الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي

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DEPARTMENT OF BIOLOGY AND ANIMAL PHYSIOLOGY

Practical Manual of Hormonal Regulation of Reproduction

Realized by Dr. Benchikh Fatima

intended for third-year Animal Biology and Physiology students.

2023/2024

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LIST OF ABBREVIATIONS

3Rs: Replace, Reduce, and Refine

ELISA: Enzyme-Linked Immunosorbent Assay

FSH: follicle-stimulating hormone

GnRH: Gonadotropin-releasing hormone

HCG: Human chorionic gonadotropin

HPG: Hypothalamic-Pituitary-Gonadal

IACUC: Institutional Animal Care and Use Committee

IUD: Intrauterine Devices

LH: luteinizing hormone

PCR: Polymerase Chain Reaction

PPE: personal protective equipment

SJL: Swiss Jim Lambert mice:

Introduction

Reproduction is a fundamental biological process in all living organisms, essential for species survival. The regulation of reproductive functions in both males and females is complex and finely tuned by various hormones. These hormones orchestrate the growth, development, and function of reproductive tissues, and they ensure successful conception, pregnancy, and birth. In this laboratory course, we will explore the pivotal role that hormones play in regulating reproductive cycles and functions. Through a series of experiments, students will investigate how specific hormones, such as estrogen, progesterone, testosterone, and gonadotropins, contribute to reproductive health and how imbalances in these hormones can lead to reproductive disorders.

Objectives:

- To understand the biochemical pathways of key reproductive hormones. Students will learn how hormones are synthesized, secreted, and their mechanisms of action in target tissues.
- To study the hormonal regulation of the menstrual cycle in females and spermatogenesis in males. Practical sessions will include assays to measure hormone levels during different phases of the reproductive cycle.
- To analyze the effects of hormonal imbalances on fertility. Through experiments, students will explore how variations in hormone levels affect the reproductive health of model organisms.

- To gain hands-on experience in techniques used to study endocrine functions related to reproduction. Techniques such as ELISA (Enzyme-Linked Immunosorbent Assay), PCR (Polymerase Chain Reaction), and histological examinations will be employed to investigate hormonal effects on reproductive tissues.

Significance

This lab work not only enhances theoretical knowledge but also provides practical skills in reproductive endocrinology, preparing students for advanced studies and research in biological and medical sciences. Understanding hormonal regulation is crucial not only for academic purposes but also for its applications in medical treatment strategies for fertility management and hormonal therapies.

Background about Hormonal regulation of reproduction

Hormonal regulation of reproduction is a complex process that involves multiple hormones working in concert across different organ systems to control sexual development, reproductive cycling, and fertility in both males and females. Here's an overview of how hormones regulate reproduction:

In Females

Hypothalamic-Pituitary-Gonadal (HPG) Axis:

- The process begins in the hypothalamus, which secretes gonadotropin-releasing hormone (GnRH).
- GnRH stimulates the anterior pituitary gland to produce and release follicle-stimulating hormone (FSH) and luteinizing hormone (LH).
- FSH and LH act on the ovaries, promoting follicle development and the secretion of estrogen and progesterone.

Ovarian Hormones:

- Estrogen: Primarily responsible for the development of female secondary sexual characteristics and the regulation of the menstrual cycle. It prepares the endometrium for potential pregnancy.
- Progesterone: Produced after ovulation by the corpus luteum; it enhances the endometrium to support implantation and is crucial for maintaining early pregnancy.

Menstrual Cycle:

- Divided into the follicular phase, ovulation, and luteal phase, controlled by varying levels of estrogen and progesterone.
- The rise in LH triggers ovulation—release of an egg from the dominant follicle.
- If the egg is not fertilized, hormone levels drop, leading to menstruation.

Pregnancy:

- If fertilization occurs, the embryo produces human chorionic gonadotropin (hCG), which maintains the corpus luteum and its production of progesterone, preventing menstruation and supporting pregnancy.

In Males

Hypothalamic-Pituitary-Gonadal Axis:

- Similar to females, the hypothalamus releases GnRH.
- This hormone stimulates the pituitary to release LH and FSH.
- LH stimulates testosterone production from the Leydig cells in the testes, while FSH, along with testosterone, supports the production of sperm in the seminiferous tubules.

Testosterone:

- Key male hormone responsible for the development of male secondary sexual characteristics, such as increased muscle and bone mass, and the growth of body hair.
- Regulates libido, erectile function, and sperm production.

Feedback Mechanisms:

- Both male and female reproductive systems use negative feedback to regulate hormone levels and maintain homeostasis.
- For example, high levels of sex hormones (estrogen in females, testosterone in males) typically inhibit the release of GnRH, LH, and FSH, preventing overproduction.

Regulatory Disorders

- Polycystic Ovary Syndrome (PCOS): Characterized by excess androgen production, which disrupts normal ovulation.

- Hypogonadism: Occurs in both males and females; characterized by insufficient sex hormone production due to pituitary or gonadal dysfunction.

The hormonal regulation of reproduction is essential for sexual development, reproductive health, and successful reproduction. Hormones like GnRH, LH, FSH, estrogen, progesterone, and testosterone play critical roles in these processes, with precise control exerted through complex feedback mechanisms. Disorders in these hormonal pathways can lead to various reproductive health issues, making understanding these systems crucial for both healthcare and research.

Background on Laboratory Animals

Laboratory animals are vital in research for understanding disease mechanisms, testing pharmaceuticals, and exploring physiological phenomena. Different species are used based on their unique biological traits, ease of handling, ethical considerations, and relevance to specific studies.

Common laboratory animals

- **Mice:** The most widely used due to their small size, short reproductive cycles, and genetic similarities to humans.
- **Rats**: Larger than mice, they offer easier handling of organs and blood samples, and are commonly used in neuroscience and behavioral studies.
- **Rabbit**s: Used in immunology and toxicity studies because of their size and immune system characteristics.

- **Guinea Pigs**: Historically significant in nutrition and immunology research.
- **Zebrafish:** Transparent embryos allow real-time observation of developmental processes, useful in genetics and developmental biology.
- **Fruit Flies** (Drosophila melanogaster): Excellent for genetic studies due to their simple genome and short life cycle.
- **Frogs** (e.g., Xenopus): Widely used in embryonic development studies due to their large, easily manipulated eggs.

