Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"

	2024 11, 19
Affiliation/Position	Wildlife Research Center/M1
Name	Azimey Xorlali

1. Country/location of visit

Wildlife Research Center (WRC), Laboratory

2. Research project

Advanced laboratory skills in field biology Course II for M1 students

3. Date (departing from/returning to Japan)

2024. 11. 11 – 2024. 11. 08. 15 (5 days)

4. Main host researcher and affiliation

Professor Miho Murayama & Dr. Mayako Fujihara, WRC, Kyoto University

5. Progress and results of your research/activity

Training Report: Advanced laboratory skills in Field Biology Course II

Aim of the Course:

This course aimed to introduce students to how oocytes and ovarian tissues are handled for practical use like Invitro Fertilization (IVF).

(1) **Briefing Session**

The Course began with an introductory session where Fujihara-sensei introduced participants of the course to the content like the structure and expected outcomes. A self-introduction session was then conducted for us to get to know one another.

Next, we observed the diagram of the structure of the Ovary that would be later observed in the practical session (Histological Assay).

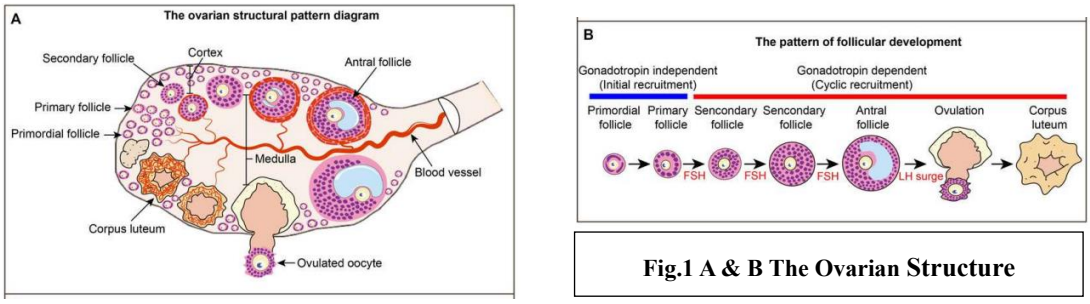

Fig.1 A & B The Ovarian Structure

(2) **Lab Session**

We proceeded to the lab session where each student was tasked to extract the ovaries (ovary dissection) from an already obtained structure that included other reproductive tracts like the uterus. This process required careful cutting and precision so as not to damage the ovary in the process. The uterus and fat layers were trimmed. (*I found it challenging at first but became interesting afterwards*). The samples were collected from cats and dogs.

The procedures are described as follows:

- Oocyte collection and evaluation: After obtaining the ovary, it is placed in a petri dish and measured (length, width and height). It was recommended that the procedure be carried out within a temperature of about 37°C, room temperature as long exposure to high temperatures can damage the follicles.

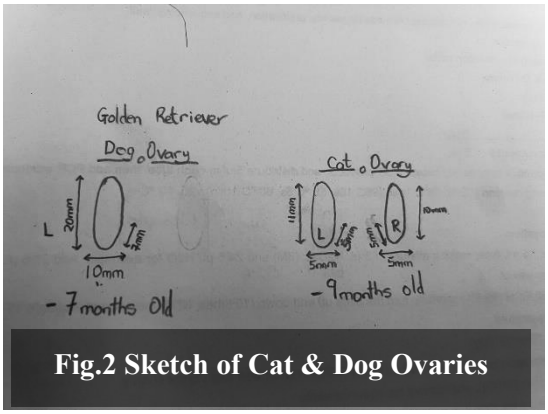

Fig.2 Sketch of Cat & Dog Ovaries

The collected ovaries are then weighed and recorded. For these ovaries, despite the dog being 7 months old and the cat being 9 months old, the dog's ovaries (0.7710g) weighed more than the cat's (Left 0.1444g & Right 0.1271g).
(*This was an interesting observation as also the ovaries of the dog were more folded and difficult to locate in comparison with the cats.)*
It was highlighted that the weight of the ovaries varies and can be affected by several factors including species.

The samples collected were washed in a medium and sliced carefully at several points to create an opening to observe the oocytes under the microscope. Each student observed their sample to determine the quality of the oocytes.

