ASIAN CONGRESS
ON VETERINARY
ANATOMICAL,
HISTOLOGICAL
AND CYTOLOGICAL
SCIENCES

The First Conference of the Asian Association
of Veterinary Anatomists

PROCEDINGS

Tsukuba International Congress Hall
Ibaraki-Tsukuba, Japan
19th - 21st March, 2006

(http://www.jpn-ava.com)
LECTURE PROGRAM
21st March, 2006

9:00-9:05: Opening remarks
Dr. Masahito UEHARA (Tottori University)

9:05 – 12:00 Lectures
Chair person: Dr. Kazuyuki TANIGUCHI (Iwate University)

9:05 - 9:25: The education and research of veterinary anatomy in China
Dr. Yaoxing CHEN, Department of Veterinary Anatomy, College of Veterinary Medicine, China Agricultural University (Beijing, People’s Republic of China)

9:25 - 9:45: Teaching of veterinary anatomy in Korea
Dr. Heungshik S. LEE, Department of Veterinary Anatomy, College of Veterinary Medicine, Seoul National University (Seoul, Republic of Korea)

9:45 - 10:05: Research fields of veterinary anatomy in Chungbuk national university, Korea
Dr. Sang-Yoon NAM, Research Institute of Veterinary Medicine and Department of Veterinary Anatomy, College of Veterinary Medicine, Chungbuk National University (Chungbuk, Republic of Korea)

10:05 -10:25: The education and research of veterinary anatomy in Bangladesh
Dr. Mohammed Zahirul Islam KHAN, Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University (Mymensingh, Bangladesh)

10:25 -10:45: Veterinary anatomy education in Indonesian veterinary school: Present status and future recommendation
Dr. Srihadi AGUNGPRIYONO, Department of Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University (IPB) (Bogor, Indonesia)

10:44 -11:05: Para Rubber Models for Veterinary Anatomical Teaching Aid
Dr. Teerasak PRAPONG, Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University (Bangkok, Thailand)

Dr. Worawut RERKAMNUAYCHOKE, Department of Anatomy, College of Veterinary Medicine, Kasetsart University (Bangkok, Thailand)
11:25 - 11:45: The education and research of veterinary anatomy in Japan  
Dr. Kazuyuki TANIGUCHI, Laboratory of Veterinary Anatomy, Faculty of Agriculture, Iwate University (Morioka, Japan)

11:45 - 11:55: General discussion

11:55 - 12:00: Closing remarks  
Kazuyuki TANIGUCHI (Iwate University)

CONFERENCE COMITTEE

President: Dr. Kazuyuki TANIGUCHI, Iwate University
Vice President: Dr. Masahito UEHARA, Tottori University
Committee members: Dr. Kazunari TAKEHANA, Rakuno-gakuen University  
Dr. Kazumi TANIGUCHI, Kitasato University  
Dr. Eime SATO, Tohoku University  
Dr. Eiichi HONDO, Yamaguchi University
Secretary-general: Dr. Noboru MANABE, The University of Tokyo
Treasurer: Dr. Masamichi KUROHMARU, The University of Tokyo
Honorary presidents: Dr. Jynzo YAMADA, Professor Emeritus of Obihiro University  
Dr. Yoshihiro HAYASHI, The University of Tokyo  
Dr. Takashi MAKITA

The conference is financially aided by Japanese Association of Veterinary Anatomists and partly by a Grant-in-Aid for Creative Scientific Research (13GS0008) to N.M. (Secretary general) from the Japan Society for the Promotion of Science.
THE EDUCATION AND RESEARCH OF VETERINARY ANATOMY IN CHINA

Yaoxing CHEN

Department of Veterinary Anatomy, College of Veterinary Medicine,
China Agricultural University, Beijing 100094, People's Republic of China
Tel:+86-10-6273-3013, E-mail: yxchen@cau.edu.cn

Up to now, there are forty-three universities owned faculty of veterinary medicine in China. The period of schooling is usually four years, but also has five years in the five universities such as China Agricultural University, Nanjing Agricultural University, Huazhong Agricultural University, South China Agricultural University and Northwest China Agricultural University. From these schools, around 5,000-6,000 vets are graduated every year. They were awarded the Agricultural Bachelor degree. The main goal of veterinary education is to prepare students with “broad foundations, strong competence, fine makings and well adaptabilities”. Therefore, all students have to complete a total of 230 credits and graduation thesis. Of them, credits of the basic courses (including anatomy) are about 55%. The course of veterinary anatomy consists of macroscopic anatomy (6.5 credits) and histology & embryology (5 credits). Two courses are given subsequently within 2 semesters, i.e. in 3rd and 4th semester. In recent years, with the development of anatomy and higher demand of veterinary specialists, educations of veterinary anatomy emphasized further the training and practicing of skill for students. In China, the research fields of veterinary anatomy includes Neurobiology, Mucosal Immunology, Reproduction and Biology, and Embryogenesis, etc. Some morphological methods such as electron microscopy, confocal microscopy, immunohistochemistry, and the molecular biological techniques such as gene cloning, RT-PCR, Northern blotting and in situ hybridization, were used. In addition, the Conference for Chinese Association of Veterinary Anatomists is convened once every two years. The education and research of veterinary anatomy were discussed in the conference.
TEACHING OF VETERINARY ANATOMY IN KOREA

Heungshik S. LEE

Department of Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea
Tel: +82-2-880-1275, E-mail: leehss@snu.ac.kr

In Korea, Veterinary education is 6-year-system composed of 2 years pre-veterinary and 4 years veterinary course. Currently, total teaching hours in veterinary course is about 4,100 hours. The teaching hours of veterinary anatomy, histology and embryology are about 11% of total teaching hours. In other words, total lecture hours are about 200 hours and Lab 250 hours. And the credit point is 8 ~ 10 and 2 ~ 4, respectively. The Korean Association of Veterinary Anatomists (KAVA) recommend 1 cow, 5 dogs, 2 pigs, 5 goats and 20 chickens per 20 students to be used for the dissection. Enrolled student is 43 to 111 per class depending on school. The ratio of male and female students is about 7:3 and the ratio of teaching staff to students is 1:25. For evaluations of student’s performance, we use written test, oral test, lab practice and quiz. About 10% of enrolled students are failed, annually. We have been doing our best to improve teaching quality such as increasing staff and introducing PBL.
It is a great pleasure to be here with you to deliver this presentation at this meeting as a President of KAVA. First of all, I want to extend my sincere appreciation to the President of JAVA and JAVA members who devoted their precious time and effort to put all pieces together to organize this meeting possible and successful.

There are 10 veterinary schools in Korea. One is private and nine is national schools. Four hundred fifty students graduate vet schools every year. Veterinary education is composed of 4 year-veterinary courses and 2 year-preveterinary courses.

In 2 year-preveterinary course, physics, chemistry, biology, mathematics, animal husbandry, nutrition, environmental or ecology, philosophy, history and logics are provided to students. In 4 year-veterinary course, twenty three lectures are provided to the students, which are to meet the global standardization of Veterinary Education Minimum Requirement guided by WVA (World Veterinary Association). They are anatomy, physiology, pathology, microbiology, internal medicine, surgery, radiology, ophthalmology, dermatology, neuroscience, and theriogenology, and etc.

Now 9,039 veterinarians are doing their jobs in Korea. The 36% of them are clinical veterinarian. Another 64% of them have their jobs in government organization, university, research institutes and private companies. This is quietly different situation compared with Europe or USA. In Korea, there are 2,700,000 pets, 11,200,000 edible domestic animals, 127,500,000 chickens and 2,700,000 pets. Livestock unit is just only 419 per veterinarian in Korea. This is also quite different from Europe.

These days, the veterinary education of Korea is facing on turning point due to changes of social and economic conditions. During the last 10 years, they have been increasing about 4% every year, but
currently these conditions are stagnating. Population of old age augment in an alarming way together with rising incomes, and singles and nuclear family is also increased. In addition, the species of pet animal become more diverse. Traditionally, almost Korean is vegetarian, but these life style has been changing due to economic growth and westernization. Therefore, consumption of meat, egg and milk has been increasing.

The biotechnology boom is due to increase investment of R&D by public and private projects and to asking industrial and social needs. It is rapid increase of private and public investment to biotechnology fields. During the next 5 years, they will invest 10 billion US dollar. This amount may not as big as those in the USA or Japan. But the annual growth rate is very high approaching to 70%. So we expect shortage of DVM by the increasing contribution of veterinary science and shortage of MS and Ph.D manpower in biotechnology fields until the year 2010. Shortage rate is 68% on average per year. In these fields, each year about 150 veterinarians will be on an average.

So, we have been reformed the veterinary curriculum to satisfy the above mentioned conditions 9 years ago. For that reasons, we have been reinforced paraclinical and clinical course. Therefore, during recent 5 years, 10% of lecture time has been diminished in anatomy, histology and embryology, the contents of them have been reinforced. Now 96~128 hours for anatomy, 64~96 for histology, 32~64 for embryology and 15~30 for neuroscience are dedicated. Total teaching hours in veterinary course is about 4,100 hours. The total teaching hours for anatomy, histology and embryology are about 11% of them. The total teaching hours for anatomy, histology and embryology are about 200 hours, and its lab practice about 250 hours. The credit point of anatomy is to 5~6, histology 2~3 and embryology 1~2, and that of lab is 1~2, 1~2 and 1, respectively.

Currently, 43 to 111 students per a class are enrolled depending on schools. The ratio of male and female students is about 7:3. The ratio of anatomy faculty to students is 1:25. Dog, cow, pig, goat and chicken are used for gross anatomy. One cow, 5 dogs, 5 goats, 2 pigs and 20 chickens are used per 20 students to meet the standardization of "Minimum Recommended Unit for Dissection Lab" edited by KAVA.

Gross anatomy is taught by organ system, and histology and embryology lecture is same as gross anatomy.
For evaluations of student’s performance, we use written test, oral test, lab practice and quiz. About 10% of the enrolled students are failed, annually. Regular examination are composed of multiple choice question test, essay type, short answer type, completion type, subjective question test and objective question test. Generally, actual observation on microstructure under microscope is key point to get good score for veterinary histology.

For veterinary anatomy textbook, "Textbook of Veterinary Anatomy" in Korean edited by Dyce, Sack and Wensing, and "Canine Anatomy" edited by Adams has been widely used. For laboratory course of veterinary anatomy, "Guide to the Dissection of the Dog" in Korean edited by Evans and de Lahunta have been used.

As veterinary histology textbook, "Textbook of Veterinary Histology" in Korean edited by Dellman and Eurell, has been widely used. For laboratory course of veterinary histology, "Color Atlas of veterinary histology" in Korean edited by Bacha Jr. has been used. "Neuroscience" edited by Bear, Connors and Paradiso has been used for Veterinary Neuroscience Course.

Recently, in Korea, newly established education system is widely introduced for better and effective education in the field veterinary medicine. Block lecture based on organ systems or systematic
lecture system from basic to clinics are prepared to introduction to regular course. Problem based learning (PBL) or Objective Structured Clinical Examination (OSCE)/ Clinical Performance Examination (CPX) system will be introduced in near future.
RESEARCH FIELDS OF VETERINARY ANATOMY IN CHUNGBUK NATIONAL UNIVERSITY, KOREA

Sang-Yoon NAM

Department of Veterinary Anatomy, College of Veterinary Medicine, Chungbuk National University, Cheongju 361-763, Republic of Korea
Tel: +82-43-261-2596, Fax: +82-43-271-3246, Mobile: +82-11-9708-6716 E-mail: synam@cbu.ac.kr

We have been studying in the functional analysis of genes of antioxidative enzymes during mammalian embryogenesis and the development of a novel biomarker for endocrine disruptors in male reproductive organs and its application. For these research purposes, we have used the morphological methods such as electron microscopy, confocal microscopy, and immunohistochemistry, and the molecular biological techniques such as gene cloning, RT-PCR, Northern blotting, whole embryo culture, whole mount and tissue in situ hybridization, and RNAi. Spatio-temporal analysis on the gene expression of antioxidant enzymes during mouse embryogenesis and studies on the endocrine disruptors in male reproductive organs will be discussed.
RESEARCH FIELDS OF VETERINARY ANATOMY IN CHUNGBUK NATIONAL UNIVERSITY

Sang-Yoon NAM, DVM, PhD

Department of Veterinary Anatomy, College of Veterinary Medicine, Chungbuk National University
Cheongju 361-763, Korea

We have been studying in the functional analysis of genes of antioxidative enzymes during mammalian embryogenesis and the development of a novel biomarker for endocrine disruptors in male reproductive organs and its application. For these research purposes, we have used the morphological methods such as electron microscopy, confocal microscopy, and immunohistochemistry, and the molecular biological techniques such as gene cloning, RT-PCR, Northern blotting, whole embryo culture, whole mount and tissue in situ hybridization, and RNAi.

Spatio-temporal analysis on the gene expression of antioxidant enzymes during mouse embryogenesis

Reactive oxygen species (ROS) can be produced by normal embryo metabolism during embryogenesis. The production of ROS may interfere with the embryo redox status, thereby causing 'oxidative stress' which may alter essential cellular functions such as gene expression. The oxidative stress in embryos is known to stimulate gene expression of antioxidant enzymes which protect early gametes and embryos against ROS damage during embryo development. Most of selenoproteins also participate in scavenging ROS. Previous reports demonstrated that selenoproteins are essential for early embryonic development. Homozygous mutation of thioredoxin gene in mice caused embryonic death in the preimplantation stage. In addition, thioredoxin-2 mutant embryos showed massively increased apoptosis at Ed 10.5 and completely disappeared at Ed 12.5 from placenta. On the other hand, mice inactivated by homologous recombination for GI-GPx and selenoprotein P showed no developmental defects. Furthermore, deficiencies of extracellular SOD and CuZnSOD did not affect the normal process of mammalian development, while MnSOD mutation induced the growth retardation in C57BL/6J mice. These findings suggest that the antioxidant enzymes may be regulated in various manners during embryogenesis. Recent studies have shown that genetic inactivation of cGPx induced by high oxidative stress resulted in growth retardation in adulthood due to reduction of mitochondrial energy production, indicating that the expression of cGPx may be essential for normal cellular function. In our studies, cGPx mRNA level was investigated at the ontogenic stage of mouse embryos using semiquantitative RT-PCR analysis. The cGPx mRNA was constantly expressed throughout all the embryonic stages. These findings suggest that the defence system of cGPx is conserved essentially for all steps of embryonic development. Esposito et al (2000) reported that X-Gal staining of C57BL/6 mouse embryos at day 13.5 revealed GPx1tm2Mgr expression in the liver, spinal cord, and eye and a distinctive pattern in brain consistent with brain stem. Analysis of adult tissues revealed highest levels of expression in liver and kidney cortex. In our studies, cGPx mRNA was highly expressed in vibrissae, forelimb and hindlimb plates, tail, and spinal cord at Ed 13.5 and in most of organs including liver, kidney, and brain at Ed 17.5. These findings indicate that cGPx is expressed tissue-specifically on the ontogenesis of mouse embryos. The brain has been characterized by comparatively high SOD activity during mouse embryogenesis, but the cGPx and CAT activities are low throughout development. In adult brain, cGPx activity increases with aging, suggesting an increase in organic hydroperoxide formation during aging. Fetal exposure to many substances including cocaine, phenytoin, calcium channel blockers, and nitric oxide synthase inhibitor caused transient uteroplacental hypoperfusion and induced the malformations of limb and central nervous system. In our studies, cGPx mRNA was mainly expressed in limb buds, dorsal neural tube, and brain at Eds 10.5-13.5. The expression of cGPx mRNA during the development of limb appeared first in the margin of limb bud and spread out to foot plate. The cGPx mRNA expression during the development of brain was first detected in mesencephalon and thereafter observed in all central nervous system (CNS) regions. These findings indicate that cGPx may act as an important antioxidant in limb and CNS development. Lungs are exposed to relatively high oxygen tensions, especially at birth, which causes oxidative injury in the neonate who is more susceptible to oxidative stress than in adult. Therefore, cGPx may act as an major protector against ROS massively produced by lung development. From our results, cGPx mRNA was strongly expressed in developing lung tissues. In addition, the skin becomes the primary outer protective barrier. UV light induces oxygen radicals responsible for adverse effects on the skin, including carcinogenesis.
Selenium plays a protective role in UV-A damage of cultured skin fibroblasts. The protective effect in skin may be mediated, in part, by the selenium-dependent synthesis of GPx. These findings indicate that selenium-dependent GPx may be effective for the prevention of oxidative damage in embryo skin. cGPx mRNA was significantly expressed in epithelial cells of skin and whisker follicles. The signal was remarkably increased in the skin epithelium between Ed 14.5 and Ed 17.5. After Ed 14.5, cGPx mRNA expression was detected in various tissues including stomach, intestine, pancreas, kidney, lung, heart, liver, thymus, and Rathke's pouch. The cGPx mRNA was expressed in the glandular region and surface epithelium of stomach and intestinal villae of small intestines. The intestinal crypt epithelial cells are very sensitive to abdominal and pelvic radiation therapy. The damage in the mucosal epithelium can result in a variety of symptoms, including diarrhea, electrolytic imbalance. The expression of cGPx mRNA in renal tubules, glomeruli, and cortex was higher than mesenchyme and medulla. Ischemia/reperfusion induces oxidative stress and consequently damages the proximal convoluted tubules of kidney in rats. In our studies, cGPx mRNA expression was massively found both in tissues in contact with the external environment and in additional epithelial tissues involved in absorption, transport, and excretion. These data imply that cGPx is expressed mainly in metabolically active sites. Higher metabolic rates may result in the generation of ROS. cGPx activity was high in the epithelial linings of fetal and neonatal rat tissues. These facts could be explained that cGPx is a general antioxidant enzyme in embryo. The spatio-temporal expression of cGPx mRNA was observed in the forelimb and hindlimb buds, CNS, active metabolic tissues, and developing epithelial tissues. These findings are consistent with the hypothesis that cGPx functions to protect the embryo from oxygen radicals and/or hydroperoxides induced by the intra- or extracellular environment. A specific role of cGPx relative to other antioxidants on the ontogeny of embryos remains to be further studied in future.

