then the value of MCH[Gf] increased gently from 5weeks, its difference showed conspicuously.

T-CHO and, Ca, Na, K, Cl values of MCH[Gf] were higher than the values of MCH[SPF] as the tendency.

From these results, it's supposed that intestinal bacteria influence the hematopoietic function and lipid and carbohydrate metabolism at the time of weaning and growth.

The relationship between the number of newborns and the ovaries of the ultra immunodeficient NOD/SCID/JAK3null Mouse Strain

3B04

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【Introduction】 NOD/SCID/JAK3null ^{null} (NOJ) is prepared by crossbreeding an NOD/SCID mouse with a JAK-3 deficient mouse for 10 generations.
It is a highly immunodeficient mouse strain whose immune function is lower than conventional immunodeficient mouse strains. These NOJ mice are expected to be applied to research on human diseases such as human-specific infections and cancers. Unfortunately, breeding this mouse strain is difficult because the breeding efficiency of NOJ females via natural mating is low. We demonstrated in our previous report that *in vitro* fertilization and embryo transplantation could improve the breeding rate of NOJ mice in situations where breeding was difficult. However, we also found that many fertilized eggs with abnormal cleavage were seen during *in vitro* fertilization. In this report, we evaluated the functions of the ovary and the testis respectively, based on the hypothesis that frequent abnormal cleavage is one of the causes of low breeding efficiency.
【Methods】 We used 10-week-old male and female NOJ or ICR (control group) mice raised in a vinyl isolator. Ovaries and testes were soaked in 10% formalin in 0.1 M phosphate buffer and fixed. Subsequently they were cut out, soaked in paraffin following the conventional method and subsequently stained using hematoxylin & eosin (hereafter called HE). Thereafter we compared the ovaries and testes of NOJ mice with those of ICR mice with a normal immune system.
【Results and Discussion】 We observed a reduction in the number of mature follicles in the ovaries of the NOJ mouse group compared to the control group. This result suggests that there were reduced number of matured ova in the NOJ mouse group. Similarly, we observed an increase in the number of interstitial cells in the seminiferous tubule in the NOJ mouse group compared to the control group, suggesting that there were less spermatozoa in the NOJ mouse group. Overall, these results suggest that fertilization of NOJ mice is affected by a reduced number of mature ova and sperm. Hereafter we will measure the viability of the spermatozoa and analyze the functions of follicles and ovulated eggs.

SLA class II haplotype frequencies for seven years in a miniature pig line, Microminipig

3B05

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We have previously found and characterized eight swine leukocyte antigen (SLA) class I and II haplotypes, Hp-6.7, 10.11, 31.13, 16.16, 17.17, 20.18, 35.23 and 43.37, from extremely small-sized miniature pigs designated as Microminipigs (MMPs). In this study, we analyzed frequencies of the eight class II haplotypes in 2,230 MMPs that were bred for seven years (from January 2010 to August 2016). Hp-0.23 was the most frequent haplotype during the first three years in the period, and then gradually decreased. In contrast, pigs with any of Hp-0.37, 0.17 or 0.11 tended to increase during the late four years. Pigs with homozygous haplotypes continued to increase until 2012, and percentages of the homozygotes were kept around 25% after 2013. SLA class II homozygotes with Hp-0.23 were frequent, and homozygotes with Hp-0.37, 0.17 and 0.11 gradually increased after 2014. These SLA homozygotes will be valuable experimental materials for transplantation and immunological studies. These results of the class II haplotype frequencies in the MMP line suggested that in the selective breeding process, pigs with Hp-0.23 might be preferentially selected for the smaller body sizes during the first three years. Furthermore, it was also deduced that pigs with Hp-0.17 or 0.37 that had relatively large body sizes might be subsequently selected for more efficient breeding. We are going to clarify relationships between SLA class II haplotypes and biological traits in MMPs.

3B06 The relation between inbreeding coefficient and body morphometry with generation in inbred microminipigs