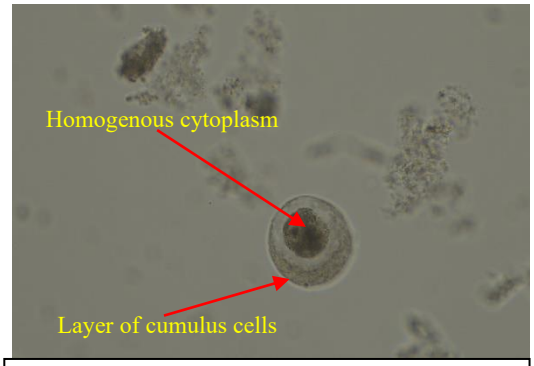

Fig.3 An example of a good Cat oocyte with homogeneous cytoplasm and layers of cumulus cells

Fig.4 An example of a bad Cat oocyte lacking significant cumulus cells layers

The next session aimed at exposing students to Histological Assay through the use of sectioning and staining of paraffin-embedded tissues. Fujihara-sensei explained and guided students in detail on the procedure for sectioning the paraffin-embedded oocytes obtained from different species at different sections of the ovary like the Cortex and follicles. These samples were trimmed and stained Hematoxylin and Eosin meticulously and were then placed on slides stored at room temperature overnight to dry up.

The dried slides containing the samples were cleaned off from any excess mounting fluid and were placed under a microscope for observation. The evaluations were made based on the distinctive characteristics associated with each development stage of the follicle and their structural normality. The development stages included Primordial, primary, secondary, antral, ovulation and corpus luteum. As a result of the period of maturity of the ovary before harvesting, no ovulation or corpus luteum stages were recorded among my samples.

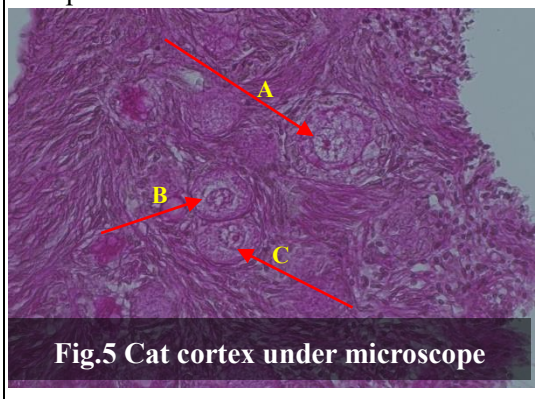

Fig.5 Cat cortex under microscope

Fig. 5 shows the structure "A" -a cat cortex in its primary stage however appears not to be in a normal structure since the oocyte is not in good condition.
Structures B & C seem to appear to be one oocyte split and developed separately which could be a twin birth if the follicles develop well.

Fig 6 Dog cortex under microscope

Fig. 6 Shows multiple oocytes observed in the cortex with the cytoplasm absent or damaged which is an indication of abnormality. The structures appear to be in the primordial stage which is the first part of the follicular development.

Several other samples were observed at different follicular growth stages including the secondary and Antral stages.
The final session of the training was about operating the microscope and how the data obtained can be interpreted depending on the aims of the research.

Conclusion:
This training course was an opportunity for me to experience the practicality of the processes used in preparing samples for in vitro fertilization. I also got the chance to solidify my knowledge of lab safety protocols since the processes involved in handling the ovaries required attention and carefulness.

Fig 7 Xorlali observing sample during the sectioning stage

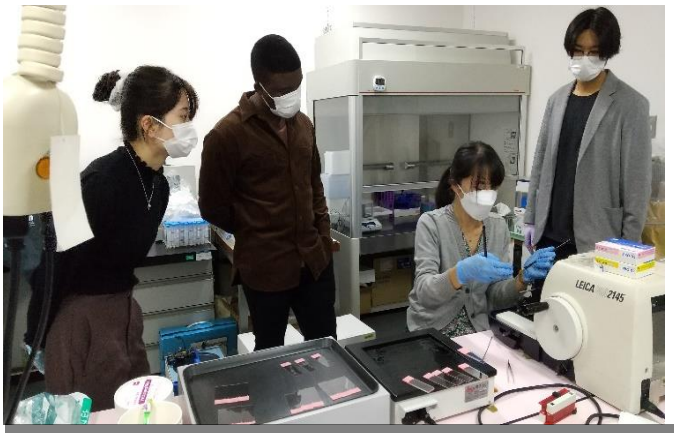

Fig.8 Students in a session with Fujihara-sensei

6. Others

Acknowledgement

- I want to extend my gratitude to Professor Murayama and Dr. Fujihara for the knowledge imparted during the training.
- I want to appreciation to the PWS team and the WRC team for making this experience a possibility.
- Also, a big thank you to Nishimoto Chinatsu, Nakamura Hizuki and Goto Yuzuki for their wonderful teamwork.