At present, we have investigated the gene expression pattern of superoxide dismutase (SOD) family in mouse embryogenesis.

**Studies on the endocrine disruptors in male reproductive organs**

It is widely accepted that estrogen is a typical female hormone. In the male, an exposure to estrogen compounds can lead to reduction in gonad size, feminization of genetic males, low sperm count and/or motility. However, it has been reported that estrogen is present in some male vertebrates and that it is normally expressed in the male reproductive organs. In mammals, estrogen appears to regulate the reabsorption of luminal fluid in epididymides and to affect sexual behavior. 17β-estradiol (E2), an intrinsic estrogen in vertebrates, is present in the serum and its receptor is expressed in the testis during the whole process of spermatogenesis. Even though there has been a noticeable increase in reports analyzing the actions of estrogen in the testis, the clear functions of estrogen in male reproduction have not been clearly established. The selenoprotein phospholipid hydroperoxide glutathione peroxidase (PHGPx) is highly expressed in testes under gonadotropic control. The expression patterns of PHGPx mRNA by 17β-estradiol (E2) as an estrogen and tamoxifen (Tam) as an estrogen antagonist were investigated in the reproductive organs of male rats. Twelve-week-old male Sprague-Dawley rats were subcutaneously injected with E2 (7.5 µg/kg/day) or Tam (5 mg/kg/day) for 1 week. The treatment of E2 significantly increased the levels of PHGPx mRNA in both testes and prostates, while the treatment of Tam significantly decreased the levels of PHGPx mRNA, compared to vehicle control (p<0.01). The treatment of E2 or Tam slightly decreased the levels of PHGPx mRNA in epididymides. In histopathological examination, severe vacuolization and depletion of germ cells in seminiferous tubules, cell debris in the tubular lumen, and mild proliferative changes in interstitial tissues were observed in the testes of Tam-treated rats, while only mild spermatogonial proliferation was observed in the seminiferous tubules of E2-treated rats. There were no typical histopathological changes in the epididymides of all experimental rats but a mild epithelial proliferation in the prostates of E2- or Tam-treated rats. From these results, we conclude that exogenous estrogens increase the PHGPx gene expression by forming a complex with ERβ, then binding to estrogen-responsive elements of PHGPx gene with unknown coactivators, which regulate the expression of PHGPx. In contrast, Tam blocks the binding of estrogen with ERβ thereby reducing the expression of PHGPx. To elucidate the correlation of PHGPx with ERβ, more detail studies remain to be performed in near future.
THE EDUCATION AND RESEARCH OF VETERINARY ANATOMY IN BANGLADESH

Mohammed Zahirul Islam KHAN

Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh
Tel: +88 091 55695-2589, Fax: +88 091 55810
E-mail: mzikhan1957@yahoo.co.in

The veterinary anatomy has been incorporated as an important basic subject of Veterinary Science, in Bangladesh. Therefore, prior to the presentation regarding the education and research of veterinary anatomy, it is important to describe in short history of Veterinary Science in Bangladesh. History of veterinary science in Bangladesh (establishment of Bangladesh Agricultural University and establishment of Veterinary Colleges and Faculties in the private Universities), education of veterinary anatomy in Bangladesh (undergraduate course, master degree course, and doctor course), the teachers and their abilities, cooperation with the governmental and non-governmental organization, research in the department of veterinary anatomy (studies on the immune system of the chicken, studies on the aortic heart valve of indigenous cattle for bioprosthetic purpose) will be discussed. In conclusion, the department of Anatomy and Histology is existing in 4 government colleges, and both in a private and public University with standard syllabus and academic courses in Bangladesh. The members of the department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University not only engaged with their intra-faculty academic activities they have also been carried out an academic extension program with different NGO’s, and having linkage program with Medical colleges, Veterinary colleges and Bangladesh Heart Research Association.
The veterinary anatomy has been incorporated as an important basic subject of Veterinary Science, in Bangladesh. Therefore, prior to the presentation regarding the education and research of veterinary anatomy, it is important to describe in short history of Veterinary Science in Bangladesh.

History of Veterinary Science in Bangladesh

Establishment of Bangladesh Agricultural University

With the partition of India in August 1947 and consequence emergence of Pakistan the Muslim Veterinarians move to the East Pakistan and established a Veterinary College at the outskirts of Comilla town on 7th December 1947 with a three years diploma course known as DVMS (Diploma in Veterinary Medicine and Surgery). The Directorate as well as the Veterinary College then got shifted from Comilla to Dhaka in 1950 and five years B. Sc. (AH) degree course was introduced under the affiliation of the University of Dhaka in 1951. From Dhaka the Veterinary College was shifted to Mymensingh towards the close of 1955. The degree was renamed as B.Sc (Vet. Sci. & AH) in 1957. In 1961, the East Pakistan Agricultural University (now Bangladesh Agricultural University) was established taking the existing East Pakistan College of Veterinary Science and Animal Husbandry as its nucleus. The activities of the former College of Veterinary and Animal Husbandry taken over by the two faculties, viz. Faculty of Veterinary Science and Animal Husbandry. The complete Veterinary degree of B.Sc. (Vet. Sci. & AH) was bifurcated at that time into two separate degrees, namely DVM (Doctor of Veterinary Medicine) and B. Sc. (AH) in 1963 of 4 years course.

Establishment of Veterinary Colleges and Faculties in the Private Universities

With the demand of the country, four Government Veterinary colleges have been established in Bangladesh, at Sylhet (1994), Chittagong (1996), Dinajpur (2003), and Barisal (2003). A complete Veterinary Faculty has also opened in University of South Asia located at the heart of the capital of Bangladesh very recently. Naturally anatomy departments are also there.

EDUCATION OF VETERINARY ANATOMY IN BANGLADESH

The academic courses and curricula regarding anatomy and histology at undergraduate level are same in Bangladesh Agricultural University (BAU), four colleges, and in private University. Variation exists only in that the colleges are run by undergraduate courses only, in contrast, the BAU is run by both undergraduate and graduate (masters and Ph.D) courses. Therefore, in this paper BAU is focused as a model for education and research of veterinary anatomy in Bangladesh.

1) Undergraduate course

(a) Duration of the course

The veterinary degree (DVM) course offered by BAU is of 4 years duration under semester system. It is consisting of 4 levels having a total 8-course semester (each semester is for 6 months) and 1 extra semester for internship program. In each semester 4 months is scheduled for theory and practical classes and rest of the 2 months is for examination, tabulation, and publication of the results. Teaching of anatomy courses is confined from level 1 to level 3 of the DVM course only. The courses of the anatomy subjects of the Faculty of Animal Husbandry, Bangladesh Agricultural University is also under the jurisdiction of the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.

(b) The courses, total marks, credit hours and contact hours

We are teaching different branches of anatomy (Table 1) to the students (about 100 students are getting their admission per year) in well equipped modern class rooms and laboratories in such a way that a student can understand correlated subjects in the subsequent semester also, e.g. pathology, physiology, medicine, surgery and other subjects of the veterinary sciences. The teachers teaches the student in theory classes using black board, overhead projectors and multimedia projectors.
Table 1. Name of the subjects, courses, credit hours, contact hours, and marks

<table>
<thead>
<tr>
<th>Department</th>
<th>Subjects</th>
<th>Title of the courses, Level and Semester</th>
<th>Credit hrs. (T+P)</th>
<th>Contact hrs.</th>
<th>No. of courses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomy and Histology</td>
<td>1. Gross Anatomy</td>
<td>1. Anatomy (osteology, arthrology, myology. Course of level 1 semester 1</td>
<td>2+1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Anatomy (splanchnology, angiology, aesthesiology). Course of level 1 semester 2</td>
<td>2+1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Comparative and Neuroanatomy. Course of level 2 semester 1</td>
<td>0+1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Topographic and surgical anatomy. Course of level 3 semester 1</td>
<td>0+1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2. Histology and Embryology</td>
<td>1. Histology. Course of level 1 semester 1</td>
<td>3+1</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Embryology. Course of level 1 semester 2</td>
<td>1+1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>8+6</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Total marks: 10 subjects (4 theory + 6 practical) × 100 = 1000

2) The master degree course

M. S course having 3 semesters (6 month each), which is comprising 2 course semester and 1 research semester. The course semester is oriented in such a way that a student should take compulsory and optional subjects (Table 2).

Table 2. Design of courses of M.S. in Anatomy

**January –June semester**

**Compulsory course:**

<table>
<thead>
<tr>
<th>Course code</th>
<th>Course title</th>
<th>Credit hour (T + P)</th>
<th>Total credit and Marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAH 501</td>
<td>Ruminant Anatomy</td>
<td>1 + 1</td>
<td>8 credit 50x8 = 400</td>
</tr>
<tr>
<td>VAH 502</td>
<td>Veterinary Histology</td>
<td>1 + 1</td>
<td></td>
</tr>
<tr>
<td>VAH 503</td>
<td>Developmental Anatomy</td>
<td>2 + 0</td>
<td></td>
</tr>
<tr>
<td>VAH 504</td>
<td>Evolutionary Anatomy</td>
<td>2 + 0</td>
<td></td>
</tr>
</tbody>
</table>

**Elective courses:** The students select two subjects as an elective course from the following:

<table>
<thead>
<tr>
<th>Course code</th>
<th>Course title</th>
<th>Credit hour (T + P)</th>
<th>Total credit and Marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAH 505</td>
<td>Equine Anatomy</td>
<td>1 + 1</td>
<td>4 credit 50x4 = 200</td>
</tr>
<tr>
<td>VAH 506</td>
<td>Comparative Anatomy of Locomotive System</td>
<td>1 + 1</td>
<td></td>
</tr>
<tr>
<td>VAH 507</td>
<td>Comparative Anatomy of Digestive and Respiratory system</td>
<td>1 + 1</td>
<td></td>
</tr>
<tr>
<td>VAH 508</td>
<td>Anatomy of Immune system</td>
<td>2 + 0</td>
<td></td>
</tr>
<tr>
<td>VAH 509</td>
<td>Comparative Anatomy of Urogenital system</td>
<td>1 + 1</td>
<td></td>
</tr>
<tr>
<td>VAH 510</td>
<td>Canine and Feline Anatomy</td>
<td>1 + 1</td>
<td></td>
</tr>
</tbody>
</table>

**July December semester**

**Compulsory course:**

<table>
<thead>
<tr>
<th>Course code</th>
<th>Course title</th>
<th>Credit hour (T + P)</th>
<th>Total credit and Marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAH 511</td>
<td>Avian Anatomy</td>
<td>1 + 1</td>
<td>8 credit 50x8 = 400</td>
</tr>
<tr>
<td>VAH 512</td>
<td>Endocrine Anatomy</td>
<td>2 + 1</td>
<td></td>
</tr>
<tr>
<td>VAH 513</td>
<td>Neuro Anatomy</td>
<td>1 + 1</td>
<td></td>
</tr>
<tr>
<td>VAH 514</td>
<td>Histochemistry</td>
<td>1 + 0</td>
<td></td>
</tr>
</tbody>
</table>
Elective courses: The students select two subjects as an elective course from the following:

<table>
<thead>
<tr>
<th>Course code</th>
<th>Course title</th>
<th>Credit hour (T + P)</th>
<th>Total credit and Marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAH 515</td>
<td>Comparative Anatomy of Blood Vascular and Lymphatic System</td>
<td>1 + 1</td>
<td>4 credit Marks: 50x4=200</td>
</tr>
<tr>
<td>VAH 516</td>
<td>Histology of Digestive and Respiratory System</td>
<td>1 + 1</td>
<td></td>
</tr>
<tr>
<td>VAH 517</td>
<td>Histology of Blood Vascular and Lymphatic System</td>
<td>1 + 1</td>
<td></td>
</tr>
<tr>
<td>VAH 518</td>
<td>Histology of Urogenital System</td>
<td>1 + 1</td>
<td></td>
</tr>
<tr>
<td>VAH 519</td>
<td>Comparative Anatomy of Sense Organ</td>
<td>1 + 1</td>
<td></td>
</tr>
<tr>
<td>VAH 520</td>
<td>Anatomy of Laboratory and Zoo Animals</td>
<td>1 + 1</td>
<td></td>
</tr>
</tbody>
</table>

Thesis Semester

<table>
<thead>
<tr>
<th>Course code</th>
<th>Course title</th>
<th>Total credit</th>
<th>Marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAH 551</td>
<td>Research</td>
<td>5 credit</td>
<td>5 x 50 = 250</td>
</tr>
<tr>
<td></td>
<td>Examination of Thesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thesis Defense</td>
<td>3 credit</td>
<td>3 x 50 = 150</td>
</tr>
<tr>
<td></td>
<td>Satisfactory/Unsatisfactory</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PhD Course

We are offering research oriented PhD course, however, due to absence of advanced technologies few students are taking their admission here, and most of the graduates are interested to obtain their higher studies from an advanced technology based countries like Australia, Canada, Japan and United Kingdom. So far we have offered 2 PhD degrees from our present department.

The teachers and their abilities

At present the department of Anatomy and Histology of Bangladesh Agricultural University have 4 Professors, 2 Assistant Professors and 4 lecturers. Most of the teachers are taking their PhD from Japan followed by United Kingdom and Australia. The teachers are skilled in delivering their lectures in English and they have also ability to conduct high quality research.

Cooperation with the governmental and non-governmental organization

Our countries governmental and non-governmental organizations (NGO’s) taking part in the alleviation of rural poverty in different ways including poultry and livestock development of Bangladesh. Their activities initiate improvement of countries economy. The BRAC (Bangladesh Rural Advancement Committee), Proshika, and Agricultural Extension Education System (AEES) are the best examples. In connection of the artificial insemination (AI) program every year around 160-field assistant from respective NGO’s are coming to the department of Anatomy and Histology to learn the anatomy of the reproductive system of the bull and cow and topographic anatomy of the animals.

RESEARCH IN THE DEPARTMENT OF VETERINARY ANATOMY

For research purpose, the graduate students and teachers of the department of Anatomy and Histology, BAU have been following the histology, histochemistry and immuno-histochemistry (using paraffin sections) of the different organs of animals including birds. Due to lack of frozen microtome and electron microscopy it is not possible to do advanced research here. However, now a day we improve our research a little. Moreover, we are strictly following the need-based research of country and R & D (Research and development) system. At present we are engaged with the research of the chicken’s immune system of different farms of Bangladesh, and have been studying the aortic heart valve of the indigenous cattle of Bangladesh for future bioprosthetic purpose.

Studies on the immune system of the chicken

Bangladesh has now 0.3 millions broilers and layer farms both in small and large scale (Khamer, 2005). These chickens are playing a significant role in poverty alleviation as well as in improving country economy by supplying eggs and meat. However, the viral diseases, respiratory diseases, vitamin deficiencies, and the effect of aflatoxin in the food creating common constraint in developing the poultry farms in Bangladesh. Most of these diseases affect the immune system and disorganize the histological structure of the lymph node and mucosa associated lymphatic organs leading to poor production of meat,
egg and even mortality rate of the chicken increased significantly. Concerning this point we are focusing our attention towards immunomorphological studies of the local breed of chickens as well as broilers and layers to understand the frequency of lymphocyte’s population and immunoglobulin containing plasma cells; enhancement of the activities of immunocompetent cells by hormone, drugs (Vitamin E and Selenium), and vaccination in the major lymphoid organ and mucosa associated lymphatic organs. The results showed that the chicken IgG cells in different tissues were numerous followed by IgM and IgA. In the female their population correlates estrogen hormone, and, both vitamin E and selenium increased the population of immunocompetent cells in male and female birds.

Studies on the aortic heart valve of indigenous cattle for bioprosthetic purpose

Now a day aortic heart valve diseases is the most common disease of the heart and open-heart surgery becomes an acceptable means of treatment of congenital, rheumatic and ischemic heart diseases of human being. At present, impaired/defective valves in cardiac patient of human being are replaced with mechanical or tissue valves of pig and bovine pericardium origin throughout the world. These imported valves are very expensive. Most of the village people of Bangladesh cannot buy it for their treatment purpose. Concerning with this point Prof. Dr. Sirajur Rahman Khan, Cardio thoracic surgeon of Bangladesh Heart Research Association (BHRA) collaborately worked with us, and we carried out an initial research on the aorta of medium sized indigenous cattle (2–4 years of age) of Bangladesh. We observed that the orifice diameter of the aortic valves were ranges from 22.75-35.00 mm. in this animals. This is the common range of human aortic valve also. These results initiate us to study the aortic valve of the indigenous cattle of Bangladesh with the collaboration of Bangladesh Heart Research Association (BHRA) for the future bioprosthesis. In this regard F n F Pharmaceuticals Company financed for the whole project including cost for a PhD student. In order to carry out the research a total of 32 bovine heart specimens of both sexes were collected for the study of gross morphological component, microscopic composition and its histochemistry. The gross study revealed the presence of lunulæ, corpora arantii, fibrosa, ventricularis, commissures, valsalva’s sinuses, of the three leaflets of the valve; sinotubular junction, annulus and inter leaflet triangle of the aortic root of the heart of indigenous cattle of Bangladesh. The gross results showed the similarities of these components with other animals including human.