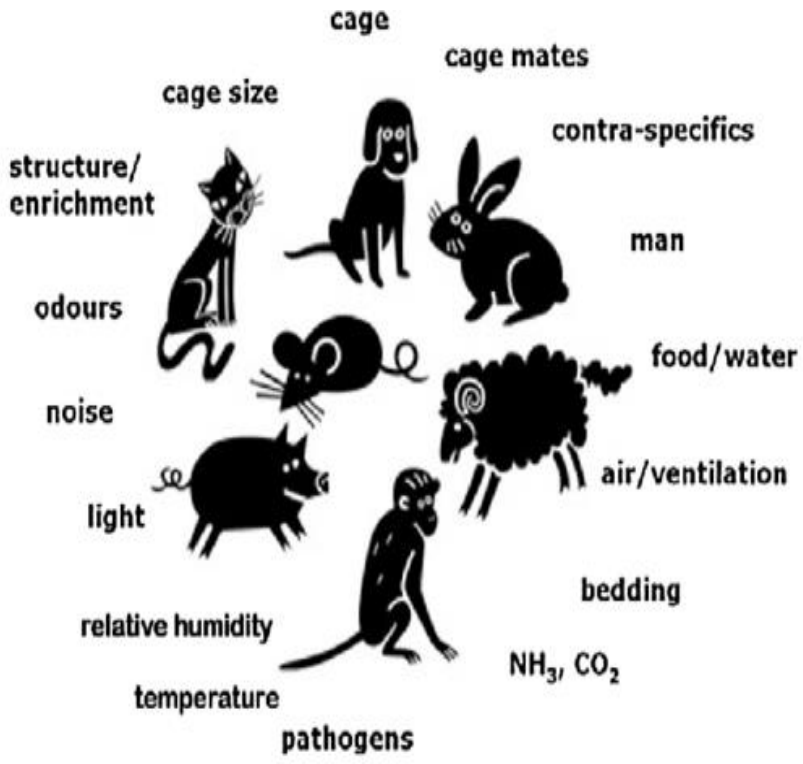

Figure 1:The environment of the laboratory animal.(Balls & Combes, 2016)

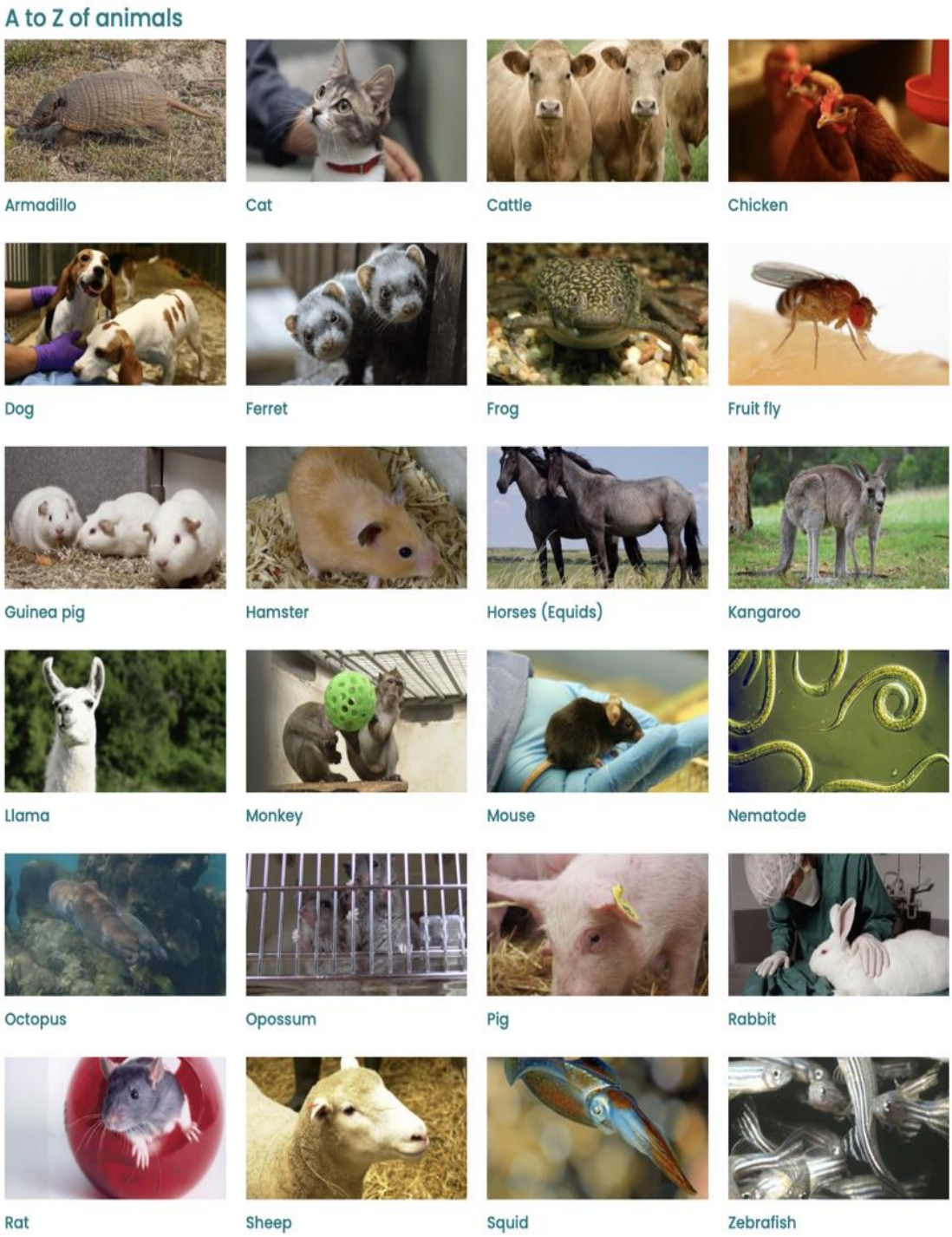

*Figure 2:An A-Z of animals used in research from Understanding Animal Research (uar.org.uk)(*Laboratory Animals Used in Research*, n.d.)*

WHY MICE?

Genetic Similarity to Humans

- Mice share approximately 95% of their genes with humans, making them excellent models for human diseases. This genetic similarity allows for the study of human-like pathologies under controlled laboratory conditions.

Advantages of Using Mice in Research

- Genetic Manipulability: The advent of technologies like CRISPR and other genetic engineering tools has made it easier to edit the mouse genome, allowing the creation of transgenic models to study specific diseases.
- Reproductive Efficiency: Mice have a short gestation period (about 19-21 days) and can reproduce quickly and frequently, facilitating generational studies and rapid propagation of genetic traits.
- Cost-Effectiveness: Their small size requires less space and resources compared to larger animals, making them economically viable for many labs.
- Extensive Background Data: There is a vast amount of historical data on mice, including a wide range of established disease models and well-characterized genetic strains.

Ethical Considerations:

- The use of mice aligns with the "3Rs" principle in animal research: Replace, Reduce, and Refine. They help minimize the number of larger animals used and improve the ethical aspects of biomedical research.

Applications: Mice are used across various fields:

- Medical Research: Disease models for cancer, diabetes, cardiovascular diseases, and neurological disorders.
- Genetic Studies: Understanding gene functions and interactions.
- Drug Development: Testing efficacy and safety of new pharmaceuticals.
- Behavioral Science: Studying learning, anxiety, and other psychological traits.

The choice of laboratory animal depends on the specific scientific questions and the biological relevance of the animal to the system being studied. Mice, with their close genetic relationship to humans, ease of handling, and economic viability, remain the cornerstone of biomedical research, providing invaluable insights that guide human medicine.

1. Comparative Dissection of Male and Female Mice

PRACTICAL WORK: COMPARATIVE DISSECTION OF MALE AND FEMALE MICE

Objective

To provide students with a comprehensive understanding of the anatomical structures of male and female mice, emphasizing reproductive anatomy and other organ systems.

Materials Needed

- ✓ Specimens: Male and female mice, preserved for dissection. Ensure that these specimens are obtained in accordance with ethical standards and legal requirements.
- ✓ Dissection Kits: Scalpels, scissors, forceps, pins, dissection trays, and gloves.
- ✓ Magnification Tools: Handheld magnifiers or dissecting microscopes.
- ✓ Protective Gear: Lab coats, gloves, and safety goggles.
- ✓ Disposal Bins: For the proper disposal of biological waste.
- ✓ Reference Materials: Anatomical charts and guides specific to mice.

