Consequently, it was demonstrated that ascorbic acid was also an inhibiting effect on angiogenesis effect *in vivo*.

SPERM STORAGE IN THE ISTHMUS OF THE MAMMALIAN OVIDUCT

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The main sperm storage site is considered to be the caudal isthmus of the oviduct. The variety of different possible modes of sperm storage is observed in the mammals. Suncus spermatozoa generally displayed a slow languid movement within the crypts and were not adherent to the epithelium, and remained for more than 30 h after insemination. In the Japanese long-fingered bat, the spermatozoa clustered with their heads oriented in parallel towards the epithelial cells, and established associations with the microvilli and/or indentations of the epithelial cells. The spermatozoa remained for more than 7 days after insemination. In the Japanese house bat, the heads of spermatozoa were lodged in indentations of the epithelial cells, and stored for more than 160 days after insemination. The functions of the isthmus of the oviduct for sperm storage are discussed.

IDENTIFICATION OF THE FOLLICLES DIGESTION FACTOR USING THE MEDAKA FISH *IN VITRO* OVULATION SYSTEM

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The breakdown of the extracellular matrix is associated with normal ovarian function, such as ovulation and luteolysis . Matrix metalloproteinases (MMPs) are thought to play a crucial role in these events. A number of studies have been carried out, mainly using mammalian ovaries, to address the mechanism of follicle rupture during ovulation, but biological functions of the MMP family members in this process is not fully understood. In the present study, we used the medaka fish, *Oryzias latipes*, to explore the role played by MMPs in the ovulation.

HATCHING ENZYMES OF BIRD AND REPTILE, AND THEIR EXPRESSION

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The hatching enzyme of medaka, *Orizias latipes*, is composed of two enzymes, HCE and LCE, and both belong to the astacin family, a subgroup of zinc-metalloproteases. Based on information from molecular structures of medaka hatching enzyme and other astacin family proteases, we cloned a cDNA for hatching enzyme of Chinese softshelled turtle (*Pelodiscus sinensis*), by RT-PCR and 3'- and 5'-RACE method. A 410-amino acid sequence encoded by the cDNA consisted of an astacin protease domain and a CUB domain, and its overall similarity to the quail enzyme was 56.9%. Molecular phylogenetic analysis revealed that hatching enzymes including the turtle enzyme were grouped into the same branch and clearly discriminated from other astacin proteases such as BMP-1 and Meprin. In the group of hatching enzymes, *Xenopus*, quail and turtle enzymes were further divided from the fish hatching enzyme group and form a branch. We will discuss molecular characters of the bird and reptile hatching enzymes as comparing them with hatching enzymes of other animals.

COMAPARATIVE STUDY OF EXPRESSION AND CHANGE OF THE DNA BINDING CAPACITY OF THE *XENOPUS* KU PROTEINS IN THE GONAD AND OTHER ORGAN

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The role of the Ku protein which is a kind of autoantigen observed to the autoimmune patient was investigated in the process of cell differentiation. The genes of the *Xenopus* Ku proteins were separated, and the base composition was clarified. Then, analysis on differences of expression level and phosphorylation of the Ku protein of various *Xenopus* organs was carried out. The result showed that there was the increase of the remarkable expression level in gonad (ovary, testis), and we have obtained evidence that phosphorylation and DNA binding capacity of Ku 70 kDa protein are different from other organs.

THE ANCHORING MECHANISM OF SPINDLE POLE UNEQUAL CYTOKINESIS OF GRASSHOPPER NEUROBLASTS

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The grasshopper neuroblasts perform unequal division to produce small ganglion cells (GC). In the unequal division, it is necessary to shift the spindle toward the GC-side and to anchor to the GC-side cortex. An Ar-laser microbeam was irradiated either to the cap cell side spindle pole or the GC-side one at metaphase. The irradiation induced shortening of spindle body, and abnormal karyokinesis. In the irradiated cell, the anchoring to the GC-side cortex was prevented. However, a change in GC-side cell cortex occurred to form a small bud at middle anaphase. The present experiment suggests the presence of the original clock for changes in both cell cortex and karyokinetic behavior. The bud induced by the irradiation to the spindle pole appears to participate in the spindle anchoring; it may be a structure to grip the GC-side spindle pole for establishing unequal cytokinesis.

ROLE OF ZP3 IN CARP EGGS

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It is well known that ZP3 is the sperm receptor in mammalian eggs. However, ZP3 in fish eggs is not necesssary for the sperm receptor, because of the presence of a micropyle. Nevertheless, ZP3 exists in the vitelline envelope of fish eggs. Therefore, we addressed the role of ZP3 using carp ZP3 and anti-carp ZP3 antibody. The extracellular exudate from carp eggs artifitially activated contained a great amount of ZP3. The ZP3 purified from the extracellular exudate had the ability to agglutinate different species of fish sperm and bacteria. The agglutinated bacterial cells, all not, changed the shape into an ellipse or a rugged surface within 30 min after beginning of the experiment. The agglutination was inhibited by the presence of D-galactose or L-fucose in Gram-positives and sialic acid in Gram-negatives. Antibacterial effect of ZP3 was bacteriostatic against Staphylococcus aureus (MRSA) and *Aeromonas hydrophila* with survival rate of about 15 or 40% respectively. This suggests that ZP3 in the vitelline envelopes of carp eggs may play an important role to protect fertilizing eggs from bacterial infection by purging away bacteria for cleanliness of their surroundings.

AQUATIC ANIMAL EXPERIMENT FACILITY FOR SACE UTILIZATION RESEARCH WITH SMALL FRESHWATER FISH AND AMPHIBIAN

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National Space Development Agency of Japan (NASDA) has developed aquatic animal experiment facilities for NASA Space Shuttle use and has conducted many experiments in space with various aquatic animals. Currently, we are studying the next-generation aquatic animal experiment facility or the Aquatic Habitat (AQH) for both Space Shuttle and Space Station use. The AQH will have the capabilities to accommodate three-generations of small freshwater fish (medaka and zebrafish) and egg through metamorphosis of African clawed frog (*Xenopus*). A prototype breeding system was manufactured and basic tests were performed with medaka and *Xenopus*. The medaka mating and spawning behavior were performed successfully, medaka larvae grew into adult fish in approx. 1.5 months, and also *Xenopus* metamorphosis completed in the specimen chamber with small water volume, although some problems that should be resolved were clarified.

EXPRESSION OF GENES RELATING TO α-MSH SIGNALING IN THE PIGMENT PATTERN FORMATION OF FEATHER BUDS IN QUAIL EMBRYOS

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The plumage on the dorsal trunk of Japanese quail (*Coturnix japonica*) embryos exhibits longitudinal black and yellow stripes of pigments produced by melanocytes. Although the mechanism of this pigment pattern formation has been analysed by using quail-chick chimeras, it is largely unknown. In recent years, genes relating to α-MSH signaling, which plays important roles in the pigment synthesis in melanocytes, have been identified in mice, and this signaling may be also involved in regulation of avian feather color. In this study, we focused on this signaling system and examined mRNA or protein expression of genes relating to this signaling system during plumage pigment pattern formation of quails. Although α-MSH peptide was not immunohistochemically detectable, mRNAs of POMC, which is the precursor of α-MSH, and PC1 and PC2, which digest POMC to produce α-MSH, were expressed in the dorsal skin of 10-day-old embryos. mRNA of Mc1r, which is a receptor of α-MSH, was also expressed in the skin of 10-day-old embryos and cultured melanocytes. These results suggest that α-MSH signaling may be involved in the pigment pattern formation of feather buds in the quail.