The assessment of the anatomical characters of the bovine heart valve is important for the probability of the future bioprosthesis. The characteristics assessed related to sex and age were the height of the cusps, size of the lunulæ, intercommissural distance, position of the coronary ostium in relation with the correspondent Valsalva’s sinus and commissures (left and right), position of the ventricular septum in relation to the aortic valve, aortic diameter of the heart and weight of the heart in relation to sex and age of animals. The results showed that among three cusps, the height and size of the lunulæ was insignificantly higher in the noncoronary cusp (NCC) followed by left coronary cusp (LCC), and right coronary cusps (RCC) regardless to the sex and age. The mean height of the cusps, width of the commissures, length of the free margin of the cusps in all the animals studied was ranges from 14.25± 2.60 to 15.19± 1.75, 4.41± 0.50 to 4.72± 0.46, and 31.50± 4.31 to 32.22± 3.10, respectively. It was evident here that the length of the free margin of the cusps was greater than its height. The external intercommissural distances were higher in all the cusps of the valve rather than the internal intercommissural distances. Irrespective of the sex and age of the animals, the external intercommissural distances were higher in the NCC followed by LCC and RCC, and the mean value was 35.06 ± 4.29, 34.78± 3.38 and 34.38± 3.67, respectively. In contrast, the intercommissural distances were insignificantly higher in the LCC followed by RCC and NCC, and the value was 22.09± 4.65, 21.56 ± 4.79 and 21.13 ± 4.44, respectively. In regard to the position of the ostia and distances of commissures (left and right) and Valsalva’s bottom from ostia, it was found that the distance between left commissure and coronary ostium of the RCC was insignificantly higher than the LCC. In contrast, the distance between the coronary ostia and the right commissure and to the Valsalva’s bottom was higher in LCC rather than RCC. The mean diameter of the aortic valve of indigenous cattle of Bangladesh was 23.28 ± 2.85, and there was no significant sexual difference, but the diameter increased progressively with the increase of age of animals. The results of these assessments in the indigenous cattle of Bangladesh are close to the human aortic valve as indicated by Jatene et al. (1999). The mean weight of the heart in male was 609.38 gm, and in female it was 603.13 gm. Although the weight of the bovine heart in the present study increased with age, however, differences between male and female was not significantly varied. The histological and histochemical study revealed that the leaflets of the aortic valve were consisting of fibrosa, spongiososa and ventricularis. These three layers contain abundant collagen and elastic fibers.

The gross morphology, assessment of different anatomical characters, and histological
composition of the aortic heart valve of 2-4 years of indigenous cattle of Bangladesh is nearly similar to that of human as assessed by Jatene et al. (1999). However, further research regarding testing of the valve, immunological compatibility, and rejection of the valve by human is essential prior to bioprosthesis.

**Conclusion**

In conclusion, the department of Anatomy and Histology is existing in 4 government colleges, and both in a private and public University with standard syllabus and academic courses in Bangladesh. The members of the department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University not only engaged with their intra-faculty academic activities they have also been carried out an academic extension program with different NGO’s, and having linkage program with Medical colleges, Veterinary colleges and Bangladesh Heart Research Association.
VETERINARY ANATOMY EDUCATION IN INDONESIAN VETERINARY SCHOOL: PRESENT STATUS AND FUTURE RECOMMENDATION

Srihadi AGUNGPRIYONO1, Teguh BUDI POJO2, Chairun NISA1, Erdiansyah RAHM3

1 Department of Anatomy Physiology and Pharmacology Faculty of Veterinary Medicine, Bogor Agricultural University (IPB), Bogor, Indonesia
Tel: +62-251-62-9469, Fax: +62-251-62-9459, E-mail: ysrihadi@ipb.ac.id
2 Department of Anatomy, Faculty of Veterinary Medicine, Gadjah Mada University (UGM), Yogyakarta, Indonesia
3 Department of Anatomy, Faculty of Veterinary Medicine, Syiah Kuala University (UNSYIAH), Banda Aceh, Indonesia

Veterinary education in Indonesia has been initiated since a hundred years ago. Up to now, there are five faculties of veterinary medicine in Indonesia. From these schools, around 300-500 vets are graduated every year. The curriculum for the veterinary education in Indonesia is made based on the competency of the graduate. All 5 vet schools use similar national curriculum for veterinary education, with a total of 148-155 credits. The education process is basically divided into 2 steps; a 4 years course towards Bachelor degree and followed by an additional 1-2 years internship course to get the doctor of veterinary medicine (DVM) degree. Veterinary anatomy is divided into veterinary (macroscopic) anatomy (8 credits), histology (4 credits), and embryology (2 credits), and is given subsequently within 3 semesters, i.e. in the 3rd, 4th and 5th semester. Before taking anatomy, the students are required to take Animal Biology in the 2nd semester. The present and future development of veterinary anatomy is closely related with the competency of the graduates and the need from global market. The present global market needs fresh and young graduates with a good skill in veterinary profession, good entrepreneurship and be fluent in English. Therefore, in addition to the regular teaching and learning method, additional activities are needed to fulfill the above competency. The additional activities proposed include training and practice through the use of mobile clinic units, the use of student clubs for conducting a series of certified courses in certain fields, teaching certain subjects (including anatomy) in English and conducting a continuing education on certain topics such as (in anatomy) ophthalmology, radiographic anatomy, surgical anatomy, anatomy of the horse, etc. Strategy for the development of anatomy shall be different between undergraduate level and postgraduate level. In the teaching and learning process at the undergraduate level, an integrated method should be adopted to facilitate the students with a better understanding of structure and function of the body. This method puts anatomy as one or part of the subjects that together with the other subjects provide an integrated and comprehensive review on certain topic of the structure and function which may also with a bit discussion on its possible pathological condition. On the other hand, the development of *anatomia per se* as knowledge has to be done continuously through research and scientific courses at postgraduate level.
Brief History of Veterinary Education in Indonesia

Veterinary education in Indonesia has been initiated since 1861 in Surabaya by the establishment of a 2 years veterinary course. This course was founded by Dutch government in order to meet the demand of veterinarian during the endemic of many livestock diseases in Indonesia. This course was, however, closed in 1875 as there were only 8 students during the period of 9 years. In 1907, a four years course of veterinary medicine, namely *Cursus tot opleiding van Indlandsche Veeartsen* was established in Bogor (Buitenzorg). Along the development of its curricula, the name of the course was changed into *Indlansche Veeartsenschool* in 1910 and then into *Nederlands Indische Veeartsenschool* (NIVS) in 1914. The school was changed into *Bogor Senmon JyuiGakko* in 1942-1945 during Japanese occupation. In 1946 after 2nd world war, the school was changed into *Balai Perguruan Tinggi Kedokteran Hewan Indonesia* (BPTKHI) with 5 years course. In 1949, a branch of the school was opened in Yogyakarta and this branch was then developed into a faculty under the University of Gadjah Mada, while the school in Bogor was also developed into a faculty and joined with other faculties under the University of Indonesia in 1950. In 1963, 2 faculties, Faculty of Agriculture and Faculty of Veterinary Medicine were separated from The University of Indonesia and were developed under Bogor Agricultural University (IPB). After 1960, the need of veterinarian was increase due to the rapid development of livestock population and health in Indonesia. Indonesian government established Faculty of veterinary medicine in Banda Aceh under Syiah Kuala University in 1961, in Surabaya under Airlangga University in 1972 and in Denpasar Bali under the University of Udayana in 1988. Therefore, at present there are 5 faculties of veterinary medicine (FVM) in Indonesia, in Syiah Kuala University (UNSYIAH), Banda Aceh, in Bogor Agricultural University (IPB) Bogor, in Gadjah Mada University (UGM), Yogyakarta, in Airlangga university (UNAIR), Surabaya, and in Udayana University (UNUD), Denpasar. Every year about 300-500 veterinarian are graduated from these faculties.

Standard of Competency of Indonesian Veterinarian

In Indonesia, veterinary medical education is run under the collaborative management between the FVM and the Indonesian Veterinary Medical Association. Both institutions established the curriculum based on a standard of competency of the veterinarian. The competency of Indonesian veterinarian requires the graduates to have a good skill in veterinary medical profession, knowledge and technology in the veterinary medicine to do the job related with the veterinary authorities in biomedical, animal health management, population and environmental health and in the quality assurance of food of animal origin.

Curriculum and the subject of Veterinary Anatomy

The curriculum for FVM is established as a competency-based curriculum. Through an agreement which was signed in the year 2000 in Surabaya, all the 5 FVM in Indonesia conduct the same curriculum namely *Kurikulum Nasional Kedokteran Hewan* (National Curriculum for Veterinary Medical Education). Total credit of the subject to complete the education of veterinary medical doctor (veterinarian) varies within 148-155 credits. Among these, subject with 97 credits are similar in all faculties. The remaining credits are given along the interest and specific competency of each faculty. The education process of FVM is basically divided into 2 steps: (1) a 4 years course towards Bachelor degree and followed by and (2) an additional 1-2 years internship course to get the doctor of veterinary medicine (DVM) degree. A Bachelor of Veterinary Medicine can also pursue their education towards the postgraduate/Master program.

It has been known that anatomy is one of the important subject not only in the support of the medical education including veterinary medicine and dentistry, but also in other certain fields such as archeology, animal and plant biology, and even in the field of socio-cultural, art and engineering. In FVM, anatomy benefits the students to understand (1) the structure and the function of the body and (2) the comparative of the structure and function among animals, and (3) the basic knowledge to learn preclinical
and clinical subjects at the next stages.

In the curriculum of Indonesian FVM, the subject veterinary anatomy is divided into veterinary (macroscopic) anatomy (8 credits), histology (4 credits), and embryology (2 credits), given a total of 14 credits (Table 1). All are given subsequently within 3 semesters, i.e. in the third, fourth and fifth semester. The subjects of macroscopic anatomy include arthrology, systematic anatomy and organology (6 credits in 2 semesters) and topographic anatomy (2 credits in 1 semester).

Table 1. Subject of Veterinary Anatomy in the Faculty of Veterinary Medicine, Indonesia

<table>
<thead>
<tr>
<th>No</th>
<th>Subject</th>
<th>Credits</th>
<th>Lecture* hours per week</th>
<th>Practice* hours per week</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Veterinary anatomy I</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>Arthrology, osteology, angiology</td>
</tr>
<tr>
<td>2.</td>
<td>Veterinary Anatomy II</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>Organ system</td>
</tr>
<tr>
<td>3.</td>
<td>Topographic anatomy</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>Horse anatomy, applied surgical anatomy</td>
</tr>
<tr>
<td>4.</td>
<td>Histology I</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>Basic tissue</td>
</tr>
<tr>
<td>5.</td>
<td>Histology II</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>Organ system</td>
</tr>
<tr>
<td>6.</td>
<td>Embryology</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>Embryogenesis, organogenesis, teratology</td>
</tr>
</tbody>
</table>

* 1 hour of lecture = 50 min, 1 hour of practice = 150 min

In USA, for example, there is premedical stage before entering the faculty of medicine or veterinary medicine. During this period, which is commonly 4 years, students learn zoology (comparative anatomy), genetic, evolution, ecology and physiology (functional anatomy) and other subjects. The graduates become students of medical or veterinary medical schools and learn anatomy in 1 or 2 semesters. In Indonesia, before taking the anatomy in the 3rd semester, the students have to take animal biology and biochemistry during their first and second semester (1st year).

Competency of the Graduates versus the Global Market

Present globalization has effect to the policy of higher education in Indonesia as well as veterinary medical education. Graduates are required not only to have a better skill in the veterinary profession but should also have a good entrepreneurship and able to make communication in English. Refreshing and reevaluation of the curriculum has been done in order to increase the competitiveness of the graduates to regional (Asia) and international levels. Furthermore, global market requires young, fresh and smart graduates. Therefore, a long time of study period due to much time need for too many things that we think have to be taught and delivered to the students is not relevant anymore in the meantime. To meet this market demand, FVM has to change its traditional method and begin produce a skillful, young and fresh graduates within a relatively short period, but without sacrificing the quality of teaching and learning process.

Future view of Education and Research in the Veterinary Anatomy

Subjects in the medical curriculum are closely related each other. All subjects contribute to a whole understanding about normal or abnormal condition of the body, pathogenesis, mechanism of drug or treatment etc.

To increase the quality and competency of the graduates to meet the market demand as mentioned above, some modifications in the teaching and learning activities in FVM has to be created in addition to the regular teaching and learning process. In order to increase the quality of graduates, in the FVM IPB there are some activities in addition to the regular activities of teaching and learning as follow:

1. To increase the skill in the veterinary practice, in addition to regular laboratory and field practice, students are given additional training and practice through the use of mobile clinic units which bring regularly the students to the field to actively find the cases and do diagnose and treatment
2. The use of student clubs for conducting a series of certified courses in certain fields (avian medicine, wild animal medicine, aquatic medicine etc)
3. To push the students to be familiar and able to make communication in English, chapters of
certain subjects (including anatomy) are taught and given in English. For that, firstly the teachers are assigned to learn how to teach in English in a professional English school. English student club

4. As for veterinary anatomy, certain topics of veterinary anatomy are taught in a service course for the graduates/ veterinarian. The course is a continuing education and is conducted regularly each year. Participants are the graduates that need additional knowledge on certain topics such as ophthalmology, radiographic anatomy, surgical anatomy, anatomy of the horse, etc.

The length of study has to be punctual. This is related with many factors such as student, curriculum, teacher, facilities and supporting equipment, management system etc. Big number of subject and credits and the difficulties of (to understand) the subject will contribute to the length of study period. To conduct veterinary education in a relatively short time it is important to create or modify the method of delivering the knowledge to the student.

The development of veterinary anatomy in the veterinary medical education is closely related with the development of the other subjects. In the learning of veterinary anatomy, the use of life animals, models, visual aid, microscopes, radiograph, computer images, film, cadavers, etc provides the student a better understanding on the related matter. However, the method to deliver anatomy is not only limited with the use of models and aid materials mentioned above.

At present, actually we teach the anatomy by teaching the knowledge of anatomy itself (anatomia per se) and mostly with too many definitions. For example, we teach that the body is consisted of this and that, the skin layers are this and that, this is the macroscopic anatomy of A, this is the histology of B etc, but we do not or seldom tell about comprehensive story or process for instance of a phenomenon of a body function. The students "need time" to arrange the mosaic they got from each subject as well as anatomy to construct a right story that they can finally understand. Some students may get success to produce a good form of mosaic arrangement and then he or she can understand about the story. However, indeed many more students are still puzzled and they are still like this when entering higher semesters to learn para-clinical or clinical subjects. Why do we teach anatomy like this? may be because we are anatomist, so our teaching method is derived rigidly from our subject-egoism in our mind. Simultaneously, all subjects actually tend to give similar problems. This became a hard work for the students to harmonize all the information they got from each subject to understand the animal body and function also the correlation among the subjects in the curriculum. This, in fact, is one of the problems in making a longer period of study and so far has also created a big inefficiency in the teaching and learning process. Students are trained to think in cluster, and most of them are not able to make integration or understand the correlation among subjects. Therefore, in the present time, teaching anatomia per se as knowledge may be not relevant for undergraduate level. An integrated method is one possible answer to solve this problem. Teachers have to collect and mix various information, make a good composition and story and then deliver the whole story to the students as an integrated and comprehensive understanding. For example, when we teach about the liver to the students, a comprehensive story including its macroscopic and microscopic structure, function, metabolism and its relation with the organ system will be interesting and easily understood by the students. A bit of additional information regarding its possible pathological condition and disease will be very positive. In this case, pieces information of embryology, gross anatomy, histology, physiology, biochemistry and pathology or clinic are gathered together to support a complete story about the liver.

Actually, integrated method will ease the students as well as the teachers because the medical science is a comprehensive knowledge. Each subject is closely related each other and contribute to the whole understanding on the body system and disease.

However, there are still some factors to be cleared out before the implementation of the integrated method, which are as follow:

1. Tradition teaching method, in fact, has been used for a very long time and is very difficult to change. Each subject has been developed and taught rigidly based on subject and personal interest.
2. Up to now, only very few textbook available for integrated subjects.
3. In its implementation, the integrated method can not be done only by one subject. It should be conducted together.

A common understanding and agreement from the institution and teaching staffs to reevaluate the curriculum and to adopt the integrated method is needed. As far as we know, the integrated method is being applied in the Royal (Dick) School of Veterinary Studies, The University of Edinburgh, in the United Kingdom.
Table 2. Correlation between Anatomy and other subjects in the veterinary medicine curriculum

<table>
<thead>
<tr>
<th>Topic / subjects</th>
<th>Applicative aspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure and function</td>
<td>Topographic orientation, Understanding the body system</td>
</tr>
<tr>
<td>Clinical diagnostic</td>
<td>Age determination</td>
</tr>
<tr>
<td></td>
<td>Normal and abnormal sign of the body</td>
</tr>
<tr>
<td></td>
<td>Sampling of blood or body fluid</td>
</tr>
<tr>
<td></td>
<td>X-ray, MRI, CTScan</td>
</tr>
<tr>
<td>Pathology</td>
<td>Organ and tissue sampling</td>
</tr>
<tr>
<td></td>
<td>Position and abnormal signs of organs</td>
</tr>
<tr>
<td>Clinics</td>
<td>Injection area</td>
</tr>
<tr>
<td></td>
<td>Anesthetic injection</td>
</tr>
<tr>
<td>Reproduction</td>
<td>Pregnancy examination</td>
</tr>
<tr>
<td></td>
<td>Delivering</td>
</tr>
<tr>
<td>Surgery</td>
<td>Therapeutic surgery</td>
</tr>
<tr>
<td></td>
<td>Cosmetic surgery</td>
</tr>
</tbody>
</table>

On the other hand, anatomy is indeed a very important and dynamic knowledge. As a basic science, its support to the other related fields will be better if anatomy itself is well developed. Therefore, the development of *anatomia per se* as knowledge is also very important to be carried out continuously. We in the department or laboratory of veterinary anatomy should plan about the future of our direction. In some countries, the name of department or laboratory of Anatomy has changed into: morphology and molecular anatomy, evolutionary morphology, evolutionary and applied biology, development anatomy and morphometry, morphology and cellular biology *etc.*, which reflect wide development of anatomy.