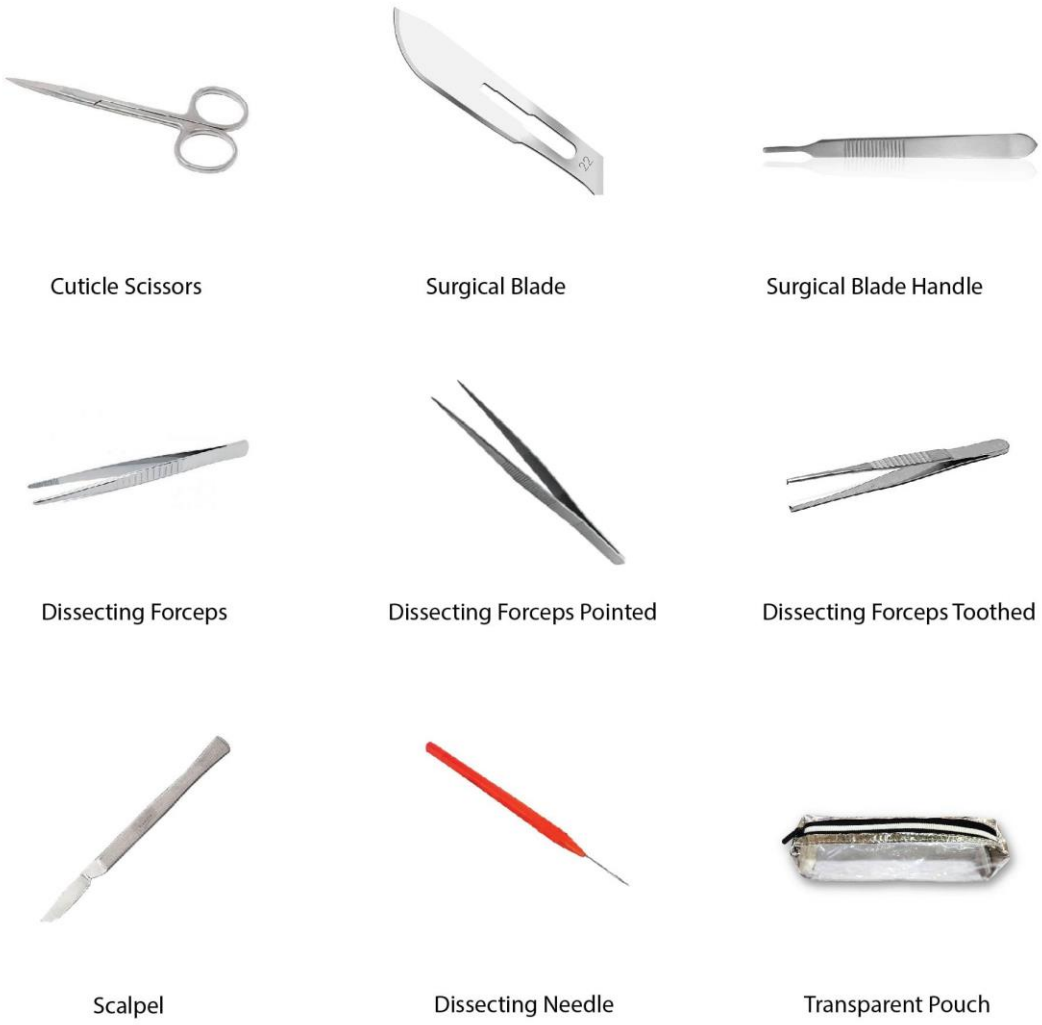

*Figure 3: dissection tools.(*Dissection Tools - Google Search*, n.d.)*

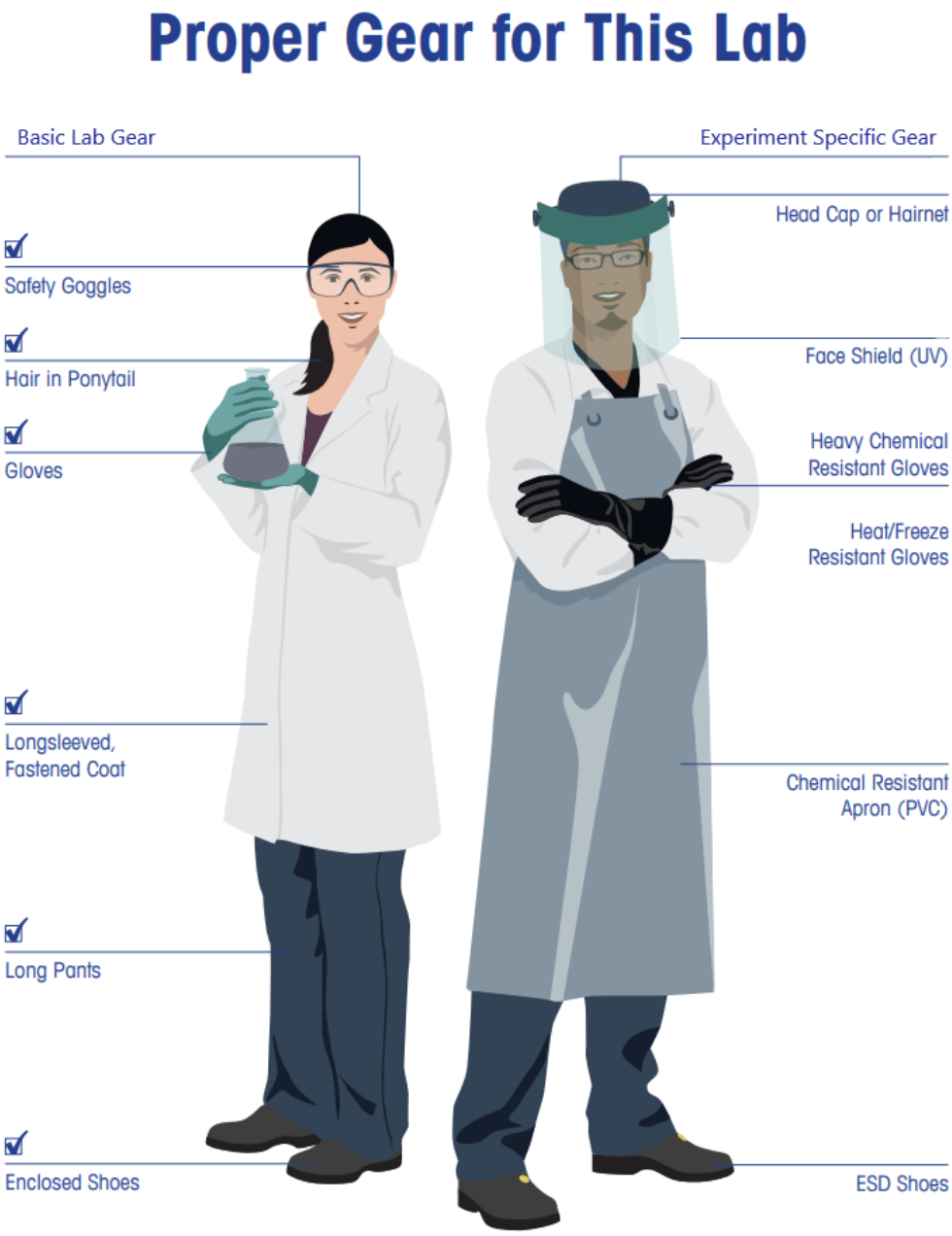

*Figure 4 :Lab Safety (*Because in Laboratory Safety Comes First - Personal Protective Laboratory Equipment Poster, *n.d.)*

Methodology

1. Preparation Phase

- Ethical Considerations: Begin with a briefing on ethical practices, including the rationale behind using animals in research and education, the importance of minimizing suffering, and proper handling techniques.