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【Background and Purpose】 Microminipig (MMP) is produced by Fuji Micra Inc. and used for experimental applications. MMPs weigh around 10 kg at 6 to 7 months of age, when sexual maturity occurs, and have variations in coat color and SLA (Swine Leukocyte Antigen) haplotype. However, genetically consistent microminipigs can be useful for the future experiments requiring more precise outcomes. Inbred microminipigs (IB-MMP) family was produced and individual traits were evaluated in this study. [Materials and Methods] A pair of male and female MMP (1st generation) was selected from the population, and then up to 5th generation was produced by brother-sister or male cousin-female cousin mating at each generation. Inbreeding coefficient of each generation was calculated using CoeFR (Sato) analysis software. Body weight, body length, and withers height were measured at 6 months of age to find out any changes in shape. The coefficient of variation was also evaluated between generations. In addition, the number of pigs born alive was recoded. 【Results and Discussion】 The inbreeding coefficient increased with generation: 1st = 0% or 12.5%, 2nd = 18.8%, 3rd = 35.9%, 4th = 47.7%, 5th = 52.7%. No significant differences between generations were observed in body weight and body length. Although the withers height was significantly lower ($p<0.05$) in 4th generation than that of 2nd, there was no tendency through generations. The coefficient of variation of body weight, body length, and withers height seemed to decrease with generation. The data regarding reproduction is now being gathered. These results may indicate that IB-MMP is comparable to present MMP in body shape, and that more physically consistent inbred family can be established by increasing inbreeding coefficient with further generations.

3B07 Experimental animal websites at Kumamoto University

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In order to perform animal experiments appropriately and efficiently, researchers need a lot of information. To provide this information, the Center for Animal Resources and Development (CARD), Kumamoto University operates a number of dedicated websites, concerning (1) animal facilities, (2) our mouse bank system, (3) reproductive engineering techniques, and (4) training courses on reproductive engineering techniques. (1) - (3) were made locally using Dreamweaver, while (4) was made using a content management system (weebly.com). Using Google Analytics, we analyzed access to our websites from May 2015 to Dec 2016 (1) - (3) and from Dec 2015 to Dec 2016 (4), with the goal of improving website operation efficiency.

We discovered that the total access counts/total number of pages for each website were (1) 51,691/58, (2) 25,412/42, (3) 50,419/23, and (4) 7,035/5 respectively. Moreover, the most frequently accessed pages within each website were (1) Natural mating method for mice (7,986 hits), About the Kumamoto earthquakes (3,195 hits); (2) Public mouse bank system (2,835 hits), Mouse transportation method (1,432 hits); (3) Ultra-superovulation method (5,167 hits), *In vitro* fertilization (4,418 hits); and (4) The history of our training courses (1,139 hits), and Joining a training course (1,131 hits).

Furthermore, we found that the proportion of traffic to each website from within Kumamoto University were (1) 24.5%, (2) 25.3%, (3) 4.6%, and (4) 36.9% respectively.

Finally, we found that hosting (4) on weebly.com cost us almost nothing in terms of website creation and maintenance costs.

This research highlighted the most frequently accessed web pages within our websites. This information will contribute to the sharing of valuable information concerning animal experiments through the realization of a website with frequent access and low maintenance costs.

Mice breeding Study using a cage lid made of resin (Safety Resin Lid)

3B08

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A stainless wire has been used for the material of the cage lid for mouse or rat. A stainless wire cage lid has durability, but it has been pointed several problems such as occupational safety and influence to an animal. It's expected that the cage lid made of the glass-reinforced resin (Safety Resin Lid, GROWBIC CO., LTD.) settles the various problems. In this study, we examined influence of Safety Resin Lid to growth, feed intake and water intake of mice breeding with the lids. Male C57BL/6N mice (5 weeks old; n=30) were used for this study. The mice were divided into two experimental groups: one group was breeding with Safety Resin Lid (resin lid group) and the other group was breeding with stainless wire lid (wire lid group). Each group was prepared 6 cages (5 mice/cage). We measured body weight, food intake and water intake of the mice once a week for 8 weeks. In addition, we observed Safety Resin Lids whether mice damage the lids by biting once a week.

Bite marks were not observed in Safety Resin Lid after 8 weeks breeding. There was no significant difference in the body weight between resin lid group and wire lid group. However, feed intake and water intake were higher in wire lid group compared to resin lid group. From these results, Safety Resin lid may have calming effect compared to the conventional stainless wire lid.

A novel *in vivo* model for predicting myelotoxicity of chemotherapeutic agents using IL-3/GM-CSF transgenic humanized mice