Anatomy has developed in line with the recent advances in the molecular biology, molecular genetic, cytology and immuno(cyto)chemistry. This has promoted a considerable number of research activities and publications. While in the undergraduate level an integrated method in the teaching and learning is the best choice, the development of anatomy has to be done through intensive research activities in the postgraduate level. In Indonesia, the subjects of molecular and cellular biology, immunohistochemistry, development anatomy are taught and more intensely discussed in the postgraduate program. In addition to that, in FVM IPB, special short courses as a continuing education in the scientific field on the molecular biology, histochemistry, and cell culture or development anatomy are also conducted regularly.

**Conclusion**

Veterinary anatomy is part of the subjects in the competency-based curriculum in FVM. Strategy for the development of anatomy would be different between undergraduate and postgraduate level, and closely related with the competency of the graduates and demand from the global market. In the teaching and learning process at undergraduate level, we may adopt integrated method, put anatomy as one of the subjects that together with the others provide an integrated and comprehensive understanding of structure and function of the body. Addition of continuing education on certain topics will be very useful to improve the ability of fresh graduates. On the other hand, the development of *anatomia per se* as knowledge has to be done continuously through research and scientific courses at postgraduate level.

**References**


Ind Bl Diergeneesk 40:1-10
PARA RUBBER MODELS FOR VETERINARY ANATOMICAL TEACHING AID

Teerasak PRAPONG, DVM, PhD

Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University  
50Paholyothin Rd., Chatuchak, Bangkok 10900, Thailand  
fvettsp@ku.ac.th

Today the use of animals in anatomical teaching for veterinary students is concerned with the experimental animal ethics and animal welfare issues. Developing of teaching aid models is an alternative of using live animals for anatomical specimens. In the past all teaching aid models were imported from abroad. These models have two disadvantages; they have high cost and are made of hard silicone. To solve these problems, the Department of Anatomy, Faculty of Veterinary Medicine at Kasetsart University in Thailand uses para rubber for making anatomical teaching aid models. Supporting by research fund from National Research Council of Thailand and from Kasetsart University, the pilot factory of para rubber teaching aid model has been established in 2003. Various anatomical teaching aid models have been developed. These para rubber models are used in anatomical laboratory classes for teaching and self-reviewing of veterinary students. Unlike hard silicone or plastic models, the para rubber models are soft, unbreakable and have light weight. Not only teaching aid models, some of medical practicing models such as artificial intestines and suture practice skins were also invented.

Keywords: Para rubber models, Veterinary Anatomical teaching aid.
At present, there are 95 institutes/universities in Thailand of which 66 are public institutes (60 limited admission universities, 2 open universities and 4 autonomous universities) and the rest are private institutes/universities. Currently there are 6 universities offering veterinary education, 5 governmental (Chulalongkorn: CU, Kasetsart: KU, Khon Kaen: KKU, Chiang Mai: CMU and Mahidol University: MU) and one private university (Mahanakorn University of Technology: MUT). From their first establishment of veterinary schools in the year of 1935 to the year of 2001, a total numbers of 3,907 veterinarians are generated, and by the year 2004, the numbers has reached 5,000. Approximately 35% of veterinary graduates are in governmental sectors (mainly universities and the Ministry of Agriculture) and the other 59% are in private sectors of which 20% are in private animal clinics. Now, approximately 500 veterinarians are graduated each year. Curriculum structure of Veterinary Medicine program consists of 240–248 credits of which 30–37, 190–204, and 6 credits are general education courses, veterinary courses, and elective courses respectively. KU and CU have their plans for higher education programs and research, such as Ph.D. Program in Biomedical Science (Interdisciplinary) in year 2006, M.Sc. Program in Applied Veterinary Anatomy in year 2007 and Ph.D Program in Anatomical Science in year 2008.
THE EDUCATION AND RESEARCH OF VETERINARY ANATOMY IN JAPAN

Kazuyuki TANIGUCHI

Laboratory of Veterinary Anatomy, Faculty of Agriculture, Iwate University, Morioka 020-8550, Japan, Tel: +81-19-621-6207, Fax: +81-19-621-6209, E-mail: anatomia@iwate-u.ac.jp

In Japan, veterinary treatment was carried out from ancient times. Even in the Chronicles of Japan (720), the oldest official history of Japan, we find the sentence as follows: “Oonamuchinomikoto” and “Sukunahikomonomikoto” managed the whole country with one accord, and laid down remedies for diseases of men and animals. Thereafter, the traditional veterinary treatment remained in existence with the accumulation of improvements in the remedy until the Meiji era. On the other hand, the education for modern veterinary medicine started in the early years of the Meiji era. In 1874, the Institution for Agriculture was established. In 1876, the Sapporo Agricultural School, the predecessor of the present Faculty of Veterinary Medicine, Hokkaido University, was established. The Institution for Agriculture was renamed as the Agricultural School in 1877, and renamed again as the Komaba Agricultural School, the predecessor of the present Faculty of Agriculture, the University of Tokyo, in 1882. In these days, foreign teachers were essential for the education of modern veterinary medicine. In 1878, John C. Cutter arrived in the Sapporo Agricultural School from the USA. In 1880, Johannes Ludwig Janson arrived in the Agricultural School. They laid the foundations of the education of modern veterinary medicine. Thereafter, as the militarism was prevalent in Japan after the Sino-Japanese War (1894-95) and the Russo-Japanese War (1904-05), horses were mainly used as materials in the education of veterinary medicine because they were militarily important. After the Second World War (1941-45), the materials were shifted from horses to cattle and dogs. As for the veterinary anatomy, the education of veterinary anatomy seemed to be started simultaneously with the arrival of Cutter and Janson in Japan, although the department of veterinary anatomy was first established in 1893 in the University of Tokyo. As for the activity of the society of veterinary medicine, the Great Japanese Association of Veterinary Medicine was established in 1885, and renamed as the Central Association of Veterinary Medicine in 1887. On the other hand, the Japanese Society of Veterinary Science was separately established from the Central Association of Veterinary Medicine. In 1938, the Central Association and the Japanese Society united to form the Great Japanese Society of Veterinary Science, and the first volume of the Japanese Journal of Veterinary Science was published at the same time. The former was renamed as the Japanese Society of Veterinary Science in 1948 and remained until now.
1. History of Veterinary Medicine in Japan

In Japan, veterinary treatment was carried out from ancient times. Even in the myth, we found several articles concerning the veterinary treatment as follows:

- Ookuninusinomikoto 大国主命 cured the injury of a white rabbit hurt by sharks in Inaba province 古墳の白兎. "the Ancient Chronicle 古事記"
- Oonamuchinomikoto 大己貴命 and Sukunahikonanomikoto 少彦名命 managed the whole country with one accord, and laid down remedies for diseases of men and "animals". "the Chronicles of Japan 日本書紀"

In these days, at least 30 kinds of roots of grass and bark were thought to be used for medical purposes. In the history of Japan, many veterinary articles were recorded. I selected and arranged them chronologically with occasional descriptions about the great incidents in the Japanese history.

In the Ancient Times

- In 456, we found the record of tenotomy 切髪術 in the horse. "Satsuma Topography 薩摩風土記"
- In 595, the remedy for horses was introduced from the ancient Korea 百済. It was handed down as the Crown Prince school 太子流.
- In 645, the Reformation of the Taika era 創仏の改新 was performed.
- In 701, Taihou Laws 大宝律令 were enacted. In them, we found the official title of "the doctor of horses 馬医師".
- In 710, the capital was settled in Nara 奈良.

In the Heian Era

- In 794, the capital was moved to Kyoto 京都 (beginning of the Heian era 平安時代).
- In 804, Nakakuni Taira 平仲国 went over to China to learn the remedy for horses and taught it to his sons as the Nakakuni school 仲国流 after he returned to Japan. He wrote "the Hundred Questions and Answers by Nakakuni 安騏集". This is the first veterinary book in Japan. He used the old Chinese Book of Remedy for Horses 安騏集 as the textbook. This book was very bulky and consisted of 60 volumes.
- In 907, Engi Laws 延喜式 were issued. In them, we found articles about medicines and feeding standards for horses.

In the Kamakura Era

- In 1192, Yoritomo Minamoto 源頼朝 established the Shogunate government 幕府 in Kamakura 鎌倉 (beginning of the Kamakura era). In the Kamakura era, horses were regarded as very important for battles in the military class. As horses increased in number, many horse experts 伯楽 appeared in various districts and were engaged in the remedy for horses. We found the following article in the History of the Kamakura Shogunate 東鍛. A horse expert removed the arrow from the deep part of the horse body and cured the wound during the battle in 1221.
- In 1267, the Picture Scroll for Horse Doctors 馬医絵巻 was written. This scroll contained explanations and drawings of 17 kinds of medicinal herbs.
- In 1268, the first horse hospital was founded in the Paradise Temple 極楽寺 in Kamakura 鎌倉.
- In 1310, the Ten Pictures of Country Cattle 国牛十図 was written. This book described the features of 10 kinds of country cattle.
**In the Muromachi Era**

- In 1338, Takauji Asikaga 足利尊氏 established the Shogunate government in Kyoto (beginning of the Muromachi era 室町時代).
- From 1467 to 1477 The civil war of the Ounin era 応仁の乱 (beginning of the long war time 戦国時代).
- In 1505, the Notes for Sick Horses 病馬覚書 was written.
- In 1551, the book named the Essentials for Horse Doctors 馬医齋闘 was transcribed for public use. This book was edited by Nakakuni Taira 平仲国 in the Heian era.
- In 1573, the Illustration of Remedy for Horses 療馬図説 was written. This book gave the names of parts of horse body and points for acupuncture.
- In 1593, the Book of Secrets for Remedy for Horses 馬療秘伝書 was published.
- In 1595, the Scroll of Remedy for Horses 馬療書巻物 was published.
- In 1596, the Secret Acupuncture for Horses 馬の秘針書 was copied for public use. This book was edited by Nakakuni Taira 平仲国 in the Heian era.

**In the Edo Era**

- In 1603, Ieyasu Tokugawa 徳川家康 established the Shogunate government in Edo 江戸 (old name of Tokyo) (beginning of the Edo era). During this era, the books for remedy for horses increased in number to result in the improvement in quality and quantity of horse doctors.
- In 1604, the Kana Book of Old Chinese Remedy for Horses 仮名安駄騏 was published.
- In 1616, the Book of Dogs 犬の書 was published.
- In 1617, the Verbatim Note for Horse Medicine 馬薬聞書 was published.
- In 1620, the Pathology by Horse Experts 伯楽病理論 was published.
- In 1621, the Instruction of the Deified Spirit for Horse Experts 伯楽天神伝書 was published.
- In 1638, the Collection of Wonder Drugs for Horse 馬の妙薬集 was published.
- In 1647, the Hundred Pictures of Horses 百馬図 was published.
- In 1657, the Collection of Common Explanations for Sick Horse 病馬俗解集 was published.
- In 1659, the Book of Bovine Disease 牛病書 was published.
- In 1665, the Book of Secrets for Horse Diagnosis 馬見様の秘伝 was published. In this book, development and abration of teeth was illustrated in horses from 1 to 24 of age.
- In 1680, the stable for sick horses 病馬厩 was founded in Yotsuya 四谷, Edo.
- In 1685, Tsunayoshi Tokugawa 徳川家綱 enacted the Law of Compassion for Living Things 生類憐みの令. By this law, dogs were especially protected and the organization of dog doctors 犬医師 was established. In contrast, this law was very notorious for severe prohibition on hunting and fishing and rigid rules to protect dogs. This law was abolished immediately after the death of Tsunayoshi in 1709.
- In 1688, the Collection of Top Secrets for Horse Care 要馬秘極集 was published.
- In 1717, the Essentials for Military Horses 武馬必要 was published.
- In 1725, five horses were imported from Holland. At the same time, Hans Jurgen Keijser (1697~1735) was invited to Japan from Holland for remedy for horses. He stayed in Japan until 1735 for 11 years and taught the European style of horseback riding, remedy for horses, hoof cutting and shoeing. Although he was not to be called as veterinarian because the educational system for veterinary medicine was not established in these days in Europe, he must be the first one to introduce the European style of remedy for horses into Japan.
- In 1730, the Dutch books for remedy for horses brought by H. J. Keijser were translated and edited into Japanese and published as the Book of Dutch Horse Care 和蘭馬養書. Note it was 44 years before the translation and publication of the Tafel Anatomia 解体新書 in 1774 by Genpaku Sugita 杉田玄白.
- Around 1730, the Complete Work of Remedy for Horses 馬経大全 was published. In this book, names of horse diseases were described in a dialect.
- In 1732, the Manual for Remedy for Horses 馬療辯解 was published.
- In 1756, the Convenient Book for Remedy for Cattle 牛療治調法記 was published.
- In 1758, the Daily Handbook for Remedy for Horses [日用馬療医便] was published.
- Around 1785, the Creative Considerations on Quivering Dogs [痙狗創考] was published.
- In 1791, the Book of Secrets for Horse Experts [伯楽秘書] was published. This book carried the excellent illustrations of horse anatomy.
- In 1806, the New Theory for Horses in the Stable [厩馬新論] was published. This book was based on rational theories acceptable in modern veterinary medicine.
- In 1851, the New Book of Horse Anatomy [解馬新書] was published. This is the first book of veterinary anatomy in Japan. Thereafter, the traditional veterinary treatment remained in existence with the accumulation of improvements in the remedy until the Meiji era.

2. Education of Veterinary Medicine after the Meiji Restoration

The education for modern veterinary medicine started in the early years of the Meiji era and continues up to now. Many veterinary schools were established and renamed or closed until now as listed below. In the following articles, the predecessors are restricted to the Department of Veterinary Medicine of the concerned universities.

- In 1868, the Meiji Restoration [明治維新] was performed.
- In 1874, Institution for Agriculture [農事修學場] was established.
- In 1876, Sapporo Agricultural School [札幌農學校], the predecessor of Hokkaido University [北海道大学], was established.
- In 1877, Institution for Agriculture [農事修學場] was renamed as Agricultural School [農學校].
- In 1881, Private Veterinary School [私立獣醫學校] the predecessor of Nippon Veterinary and Animal Science University [日本獣医学習大学], was established (closed in 1889).
- In 1882, Agricultural School [農學校] was renamed again as Komaba Agricultural School [駒場農學校], the predecessors of the University of Tokyo [東京大學] and Tokyo University of Agriculture and Technology [東京農工大學].
- In 1888, Osaka Prefectural School of Agriculture [大阪府立農學校], the predecessor of University of Osaka Prefecture [大阪府立大学], was established.
- In 1892, Private Tokyo Veterinary School [私立東京獣醫學校], the predecessor of Nippon Veterinary and Animal Science University [日本獣医学習大学], was established (closed in 1902).
- In 1894, Private Azabu Veterinary School [私立麻布獣醫學校], the predecessor of Azabu University [麻布大学], was established.
- In 1902, Morioka Higher Agricultural and Forestry School [盛岡高等農林學校], the predecessor of Iwate University [岩手大学], was established.
- In 1907, Private Tokyo Veterinary School [私立東京獣醫學校] the predecessor of Nihon University [日本大学], was established.
- In 1911, Private Nippon Veterinary School [私立日本獣醫學校], the predecessor of Nippon Veterinary and Animal Science University [日本獣医学習大学], was established.
- In 1939, Kagoshima Higher Agricultural and Forestry School [鹿兒島高等農林學校] the predecessor of Kagoshima University [鹿兒島大学], was established.
- In 1939, Tottori Higher Agricultural School [鳥取高等農業學校], the predecessor of Tottori University [鳥取大学], was established.
- In 1940, Utsunomiya Higher Agricultural and Forestry College [宇都宮高等農林專門學校], was established (closed in 1949).
- In 1940, Gifu Higher Agricultural and Forestry School [岐阜高等農林學校], the predecessor of Gifu University [岐阜大学], was established.
- In 1940, Miyazaki Higher Agricultural and Forestry School [宮崎高等農林學校], the predecessor of Miyazaki University [宮崎大学], was established.
- In 1941, Obihiro Higher Veterinary School [帯広高等獣醫學校], the predecessor of Obihiro University of Agriculture and Veterinary Medicine [帯広畜產大学] was established.
- In 1944, Yamaguchi Higher Veterinary School [山口高等獣醫學校], the predecessor of Yamaguchi University [山口大学], was established.
- In 1944, Keio Veterinary and Zootechnical College [慶應義塾動物畜産専門学校] was established (closed in 1949).
- In 1964, Rakuno Gakuen University [酪農学園大学] was established.
- In 1966, Kitasato University [北里大学] was established.

In the early years of the Meiji era, foreign teachers were essential for the education of modern veterinary medicine. In 1878, John C. Cutter arrived in the Sapporo Agricultural School [札幌農学校] from the USA. In 1880, Johannes Ludwig Janson arrived in the Agricultural School [農学校] from Germany. They laid the foundations of the education of modern veterinary medicine.

Thereafter, as the militarism was prevalent in Japan after the Sino-Japanese War [日清戦争] (1894-95) and the Russo-Japanese War [日露戦争] (1904-05), horses were mainly used as materials in the education of veterinary medicine because they were militarily important. A boom in the establishment of the veterinary schools and colleges, i.e., 8 schools and colleges during 6 years from 1939 to 1944, must also be understood in this context.

After the Second World War [第二次世界大戦] (1941-45), the materials were shifted from horses to cattle and dogs. As for the veterinary anatomy, the education of veterinary anatomy seemed to be started simultaneously with the arrival of Cutter and Janson in Japan, although the department of veterinary anatomy was first established in 1893 in the University of Tokyo.

