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(Abroad • Domestic) Internship report form (Student) 2024/06/11 (Year/Month/Day)

Name	Md. Zahir Uddin Rubel
Laboratory	Anatomy
Year (Grade)	D3
Internship institution	Faculty of Veterinary Medicine, University of Milan
Internship period	Internship period: 05/03/2024 - 05/29/2024 (Departure Date from Sapporo: 05/02/2024. Arrival Date in Sapporo: 05/30/2024)
Purpose	The purpose of these goals is to improve knowledge of mucosal immunity with new microvesicles analysis techniques, to understand immunity differences in various species by studying unique animal samples and use internship experiences to guide career choices and create professional connections.

- The reason why you chose this institute

The University of Milan, renowned as one of the top 100 emerging universities globally, particularly distinguishes itself through its faculty of veterinary medicine. The laboratory led by Prof. Cristina specializes in veterinary pathology, with recent research focusing on exosomes (Exos) in various contexts, such as tumors (Front Vet Sci 2023;10:9:1083174). Recent studies in animals have highlighted the significant pathogenic role of Exo-derived molecules, such as RNA and proteins in body fluids, in inflammatory diseases. There is potential for these molecules to serve as biomarkers (Biomedicines 2021;9:8:1061).

Given that my current research for my PhD thesis primarily employs histopathological methods, I have chosen this institute to further develop my skills and knowledge. Specifically, I aim to merge my existing histological techniques with the molecular analysis of Exos, particularly focusing on mucosal immunity. This collaboration will provide me with a comprehensive understanding of veterinary pathology and immunology, aligning with the cutting-edge research conducted at the University of Milan.

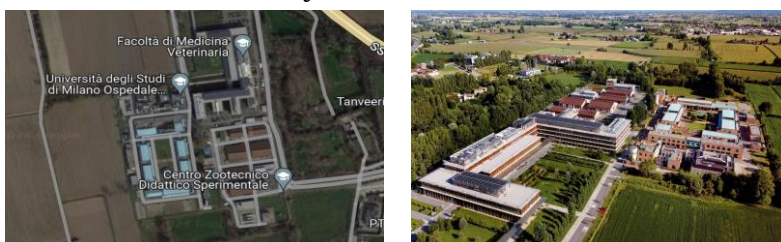


Figure 1: Location of the faculty of Veterinary Medicine, University of Milan.

-Result of the activity

During my internship period, I was involved in the veterinary molecular pathology group, encompassing a range of tasks focused on studying and analyzing microvesicles and neutrophils derived from bovine biofluids (milk and blood). I primarily worked on an ongoing project titled “Resiliency to Heat Stress: A Systems Biology Approach,” which included heat stress (HS) and normothermia (NT) conditions. This experiment used two different breeds: Brown Swiss (BS) and Holstein Friesian (HF).

These tasks were crucial for advancing our understanding of veterinary molecular pathology and immunology, with specific emphasis on the following activities:

1. **Training Class on Peripheral Blood Mononuclear Cells (PBMC) Purification:**
Before engaging in the main experiment, I attended a training class where I learned to purify bovine PBMC from whole blood samples, a crucial part of the main project. Photos from this training session are included in Figure 2.



Figure 2: Training Class on Peripheral Blood Mononuclear Cells (PBMC) Purification.

2. **Isolation of Microvesicles from Bovine Biofluids:** I employed advanced techniques such as ultracentrifugation and size exclusion chromatography (SEC) to isolate microvesicles from bovine blood plasma and milk (Figure 3). During my internship, I processed around 30 samples for the isolation of microvesicles. This process is vital for obtaining pure microvesicle samples for subsequent analysis and research.

