



# HOKKAIDO UNIVERSITY

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## Laboratory of Theriogenology

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Laboratory of Theriogenology was established in 1954 to undertake research on artificial insemination in domestic animals. The studies at early days of the laboratory were mainly on the cellular genetics and the morphology and physiology of sperms. From early 70s, bovine embryo transfer became the main field of research. Studies were made to develop and improve various aspects of embryo transfer procedures and *in vitro* production of bovine embryos. The other important line of works were a series of studies on reproductive physiology in the Hokkaido brown bear, a wildlife species inhabiting in Hokkaido. Earlier of this year, new head of the laboratory : Professor Yoshiyuki Takahashi, has been appointed and changes in laboratory activity are taking place at present. The main ongoing studies at the laboratory are as follows :

### 1. *In vitro*/laboratory production of cattle and pig embryos

We have developed *in vitro* production system of bovine embryos that include the procedures for *in vitro* oocyte maturation, fertilization and embryo culture<sup>3,4</sup>. The system becomes a standard method for *in vitro* bovine embryo production. Lately, development of cloning technology using nuclear transfer technique is becoming a major part of the project. We have already produced cloned offspring by using nuclear transfer technique with blastomeres of preimplantation embryos as nuclear donors in mice<sup>1</sup>) and cattle<sup>5</sup>). Last year, Wilmut *et al.* first succeeded to produce lamb derived from adult mammalian cells<sup>7</sup>). The study demonstrated totipotency of somatic cells from adult animals and caused a great sensation to the reproductive and developmental biologists. Many laborator-

ies have been following their procedures and to date some offspring have been produced in cattle but not in pigs. We are currently establishing the cloning technique using somatic cells as nuclear donors in the cattle and pig. This project will be extended further in combination with gene manipulation to establish multiple copies of transgenic animals that produce drugs and other bioactive agents for clinical and research use and provide the organs for transplantation medicine.

### 2. Primordial follicle culture system

The number of follicles (*i.e.*, oocytes) peaks at the late stage of fetal life in cow. However, the most of the follicles are unusable and lost before or during follicular development or remain at storage till the animals are culled. Utilization of these follicles provides various benefits in animal production that include enhanced production and an increased rate of genetic improvement. The follicle culture system also provides *in vitro* grown and matured oocytes that will be used as nuclear recipients in nuclear transplantation for cloning. From the practical aspect, this project will determine the conditions in which primordial or preantral follicles can grow and mature to provide viable oocytes for fertilization and subsequent development. In addition to the practical aspect of utilizing unusable follicles, this project will contribute to enhance our understanding in follicular development and atresia. Currently the role of gonadotropins, growth factors and other bioactive agents in follicular development and atresia are being determined.

### 3. Reproductive physiology in the brown bear

A recent study has shown decreased genetic variability in the local population of the brown bear<sup>6</sup>) and stressed the importance of reproductive management to maintain genetic variability to prevent this species from extinction. One of the two projects regarding the brown bear is to understand the reproductive cycle of this animal. The project aims to elucidate the mode of

ovulation and the ovarian cycle by using endocrinological and ethological approaches. This study, in conjunction with the previous work in males<sup>2)</sup> will provide precious information to develop an artificial breeding procedure in the brown bear and related species. The other project in the brown bear is aiming to develop non-invasive method for individual identification of the brown bear using feces. Feces provide various information to the scientists studying physiology and ecology of wildlife. With identification of the individuals for given feces, the value of the information from feces greatly increases.

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