3C01

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Myelotoxicity, the suppression of myelopoiesis in the bone marrow (BM), is a serious adverse effect of chemotherapy. Evaluating myelotoxicity is essential for ensuring the safety of novel drugs before they are approved for clinical applications. Although *in vivo* prediction of the maximum tolerated doses (MTDs) of anticancer drugs is usually performed in rodents, the results are not always applicable to clinical treatment because drugs may have different effects in human and rodent cells. Previously, we generated a human IL-3 and GM-CSF transgenic humanized mouse (hu-IL-3/GM Tg), in which human granulocytes effectively differentiated in the BM and circulated in the peripheral blood (PB) after hematopoietic stem cell transplantation. In this study, we established a novel *in vivo* preclinical evaluation model for predicting human myelotoxicity using these hu-IL-3/GM Tg mice. The myelotoxicity of three kinds of cytotoxic anticancer drugs (topotecan, oxaliplatin, and paclitaxel) was investigated by kinetic flow cytometry of human or murine granulocytes in PB and by colony-forming unit granulocyte/macrophage (CFU-GM) assays using BM cells from the humanized mice. In both *in vivo* and *in vitro* analyses, topotecan was more myelotoxic to human than murine granulocytes. In contrast, oxaliplatin was more myelotoxic to murine granulocytes. The level of myelotoxicity of paclitaxel treatment was comparable between human and mouse cells. These results demonstrate that our humanized mouse model can simultaneously evaluate myelotoxicity against human and mouse cells *in vivo*, and provides an effective preclinical tool for predicting appropriate doses of anticancer agents for clinical treatment.

3C02 Anti-tumor effect and safety of PI polyamide alkylating agent targeting the amplified MYCN gene in neuroblastoma

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The proto-oncogene *MYCN* is frequently amplified in neuroblastoma and responsible for the development of the aggressive disease. MYCN is an important therapeutic target although no drugs directly targeting MYCN have not been identified. We have previously reported that a pyrrole-imidazole (PI) polyamide indole-seco-CBI conjugate designed to bind to the coding region of the *MYCN* gene (MYCN-A3) significantly suppresses *MYCN* gene expression and cell proliferation in neuroblastoma cells with *MYCN* amplification. In the present study, we further confirmed its anti-tumor activity in xenograft mouse models using different *MYCN*-amplified neuroblastoma cell lines by intravenous administration at 0.3 mg/kg BW without affecting weight gain of mice. To determine maximum tolerated dose (MTD), we performed administration in ICR mice of escalating intravenous dose of MYCN-A3. Blood biochemical analysis and histology have indicated that MTD of MYCN-A3 is 3 − 30 mg/kg BW in ICR mice. Acute and chronic toxicity tests combined with the modified-SHIRPA protocol after a single administration at 3 mg/kg BW revealed that MYCN-A3 had no serious adverse effect at the dose ten times higher than the effective dose. Intriguingly, MYCN-A3 treatment diminished probe hybridizations at *MYCN* loci by FISH and southern blotting analyses. These data suggest that MYCN-A3 is a promising drug candidate for the treatment of *MYCN*-amplified neuroblastoma with low systemic toxicity.

3C03 Use of secNluc to evaluate drug efficacy in an orthotopic mouse model bearing human leukemia cells

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Although survival can be used as an index to evaluate drug efficacy in an orthotopic mouse model bearing human leukemia cells, unexpected toxicity to a drug occludes the relationship between survival and drug efficacy. Thus, the method of evaluating drug efficacy using secNluc, a kind of a secretional luciferase, was tested as a new index. U937-secNluc, a human leukemia cell line that secretes secNluc, was injected to the tail vein of irradiated SCID beige mice. SecNluc activity in plasma (RLU value) on the 7th day after injection was used to form groups (N=5/group) for vehicle, triptolide (TPL) 0.05 mg/kg, and TPL 0.15 mg/kg, which were then administered intraperitoneally once a day for 14 days. Compared with the vehicle group, the RLU values for the TPL 0.05 mg/kg and 0.15 mg/kg groups on the 4th day of administration were low and were statistically significant. The RLU value for the TPL 0.15 mg/kg group on the 8th day of administration was confirmed to have decreased to the level seen before implantation. These results suggest that secNluc can be used as an index to evaluate drug efficacy in an orthotopic mouse model bearing human leukemia cells.

An approach of dermatitis treatment in laboratory mouse using ointment

3C04

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Dermatitis in mice by over grooming or by fighting, might lead to an appearance of abnormal behavior, weight loss, or inflammatory reaction. These factors may cause an unpleasant influence in research testing. In worst cases, euthanasia is being considered. The Guide for the care and use of Laboratory Animals, says that "a veterinary program that offers a high quality of care and ethical standards must be provided, regardless of the number of animals or species maintained." . To provide a superior veterinary program, we challenged to treat C57BL/6J mouse by using ointment, referencing the guidelines of human pressure ulcers.

【Method】 To mouse with dermatitis, hair around the wound and dead tissues were removed. After then, ointment was applied. When applying, methodological approaches, applying frequency, and curing process were checked.