- Tool Preparation: Ensure all dissection tools such as scalpels, scissors, forceps, pins, and dissection trays are clean and ready for use.
- Safety Measures: Distribute protective gear like gloves, lab coats, and safety goggles to all participants.
- Specimen Preparation: Thaw specimens if preserved and lay them on the dissection trays. Identify and record any external anomalies or distinguishing features.

2. External Examination

- Identify Sex: Examine external genital structures to differentiate between male and female mice.
- Document External Features: Note features such as fur color, body size, and any signs of disease or injury.

3. Opening the Body Cavity

- Initial Incision: Make a ventral midline incision starting from just below the chin extending to the lower abdomen, being careful to cut only skin deep.
- Expose Muscular Wall: Reflect the skin to expose the muscular layer beneath. Make a similar incision through the muscle to access the internal cavity.
- Pin Back the Flaps: Use pins to secure the skin and muscle flaps to the dissection tray, providing clear visibility and access to the internal organs.

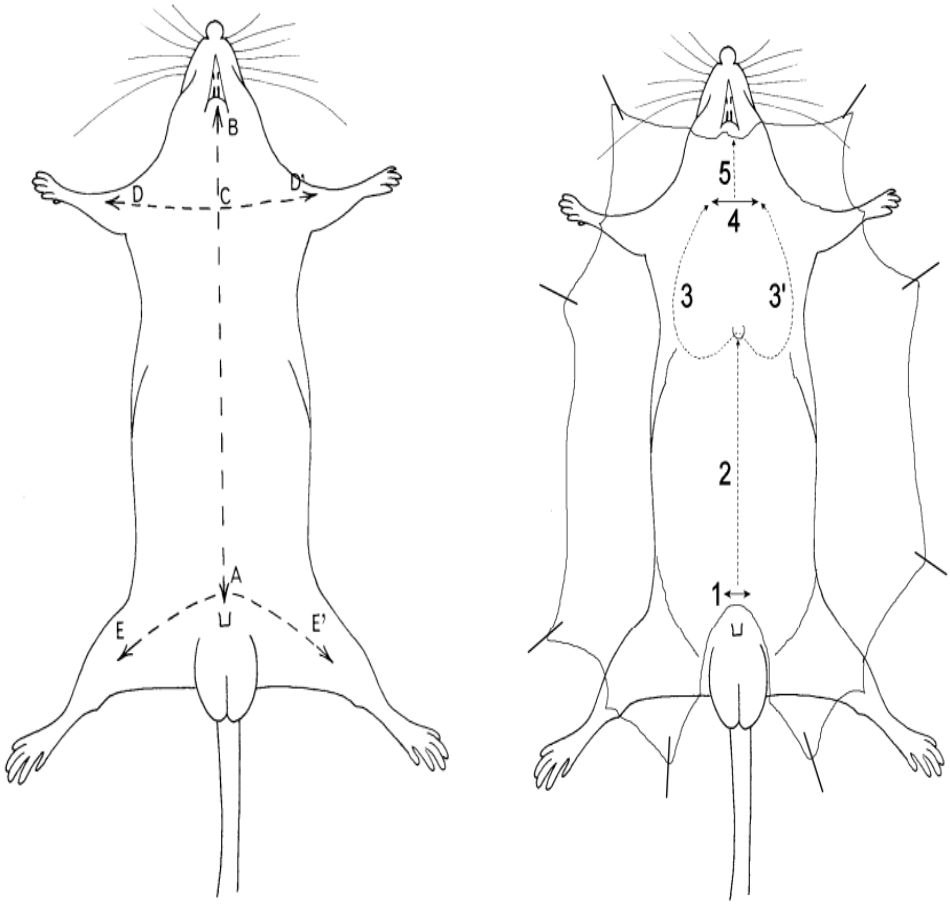

*Figure 5 : Flow diagram of the procedures for mouse dissection.(*Protocols for Field and Laboratory Rodent Studies*, n.d.)*

4. Systematic Exploration of Organs

- Digestive System: Locate and identify the liver, stomach, intestines, and pancreas. Observe the connection between these organs.
- Circulatory and Respiratory Systems: Identify the heart and lungs. Note the position of the heart between the lungs and observe the major arteries and veins.
- Reproductive System:
- In males, identify the testes, seminal vesicles, and vas deferens.
- In females, find the ovaries, uterus, and fallopian tubes.
- Urinary System: Locate the kidneys and urinary bladder.