As for the activity of the society of veterinary medicine, the Great Japanese Association of Veterinary Medicine [大日本獣医会] was established in 1885, and renamed as the Central Association of Veterinary Medicine [中央獣医会] in 1887. On the other hand, the Japanese Society of Veterinary Science [日本獣医学会] was separately established from the Central Association of Veterinary Medicine in 1921. In 1938, the Central Association and the Japanese Society united to form the Great Japanese Society of Veterinary Science [大日本獣医学会], and the first volume of the Japanese Journal of Veterinary Science [日本獣医学雑誌] was published at the same time. The former was renamed as the Japanese Society of Veterinary Science [日本獣医学会] in 1948 and remained until now.

3. Current Problems in the Education and Research Works of Veterinary Medicine in Japan
- Shortage in the number of teaching and research staff
  about 3 persons per one laboratory
  about 30 to 50 persons per one university
- Shortage in the budget especially in the national universities
  about 300 to 500 thousand yen per one person
- Insufficiency in the equipment especially in the national universities
The First Conference of The Asian Association of Veterinary Anatomists

Tsukuba International Congress Hall
Ibaraki-Tsukuba, Japan
19th - 21st March, 2006
(http://www.jpn-ava.com)

POSTER SESSION
19th - 21st March, 2006
Distribution of BrdU labeled cells in the olfactory bulb and Changes in lectin binding patterns in nasal epithelia of the lateral ventricle of Xenopus laevis. of Xenopus laevis during metamorphic period

Shouichiro SAITO, Yasuro ATOJI, Daisuke ENDO, Yoshio YAMAMOTO, Veterinary Anatomy, Faculty of Applied Biological Science, Kazuyuki TANIGUCHI (Laboratory of Veterinary Anatomy, Iwate University)

The olfactory bulb (OB) is provided with neurons and glial Nasal cavity of Xenopus laevis consists of principal, middle, and cells from the rostral part of the lateral ventricle (LV) via rostral inferior chamber, and is covered with olfactory epithelium (OE), migratory pathway throughout life. This neurogenesis and middle chamber epithelium (MCE), and vomeronasal epithelium migratory process is revealed mainly in murine brain, but still (VNE), respectively. It has been reported that juvenile nasal remains unclear in most vertebrate species. In the present epithelia are changed to adult type during metamorphic period. study, proliferating cells in OB and LV of adult Xenopus laevis In the present study, we examined changes in lectin binding were examined by the injection of BrdU, an analog of thymidine. patterns in nasal epithelia of Xenopus laevis during metamorphic period. Fifty mg/kg of BrdU (Sigma-Aldrich, MO) was injected into the adult male Xenopus intraperitoneally for 3 days, a single injection each day. The animals were euthanized and fixed by 4% paraformaldehyde in phosphate buffered saline. The forebrain was routinely embedded in method with 22 biotinylated lectins. paraffin and cut at 5 microm in thickness. Sections were When WGA, LEL, DSL, VVA, and Jacalin were applied to the OE deparaffinized, heated by microwave and incubated with the and MCE, basal cells and sensory cells were negative at stage 55 primary antibody against BrdU (NA20, EMD Biosciences, CA); and stage 57. At stage 59 or stage 61 some basal cells were positive at stage 63. At stage 66 and adult, most basal cells and cells were few in OB and the epithelium of the rostral part of LV enters OB and is encircled by positive, and most basal cells and some sensory cells were the numerous number of the granule cells of OB. BrdU labeled positive at stage 63. At stage 66 and adult, most basal cells and cells were few in OB and the epithelium of the rostral part of LV. sensory cells were positive. When UEA-I was applied, every basal cell in OB and the epithelium of the rostral part of LV were weakly positive at stage 66. This study suggested that active proliferation stage 66 and adult, occurred in a part of LV distant from the OB in Xenopus laevis. It is suggested that juvenile sensory cells in the nasal cavity of Xenopus laevis seems to start to change at stage 59 and replace to adult type at stage 66.
Respiratory depression circuit revealed by FOS immunoreactivity Fine structure of two types of olfactory organs in under l-menthol stimulation to rat’s nasal mucosa Trachmys scripta elegans

Jun WAKAI, Yoshio YAMAMOTO, Toshikiro OIKAWA, Hiroyasu ITOU, Yoshio YAMAMOTO, Kazuyuki TANIGUCHI, Kazuyuki TANIGUCHI
(Laboratory of Veterinary Anatomy, Iwate University)

It has been reported that stimulation to the nasal mucosa by In vertebrates, at least two types of olfactory organs can occur, cooling air and l-menthol activates trigeminal cold receptor and olfactory epithelium (OE) and vomeronasal organ (VNO). In this induces respiratory depression. The aim of the present study study, the fine structure of olfactory organs was examined in rats to reveal respiratory depression circuit by use of aquatic turtle, Trachmys scripta elegans, as part of a phylogenetic immunohistochemistry for FOS protein as a maker of neuronal study of the olfactory organs in vertebrates. activation. Six adult animals were anesthetized with pentobarbital, and Wistar rats were anesthetized by urethane, and 10 microl of 0.4 M sacrificed by cardiac perfusion with saline followed by a l-menthol in paraffin oil was injected to the right nasal cavity. Karnofsky’s solution. Materials were post-fixed and embedded. One hour after the stimulation the rats were fixed with Ultrathin sections were stained and observed under H-7100. Zamboni’s fixative, and then brains were dissected out. Serial The entire nasal cavity consisted of two diverticula transverse sections (50 microm) were cut with the cryostat and communicating each other, the upper and lower diverticula. The processed for immunohistochemistry using the avidin-biotin upper deverticulum opened anteriorly at the external naris, and peroxidase complex method with antibody directed against FOS was lined with the OE. Olfactory cells of the OE were bipolar protein. neurons. Their dendrites extended up to the lumen to form In rats stimulated by l-menthol, numerous FOS immunoreactive olfactory vesicles and possessed long cilia on their free surface. cells were observed in locus coeruleus, area postrema, nucleus The supporting cells of the OE were equipped with microvilli tractus solitarius (NTS), nucleus ambiguus and spinal trigeminal and possessed secretory granules in the apical cytoplasm. The nucleus (SPV). In NTS, FOS immunoreactive cells were mainly lower deverticulum opened posteriorly at the choana, and was distributed caudal to obex in medial and commissural subnuclei. lined with lower deverticulum epithelium (LDE). The sensory cells In SPV, FOS immunoreactive cells were mainly observed in the of the LDE possessed long microvilli on their surface and caudal part. contained centrioles in dendrites as well as sensory cells of the The expression sites of FOS-positive cells after l-menthol VNO in other species. The supporting cells of the LDE were stimulation to the nasal mucosa were similar to those after equipped with microvilli and possessed secretory granules in the nociceptive stimulation by ammonia or mustard oil. apical cytoplasm.
TREK is one of subfamily of tandem pore domain K+ channel, and can be divided into three members, TREK-1, TREK-2 and TRAAK. It has been reported that TREK-1, TREK-2 and TRAAK are sensitive to temperature in addition to polyunsaturated fatty acid, pH cellular volume and general anesthetics. We examined colocalization pattern of TREK-1, TREK-2 and TRAAK, with thermosensitive Ca2+ channels, TRPV1, TRPV2 and TRPM8, in the rat trigeminal sensory neurons. Trigeminal ganglia of Wistar rats were fixed with Zamboni’s fixative or shock-frozen. Eight microm cryostat sections were stained by double-immunofluorescence for TREK channels (TREK-1, TREK-2 and TRAAK) and TRP channels (TRPV1, TRPV2 and TRPM8) . In the trigeminal ganglia, 24, 30 and 33 % of neurons were immunoreactive for TREK-1, TREK-2 and TRAAK, respectively. Average sizes of TREK-1, TREK-2 and TRAAK immunoreactive neurons were 447 ± 185, 445 ± 23 and 492 ± 12 microm2, respectively. TREK-1 immunoreactive neurons were colocalized with TRPV1 (57 %), TRPV2 (11 %) and TRPM8 (33 %) . TREK-2 immunoreactive neurons were colocalized with TRPV1 (33 %), TRPV2 (9 %) and TRPM8 (19 %) . TRAAK immunoreactive neurons were colocalized with TRPV1 (47 %), TRPV2 (10 %) and TRPM8 (22 %) . The present results suggest that TREK-1, TREK-2 and TRAAK modulate properties of TRP channels in trigeminal thermosensitive sensory neurons.
Effect of Neurotrophic factors on Methylmercury-Induced Neurotoxicity in Primary Culture Method of Rat Cerebellar Neurons

Motoharu SAKAUE, Misato MAKITA, Naoko MORI, Kou SAKABE
(Department of Public Health and Molecular Toxicology, School of Pharmaceutical Sciences, Kitasato University)

[Background] Neurotrophic factors mediate differentiation, growth, apoptosis and survival of neurons by binding to the tyrosine kinases, such as TrkA, TrkB and TrkC, or to the p75 low-affinity neurotrophin receptor. Because of MeHg is widely acceptable as a neurotoxin, we have studied mechanisms of methylmercury (MeHg) -induced neurotoxicity using primary-cultured cerebellar neurons. In the present study, we investigate whether neurotrophic factors have any effects on neurotoxicity by low dosage MeHg treatment. [Methods] Primary cultures of cerebellar neurons were prepared from Wistar rats (Jcl:Wistar) within 24 hrs after birth. After 2-days pre-incubation, MeHg and/or neurotrophic factors, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), were added into medium of the primary cultures. The cultures were fixed 48 hrs after MeHg treatment and the number of viable cell was estimated by crystal violet staining method. [Results and Discussion] MeHg treatment for 48 hrs induced cell death of rat cerebellar neurons at 30 nM in vitro. NGF had no effect on the cytotoxicity. However, BDNF exacerbated the cell death during MeHg treatment to the cells in a concentration dependent manner unexpectedly. Furthermore, The BDNF effect was attenuated though absorption using anti-BDNF antibody. These results suggested that BDNF promotes the MeHg-induced cell death of rat cerebellar neuron in vitro.
Comparative morphology of the mammalian facial nucleus

Rui FURUTANI, Masahiko TAKADA, Shoei SUGITA
(Function and Morphology, United Graduate School of Animal Science, Tokyo University of Agriculture and Technology)

The facial movements are primarily innervated by the facial nucleus. We attempted to identify a transneuronal pathway into the nasolabial motoneurons in the rat by the injection of a rabies virus into the musculus levator nasolabialis and the levator labii superioris. Localization of the immunoreactive neurons shows that nasolabial movement is innervated by nigrostriatal fibers via central nucleus of amygdala. Similar descending fibers are also found in the monkey. Although the facial musculature shows a difference between the rat and monkey, similar multisynaptic inputs into facial motoneurons are retained in both species. At the same time, the morphology of mammalian facial nucleus as a column of primary motoneurons were investigated in the rat, rabbit, ferret, goat, bovine, pig, horse, and Japanese monkey. The facial subnuclei of those mammals were ordinary localized medial to lateral on the transverse plane showing a species-specific difference. The dorsolateral subnucleus (DL) innervating nasolabial movement was observed in the facial nuclei of all species except monkey. Those cells of DL in the monkey were involved into the intermediate subdivision corresponding to the zygomatic movement.
Topography of Ganglion cells in the Retina of the Duck (Anas platyrhynchos var.domesticus)

Lutfur RAHMAN, Masato AOYAMA, Syouei SUGITA
(Function and Morphology, Utsunomiya University)

The distribution and soma diameters of retinal ganglion cells have been examined in whole mounted retinas of mallard ducks (Anas platyrhynchos var.domesticus). Map of the distribution of ganglion cells was produced from the Nissl-stained retinal whole mount. The topography of retinal ganglion cells was described in the area centralis, temporal, nasal, dorsal and ventral part of the whole mount retina. The mean value of total number of ganglion cells was estimated at about 1.7 millions, whereas the maximum ganglion cell density was 15820/mm² at the area centralis of the whole mount. The concentration of the ganglion cell bodies highest at the temporal area followed by nasal, dorsal and ventral area respectively. The size of ganglion cells were presented as measured area. The range of soma size of ganglion cells varied between 56-406 microm². The histograms of soma size in each zone indicated that a population of small sized ganglion cells persists into the central area just above the optic disc, on the other hand, the largest soma area was into the ventral zone of the retina.
Cellular response to TNFalpha and signaling events mediated by TNF-Rs in tendinocytes

Yoshinao HOSAKA, Rikio KIRISAWA, Hiromi UEDA, Kazushige TAKEHANA
(Veterinary Anatomy, Rakuno Gakuen University)

To reveal cellular response to tumor necrosis factor (TNF) alpha and signaling events mediated by TNF-receptors (TNF-Rs) in tendinocytes, we focused on four signaling factors, TNF-R1, TNF-R2, TRAF2 and NF-kB. Cell numbers were counted after treated with various concentration of TNFalpha. Signaling event mediated by TNF -Rs were visualized by using an immunohistochemical method, and the amounts of these factors were determined by Western blot analysis after treated with TNFalpha. The number of tendinocytes showed increasing tendency by addition of moderate concentration of TNFalpha, and showed decreasing and maintaining tendencies by addition of high and low TNFalpha concentration, respectively. The different manner of cell proliferation, which observed in tendinocytes by the different concentration of TNFalpha was thought to be a result of ligand passing. Immunohistochemical reaction for TNF-R1 was detected in TNFalpha-treated (Tt) cells but weak reaction was in control cells. Whereas, the reaction for TNF-R2 was detected in both control and Tt cells strongly. Other factors (TRAF2 and NF-kB) in Tt cells were upregulated. Both receptors were localized in endosome-like structure in tendinocytes, and it was thought that the localization of TNF-Rs in endosome-like structure was result of internalization. The distinct response to TNFalpha and distributions of the four factors under different conditions reflect the dynamism of TNFalpha.
Advanced glycation endproducts and their receptor in early diabetic retinopathy

Kazumi TANIGUCHI, Shin-ichiro YOTSUMOTO, Masashi TSUJIO, Kazuki YOSHIOKA, Kenichiro MUTOH
(Department of Veterinary Anatomy, Kitasato University)

Retinopathy associated with diabetes may have as part of their etiology the accumulation of advanced glycation endproducts (AGE) and their receptor (RAGE). We examined an early stage of retinopathy using spontaneously diabetic Zucker Diabetic Fatty (ZDF) rats. As controls, Zucker Fatty (ZF) and Zucker Lean (ZL) rats were examined. ZF rats have homozygous mutated leptin receptor genes (fa/fa), resulting in conspicuous obesity. ZDF rats were established as diabetic by selective inbreeding of ZF rats exhibiting poor glucose tolerance, to develop Type 2 diabetes mellitus spontaneously. Both ZDF and ZF rats were derived from ZL rats (fa/+) . Immediately after diabetic symptoms became documentable in ZDF rats, retinas from all three types of rats were removed and processed for immunohistochemical observations with anti-AGE and/or anti-RAGE antibodies and observed with a confocal laser microscope by comparing differential interference contrast and fluorescent images. The density of immunopositive reactions for AGE and RAGE in endothelial cells was greater in ZDF rats than in ZF or ZL rats. The ganglion cells more intensely reacted to AGE in ZDF rats than in ZF or ZL rats. Our observations suggest that the accumulation begins at very early stage of diabetes and it may contribute to the documented development of retinopathy that is a secondary complication of diabetes.
Comparative gross anatomy around M.atlantoclavicularis in anthropoid apes

Natsuo OHSTU, Ayako ISHIGURO, Tamire KASE, Nobutune ICHIHARA, Masao ASARI, Toshiyuki HAYAKAWA
(Department of Veterinary Anatomy, Azabu University)

The orangutan is a primate which spends the majority of its life in trees. In order to examine its anatomical characteristics, we performed a gross anatomical study of the cervical structure of a male adult Orangutan, donated by the Yagiyama Zoological Park (Sendai, Japan). We confirmed that M.atlantoclavicuralis (subsequently referred to as MAC) exists in orangutans, and MAC has its origin on the transverse process of the atlas, its termination on the acromion side of the clavicle, and has innervations from C3. In addition, an examination of the documented evidence confirmed that MAC exists only in anthropoid apes, and does not exist in humans. A review of the documentation indicates that the action of MAC has not been reported thus far. It is hypothesized that it can act in two ways: 1. It raises the clavicle (if its origin is on atlas) . 2. It bends the neck forward (if its origin is on clavicle) . A histological study of MAC was performed, but provided no meaningful interpretations. In this study, the significance of the presence of MAC is discussed by investigating the morphological changes around the atlas and clavicle of various anthropoid apes, which result from the differences in their weight and action styles.
The orangutan, the only arboreal great ape in the world, has a distinctive foot structure that adjusts to the type of grip required. Therefore, when walking on the ground, it does not stand with its trunk in a vertical position and touches only the exterior of its sole to the ground, although its foot posture is of the plantigrade type. The young orangutan, however, touches all parts of its sole to the ground. For this research, the lower extremities, especially the gross anatomy of the lower legs and feet were examined in two adult orangutans, which were donated by the Yagiyama Zoological Park (Sendai, Japan). A comparative study was made with documented data including other primates. The lower leg was similar in shape to the forearm, and the relatively thin belly of the gastrocnemius extended to the vicinity of the calcaneal bone where a short Achilles’ tendon diagonally inserted to the calcaneal bone. As a result, the inside of the foot was pulled up, and the outside was depressed. The popliteus was well developed, and the plantar aponeurosis was observed to be present though the plantaris was not found. It may be the case that these morphologic findings correlate with the unique locomotion system of the orangutan. Furthermore, it is thought that the orangutan may be considered an animal that can provide an important differentiation when discussing the role of the plantar aponeurosis.
Type 2 Helper T Cells Produce Activin A upon Activation

Kenji OGAWA, Masayuki FUNABA, Masafumi TSUJIMOTO
(Laboratory of Cellular Biochemistry, RIKEN)