【Result】 Cotton swab was satisfying than using fingers directly, to control the applying area, and to control the amount of ointment. With applying frequency of over three times a week, progressive recovery was observed. Putting filtering cover on cages to moisturize the air inside, helped the skin regenerate. Also, we found out that it is possible to presume the reason of the dermatitis and it's healing stage by the area, color and condition, which was similar as in human. Additionally, there were observations of early treatment guiding to a result of early recovery.

【Conclusion】 Ointment application effects positively to dermatitis mouse. Also, treatment needs can be evaluated presuming the reason and it's healing stage by its color and condition. And when preparing a new veterinary program, early treatment should be considered.

Evaluating methods for four components of pain

3C05

○ Ryuichiro Ando, Syuki Ojima

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Pain is a subjective sensory which defined as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP, 1979) . Therefore, it is not easy for us to give an objective evaluation for pain of laboratory animals, which do not have a clear language. However, pain is known to bring about four components comprised of the sensory, affective, autonomic and motor components. Thus, we can assess pain of laboratory animals objectively and comprehensively if we perform evaluations of the four components appropriately.

We examined the effects about arterial capsaicin-induced four components measured by the electrophysiological and behavioral pharmacological techniques and then we discuss the validity of these evaluating methods in this report.

Laxative effect of saponin-enriched extracts of *Asparagus cochinchinensis* in the loperamide-induced constipation of SD rats

3C06

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Various natural products containing saponin have been reported as herbal medicines with laxative effects in the constipation model, although saponin-enriched extract of *Asparagus cochinchinensis* (SPA) in constipation has yet to be established. To investigate the laxative effects of SPA on chronic constipation, constipation phenotypes and their related mechanisms were investigated in the transverse colons of SD rats with loperamide (Lop)-induced constipation following treatment with 100 mg/kg of SPA containing a high concentration of saponin (58.67 mg/g). Although food intake and water consumption were maintained at a constant level in the subset group, the total number and weight of stools were significantly higher in Lop-induced constipation rats that were treated with SPA. Moreover, the thickness of the mucosa layer and flat luminal surface, as well as the number of goblet cells, paneth cells and lipid droplets were increased in Lop+SPA compared with the Lop+Vehicle treated group. Furthermore, the Lop+SPA treated group showed significant recovery of the mRNA expression of the muscarinic acetylcholine receptors M2 and M3 (mAChR M2 and M3), some mediators in the downstream signaling pathway of these receptors, the ability for mucin secretion and expression of the membrane water channel gene (aquaporine 8, AQP8). Finally, the activity of SPA was confirmed in primary smooth muscle of rat intestine cells (pRISMC) based on inositol 1,4,5-trisphosphate (IP3) concentration. Therefore, the results of the present study provide the first strong evidence that SPA can be considered an important candidate for improving chronic constipation induced by Lop treatment in animal models.

Improvement effects of Red *Liriope platyphylla* against loperamide-induced constipation through regulation of muscarinic acetylcholine receptors and endoplasmic reticulum stress downstream signaling pathway

3C07

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Red *Liriope platyphylla* (RLP) has long been known as an herbal medicine for treatment of chronic diseases including diabetes, neurodegenerative disorders and obesity. In this study, alterations in constipation symptoms and regulation of related mechanisms were investigated in rats with loperamide (Lop)-induced constipation after administration of extract of RLP (EtRLP). Stool and urine excretion were significantly higher in the Lop+EtRLP treated group than the Lop+vehicle treated group, although feeding behaviors were maintained at a constant level. A significant increase in the villus length, crypt layer, muscle thickness, ultrastructure of the crypt and mucin secretion in the transverse colon of the constipation model was detected in the Lop+EtRLP treated group. Alterations in the muscarinic acetylcholine receptors (mAChRs) signaling pathway and endoplasmic (ER) stress response, including the number of secretory granules and the structure of the membrane sack, were rapidly recovered in the Lop+EtRLP treated group relative to the Lop+vehicle treated group. Spicatoside A, a candidate detected in RLP, led to recovery of the level of G α expression and IP3 concentration in primary smooth muscle of rat intestine cells (pRISMC). Overall, these results suggest that EtRLP containing spicatoside A improves symptoms of Lop-induced constipation in SD rats through recovery of the mAChRs downstream signaling pathway and ER stress response.