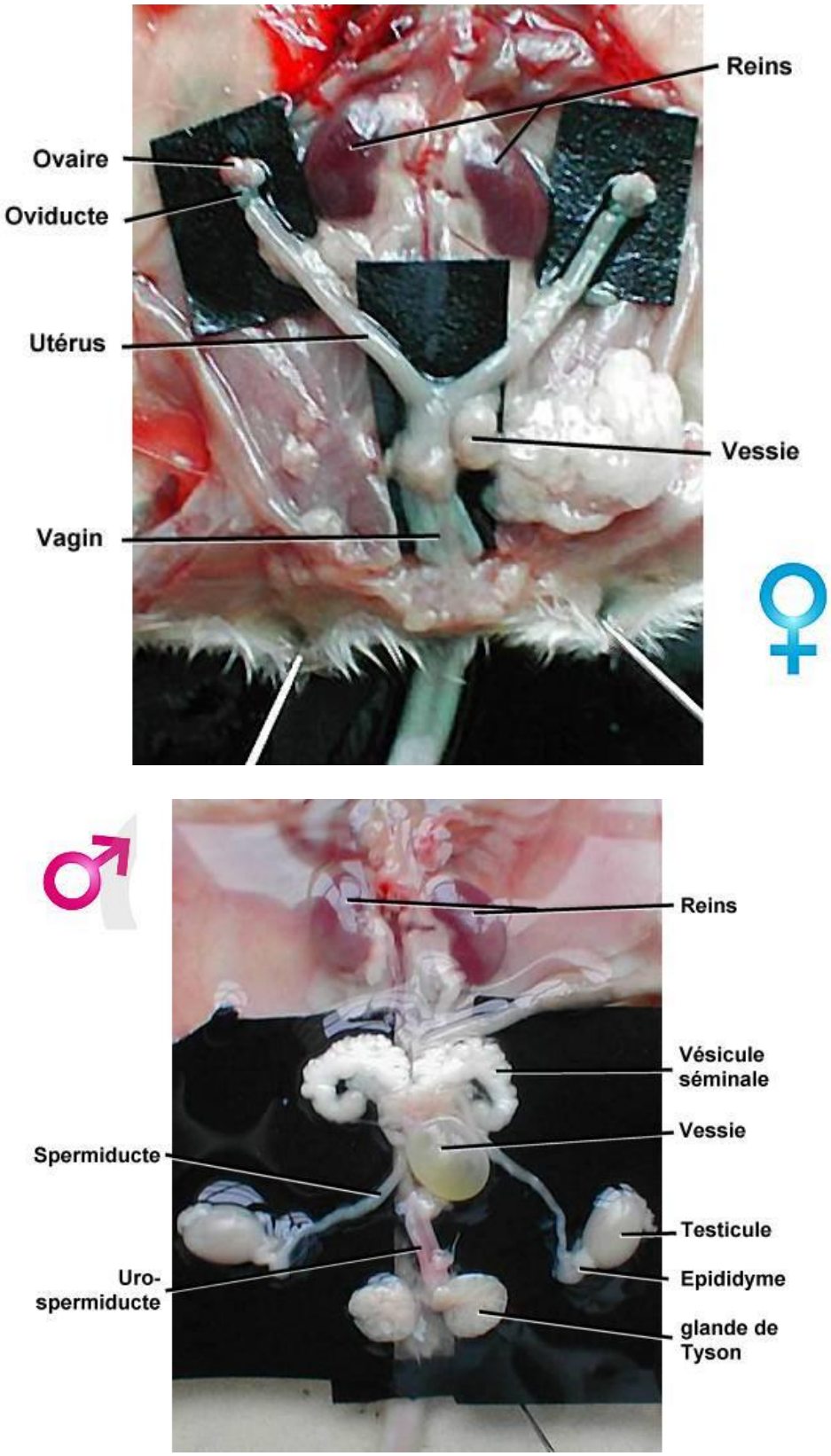

*Figure 6: Comparative Urogenital Anatomy of Male and Female Mice(*Protocols for Field and Laboratory Rodent Studies*, n.d.)*

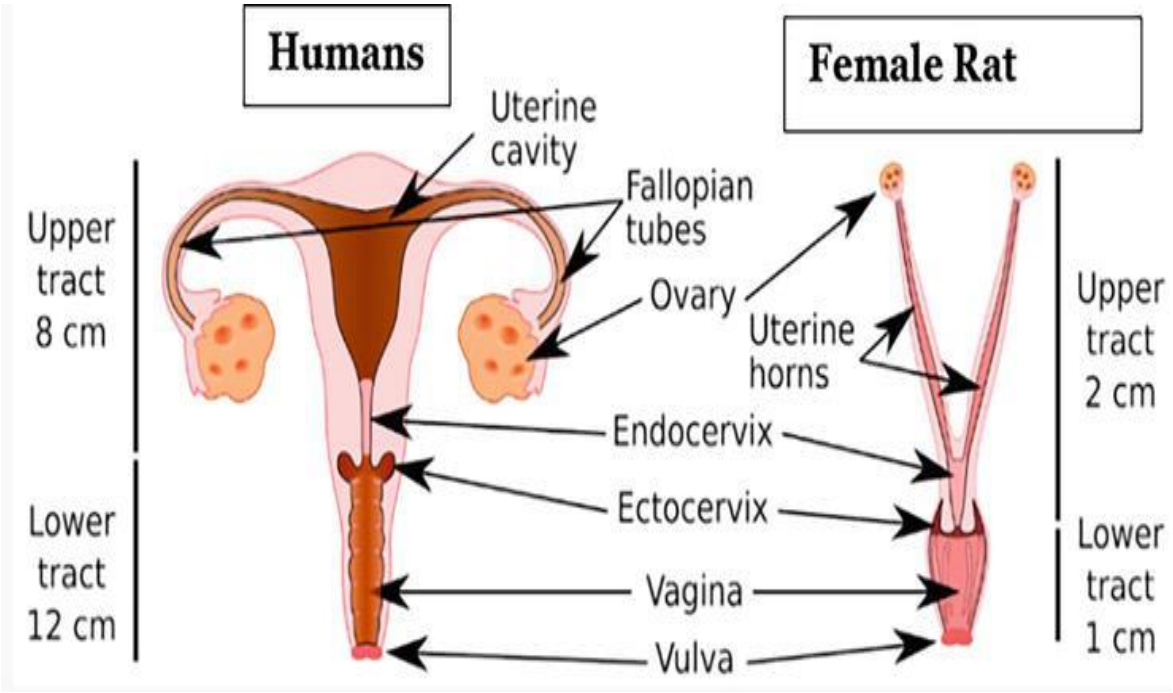

Figure 7:Anatomical feature of the reproductive system of a female rat and human.

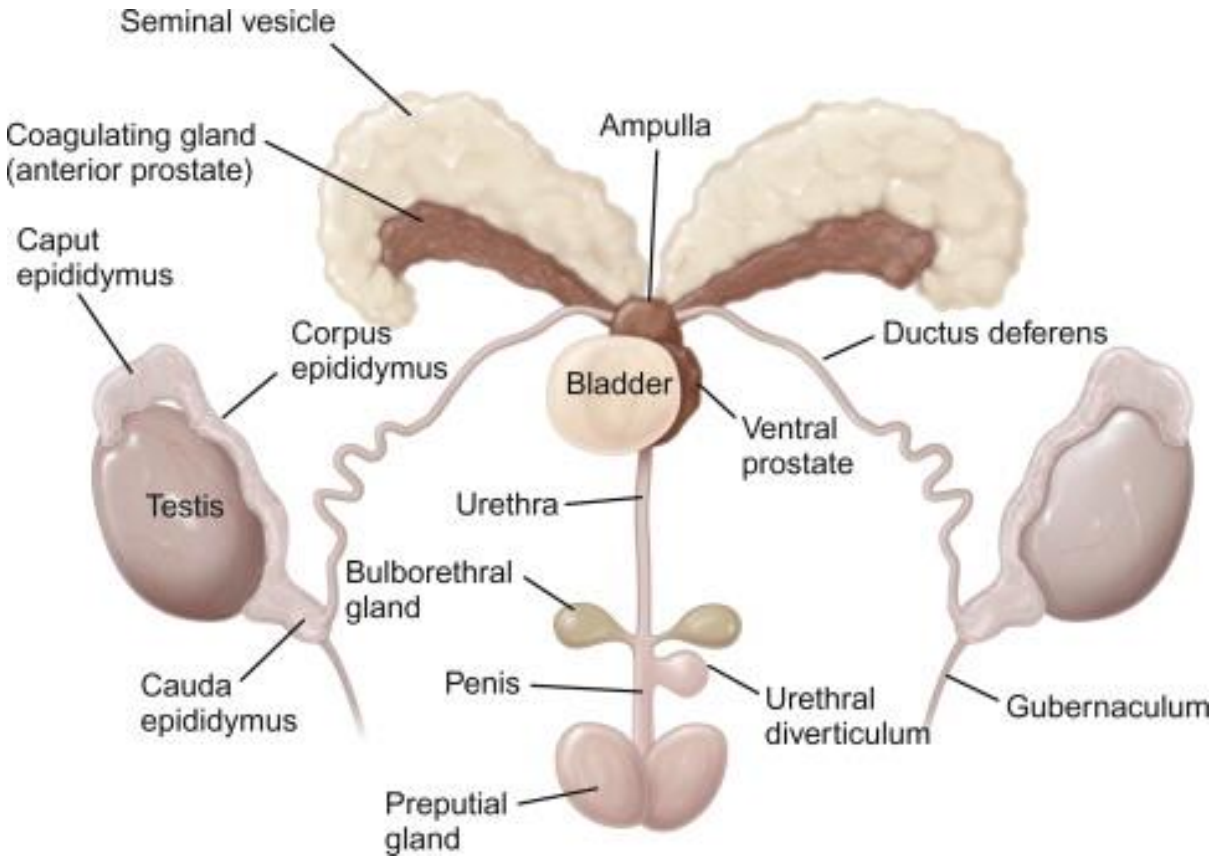

*Figure 8:Male Reproductive System(*Mouse Male Reproductive System, Illustration - Stock Image - C047/4178 - Science Photo Library, *n.d.)*

5. Detailed Organ Inspection

- Function and Relation: As each organ is identified, discuss its function and its relationship to surrounding structures.
- Organ Interaction: Highlight how different systems interact, such as the relationship between the digestive and circulatory systems.

