



Title	Pathological studies on the transmission of so-called fowl glioma
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Citation	Japanese Journal of Veterinary Research, 48(1), 80-81
Issue Date	2000-05-31
Doc URL	<a href="http://hdl.handle.net/2115/2836">http://hdl.handle.net/2115/2836</a>
Type	bulletin (article)
File Information	KJ00003408170.pdf



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## Pathological studies on the transmission of so-called fowl glioma

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So-called fowl glioma is histologically characterized by multiple nodular gliomatous growths associated with disseminated non-suppurative encephalitis. It is suggested that this disease may be a neoplasm induced by a strain of avian leukosis/sarcoma virus (ALV) group. The purpose of the present study were to examine the *in vivo* transmission of so-called fowl glioma in Japanese bantam.

Chapter 1. Twenty 1 or 2-day-old chicks of Japanese bantam were intracranially injected with brain homogenate from a bantam affected with naturally-occurring so-called fowl glioma. These bantams were necropsied at 70, 140, 220, or 365 day-old and pathologically examined. Medium (RPMI 1640) or normal brain homogenate were used as the control inoculum. Five birds of the control groups had mild perivascular lymphocytic infiltration and minimal gliosis in the brain. In contrast, all birds of the experimental group showed non-suppurative encephalitis and 7 bantams revealed multiple nodular gliomesenchymal proliferation in the central nervous system. The encephalitis was characterized by mural and perivascular lymphocytic infiltration, microglial nodules and occasional neuronophagia. These lesions were disseminated throughout the cerebrum. The nodular foci were sharply demarcated and consisted of dense aggregation of gemistocytic and fibrillary astrocytes and mesenchymal cells with reticulin fibers. Anastomosing blood vessels with infiltration of lymphocytes

mildly proliferated within the nodules. Immunohistochemically, the astrocytes had positive reactivity for glial fibrillary acid protein (GFAP) and most of these gliomesenchymal cells were positive for proliferating nuclear cell antigen and occasionally had ALV antigen. These histological findings in the experimental group were consistent with those in so-called fowl glioma. These results suggested that this disease could be transmitted with intracranial injection of the affected brain homogenate and that this disease is caused by an unidentified strain of ALV.

Chapter 2. Since the first case of so-called fowl glioma in Japan were found in a zoological garden in 1996, 17 affected birds have been totally recognized in this garden. In the present study, we pathologically examined 11 Japanese bantams having a blood relation with these affected birds in another zoological garden. These birds showed mild depression and mild discoloration of the head crest. Histologically, there were mild to moderate disseminated non-suppurative encephalitis throughout the brains in 7 of the examined birds and two bantams had multiple gliomatous nodules in the cerebrum. The inflammatory lesions consisted of intramural and/or perivascular infiltration of lymphocytes and microglial nodules. The gliomatous nodules were composed of GFAP-positive astrocytes and mesenchymal cells with blood vessel proliferation. These gliomesenchymal cells were positive for ALV antigen. The 2 birds were histologically diagnosed as so-

called fowl glioma and the remained 5 birds were suspected to be affected with this disease. In addition, lymphoproliferative foci were found in the various organs of all seven affected bantams. The normal structure was

occasionally destructed by the lymphocytic infiltration or aggregation, especially in heart and bone marrow. These results suggested that so-called fowl glioma is prevalent in the bantam flock of this zoological garden.

### In vitro growth and maturation of bovine oocytes from early antral follicles

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Recent progress in the culture system of growing bovine oocytes made it possible to use the oocytes in early antral follicles for *in vitro* production of bovine embryos. This technique is also useful as a model to study the physiology of follicular development and ovulation. The present study was conducted to examine the growth rate and maturational competence of bovine growing oocytes (<100  $\mu\text{m}$  in diameter) in early antral follicles.

First, the relationship between oocyte and follicle sizes were examined to estimate the oocyte diameter from the follicular diameter. Most (93%) of the follicles with 0.5 to 1 mm in diameter had formed antrum and contained the oocytes with 92.2  $\mu\text{m}$  in mean diameter. In this study, follicles with a diameter of 0.5 to 1 mm were defined as early antral follicles. Then, the maturation and developmental competence of oocytes from these early antral follicles were examined. Although 12% of oocytes had resumed meiosis after 24 h of *in vitro* maturation culture in TCM 199 with 10% fetal calf serum (FCS) and hormones (FSH+E<sub>2</sub>), none of them reached the metaphase II stage. Cumulus-oocyte-

granulosa complexes (COGCs) derived from early antral follicles were embedded in collagen gels and cultured for 4, 8, 10 or 14 days in TCM 199 with 10% FCS and 4 mM hypoxanthine to examine the meiotic competence of *in vitro* grown oocytes. All of COGCs formed antrum-like structures after 4 days of culture. After 4 days of the growth culture, the mean diameter of the oocytes recovered from COGCs was 102.7  $\mu\text{m}$ , and the majority (72%) remained at germinal vesicle stage after the maturation culture. The mean diameter of the oocytes from COGCs was 112.7  $\mu\text{m}$  after 8 days of the growth culture, and about 40% reached the metaphase II stage after maturation. Proportion of intact COGCs was reduced after 10 and 14 days of the growth culture due to the granulosa cell outgrowth. There was no oocyte that reached the metaphase II stage after the maturation culture.

The present study confirmed that oocytes from early antral follicles had no ability to mature without the growth culture. They need to be cultured for 8 days in the growth culture before maturation to acquire maturational competence to metaphase II stage.