3C08 Lipolytic effect of novel extracts from mulberry (*Morus alba*) leaves fermented with *Cordyceps militaris* in the primary adipocytes derived from SD rats

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Mulberry (*Morus alba*) leaves is known to have some therapeutic effects on the lipid metabolism including lipogenesis, lipolysis and hyperlipidemia. However, the identification of novel one with strong lipolytic ability among 36 extracts from the mulberry leaves fermented with *Cordyceps militaris* (EMfCs) have not yet been investigated. To achieve this, the free glycerol release were measured in the primary adipocytes of SD rats after the treatment of 36 EMfCs. Briefly, in order to prepare these extracts, the mulberry leaves powders adding four different concentrations (0%, 25%, 50% and 100%) of silkworm pupae (SWP) powder were fermented with 10% Cordyceps militaris (v/w) during three different periods (4, 5 and 6 weeks). Firstly, 36 extracts were obtained from the fermented mulberry leaves powders using three different solvents (dH2O, 50% EtOH and 95% EtOH). Among 36 EMfCs treated groups, a significant increase on the level of free glycerol was detected in primary adipocytes treated with 12 extracts when compared with Vehicle treated group. Especially, 3M2 treated group showed the highest increase of the glycerol level among 12 extracts. But, their level were not completely agreed with the non-toxicity although most extracts showed non-toxicity. Furthermore, the level of free glycerol dose were gradually increased with dose dependent manner (100, 200 and 400 ug/mL), while toxicity also enhanced with the increase of their dose. Overall, the results of this study provide strong evidence that some extracts derived from EMfCs can stimulate the lipolysis of primary adipocytes at an appropriate concentration and considered as one of lipolytic agents to treat obesity patient.

3C09 Proteomic Analysis on Cell Regulation of Vitamin C in AGS Cell

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Gastric cancer (stomach cancer) is the second leading cause of cancer-related death worldwide after lung cancer. The incidence rates are high in many Asian countries including Korea, China, Taiwan, and Japan. Some in vitro studies showed that ascorbate causes toxicity to cancer cells at concentration that do not affect normal cells. Also, ascorbate induces cell cycle arrest and apoptosis in various tumor cells. However, the molecular mechanism underlying anticancer role of vitamin C has not been fully elucidated. Thus, the present investigated the anticancer activities of vitamin C on human gastric adenocarcinoma AGS cells.

The research findings suggest that vitamin C might be a potential anticancer therapeutic agent for gastric cancer.

3D01 Applying genome editing technology to high-throughput mutant mouse production for the International Mouse Phenotyping Consortium

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RIKEN BioResource Center (BRC) participates in the International Mouse Phenotyping Consortium (IMPC) which is currently composed of 18 research institutions. Early efforts at several IMPC centers targeted critical exons to introduce small insertions or deletions (indels) by imprecise non-homologous end-joining (NHEJ) repair of Cas9-mediated double strand breaks. Because of their random nature, indel alleles are difficult to screen and quality control and cannot be standardized. The IMPC shifted its efforts to produce alleles that more closely resembled knockout alleles made in ES cells by using Cas9 to generate deletion alleles. Our approach that combines paired gRNAs and Cas9 would be reliable, cost-effective and efficient for producing knockout mice for phenotyping. We also show our recent progress on expanding the allele types applying CRISPR/Cas9 technology to genome editing of mouse embryos.

3D02 Aged change of blood & immunophenotypes in IMPC late onset pipeline

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In 2011, RIKEN BioResource Center (BRC) participated in the International Mouse Phenotyping Consortium (IMPC). As the early onset pipeline, we have performed a comprehensive phenotyping of each gene knockout mouse strain from 9 to 16 weeks of age based on the IMPC standardized procedures (IMPReSS). Additionally, IMPC has planned the age-related phenotyping pipeline from 49 to 56 weeks (Late onset). It pipeline includes behavioral, hematology, clinical blood chemistry, immuno-phenotyping and gross pathology test. So, we have compared the blood and immuno-phenotypes of 16weeks with that of 56 weeks using C57BL/6NJcl as a basal line.

Hematological and clinical blood chemistry test have been measured by whole blood and plasma respectively. Immunological test performed by use the mouse splenocyte and multicolor flow cytometry analysis, and we detected the immune cell sub populations. As a result, the hematological test, the white blood cell count, number of lymphocytes, neutrophils and monocytes has been decreased in 56 weeks old female mice. In clinical blood chemistry test, we detected LDL-C and T-CHO increased in only male mice. In the FACS analysis, there are no significant differences in the T and B cells. By contrast, we detected that the effector memory T cells (CD44+ CD62L- CD4+) have increased. Furthermore, we found that NK cells have decreased in 56 weeks old mice especially mature cells (CD11b+). Finally, we could show the age-related differences of blood rerated phenotypes in the reference strain (C57BL/6N). These data is very important to analyze an immunophenotype of each KO mouse line on late onset pipeline in IMPC.