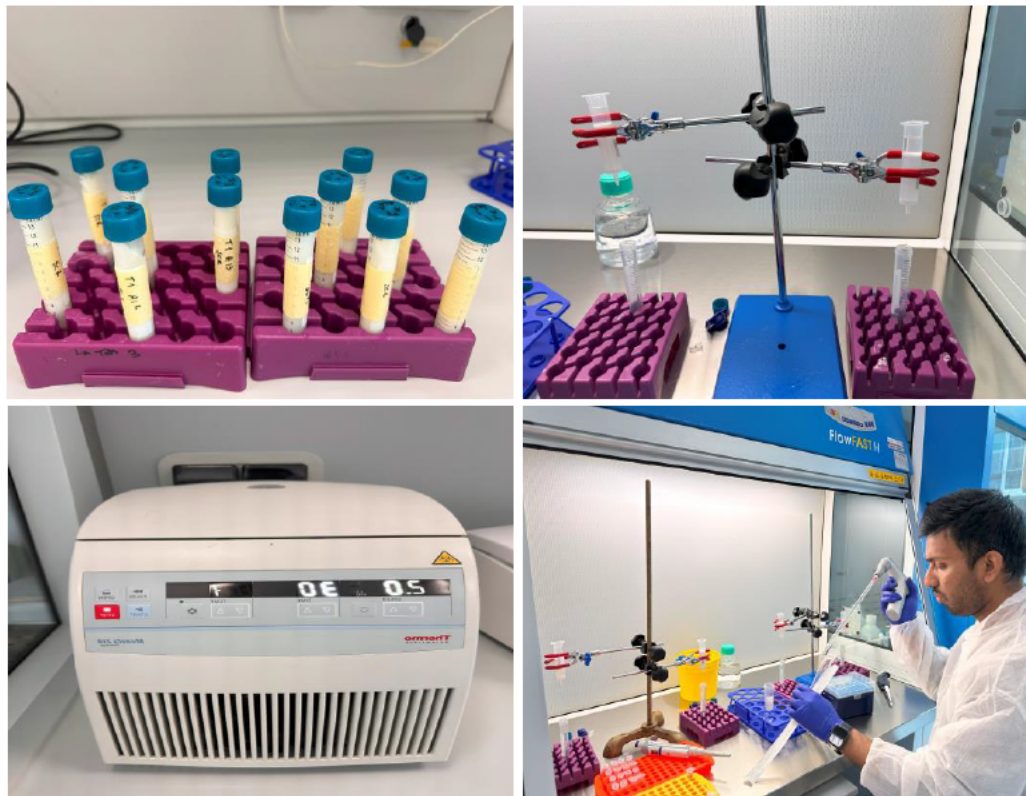


Figure 3: Size exclusion chromatography (SEC) to isolate microvesicles from bovine milk.

3. **Protein Assay:** After isolating the microvesicles, I performed a protein assay to measure the total protein concentration in the microvesicles (Table 1).

Sample	Protein [ug/mL]	Sample	Protein [ug/mL]
HS_3_BS_F3	1126.56	HS_11_HF_F3	1071.56
HS_5_BS_F3	2163.22	HS_12_HF_F3	1048.22
HS_8_BS_F3	1556.00	HS_13_HF_F3	1310.44
NT_3_BS_F3	2880.89	NT_11_HF_F3	1755.89
NT_5_BS_F3	2044.22	NT_12_HF_F3	1825.33
NT_8_BS_F3	2313.11	NT_13_HF_F3	2860.33

- 4. Isolation of Neutrophils from Whole Blood:** I learned the procedure for isolating neutrophils from whole blood samples collected from healthy cows (Figure 4). Neutrophils, a type of white blood cell, play a critical role in the immune response, and their isolation is fundamental for conducting in vitro assays.



Figure 4: Isolation of Neutrophils from Whole Blood.

- 5. Challenge of Neutrophils with Microvesicles:**

In Vitro Assays

During my internship, I conducted in vitro assays to challenge neutrophils with isolated microvesicles to evaluate cell viability. The process involved isolating neutrophils from whole blood samples collected from healthy cows, counting and adjusting them to the required concentration. The microvesicles were isolated from bovine biofluids using techniques like ultracentrifugation and SEC to ensure purity and quality. Neutrophils were exposed to varying concentrations of microvesicles in a 96-well plate format. Cell viability was assessed using assays such as MTT or trypan blue exclusion, with results meticulously recorded and analyzed to determine the impact on neutrophil survival (Figure 5).

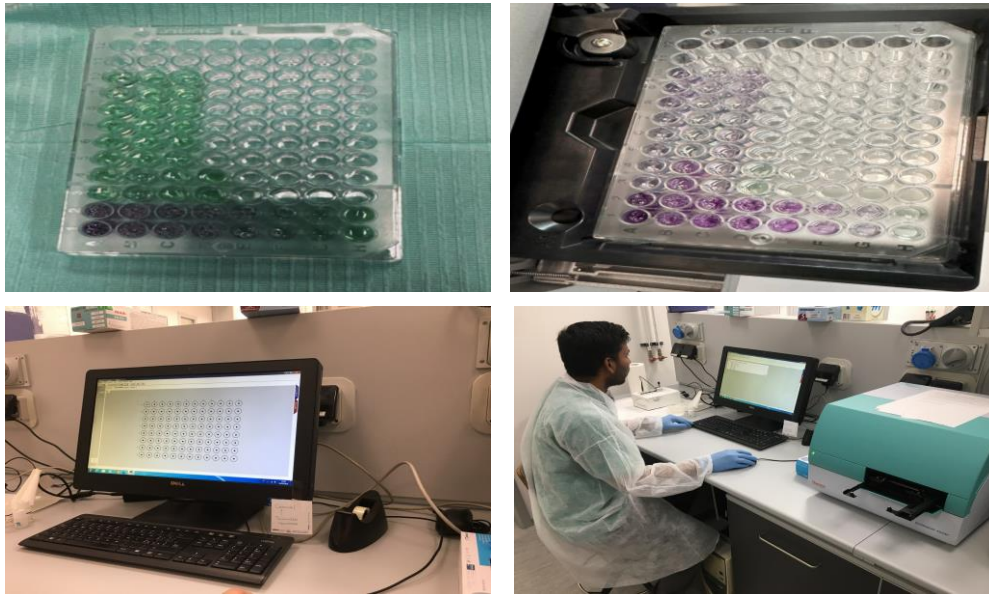


Figure 5: In vitro assays to challenge neutrophils with isolated microvesicles.