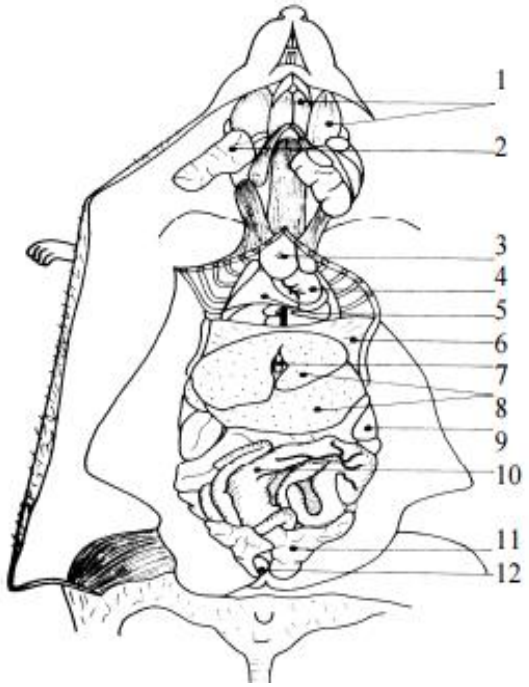

*Figure 9: Anatomy of mouse.(*Mouse Male Reproductive System, Illustration - Stock Image - C047/4178 - Science Photo Library*, n.d.)*

6. Sampling (Optional)

- Tissue Samples: If part of the learning objectives, carefully collect samples of tissues for further histological examination under microscopes.

7. Conclusion of Dissection

- Review: Go over the function of each organ system and the physiological differences observed between the sexes.

- Discussion: Encourage students to discuss their observations and any questions they might have about the dissection or the anatomy.

8. Clean-Up and Disposal

- Proper Disposal: Dispose of all biological waste according to specific guidelines to ensure biohazard safety.
- Tool Cleaning: Clean and sterilize all reusable tools.
- Sanitize Work Area: Ensure that the work area is thoroughly cleaned and disinfected.

Reflection and Feedback

- Student Reflection: Have students complete a reflection on what they learned and how they can apply this knowledge in their future studies or careers.
- Feedback Session: Provide feedback on their dissection techniques and understanding and gather feedback on the session to improve future dissections.

2. Estrous cycle

PRACTICAL WORK: OBSERVATION OF ESTROUS CYCLE

Objective

To gain a comprehensive understanding of the reproductive cycle in mammals, using mice as a model organism. This includes familiarization with the different stages of the estrous cycle and their physiological significance. A structured protocol is essential to understand the estrous cycle in laboratory mice. This protocol includes preparation, observation, and data recording phases. Here's a general guide to follow:

Materials Needed

- ✓ Female laboratory mice (preferably adult)
- ✓ A microscope
- ✓ Glass slides and coverslips
- ✓ Saline solution
- ✓ Cotton swabs or fine-tipped forceps
- ✓ Gloves and lab coat
- ✓ Recording sheets or software for data collection.

Methodology

Step 1: Preparation and Safety

- Check that all mice are healthy.
- Ensure that all participants wear personal protective equipment (PPE).
- Inform students about ethical standards and safe handling practices for mice.

Step 2: Behavioral Observation

- Observe the mice daily.
- Note behavioral and physical signs (e.g., swelling of the vulva, changes in activity).

Figure 10:Staging of The Estrous Cycle and Induction (Ajayi & Akhigbe, 2020)

Step 3: Vaginal Sampling

- Gently immobilize the mouse.
- Carefully insert the pipette containing a drop of saline solution into the vagina.
- Collect a sample of cells by gently aspirating.
- Apply the sample onto a slide.

Step 4: Slide Preparation

- Gently spread the sample on the slide.
- Allow the air to dry.

Step 5: Microscopic Observation

- Place the slide under the microscope.
- Observe and identify the types of cells present.
- Determine the phase of the estrous cycle based on microscopic observation (proestrus, estrus, metestrus, diestrus).

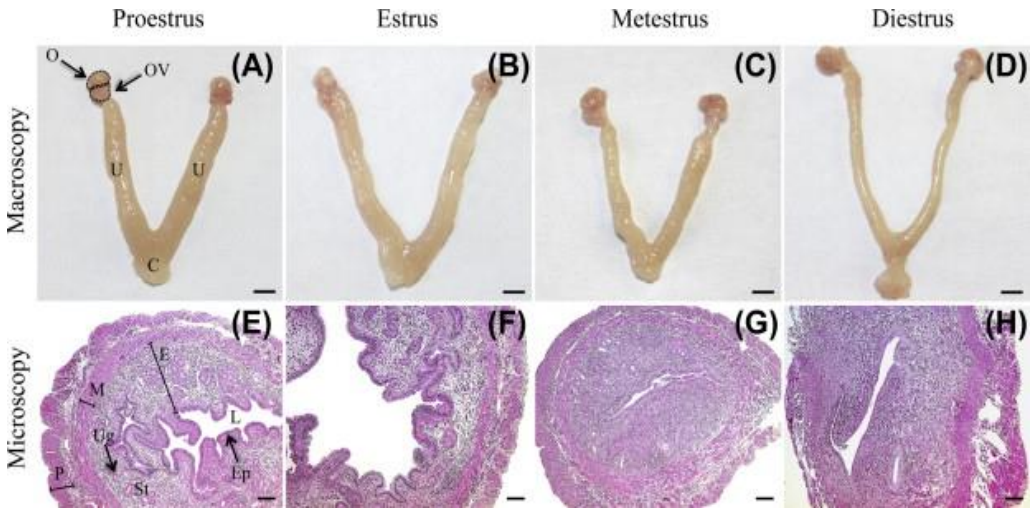

Figure 11: Uterine macroscopy and microscopy during the estrous cycle.(Ajayi & Akhigbe, 2020)