3D03 Intertrial effects on phenotypes of Calorimetry in IMPC phenotyping pipeline

○ Ikuko Yamada, Tomoko Kushida, Misho Kashimura, Tomohiro Suzuki, Hideki Kaneda, Kimio Kobayashi, Ikuo Miura, Tamio Furuse, Shigeharu Wakana

RIKEN BioResource Center

RIKEN BioResource Center Japan Mouse Clinic (JMC) has been performing systematic phenotype analysis of knock-out mice using IMPReSS (International Mouse Phenotyping Resource of Standardised Screens), which is the globally common phenotyping pipeline with standardized procedures, as a member of the International Mouse Phenotyping Consortium (IMPC).

We have conducted experiments on the basis of the procedures of IMPReSS in order to minimize variability of the data among experimental batches. We reported control data of open-field test (OF) and Pre-pulse inhibition test (PPI) in the 63nd Annual meeting of Japanese Association for Laboratory Animal Science, there was no significant difference among the batches in many parameters in OF. However, difference among batches was seen in the startle response in PPI.

In this study, intertrial effects of control data were analyzed in Indirect Calorimetry (CAL). The control data were collected from reference strain (B6/NTac) and wild type mice of each KO strains. As a result, there were no significant differences among the batches in O_2 consumption, CO_2 production and heat production. Because the variation within the batch was very small, significant difference was detected between batches in the respiratory exchange ratio (RER). On the other hand, difference among batches was seen in the ambulation, amount of food intake, and amount of water intake. From these results, it was shown that the behavioral parameters showed large variations between batches. In order to minimized influence of these data variation, it is important to compare with control data within batch.

3D04 A database showing disease-model animal relationship

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Bioresource Center, RIKEN

Recently, the requirements of contribution of model animals to medical researches becomes increased. In this background the informational system which provides information of model animals in the scene of the medical researches are desired both from medical scientists who wants to use disease model animals and biological scientists who wants know how experimental animals which they interested in contribute medical researches.

For example, in the exploring of causal gene of rare disease in which relatively larger affection of mutation of single gene is often identified, information of model animals is extremely important.

In our project, "J-Phenome" (http://jphenome.jp), we collect phenotype data from various databases of experimental animals released from Japan. We convert original phenotype data into standardized data using Resource Description Framework (RDF) which is the standardized on the Web. Phenotype data of J-phenome are standardized across species which can retrieved by the same queries.

Using phenotype data in J-Phenome, we developed data-viewer application program termed as J-Phenome Disease Model Finder (http://diseasemodel.riken.jp/) which shows phenotypic equivalence of disease and experimental animal strains. Users can retrieve experimental animals with disease, gene, tissue and phenotype names. We hope this application program contribute for provisions of experimental animal information into medical researches.

3D05 Current status of "J-phenome", a portal site of phenotype data across model organisms

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We developed an website termed as "J-Phenome" (http://jphenome. Info/) which is the portal of phenotype information produced in Japan. We collected phenotype data from various databases contain phenotype data (e.g. RIKEN BRC, NBRP Rat and NBRP Medaka) under cooperation with original databases. We managed phenotype data as secondary databases, which are converted from original databases to have ontology-based phenotype annotation and to be formalized as world-standard. As a result datasets in J-Phenome are well integrated and can be searched across datasets.

Aiming the world standard, we applied a data format, Resource Description Framework (RDF) , which enables the federation of datasets across databases. Using RDF. For example, phenotype data in J-Phenome can be used in the combination with external RDF-based databases, genome data in Ensembl (URL) , pathway data in Reactome (URL) and disease information in Mornarch Initiative (https://monarchinitiative.org/) . Broader data dissemination of Japanese bio-resource information is expected through J-Phenome.

All data sets of J-Phenome can be freely browsed, retrieved and downloaded through the function of RIKEN MetaDatabase as data infrastructure. In addition, we developed an application "J-Phenome Disease Model Finder" (http://diseasemodel.riken.jp) which enables search of disease model organisms using disease name, gene name and organ name. By using this application, it is possible to explore candidates for disease model animals across species.

Through the promotion of use of phenotype data from J-phenome, we aim to contribute to a wide range of researches including the disease researches using experimental animals.