Exo Preparation

We prepared plates with five different concentrations of protein per well, considering the different concentrations of the stock solutions. Stock solutions of Exo protein were first prepared and stored under appropriate conditions. A series of dilutions were made using the formula $C_i V_i = C_f V_f$, where C_i is the initial concentration, V_i is the initial volume, C_f is the final concentration, and V_f is the final volume. Each well was carefully loaded with 70 μL of the Exo solution and 30 μL of neutrophil suspension, making the final volume 100 μL . Experimental controls, such as wells with only neutrophils and wells with only microvesicles, were included to account for baseline effects. This meticulous preparation ensured reliable and reproducible results, providing valuable insights into the interactions between neutrophils and microvesicles.



Figure 6: Performing digital PCR for buffalo milk sample.

6. **Real-time PCR and Digital PCR:** I learned molecular biology techniques, specifically real-time PCR (Polymerase Chain Reaction) and digital PCR (Figure 6). These techniques are pivotal for quantifying nucleic acids and analyzing gene expression levels in various samples. I worked on a buffalo milk sample.
7. **Biostatistics Analysis:** I performed comprehensive biostatistical analyses on the data obtained from in vitro assays. This analysis is essential for interpreting the results accurately and drawing meaningful conclusions regarding the interactions between neutrophils and microvesicles.
8. **International Seminar Attendance:** I attended an international seminar entitled “Bile Acids: A New Approach in Animal Nutrition.” This seminar allowed me to interact with many international researchers (Figure 7).



Figure 7: Lecture of Prof. Dr. Susanne Häußler and group photo after her session.

-What do you think the positive impact of the activity will have on your further career path?

The most significant benefit from this experience is the professional connection I established with Dr. Cristina and her esteemed team, who are leaders in the veterinary pathology field. This network is likely to be invaluable for my future career, potentially opening doors to collaborative research opportunities and professional guidance. During the internship, I gained comprehensive insights into the working conditions for post-doctoral fellows in Italy, which has fueled my ambition to pursue a post-doctoral position in the country if the opportunity arises. Although the job offer was not extended, the recommendation from my host to contact principal investigators has provided me with strategic contacts that could facilitate my career advancement. Furthermore, I observed the practical integration of biofluids into clinical practice and gained a deeper understanding of the challenges associated with using biomarkers in routine clinical settings. This hands-on experience with advanced molecular techniques and exposure to the practical challenges of veterinary pathology has not only enhanced my technical skills but also broadened my understanding of translational research. Collectively, these experiences and connections will significantly enhance my career prospects, enrich my research capabilities, and align my career trajectory with cutting-edge developments in veterinary pathology and immunology.

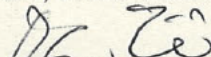
- Advice for junior fellows

Here are some tips for those who haven't completed their internships yet:

- **Take Advantage of Opportunities:** This internship allows you to explore opportunities that other graduate programs might not offer. Aim high, go to places you've always dreamed of, and you'll gain motivation to pursue your career further.
- **Reach Out to Potential Supervisors:** Don't hesitate to contact potential supervisors. Persistence is key, so follow up if you don't get a response initially.
- **Plan Early:** Start preparing early, especially for visa applications if needed. Planning ensures you have ample time to handle any unexpected hurdles.

- **Budget Wisely:** Have some savings ready for unexpected expenses, such as health checks for visas or travel costs. Financial preparedness can prevent unnecessary stress.
- **Seek Additional Funding:** If possible, secure extra funding to extend the duration of your internship. This can allow for a more comprehensive learning experience.
- **Embrace Flexibility:** Be adaptable and open to changes in your research plan or project scope. Flexibility can lead to unexpected learning opportunities and discoveries.
- **Engage with the Host Institution:** Actively participate in seminars, workshops, and other events at your host institution. This engagement can enhance your learning experience and expand your professional network.
- **Document Your Experience:** Keep detailed notes and records of your activities, methodologies, and findings. This documentation will be invaluable for future reference and for writing reports or publications.
- **Network with Peers:** Build relationships with fellow interns and researchers. These connections can provide support during your internship and become valuable professional contacts in the future.
- **Seek Feedback:** Regularly ask for feedback from your supervisors and colleagues. Constructive criticism can help you improve your skills and performance.
- **Reflect on Your Goals:** Periodically reflect on your personal and professional goals. Ensure that your internship activities align with your long-term aspirations.

By following these tips, you can maximize the benefits of your internship experience and lay a strong foundation for your future career

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