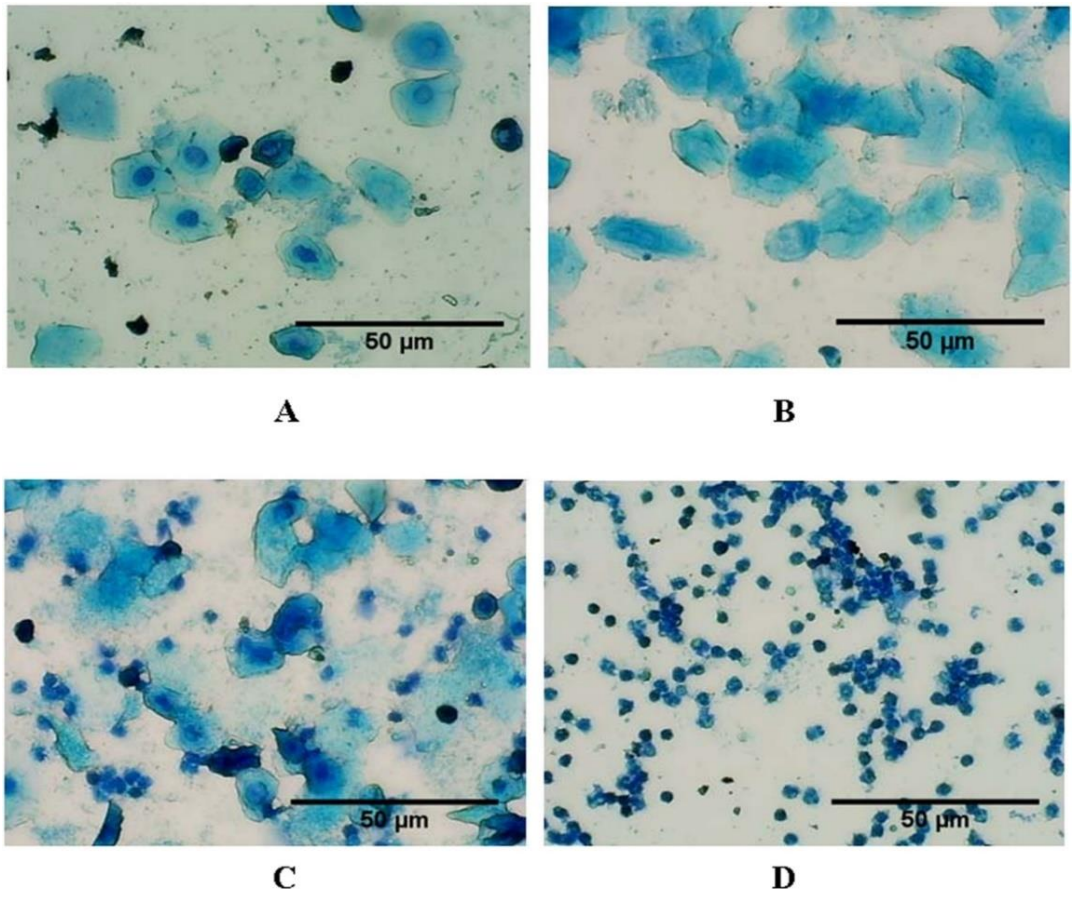

Figure 12: Photomicrographs of vaginal smear of rats showing four phases of estrous cycle (40X) in normoxic control rat. (A) Proestrous phase: nucleated epithelial cells, (B) Estrous phase: non- nucleated cornified cells, (C) Metestrous phase: nucleated epithelia(Ajayi & Akhigbe, 2020)

Step 6: Data Recording

- Document all observations and results in the logbook.
- Take microscopic photos if possible.

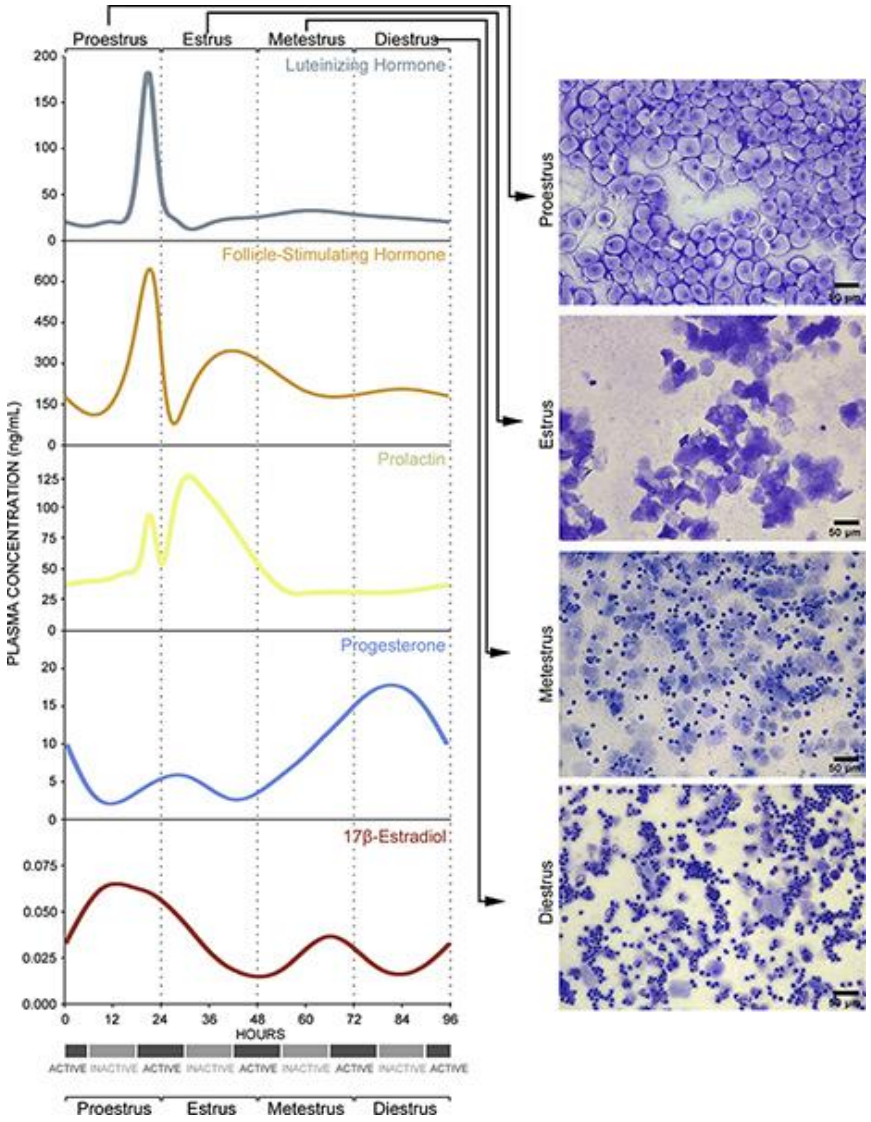

Figure 13: . Vaginal smear cytology reflects underlying endocrine events.(McLean et al., 2012)

Step 7: Analysis and Discussion

- Compare microscopic observations with behavioral observations.
- Discuss results in a group, highlighting any correlations or anomalies.