Activin A, a member of the transforming growth factor-β (TGF-β) superfamily, is a pluripotent growth and differentiation factor. The roles of activin A including regulation of expression and function in the immune cells are still largely unknown. Here we report that murine T helper 2 (Th2) cells preferentially produce activin A upon activation. Activin A expression was enhanced in CD4+T cells by anti-CD3 crosslinking. The production of activin A was elevated in in vitro differentiated Th2 cells and in activated Th2 clones. Furthermore, activin production was detected exclusively in CD4+CD25−T cells, but not in CD4+CD25+regulatory T cells even when they were activated. Activin A did not affect cell growth of CD4+CD25−T cells, although TGF-β inhibited proliferation of the cells in a dose-dependent manner. These results indicate that expression and function of activin in CD4+T cells were distinct from those of TGF-β. Activin A may function as a Th2 cytokine that promotes type 2 immune responses.
A Macroscopic Examination of the Head, Neck and Laryngeal sac in the Orangutan

Ayako ISHIGURO, Natsumi OTSU, Misato KASEDA, Nobutsune ICHIHARA, Masao ASARI, Toshiyuki HAYAKAWA
(Department of Anatomy, Azabu University)

[Purpose] The morphological features of the muscles of mastication, muscles of facial expression and suprahyoid muscles were observed, to examine whether exist correlations between the orangutan’s mastication style and the form of the head. In addition, the laryngeal sac was observed in connection with its presumed functions of vocalization and locomotion. [Method] An adult orangutan donated by the Yagiya Zoological Park (Sendai, Japan) was used and a comparative study with the documented evidence that for other primates was carried out. [Result] A well-developed pad of fat was observed in the cheek. The muscles around the mouth were also observed to be well-developed. The laryngeal sac was found to extend into the axilla, the ventral and the dorsal areas. Vessels and nerves passed through a cleft between the parts of sac. [Consideration] It is suggested that the purpose of the cheek pad which is found only in the male is to make the face appear larger and stronger. When giving expression to its feelings, the orangutan can move its mouth skillfully. It was concluded that the form of face plays an important role in communication, even though the orangutan leads a solitary arboreal life. Furthermore, the evidence indicated that the laryngeal sac works as a cushion to defend the soft tissues during brachiation. The possibility that the sac is related to locomotion was concluded to be morphologically high.
A stem/progenitor cell line derived from bovine anterior pituitary exhibits an immunomodulator function

Yasuhiro NAGAI, Hideki OGASAWARA, Yoshikazu TAKETA, Hisashi ASO, Kouichi WATANABE, shyuichi OHWADA, Takahiro YAMAGUCHI
(Laboratory of Functional Morphology, Graduate School of Agricultural Science, Tohoku University)

Inflammatory mediators regulate anterior pituitary cell function through immuno-endocrine pathway. Recent studies showed that stem/progenitor cells have been identified in many adult tissues including bone marrow, the central nervous system and skin, and act as an immunomodulator. However, the existence of stem/progenitor cell that acts as an immunomodulator has not been identified in anterior pituitary gland. In this study, we attempted to establish stem/progenitor cell line from bovine anterior pituitary gland, and investigated immunomodulatory function. A cell line from bovine anterior pituitary cloned by limited dilution methods was designated as bovine pituitary cell 1 (BPC-1). BPC-1 stably grew by repeated passage. BPC-1 was positively immunostained for pit-1, MHC class 2 and vimentin but negatively for S-100 protein. BPC-1 expressed mRNA of stem/progenitor cell-associated factors and components of Notch signaling pathway such as nestin, Oct-4, Notch1 and Hes1. BPC-1 was positive immunoreactivity for β-catenin in its nucleus. In addition, BPC-1 expressed mRNA of inflammatory cytokines such as IL-6, IL-12, IL-15 and IL-18, and phagocytosed several beads. These results strongly suggest that BPC-1 has a stem/progenitor phenotype and acts as an immunomodulator. It is expected that new aspects of the immuno-endocrine network in anterior pituitary gland are unveiled by the elucidation of BPC-1 function.
Disruption in mouse Sertoli cells treated with mono (2-ethylhexyl) phthalate in vitro

Tat Wei TAY, Bibin Bintang ANDRIANA, Ehn Kyoung CHOI, Xiao Bo ZHU, Naoki TSUNEKAWA, Yoshiakira KANAI, Masamichi KUROHMARU
(Department of Veterinary Anatomy, Graduate School of Agricultural and Life Sciences, The University of Tokyo)

Our previous studies showed that mono (2-ethylhexyl) phthalate (MEHP) caused the prominent spermatogenic cell death in mouse testes. A further exploration revealed that the Sertoli cell also went through some changes. To determine these alterations, we have investigated the effects of MEHP on 3-week-old C57Bl/6N mice Sertoli cells. In brief, testes were excised, decapsulated, minced and treated with collagenase. Single cells were obtained by gravity sedimentation and trypsin-EDTA. After incubating for 72hr, spermatogenic cells were removed with hypotonic Tris buffer. The retaining Sertoli cell-enriched aggregates were reseeded onto chamber slides for 24hr prior to MEHP treatment. The cultures were exposed to 0, 1 and 100 nmol/ml MEHP for 1hr, 6hr, 12hr, 24hr and 48hr, respectively. The Sertoli cells were stained with 0.5% toluidine blue. Thereafter, morphological changes were observed by light microscopy. Immunohistochemistry using an anti-vimentin antibody was carried out to detect the changes in intermediate filaments of Sertoli cells. As a result, the presence of vacuoles was found in MEHP-treated Sertoli cells, while immunohistochemistry showed disruption in intermediate filaments of Sertoli cells incubated for 24hr with MEHP. Next, in order to clarify the presence of any cell death initiation signals and to visualize the late stage apoptosis, annexin V-FITC and TUNEL analyses are being carried out at present.
A new rat mutant of amelogenesis imperfecta

Taku MASUYAMA, Hayato OHSHIMA, Masaru OSAWA, Norihide YOKOI, Kazuyuki TANIGUCHI
(Toxicology Research Laboratories, Japan Tobacco Inc)

A rat mutant, whitish chalk-like teeth (wct), was characterized morphologically and genetically. The mutant rats showed tooth enamel defects that were similar to those of human amelogenesis imperfecta. The wct mutation was found to disturb the morphological transition of ameloblasts from secretory to maturation stages and to induce cyst formation. This mutation also disturbs the transfer of iron into the enamel, resulting in the whitish chalk-like incisors. A genetic linkage study revealed that the wct locus maps to a specific interval of rat chromosome 14. Interestingly, the human chromosomal region orthologous to wct is a 5.5-Mb interval in human chromosome 4q21 where is a critical region for the locus of human amelogenesis imperfecta AIH2. These results strongly suggest that this wct mutant is a useful model for the identification of genes responsible for amelogenesis imperfecta and molecular mechanisms of tooth development.
Oocyte-like cells in MRL mouse testis - Its preliminary investigation

Yasuhiro KON, Akihiro KONNO, Yoshiharu HASHIMOTO, Nobuya SASAKI, Daiji ENDOH
(Laboratory of Veterinary Anatomy, Hokkaido University)

[Background] It has generally been believed that male and female animals produce sperm in testis and oocyte in ovary, respectively. During our serial reproductive investigation in MRL mice, we found a unique testicular phenotype, in which oocyte-like cells (OLCs) are detected. In the present study, frequency of these cell development was preliminarily investigated using whole-mount preparation. Additionally, we will show herewith some light and electron microscopic results obtained till date.

[Materials and Methods] Oocyte score was estimated in a whole-mount preparation of testis. After counting, the tissues were removed into phosphate buffered saline and the seminiferous tubules containing OLCs were dissected under a stereoscope. The tubules were fixed with 10% neutral buffered formalin or 5% glutaraldehyde for light or electron microscopy, respectively. [Results and Discussion] The most adequate postnatal day for observation of OLCs containing zona pellucida-like structure was Day 14 afterbirth, on which the OLCs presented the score at approximately 1.5 in one testis. In the section of single tubule preparation, OLCs showed PAS-positive zona pellucida-like structure surrounding the cytoplasm. Electron microscopic observation revealed that follicular epithelial-like cells attached at outside of zona pellucida-like structure. These results suggest a possibility that male mice produce oocytes in MRL strain.
Analysis of differentiation potency of donor XY germ cells in mouse XX/Sry testes

Mayuko ISHII, Tsuyoshi TACHIWANA, Ryuji HIRAMATSU, Tomohide KIDOKORO, Shogo MATOBA, Masami KANAI-AZUMA, Hayato KAWAKAMI, Naoki TSUNEKAWA, Yoshiakira KANAI, Masamichi KUROHMARU
(Department of Veterinary Anatomy, The University of Tokyo)

In mammals, Sry, the sex-determining gene on Y, is sufficient to induce testis formation and subsequent male development of genitalia in XX mice. However, in XX sex-reversal mice including the XX/Sry and XXY mice, germ cells disappear soon after birth due to autonomous defects of the germ cell. Therefore, it remains uncertain whether XX males have a normal testicular somatic environment to support complete spermatogenesis or not. To elucidate the potency of the somatic environment of XX/Sry males, we transplanted XY spermatogonial stem cells into the testes of XX/Sry transgenic and XY W/Wv mice. Although complete spermatogenesis was induced in the transplanted W/Wv control testes, donor XY germ cells displayed no further progress beyond the elongate spermatid stage in the XX/Sry testes. The majority of round spermatids showed detachment or pyknosis. Moreover, our ultrastructural and histochemical analyses revealed ectopic formation of ectoplasmic specialization between the round spermatid and the Sertoli cell, where F-actin bundles were highly accumulated. These findings suggest that the somatic environment of the XX testes is capable of inducing differentiation of transplanted germ cells into round spermatids, but it can not support their differentiation into spermatozoa. Such defect may be partially caused by the defective regulation of round spermatid-Sertoli cell junction in XX males.
Effect of leukemia inhibitor factor (LIF) on carbohydrate structures in murine uterus during implantation.

Shoichi WAKITANI, Tetsuya SHIMOKAWA, Eiichi HONDO, Yasuo KISO
(Veterinary Anatomy, Yamaguchi University)

Leukemia inhibitor factor (LIF) has crucial roles for embryo implantation in mice. Expression of LIF in the uterine glandular epithelium (GE) is up-regulated by estrogen at the forth day of pregnancy. LIF induces many factors in the uterine luminal epithelium (LE). Although carbohydrate structures have been shown to be involved in embryo attachment to LE, few study was made to analyze these structures in relation to LIF. Here, we studied the effect of LIF on carbohydrate structures in the murine uterus during implantation using lectin. Delayed implantation mice was treated with LIF (20mg/head). Uteri were collected at 0, 1, 3 and 6h after LIF injection. Paraffin sections were stained by 24 kinds of lectins. Apical surface of GE were strongly stained by SBA, RCA-1, WFA, LCA, UEA-1, and RCA at all stages of pregnancy. Signals of BSL-1 were exclusively positive at apical surface of GE close to the uterine lumen. Especially, STL, s-WGA, WGA and ConA were positive in leukocytes in the endometrium. Positive reactions in LE were restricted to apical parts in most lectins. It is suggested that LIF should not affect core oligosaccharides, because many lectins used in this study bind specifically to core oligosaccharide structures. LIF might enhance sensitivity of LE for embryo attachment to LE by affecting terminal residues, so that further study of terminal carbohydrate structures is required.
The multivesicular nuclear body in the Sertoli cell nucleus of Reeves’ muntjac (Muntiacus reevesi) : an ultrastructural investigation

Bibin Bintang ANDRIANA, Naoki TSUNEKAWA, Yoshiakira KANAI, Sri Supraptini MANSJOER, Junpei KIMURA, Masamichi KUROHMARU
(Department of Veterinary Anatomy, The University of Tokyo)

It is well accepted that the multivesicular nuclear body (MNB), composed of vesicles, tubules and ribosome-like structures, is commonly present within the Sertoli cell nucleus of ruminants. However, no exact information is available on the MNB of the species belonging to the Cervidae. In the present study, we tried to clarify whether the MNB is present within the Sertoli cell nucleus of the Reeves’ muntjac, (Muntiacus reevesi) . Three Reeves’ muntjac were obtained in Chiba prefecture, Japan. The testes were excised and immersion-fixed in 5% glutaraldehyde. The serial cross-sections of testes were morphologically observed using light and transmission electron microscopy. In all samples, spermatogenesis was inactive, probably due to it being the non-breeding season. The Sertoli cell nucleus of Reeves’ muntjac displayed a well-developed and large-sized MNB situated in the central region. The vesicles and irregular tubules of the MNB seem to contain some materials with low and/or middle electron density and to be surrounded by electron dense materials. The diameter of vesicles was between 180 nm and 500 nm. The MNB was frequently recognized in the Reeves’ muntjac. This finding is similar to that in domestic ruminants. This study is the first report that demonstrates the presence of the MNB in the Sertoli cell nucleus of the species belonging to the Cervidae.
Reproductive performance in perforin and b2microglobulin deficient mice

Thanmaporn PHICHITRASLIP, Eiichi Hondo, Shoichi WAKITANI, Yasuo KISO
(Department of Veterinary Anatomy, Faculty of Agriculture, Yamaguchi University)

The uterine natural killer (uNK) cell partly shares common functions with the NK cell in peripheral blood, which is not restricted by MHC class I antigen and has cytotoxic activity dependent on perforin. This study aimed to clarify the effect of beta-2 microglobulin (b-2m) and perforin on pregnancy focusing on uNK cells with their deficient models. Reproductive performance, structure of the placenta, and appearance of uNK cells during pregnancy was investigated using two knockouts and background (C57BL/6J) mice. After measuring weight of placenta in each mouse, paraffinized sections were prepared to observe histological feature of the placenta. Three implantation sites were randomly picked up in each day of pregnancy to calculate the cell density of uNK cells in the metrial gland. No detectable change was recognized between perforin deficient mice and the control. The number and the weight of fetuses in b-2m deficient mice tended to decrease compared to the control. There was no difference in weight and structure of the placenta among three kinds of mice used in this study, however, the cell density of uNK cells in b-2m deficient mice was increased in the metrial gland at mid-pregnancy. In conclusion, perforin secreted by uNK cells is not involved in maintenance of pregnancy in mice. In addition, MHC class I antigen should affect growth of the fetus and differentiation of the uNK cell.
Differential expression of motin family in the peri-implantation mouse uterus and their regulation by the ovarian steroids

Hiromichi MATSUMOTO, Yuko MIYAKE1, Hiroshi SASADA, Takiko DAIKOKU, SK DEY, Eimei SATO
(Laboratory of Animal Reproduction, Tohoku University)

Ovarian steroids and angiogenic factors in uterine vascular permeability and angiogenesis are required for implantation and decidualization. Angiomotin (Amot) is an angiostatin-binding protein that promotes endothelial cell motility and tube formation in vitro. The motin family of proteins is comprised of three polypeptides, Amot, angiomotin-like 1 (Amotl1), and angiomotin-like 2 (Amotl2), whereas the function of them in female reproductive tract remains unknown. In the present study, we examined the spatiotemporal expression of motin family in the uterus by in situ hybridization. On day 1 of pregnancy, mRNA expression of all motins was located in luminal epithelial cells. On days 2-4, Amotl1 and Amotl2 were expressed in stroma cells. While Amot was expressed in luminal epithelium on day 2, the expression was localized in stroma cells on days 3 and 4. During post-implantation, Amotl1 expression was primarily observed in decidual cells close to the implanting embryo on day 5, although the expression was restricted to the secondary decidual cells and undifferentiated stromal cells away from implanting embryo on days 6-8. Amot expression was observed in whole stroma cells on days 5-6, while the expression was similar with Amotl1 on days 7-8. Amotl2 was expressed at low level on days 5-8. We also observed that progesterone primarily up-regulates uterine expression of motins, whereas estrogen transiently stimulates them.
The expression of the motin family in mouse ovary.