3D06 Comparative analysis for reproductive ability of C57BL/6Nkorl and commercial C57BL/6N derived from different sources

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C57BL/6 is the most widely used inbred strain for a variety of research areas including cancer, cardiovascular biology, developmental biology, diabetes and obesity, genetics, immunology, neurobiology, and sensorineural research. To compare the responses of C57BL/6NKorl mice with C57BL/6Ns obtained from two different sources based on the reproductive ability, an alterations on the weight of structure of reproductive organs, the number of sperms and eggs, *in vitro* fertilization rate and fertility rate of C57BL/6NKorl mice with (Korea FDA source), C57BL/6N:A (USA source) and C57BL/6N:B (Japan source). In most group, any significant differences were not detected on the weight and histological structure of male and female reproductive organs. Also, the concentration and morphology of sperms and eggs in C57BL/6NKorl mice was very similar with those of C57BL/6N:A and C57BL/6N:B, although C57BL/6NKorl mice was more closely resembled to C57BL/6N:B. However, *in vitro* fertilization rate and fertility rate were differenced from above these. The *in vitro* fertilization rate of C57BL/6N:B were slightly higher than those of C57BL/6NKorl and C57BL/6N:A, although these increase did not show any statistical significance. Furthermore, a similar pattern detected in the results of *in vitro* fertilization rate was observed in fertility rate. But, the body weight of puppy was higher in C57BL/6NKorl and C57BL/6N:B than those of C57BL/6N:A. Therefore, these results of the present study suggest that C57BL/6NKorl, C57BL/6N:A and C57BL/6N:B derived from different sources have a similar overall response to reproductive ability, although there were a few differences in the magnitude of their responses.

The world's leading rat resource center: the National BioResource Project-Rat in Japan

3D07

○ Miyuu Tanaka, Takehito Kaneko, Birger Voigt, Yuki Neoda, Kazumi Hagiwara, Zong-hu Cui, Yuki Nakagawa, Tomomi Nagao, Satoshi Nakanishi, Ken-ichi Yamasaki, Masahide Asano, Takashi Kuramoto

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The National BioResource Project - Rat in Japan (NBRP-Rat) is one part of the NBRP in Japan for more than 20 species including animals, plants, microbes, tissues and DNAs. It is founded by the Japanese Ministry of Education, Culture, Sports, Science and Technology and started in 2002. The 3rd term of NBRP-Rat has started in 2012, following the 1st and 2nd term (2002-2011), and will finish in fiscal year 2016. The Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University (central facility) and RIKEN BioResource Center (BRC) (cooperative facility) conducted the collection, preservation and supply of rat resources and the NBRP-Rat has grown as the world's leading rat resource center. By January 2017, 824 rat strains are deposited to the NBRP-Rat and 1,163 material transfer agreements have been contracted for distribution of rat resources. Recent developments of the generation of genetically modified (GM) rats using gene editing nucleases (ZFN/TALEN/ CRISPR) technology will boost the utility of the rat as biological resource. The NBRP-Rat gives technical supports for generating GM rats based on our rat reproduction technology. From 2017, the 4th term of NBRP-Rat will start with collaboration of Kyoto University (central facility), RIKEN BRC and Osaka University (cooperative facilities).

Activity of Bio Resource Bank and Laboratory Animal Research Center at IMSUT

3D08

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Laboratory Animal Research Center of the Institute of Medical Science, The University of Tokyo (IMSUT-LARC) was established in April 1971. The whole area of a five-story building with one basement is specially designed for animal breeding and experiments. In 1998, the building has been improved to perform developmental engineering and keep bigger animals such as rabbits. It also includes infectious experiment area (P2A, P3A). Nowadays, it offers opportunity for approximately 40 laboratories to perform animal experiments.

Since techniques of generating genetically modified mice and manipulating embryos had been introduced to IMSUT, we support to preserve genetically modified mouse. In 2016, we stored 116 strains (146 lines, 1394 tubes), most of which are derived from mouse strains generated in IMSUT laboratories or transferred from other facilities. We utilize these frozen embryos for transplantation or transfer to other facilities by request from our users.

IMSUT-LARC started to open IMSUT-Bio Resource Bank (BRB) in 2013. We collect and preserve genetically modified mice strains, to accumulate bio resource (embryo, sperm, and egg). At present, 160 mouse strains are deposited to our bank. We further improve methods for animal care depending on characteristics of various mouse strains, and make efforts to increase availability of bio resources. Information on IMSUT-BRB is available from our website at http://www.ims.u-tokyo.ac.jp/larc/bank/bankindex.html.