Yuko MIYAKE, Hiromichi MATSUMOTO, Maria G. PALMERINI, Hiroshi SASADA, Guido MACCHIARELLI, Takiko DAIKOKU, Sudhansu K. DEY, Eimei SATO
(Laboratory of Animal Reproduction, Tohoku University)

[Background] Ovarian angiogenesis is involved in follicular development and corpus luteum formation. Especially, the capillary network in dominant follicles is able to acquire an increased uptake of serum gonadotropins, a variety of hormones and growth factors. A recent study identified angiomin (Amot), a novel protein that mediates the inhibitory effect of angiostatin. This protein, together with angiomin-like 1 (Amotl1) and angiomin-like 2 (Amotl2), is the member of the motin family. The aim of this study was to examine the possible role/s of the motin family in ovarian angiogenesis. To this purpose we used in situ hybridization to determine the localization of Amot, Amotl1 and Amotl2 mRNAs in the ovary. [Materials and Methods] Immature mouse ovaries were collected at 24, 48 hrs after PMSG and at 4, 8, 12, 16, 20 hrs post-hCG. Frozen sections were hybridized with 35S-labeled antisense cRNA probes and detected by autoradiography. [Results and Discussion] A high expression of Amot mRNA was detected in granulosa cells, cumulus cells and lutein cells at all stages, whereas very low to undetectable level of expression was observed in theca cells. Amotl1 and Amotl2 mRNAs showed a similar localizations of Amot, but with a weaker intensity. These results indicated that the motin family may be involved in ovarian angiogenesis, and may have important roles in follicular development and ovulation.
Sex differences in regulation of gonadotropin releasing hormone (GnRH) secretion in rat

Kouichi INAMI, Shinji TSUKAHARA, Kouzo ISHIDA, Katsuhiko WARITA, Yasushi CHIDA, Hiroshi KITAGAWA, Nobuhiko HOSHI
(Laboratory of Functional Anatomy, Kobe University)

[Introduction] Female specific GnRH surge is controlled by the neuronal network composed of the anteroventral periventricular nucleus in the preoptic area (AVPvN-POA), which has sexual dimorphism in its volume, and some nuclei, projecting directly to GnRH neurons. Although the correlation between dopamine (DA) neurons in AVPvN-POA and GnRH surge has been indicated, it was unclear what caused sex difference in GnRH secretion. [Methods] After the animals were gonadectomized and treated estradiol for promoting GnRH surge, their brains were removed for quantitative histological analysis. The activation in GnRH and DA neurons was examined by double staining for c-fos (cellular activation) & GnRH or c-fos & tyrosine hydroxylase (DA neuron). [Results] Most of GnRH neurons in the anteromedial preoptic area expressed c-fos in females, while not in males and androgenized females. c-fos containing cells in AVPvN-POA were observed more predominant in females than in males and androgenized females. c-fos immunopositive DA neurons in AVPvN-POA were rarely found in all animals. [Discussion] The sex differences in GnRH releasing function are depend on the sex differences in the activation of GnRH neurons. There is the relationship between the activation of GnRH neurons and that of the cells in AVPvN-POA. It is possible that activating cells in AVPvN-POA indirectly regulate GnRH neurons by controlling DA secretion.
Age-related change and its sex differences in histoarchitecture of the hypothalamus of F344/N rats

Kouzo ISHIDA, Shinji TSUKAHARA, Kouichi INAMI, Katsuhiko WARITA, Yasushi CHIDA, Hiroshi KITAGAWA, Shin TANAKA, Nobuhiko HOSHI
(Laboratory of Functional Anatomy, Kobe University)

[Introduction] In the mammalian brain, SCN (the circadian clock) and AVPvN-POA (sexually dimorphism) are critical to the occurrence of regular cyclic GnRH secretion. We present the age-related change and its sex differences in the hypothalamus of F344/N rats. [Methods] Animals were divided into six groups: both sexes for each of 3 age categories: young, middle, and old. For each animal, the number of neurons and glias was examined histoplanimetry by means of cresyl fast violet staining and/or immunostaining for glial fibrillary acidic protein (GFAP), a marker of astrocytes. [Results and Discussion] Loss of neurons observed in the SCN with increasing age. Neurons in the AVPvN-POA were significantly higher in young rats than in middle or old rats. While the neurons of SCN and AVPvN-POA in females gradually declined with increasing age, that in males were significantly decreased in middle and old age rats, compared to young rats. An inverse relationship between the increase in astrocytes and the decrease in neurons in the AVPvN-POA was observed. Because the proliferation of astrocytes are induced by surgical injury or chemical lesions of neurons, it is possible that the increase of astrocytes occurs in the female SCN at middle and in the AVPvN-POA at middle and old in order to diminish neuronal cell loss or remove degenerated neurons.
Changes in Expression and Localization of Connexin 43 mRNA and protein in Granulosa Cells during Follicular Atresia in Porcine Ovaries

Yuan CHENG, Naoko INOUE, Fuku MATSUDA, Yasufumi GOTO, Akihisa MAEDA, Noboru MANABE (Research Unit for Animal Life Sciences, Animal Resource Science Center, The University of Tokyo)

Gap junctions contain channels that connect neighboring cells by allowing the movement of molecules smaller than 1,000 Da. These gap junctions are formed by connexins, and play a crucial role in the regulation of apoptotic cell death. In granulosa cells of ovarian follicles, connexin-43 (Cx43) is expressed. To determine the regulatory mechanism of granulosa cell apoptosis during follicular atresia, we examined changes in the expression and localization of Cx43 mRNA and its protein in granulosa cells of porcine ovaries during follicular atresia. Stages of follicular atresia were assessed by histochemical TUNEL staining and by the ratio of progesterone and estradiol levels in follicular fluid measured by RIA. As a result, both Cx43 mRNA and protein were detected in granulosa cells of healthy, early atretic and progressed atretic follicles, but not detected in primordial or primary follicles. Western blotting showed that Cx43 protein was expressed most strongly in isolated granulosa cells prepared from healthy follicles, but only trace or slight expression was noted in those from progressed atretic follicles. That is to say, in the follicular atresia, Cx43 protein expression level was decreased. These findings indicate that Cx43 is related to granulosa cell apoptosis during follicular atresia in porcine ovaries.
Research on the methodological error of amino-acid assay occurred on deferred deproteinization-time before storage and after thawing:

Shunyu LI, Kazumi SUZUKI, Yukiyoshi KOIKE, Kiyoyoshi KUBOTA, Noboru MANABE
(Research Unit for Animal Life Sciences, Animal Resource Science Center, The University of Tokyo)

Four different times contributing to deferred deproteinization-time of variation in the determination of amino acids in plasma before storage and after thawing. In order to study the effect of deferred deproteinization-time on amino acid assay, the author divided plasma into two batches. One, the plasma was put on room temperature (24°C) and deproteinized after 0, 30, 60, and 120 min respectively. The other hand, the plasma was stored at -20°C for two weeks and just before analysis thawed (4°C, 30 min). The way of the deproteinization was the same as described above. The deferred deproteinization-time both in before storage and after thawing clearly reduced the coefficient of variation in the determination of Proline, Alanine, Methionine, and Carnosine. The C.V. of Proline, a-AAA, Lysine, Alanine, Aspartic acid, Ammonia, and Methionine were nearly 10%, however. The losses of Glycine and Cystine occurred when the sample was stored at -20°C. The concentrations of all of the most amino acids both in before and after storage significantly increased when plasma just stays at room temperature 30 min, but Aspartic acid, a-AAA, Alanine, and Cystine decreased. The concentration of amino acid significantly increased after storage at -20°C for two weeks.
Interleukin-6 regulates the expression of cellular FLICE-like inhibitory protein in granulosa cells of ovaries during follicular atresia

Akihisa MAEDA, FUKO MATSUDA-MINEHATA, Yuan CHENG, Noboru MANABE
(University of Tokyo, Research Unit for Animal Life Sciences, Animal Resource Science Center)

In mammalian ovaries, more than 99% of follicles undergo atresia, and only a few follicles grow to ovulate during ovarian follicular growth and development. Follicular selection dominantly depends on granulosa cell apoptosis, but the molecular mechanism regulating selective follicular atresia is still largely unknown. In the present study, we examined whether interleukin (IL)-6 is involved in granulosa cell apoptosis during follicular atresia or not. Both mRNAs of IL-6 and gp130 [a subunit of IL-6 receptor (IL-6R)] were detected in granulosa cells prepared from healthy, early atretic and progressed atretic follicles of porcine ovaries by quantitative real-time RT-PCR. These mRNAs were highly expressed in granulosa cell of healthy follicles and decreased during follicular atresia. IL-6 soluble receptor (IL-6sR; a subunit of IL-6R that binds with gp130) protein was detected in follicular fluids of healthy, early atretic and progressed atretic follicles by the ELISA method, and this protein level also decreased during follicular atresia. Moreover, recombinant IL-6 up-regulates cellular FLICE-like inhibitory protein long form (cFLIPL) in cultured cells derived from granulosa cells. These results indicate that IL-6 is dominantly produced in granulosa cells of healthy follicles in porcine ovaries and that IL-6 up-regulates cFLIPL and prevents apoptotic cell death.
Bid plays crucial roles in the regulation of apoptosis in granulosa cells during follicular atresia in pig ovaries

Takafumi SAI, Fuko MATSUDA-MINEHATA, Akihisa MAEDA, Yuan CHENG, Yasufumi GOTO, Sayuri ANAN, Hiroshi GONDA
(Research Unit for Animal Life Sciences, Animal Resource Science Center, The University of Tokyo)

[Aim] The apoptosis in granulosa cells plays dominant roles in atresia regulation in ovarian follicles of mammalian ovaries. However, we have hardly no information on the regulation mechanisms, especially intracelluar apoptosis-signal transducing pathway. Apoptosis-signal is initiated by activation of cell-death receptor, and then the signal is transduced by the activation of procaspase-8. To date, we know that there are two pathways, type I and II. In type I, activated caspase-8 trancate procaspase-3. In type II, Bid is trancated by activated caspase-8, and the signal is transduced into mitochondrion. Mitochondrion releases cytochrom C, and procaspase-9 is activated. Activated caspase-9 trancates procaspase-3. Here, to reveal the signaling pathway, we analyzed the expression levels of Bid and Bax, in cultured granulosa (KGN) cells.

[Methods] Bid mRNA was down-regulated by siRNA method in KGN and Hela cells, and apoptosis was induced by anti-Fas antibody and cycloheximide administration. In addition, we assessed the expression levels of Bid and Bax mRNAs in these cells.

[Results] Apoptosis was highly induced in KGN cells by down-regulation of Bid mRNA, but not in Hela cells. Expression level of Bid mRNA in Hela cells was higher than that in KGN cells, but less expression level of Bax mRNA in Hela cells was noted. Based on this and our previous data, we suppose that granulosa cells mainly use type II pathway.
Lutela cell apoptosis is regulated by soluble Fas in murine ovaries

Noboru MANABE, Fuko MATSU-MINEHATA, Akihisa MAEDA, Yuan CHENG, Takafumi SAI, Kohji KOMATSU, Junyou LI
(Research Unit for Animal Life Sciences, Animal Resource Science Center, The University of Tokyo)

In mouse luteal bodies, we demonstrated the mRNA expression of a soluble form of Fas (FasB), which binds to FasL and prevents apoptosis induction. By in situ hybridization, strong expression of FasB mRNA was observed in normal luteal bodies, in which no TUNEL-positive apoptotic cells were detected, but negative/trace expression was observed in regressing luteal bodies, in which many apoptotic cells were observed. Immunohistochemical staining demonstrated that Fas and tumor necrosis factor (TNF) alpha were localized in both normal and regressing luteal bodies, but interferon (IFN) gamma was localized only in regressing luteal bodies. Apoptosis was induced in primary cultured luteal cells, when the cells were pretreated with TNFa and IFNg, then incubated with TNFa, IFNg and mouse recombinant FasL (rFasL). However, there was no apoptosis detected in the cells when they were treated with rFasL alone, TNFa alone, IFNg alone, TNFa and rFasL, IFNg and rFasL, or TNFa and IFNg. Fas mRNA expression in cultured luteal cells was up-regulated following treatment with TNFa, IFNg or TNFa and IFNg. The expression of FasB mRNA was down-regulated when the cells were treated with TNFa and IFNg, but its expression was not changed by treatment with TNFa alone or IFNg alone.
The Cu,Zn-superoxide dismutase (SOD1) is an important antioxidative enzyme against reactive oxygen species in all aerobic organisms, but little is known about its distribution in developing embryos. We demonstrated the expression pattern of SOD1 mRNA in mouse embryos using RT-PCR and in situ hybridization analyses. SOD1 mRNA was detected in all embryos examined at embryonic days (Eds) 8.5 to 18.5 and began to decrease after Ed 15.5. SOD1 mRNA was expressed in chorion, allantois, amnion and neural folds at Ed 7.5. At Eds 9.5-12.5, SOD1 mRNA showed a high expression in sensory organs and central nervous system (CNS). At Eds 13.5-15.5, SOD1 mRNA was greatly expressed in the developing CNS, nasal chamber, lung, thymus, pancreas, intestine, metanephric kidney, adrenal gland, but was weakly expressed in the liver and heart. After Eds 16.5, the expression of SOD1 mRNA especially was greatly expressed in mucosa and metabolically active sites such as lung, kidney, stomach, and intestine and epithelial cells of skin, whisker follicles, and ear and nasal epithelia. In this study, the spatiotemporal expression of SOD1 mRNA was expressed in various cell-and tissue-specifically. These results suggest that the expression of SOD1 relates to antioxidant function during organogenesis.
The role of Sox17 and Sox18 in mouse embryonic cardiovasculogenesis.

Yohei SAKAMOTO, Toshiyasu MATSUI, Kenshiro HARA, Takumi NAGATA, Naoki TSUNEKAWA, Masami KANAI- AZUMA, Yoshiakira KANAI, Masamichi KUROHMARU
(Department of Veterinary Anatomy, The University of Tokyo)

In mouse embryo, cardiovasculogenesis occurs dynamically at 8.0 to 9.0 days postcoitum (dpc). However, the transcription factors controlled in this process are still unclear. Sox17 and Sox18 belong to the Sox (Sry-related HMG box) family of transcription factors, which are key regulators of cell-type specification, and they are co-expressed in vascular endothelial cells. Previous study indicated that Sox17 and Sox18 work complementary in the early stage of the blood vessel formation. However, the function has not been clarified. In this study, we examined a detailed expression pattern of Sox17 and Sox18 in 8.0 to 9.0 dpc mouse embryos. As a result, Sox18 was expressed in the blood vessel formation area from 8.0 dpc when the heart tube and dorsal aortae began to form. Then, it was also expressed in intersomitic vessels at 8.5 dpc and the capillary plexus of the head at 8.75 dpc. In contrast, Sox17 began to express from 8.25 dpc, in the area of the heart tube and dorsal aortae, and it was also expressed in intersomitic vessels, but not in the capillary plexus of the head. Thus, Sox17 and Sox18 are co-expressed in large blood vessels such as the heart tube and dorsal aortae, and it is speculated that Sox17 and Sox18 bear some role in the formation of these vessels. At present, to clarify the role of Sox17 and Sox18 in the embryonic cardiovasculogenesis, we try to examine the study using Sox17/Sox18 double mutants.
Evaluation of the dose-response effects of fetal and neonatal exposure of male mice to the endocrine disruptor diethylstilbestrol on fertility in adulthood

Katsuhiko WARITA, Kana ISHIHARA, Tetsuorou INAMOTO, Sachiko ONISHI, Midori YUJI, Yasushi CHIDA, Teruo SUGAWARA, Hiroshi KITAGAWA, Nobuhiko HOSHI
(Laboratory of Functional Anatomy, Kobe University)

[Introduction] Endocrine disruptors have heightened public concerns about the adverse health consequences of these chemicals for many species. We evaluated the dose-response effects of fetal and/or neonatal exposure to an estrogenic, endocrine-disrupting compound on the male reproductive tract in adult mice. [Methods] Pregnant ICR mice were given subcutaneous injections of 10 microg/day/head of diethylstilbestrol (DES) to subject the fetal mice to in utero exposure (IUE). Subsequently, the newborn male mice were treated with vehicle or 0.1-10 microg/day/head of DES as neonatal exposure. [Results] The fertility rates of each group were as follows: control, 100% (7/7); IUE only, 60% (3/5); IUE+NE 0.1 microg, 25% (1/4); IUE+NE 1 microg, 0% (0/8); IUE+NE 10 microg, 0% (0/8). In general histology, thinning of germ cell layers and atrophy of Leydig cells were observed in the groups of IUE+NE 0.1-10 microg, and the intensity of staining of Leydig cells was diminished. The FSH, LH and testosterone levels in the DES-exposed groups decreased in inverse proportion to the dose. Furthermore, the expression of ERalpha and StAR mRNA in the testis decreased in the groups of IUE+NE 1-10 microg. [Conclusion] The fertility rate was closely correlated with the testosterone levels, which were reduced by the decrease of LH and the expression of the StAR gene (the rate-limiting factor of steroidogenesis).
Theerask Prapong, Maleewan Liomsiricharoen, Sirirak Chantakru, Prapassorn Chongmeepomrisip,s Pakawadee Pongket, Apinun Suprasert
(Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University)

The histology and histochemistry of major salivary glands in Malayan pangolin (Manis javanica) were studied. Tissue samples were fixed in Carnoy’s solution before tissue preparation of paraffin technique. The 5 micron thick-sections were stained with Hematoxylin&Eosin (H&E), Alcian Blue (AB) pH 2.5, periodic acid-Schiff (PAS), AB pH 2.5-PAS, High iron diamine (HID) and HID-AB pH 2.5. Serous acini and clusters of mucous acini were found in the parotid gland together with well developed grandular ducts, modified striated duct. The sublingual gland and the submandibular gland contained exclusively tubuloacinar mucous gland. The serous acini of parotid gland stained negatively with AB pH 2.5, PAS, AB pH 2.5-PAS, HID and HID-AB pH 2.5. In contrast, the mucous cells of parotid gland, submandibular gland and sublingual gland exhibited positive reaction with all staining methods. The presence of histochemical results implied the presence of acid neutral glycoconjugates with sulfate and carboxylate groups in all the mucous cells and their secretion.
Ginseng has been widely used around the world for many years. Especially, pregnant women commonly consume ginseng. However, there are a few reports concerning the embryotoxic effects of ginseng on early developing embryo. In our study, whole embryo culture was applied to explore the effects of each ginsenosides (Rg1, Re, Rb1, and Rc) on rat embryo during their critical period of organogenesis (Ed 9.5-11.5) and RT-PCR analysis was carried out for the glutathione peroxidase (GPx) family mRNA. Median total morphological scores in embryos exposed to 5, 50 and 100 microg/ml of ginsenosides Rg1 were no significant difference compare to control group. There were no difference in the expression pattern of GPx family mRNA among control embryos and embryos exposed to 5, 50 and 100 microg/ml of Rg1. The results of other ginsenosides Re, Rb1, and Rc were similar to that of Rg1. Therefore, there was also no difference in the morphologic and GPx family mRNA level among control and experimental group exposed to 5, 50 and 100 microg/ml of Rg1, Re, and Rc. These results indicate that ginsenosides Rb1, Re, Rg and Rc did not affect on the embryotoxicity and expression of the GPx family mRNA in rat embryo organogenesis.
Anatomical characteristics of the female reproductive organs in the Malayan Pangolin

Junpei Kimura, Maleewan Liimsiricharoen, Sirirak Chantakru, Theerasak Prapong, Apinun Suprasert
(Department of Anatomy, College of Bioresource Sciences, Nihon University)

The pangolin belongs to the Order Pholidota and is the sister group to Carnivores and Perissodactyls in the recent phylogenetical analyses. Populations of Malayan Pangolin are greatly reduced, because the scales of these animals are mistakenly believed to be of medical value in China and Vietnam. This study is designed to establish the characteristics of the female reproductive organs in this animal as a basis for understanding the reproductive physiology of this endangered species. Such information is a necessary prerequisite for design of effective conservation strategies. Five dying female pangolins, seized from smugglers in Thailand, and brought to KPC wildlife breeding center were used in this study. In four individuals among five, each varied sized fetus was observed in one side of the uterine horns which can be classified as bicornuated uterus with well developed uterine glands. During pregnancy, large corpus lutea can be recognized in both sides of the ovary which has no bursa and is ellipsoidal in shape. The interstitial glandular cells are plenty in the ovary and showed positive reaction to the P450scc antibody. The interaction of maternal and fetal placenta surfaces takes place virtually over the entire sac, and so the placenta could be described as diffuse and in the epitheliochorial category. These anatomical characteristics can be recognized in the animals belonging to the Laurasiatheria clade.
Macroscopic and Microscopic Study of the Integument and Accessory Organs of Malayan Pangolin (*Manis javanica*)

Maleewan Liumsiricharoen, Theerasak Prapong, Narong Chungsamarnyart, Urai Pongchairerk, Khongsak Thiagtum, Pakawadee Pongket, Apinun Suprasert
(Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University)

The integument and accessory organs (scale, mammary gland, anal gland and glandular pad of tail) of Malayan pangolin (*Manis javanica*) were studied both in macroscopic and microscopic methods. In adult pangolin, scales from head, body, limb and tail were different in the shape and size. In newborn and fetus of pangolins, the scale pattern were developed in fetus of crown-rump length of 5 cm. and 8 cm. but not in 2 cm. The coloration, the finely striated at the base of scale and the hairs underneath scale were developed in newborn pangolin but not in fetus. Opening of anal gland was located on the skin around anus. SEM study of glandular pad under the tip of the tail showed, the entire surface was covered by numerous blunt papillae. Epidermis of pangolin skin was keratinized stratified squamous epithelium with prominent keratohyalin granules in stratum granulosum layer. The scale was the very thick keratinize layer of epidermis. Neither sweat gland nor sebaceous gland was found in dermis of pangolin integument. The hair follicles were found only the dermis of skin near the rim of scales. The mammary glands located near axilla consist of single cell layer alveoli, myoepithelial cells and connective tissue stroma similar to other mammals. The numerous large anal glands had several opening of excretory ducts for directly secreting to the superficial surface.
Histology and Glycoconjugates Histochemistry in the Small and Large Intestinal Epithelium of the Pangolin

Apinun Suprasert, Maleewan Liiumsiricharoen, Pakawadee Pongket, Therasak Prapong, Apuntee Duangngen

(Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University)

Histology and glycoconjugates histochemistry in the small and large intestinal epithelium of the Malayan Pangolin (Manis javanica) were studied by means of histological and histochemical techniques. The staining procedures employed were Hematoxylin-Eosin (H&E), alcian blue (AB) pH 2.5, periodic acid-Schiff (PAS), AB pH 2.5-PAS and lectin including penut agglutinin (PNA), Ulex europeus agglutinin-I (UEA-I) and wheat germ agglutinin (WGA) in combination with enzyme neuraminidase digestion procedures. The intestinal canal displayed no division between small and large intestine, other than a continuous enlargement, and there was no cecum. The mucous membrane of the pangolin small intestine was thrown into high villi and long crypt of Lieberkuhn. While, the mucosa of large intestine lied in a series of irregular fold, not villi. The mucosa of both intestine was covered by simple columnar epithelium in which 2 type of cells were encountered columnar and goblet cells. The mucous granule of goblet cells in small intestine were found to contain acid and neutral glycoconjugates with galactose residues. On the contrary, the mucous epithelium of large bowel involved predominantly acid glycoconjugates with fucose and sialic acid residues. Furthermore, the nature of glycoconjugates in mucous epithelium of both intestine was found to change during the upward migration from deep to superficial part.
Functional morphology of the Mole scapula with special reference to the trapezius and rhomboid muscles

Ken FUJINO, Yutaka TAKAHASHI, Akiyoshi MATSUMURA, Kunihiko KIMURA
(Laboratory Animal Science, Tokyo Metropolitan Institute of Gerontology)

The functional morphology of the scapula was analyzed with special reference to the trapezius and rhomboid muscles using three moles (Mogera wogura). The observations showed: 1. the primary role of the scapula as a mechanical base for the humeral movement is reduced; the humerus is instead stoutly linked to the cranial end of the elongated manubrium sterni via the short, quadrangle clavicle; 2. the long, rod-like scapula bridges the humeral proximal end and the dorsal surface of the rib cage, probably acting as the dynamic supporter for the cranially-shifted upper extremity during its excavating movement; 3. the trapezius develops its long, strip-like caudal portion arising from the lumber vertebrae, in contrast, its cranial one is a rather thin sheet of little muscle fibers; 4. the rhomboid consists of two portions: the cranial arising broadly from the occipital bone converges its fascicles toward insertion; the cervical sends short, obliquely running fascicles; 5. All insert on the caudal half of scapula; 6. the average fascicle length of the cranial rhomboid and the caudal trapezius amounts to 23mm, 31mm, respectively; 7. thus one of the main functions of the two is to slide scapula cranially and caudally on the dorsal surface of the thorax, without causing a rotating action, and the fascicles of the two may theoretically allow scapula to reciprocate actively in a width of 25mm at most, about 36% of the body length.
Immunohistochemical survey on the distribution of neuronal elements in the ileum of cattle

Toru IZAWA, Nobuo KITAMURA, Motoki SASAKI, Naotaka ISHIGURO, Haruo YAMADA
(Laboratory of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary medicine)

The distribution of nerve fibers and cell bodies in the ileum of calves and cows was examined in cryosections using various immunohistochemical markers: calcitonin gene related peptide (CGRP), dopamine ß-hydroxylase (DBH), galanin (GAL), methionine enkphalin (MENK), neuropeptide Y (NPY), nitric oxide synthase (NOS), protein gene product 9.5 (PGP), substance P (SP), tyrosine hydroxylase (TH), vanilloid receptor-like protein 1 (VRL1), vasoactive intestinal polypeptide (VIP), vesicular acetylcholine transporter (VAT). Abundant PGP-immunoreactive (IR) fibers were distributed in all layers and cell bodies were in the submucous and myenteric ganglia. In the mucosal layer, GAL-, SP-, VIP- and VRL1-IR fibers were abundant. Some fibers were noted in the dome region of the Payer’s Patch (PP). In the submucosal plexus, CGRP-, GAL-, SP- and VRL1-IR cell bodies and CGRP-, GAL-, NOS-, VIP and VRL1-IR fibers were frequently seen. DBH-, GAL- and TH-IR fibers were numerous around vessels. CGRP- and VRL1-IR fibers were seen around lymphatic follicles of the PP but none in the follicle. In the muscle layer, GAL-, MENK-, NOS-, SP, and VIP-IR fibers were numerous. In the myenteric plexus, NOS- and TH-IR cell bodies and GAL-, MENK-, SP-, NOS- and TH-IR fibers were abundant. The present results will be useful as fundamental database for the study of bovine gastroenterology.
Lectin binding pattern of Pacific white-sided dolphin gastric mucosa

Tetsuya SHIMOKAWA, Takuya DOIHARA, Manami MAKARA, Takehiro TERASHITA, Kyoji MIYAWAKI, Ryusuke TSUJIMURA, Shuang-yan GAO, Min WANG, Chun-yu LI, Jie CHEN, Zhong WANG, Bing XUE, Yuji MIGUCHI, Naoto KOBAYASHI, Seiji MATSUDA
(Division of Anatomy and Embryology, Department of Integrated Basic Medical Science, Ehime University School of Medicine.)

[Introduction] Dolphins have the compound stomach, defined as the forestomach, the proper gastric gland portion and the pyloric chamber. We reported about the lectin binding patterns in three stomachs, however little information is available about the cardiac glands. The aim of this study was to define cardiac glands morphologically, and to investigate the carbohydrate composition of mucins from cardiac glands and compare it with that of mucins from pyloric glands. [Methods] Three Pacific white-sided dolphin stomachs were studied. The tissues were fixed in 4% paraformaldehyde and 1.5% glutaraldehyde and routinely processed to paraffin wax. Sections were cut at 5microm thickness and stained with H-E, PAS, and Ab (pH 2.5 and 1.0) . Lectin-histochemistry was performed by the avidin-biotin complex method with 24 biotinylated lectins in the commercial lectin screening kit. [Results] Cardiac glands were confined to a very narrow region, and have a similar structure to the pyloric glands. Cardiac glands reacted with 19 lectins. A striking contrast between cardiac glands and pyloric glands was seen in DSL. [Conclusion] This observation suggests that the cardiac gland in Pacific white-sided dolphin is similar to that of terrestrial mammals, and glycoconjugates containing N-acetylglucosamine seem to play an important roll for the secretion of mucins.
Persorption mechanism of large particulates from intestinal lumen to portal blood in rats Yoshitomo FUKUI, Midori YUJI, Sachiko ONISHI, Tetsurou INAMOTO, Katsuhiko WARITA, Nobuhiko HOSHI, Hiroshi KITAGAWA
(Laboratory of Functional Anatomy, Kobe University)

[Background and Aim] Persorption of large particles from intestinal lumen to systemic blood circulation has been hitherto reported in human and animals, but the mechanisms remain unknown. Recently, however, we have proposed the persorption mechanisms of the antigenic macromolecules and small particles, mediated by both their specific antibody and Fcgamma-receptors on apoptotic intestinal epithelial cells. The both substances were transported into the systemic blood via portal vein. In this report, we present the persorption mechanisms of large particulates from intestinal lumen to portal vein. [Methods] In 5 Wistar rats with pre-oral immunization of BSA, BSA- or casein-coated polystyrene particles (av. diameter: 25microm) were administered into 2 jejunal loops under anesthesia respectively. In 5 non-immunized rats, IgG-Fc-, IgG-Fab-or BSA-coated particles were administered into 3 loops respectively. After 30 min, the extracted loops were histologically examined. [Results]In pre-immunized rats, BSA-coated particles were engulfed by the collaboration of plural epithelial cells at the apices of intestinal villi. In non-pre-immunized rats, Fc-coated particles were also engulfed by the epithelial cells in same manner. The absorbed particles were found in the venules of intestinal walls. [Conclusion]The persorption of antigenic large particulates is mediated by their specific antibody and Fcgamma-receptors.
Localization of cellular prion protein (PrPc) in murine and bovine small intestine

Kohtaro MIYAZAWA, Hisashi ASO, Takashi KANAYA, Ikuro TAKAKURA, Sachi TANAKA, Kouichi WATANABE, Shyuichi Ohwada, Suehiro SAKAGUCHI, Shigeru KATAMINE, Takahiro YAMAGUCHI (Laboratory of Functional Morphology, Graduate School of Agricultural Science, Tohoku University)

The gastrointestinal tract is thought to be the main site of entry for the pathological isoform of prions (PrPsc). Prion diseases are believed to result from the conformational change of PrPc to PrPsc. Therefore, PrPc expression is important for the infection and the spread of disease to the central nervous system. However, the distribution of PrPc in gut and its infective mechanism are still a matter of controversy. So we investigated the localization of PrPc in murine and bovine small intestine. In mouse, PrPc was expressed in some epithelial cells throughout the small intestine and in the cells like a follicular dendritic cell in germinal centre of Peyer’s patches. In cattle, PrPc positive epithelial cells were detected only in duodenum, but not in jejunum and ileum. In bovine Peyer’s patches, PrPc positive cells were distributed in extrafollicular areas, but not in germinal centre of jejunum or ileum. In this study, we showed that there were quite differences in the PrPc localization between murine and bovine small intestine. These data suggest that there may be a variety of infective routes and propagation mechanisms of PrPsc from the intestine to the brain between species.
Immunohistochemical localization of ghrelin in rodent kidneys.

Akira YABUKI, Satoshi TAHRAGUCHI, Osamu ICHII, Hiroharu MIFUNE, Masayasu KOJIMA, Yoshihiro NISHI, Mitsuharu MATSUMOTO, Shusaku SUZUKI
(Laboratory of Veterinary Anatomy, Kagoshima University)

Ghrelin is a novel peptide hormone, originally identified in the rat and human stomach, which plays various important roles. In the present study, we report the intra-renal localization of ghrelin in laboratory rodents. Kidneys from 3-month-old mice, rats and hamsters of both sexes were analyzed by immunohistochemistry. Immunoreactivity for ghrelin was clearly observed in the epithelium of the distal tubules, whereas other segments of the nephron or interstitial cells, including juxtaglomerular cells, showed negative reactions. Immunoreactivity was not detected in any animals when antiserum absorbed by excessive ghrelin peptides was used. Pre-embedding immunoelectron microscopy demonstrated ghrelin immunoreactivity exclusively on the basolateral membrane in the distal tubular cells and in the collecting ducts. Prepro-ghrelin gene expression was assessed by utilizing RT-PCR, and the expected 329 bp prepro-ghrelin mRNA was clearly detected in the kidney. In Western blot analysis, although immunoreactive band for ghrelin (app. 3 kDa) was not detected in the kidney, the expected band for prepro-ghrelin (app. 13 kDa) was clearly detected not only in the stomach but also in the kidney. This report is the first to describe the intra-renal localization of ghrelin, and findings in the present study indicate possible new roles for ghrelin in the kidney.
Aquaporin (AQP) 8 water channel was localized in the podocytes of kidney glomeruli in the adult musk shrew [the 140th JSVS, 2005, Kagoshima]. In this study, we examined the expression and localization of AQP8 in the metanephroi of the prenatal and postnatal musk shrews. The embryos (E27 and E29) and neonatal animals (P0 and P2) were fixed with Bouin’s fluid and embedded in paraffin. The sections were stained with dual-immunohistochemistry using the anti-AQP8 and anti-nephrin antibodies (Ab). In the metanephroi of the E27 and E29 embryos, anti-AQP8 Ab reactivity was detected to the developing renal vesicles. Localization was restricted to the basal site of the podocytes in the S-shaped stages of glomeruli. PAS and nephrin reactivity showed that the AQP8 expression was corresponded to the formation of the filtration slit membrane. These indicate that AQP8 may play a role in regulation of podocyte volume and/or filtration slit formation during the development of glomeruli. In the P0 and P2 kidneys, developing and matured glomeruli were stained with AQP8 Ab. The reactions to podocytes in the S-shaped stage were localized to the filtration slit, whereas those in the matured glomeruli were not localized to the basal side of the cells. This indicates that AQP8 was needed in the basal side of the developing podocytes, and was changed their localization from the basal to the intracellular region of matured podocytes.
Conservation of ancestral rRNA locus in the seven species of Old world monkey

Ryo MORISHITA, Hidesi SHIBATA, Takatoshi KANNDAA
(Department of Veterinary Anatomy, Tokyo Institute of Agriculture and Technology)

Chromosomal location of ribosomal RNA gene (rRNA) loci was determined in seven species of Old world monkey by fluorescence in situ hybridization (FISH) using 18S and 28S rDNA as probes. Seven species of primate (rhesus monkey, pigtailed monkey, Japanese monkey, crab-eating macaque, celebes crested macaque, patas monkey and African green monkey) showed single rDNA locus in the secondary constriction regions of the medium-sized submetacentric chromosomes. These regions were all differentially stained with Ag-NOR stain indicating the presence of transcriptionally active rRNA genes. To characterize the rDNA bearing chromosomes in these species, we performed comparative Zoo-FISH using human chromosome painting probes and found that the rDNA loci in these species are preferentially hybridized with the paint probe for human chromosome #22. In other species of primates such as gorilla, chimpanzee and orangutan, rRNA gene loci has been mapped to the multiple chromosomes, though one of the loci has consistent synteny with human chromosome #22 as well. These observations, together with the present study, suggest that rRNA gene loci in the old world monkey originated from the common ancestral chromosomal locus associate with the DNA segment of the human chromosome #22.
Polymorphism of rDNA loci in the dog (Canis familiaris)

Asuka SUBE, Hidesi SHIBATA, Takatoshi KANMDA
(Department of Veterinary Anatomy, Tokyo Institute of Agriculture and Technology)

In mammals, more than a hundred copies of rDNA were localized onto a single or multiple chromosomes. The dog has 78 chromosomes and the rDNA loci have been mapped to three pairs of autosome and the Y chromosome in the previous studies. However, there are still remaining discrepancies about the exact loci of rDNA due to the difficulty to distinguish each chromosome by morphological characteristics. We performed fluorescence in situ hybridization (FISH) using human 18S (5.8kb) and 28S (7.3kb) rRNA genes as probes and found polymorphisms of rDNA loci in the beagle and crossbreed dogs. The numbers of the rDNA loci in the 29 male dogs were 7 in 21 (72%), 6 in 7 (24%) and 5 in 1 (4%) individuals. In the 15 female dogs, the loci numbers were 6 in 11 (73%), 5 in 3 (20%) and 4 in 1 (7%) dogs. As the dog bears rDNA on the Y chromosome, the incidence of the polymorphism of the autosomal rDNA is almost equal in both sexes. These observations suggest that the polymorphism of rDNA clusters on the chromosomes might be occurred by the partial translocation of the rDNA from ancestral chromosomal loci to the other chromosomes, reflecting the evolutionary trail of the chromosomal rDNA loci in the dog.

We further examined a method to obtain a good yield of early metaphase from short term peripheral blood culture and found pokeweed extract (Sigma L9379, L8777) effectively stimulate dog peripheral lymphocytes to mitose.