3D09

Progress of collection, preservation and distribution of mouse resources at RIKEN BRC

○ Atsushi Yoshiki, Toshiaki Nakashiba, Shinya Ayabe, Noriko Hiraiwa, Hatsumi Nakata, Fumio Ike, Keiji Mochida, Atsuo Ogura, Yuichi Obata

RIKEN BioResource Center

RIKEN BRC has operated as the core facility of the NBRP for the mouse resources by the MEXT-AMED, Japan as well as an international hub for mouse resources together with major mouse repositories in US and Europe. Our strategy is to focus and collect strains mainly developed in Japan without duplicating overseas collections. In FY2016, the collection exceeds 8000 resources with deposits of over 200 strains including floxed Braf mutant (Hum Mol Genet 23: 6553, 2014), Amyotrophic lateral sclerosis-related patient mutation transgenic mice (Hum Mol Genet 24: 3427, 2015), Chd8-deficient autism model (Nature 537, 675, 2016). The distribution format mainly with live mice has become diversified with increase of frozen strains and organs/tissues. With approximately 400 new users per year, we have supplied mice to 5,720 domestic and overseas users in 1,200 organizations. So far, our users have successfully published 730 research papers with high impact. While developing advanced genome editing knock-in technologies by support of NBRP program, we are also developing methods of quality control of such genome-edited mice. In the International Mouse Phenotyping Consortium, we started KO mouse production by CRISPR/Cas9, based on the request of domestic scientists, and have made the KO mice publicly available for the international research community.

3D10

Experimental design-based production of genetically engineered mice using the CARD Mouse Bank System

○ Kaori Sakaguchi[1], Fumi Takahashi[1], Kiyoko Yamashita[1, 2], Tomoko Kondo[1, 2], Yukie Haruguchi[1, 2], Yumi Takeshita[1, 2], Yuko Nakamuta[1, 2], Tomoko Umeno[1, 2], Eri Ishida[1, 2], Mari Iwamoto[1], Wataru Sakamoto[1], Shuuji Tsuchiyama[1], Yoshiko Nakagawa[1], Naoko Nakamura[3], Satomi Kawabe[3], Daisuke Torigoe[3], Toru Takeo[1], Naomi Nakagata[1]

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In the CARD Mouse Bank System, we provide a range of useful services to support animal experiments using mouse reproductive techniques. Reproductive techniques facilitate the efficient production and preservation of genetically engineered mice. They allow a researcher to obtain as many animals as he or she may require via in vitro fertilization and embryo transfer. The system of experimental design-based production of genetically engineered mice expedites animal experiments and saves on labor, space and other costs related to the upkeep of animal colonies. Recently, demand for the experimental design-based production of genetically engineered mice is increasing. In this presentation, we will give an overview of this service and explain how it contributes to the efficient management of animal experiments using mouse reproductive techniques at our center.

Efficient reanimation service from cryopreserved embryo and sperm using the CARD Premium Mouse Bank System

3D11

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The Center for Animal Resources and Development (CARD), Kumamoto University provides a mouse bank service, which supports the collection, production, preservation and supply of genetically engineered mice in order to facilitate life science research using genetically engineered mice. Information concerning the archived mouse lines and strains is published on the International Mouse Strain Resource (IMSR) website. Researchers can acquire these mice under the terms of a material transfer agreement. In addition, CARD provides another type of mouse bank service, called the CARD Premium Mouse Bank System. Our premium mouse bank system offers a reanimation service, in which we reanimate genetically engineered mice from cryopreserved sperm and embryos prepared at a different institute or university. In this presentation, we will give a few examples of efficient reanimation using the reproductive techniques offered by the CARD Premium Mouse Bank System.

Long-term Hematopoietic Engraftment of Human iPS Cell-Derivatives After Sheep in Utero Transplantation

3D12

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Generating hematopoietic stem cells (HSCs) from human iPSCs has been a great challenge. Nobody has successfully achieved it thus far. We hypothesized some key environmental factors are missing *in vitro*. Therefore, we tried *in vivo* generation of HSCs from human iPSCs. In this study, we generated human/sheep hematopoietic chimera; that is, sheep that have human HSCs derived from human iPSCs. Human iPSCs were cultured on murine stromal OP9 cells with multiple cytokines for 6 days for differentiation to a hematopoietic lineage. The cells were then transplanted into the liver of busulfan-conditioned fetal sheep (day 47 - 63, full term 147 days). The animals were delivered at full term, and the engraftment of human hematopoietic cells was quantitatively evaluated by PCR of colony-forming units (CFUs) positive for human-specific ND5 gene sequence. In the lambs after birth, human CFUs were detected in the bone marrow at levels of 2.3% to 6.3% (n = 4), and they were still detectable at present (at 26 months post-transplantation). Considering that many researchers have long failed to generate engraftable HSCs from human iPSCs, the data here imply that the acquisition of long-term hematopoietic engraftment ability of human iPSCs may depend on the *in vivo* microenvironment such as in the fetal sheep liver. We are now taking out human HSCs from the sheep.