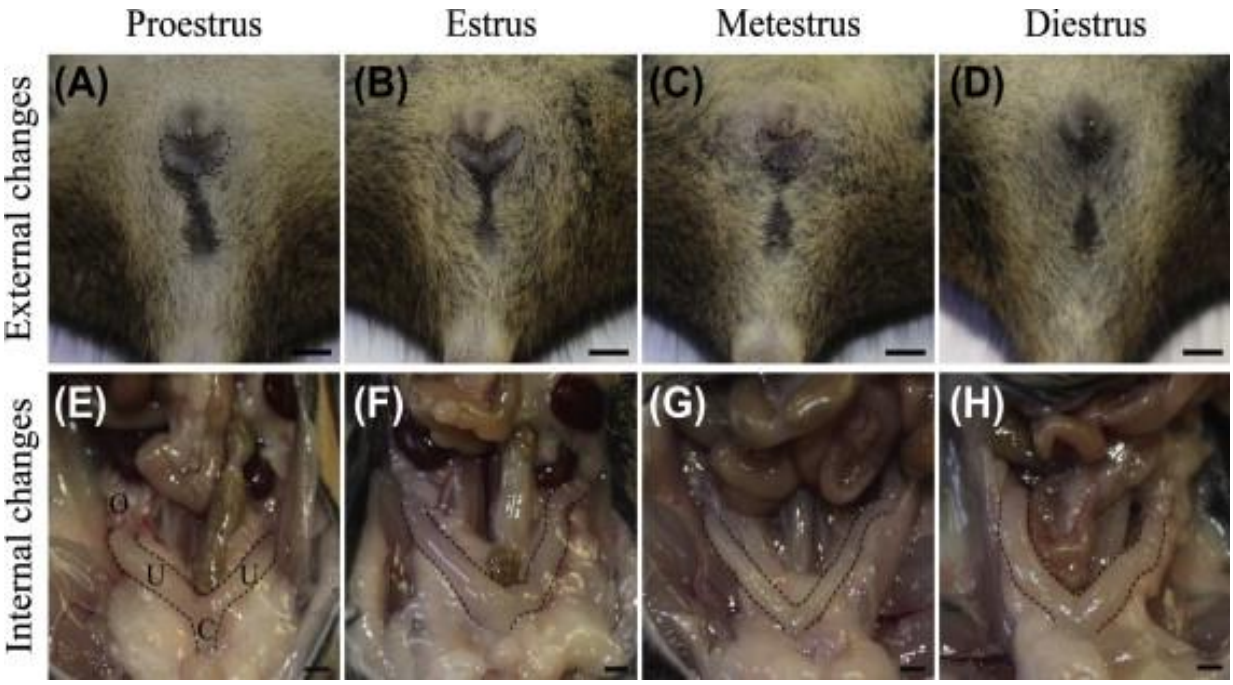

Figure 14: Reproductive Tract Changes during the mouse Estrous Cycle(McLean et al., 2012)

Step 8: Cleaning and Storage

- Clean all equipment thoroughly.
- Ensure that the mice are safely returned to their environment.
- Ethical Considerations

Ethical Considerations

- Clean all equipment thoroughly.
- Ensure that the mice are safely returned to their environment.
- Ethical Considerations

Conclusion

Students must prepare a report or deliver a presentation about their results, emphasizing the connections between theory and actual observations.

3. Endocrinology of reproduction

PRACTICAL WORK: ENDOCRINOLOGY OF REPRODUCTION

Objective

This practical work aims to understand the endocrinology of reproduction, focusing on the gonadotropic axis. This includes studying the hormonal regulation of the reproductive system and the interaction between the hypothalamus, pituitary gland, and gonads. By exploring the roles of key hormones like GnRH, FSH, LH, estrogen, and testosterone, students will gain insights into how these hormones influence reproductive functions:

Materials Needed

- ✓ Laboratory animals (e.g., mice or rats)
- ✓ Hormone assay kits (for FSH, LH, estrogen, testosterone)
- ✓ Microscopes and slides
- ✓ Blood collection equipment
- ✓ Dissection tools
- ✓ Protective gear (gloves, lab coats, eye protection

Methodology

Step 1: Hormone Sampling

- Collect blood samples from laboratory animals at different stages of their reproductive cycle.
- Measure the levels of FSH, LH, estrogen, and testosterone using hormone assay kits.

Step 2: Gonad Examination

In this step, the focus is on the direct examination of the gonads, which are the primary reproductive organs: the testes in males and ovaries in females. Here's a breakdown of what this step entails:

- Dissection: This involves the careful dissection of laboratory animals to access the reproductive organs. It's important to do this methodically and ethically, minimizing any potential suffering for the animals.
- Observation of gonads:

✓ In males, the testes are examined. Key aspects to look at include the size, texture, and any noticeable abnormalities. The testes are responsible for producing sperm and testosterone, a crucial hormone in the male reproductive system.

✓ In females, the ovaries are the focus. Observations might include the size of the ovaries, the presence and stages of follicular development, and any signs of ovulation or corpus luteum formation. The ovaries produce eggs and secrete hormones like estrogen and progesterone.

- ✓ Correlation with Hormone Levels: Observations from the gonad examination are correlated with hormone levels measured in the blood. For example, high levels of FSH and LH might correlate with active spermatogenesis in the testes or follicular maturation in the ovaries.
- Dissect the animals to examine their gonads (testes in males, ovaries in females).
- Observe the morphology of the gonads and any changes corresponding to different hormone levels.

Step 3: histological analysis.

- Tissue Section Preparation: Gonadal tissues are carefully sectioned into thin slices. These slices are treated with various stains to highlight different cell types and structures under a microscope.
- Microscopic Examination:

➢ In the testes, look for structures like seminiferous tubules, where sperm production occurs. You might assess the presence and stages of sperm cells, Sertoli cells (supporting cells), and Leydig cells (testosterone-producing cells).

➢ In the ovaries, focus on identifying different stages of follicle development from primordial follicles to mature Graafian follicles. Observing corpus luteum formation post-ovulation can also be insightful.

➢ Understanding Hormonal Influence: This step is crucial to understand how different hormones influence the structure and function of gonadal tissues. For instance, changes in the seminiferous tubules or follicles can be linked to fluctuations in FSH and LH levels.

Step 4: Hormone Manipulation

- Administer exogenous hormones (e.g., GnRH analogs, FSH, LH) to a group of animals.
- Observe and record the effects of hormone administration on the reproductive system.

Step 5: Data Analysis

- Analyze the collected data to understand the relationships between different hormones in the gonadotropic axis.
- Correlate hormone levels with gonadal morphology and reproductive cycle stages.

Step 6: Report Writing

- Compile the findings in a detailed report.
- Discuss the role of the gonadotropic axis in regulating reproductive functions.

Ethical Considerations

- Ensure all procedures comply with ethical guidelines for animal handling and experimentation.
- Minimize discomfort and stress to animals during the experiments.

Conclusion

This practical work offers students hands-on experience in understanding the endocrinology of reproduction, particularly the gonadotropic axis. It emphasizes the importance of hormonal regulation in reproductive health and disease. Students should be able to explain how alterations in hormone levels can affect reproductive functions and the potential implications in clinical settings.

4. Castration and Ovariectomy

PRACTICAL WORK CASTRATION AND OVARIECTOMY

Objective

To explore the surgical techniques of ovariectomy and castration in mice and to study the physiological and behavioral effects of these procedures.

Materials Needed

- ✓ **Lab Mice:** male and female mice.
- ✓ **Surgical Suite:** Sterilized environment suitable for small animal surgery.
- ✓ **Anesthesia and Analgesia:** Isoflurane for anesthesia, along with appropriate analgesics for post-operative care.
- ✓ **Surgical Tools:** Scalpels, scissors, forceps, sutures, and needles.
- ✓ **Post-operative Care Supplies:** Heating pads for recovery, antiseptics for wound care.
- ✓ **Monitoring Equipment:** For recording physiological data such as heart rate, respiratory rate, and possibly hormone levels pre- and post-operation.
- ✓ **Blood Collection Supplies**: Syringes, needles, and microtainers for blood sampling.
- ✓ **Centrifuge**: For serum separation.
- ✓ **ELISA Kits**: For quantitative determination of specific hormones (e.g., estrogen, testosterone, progesterone, LH, FSH).

- ✓ **Refrigerator or Freezer:** For storing blood samples if immediate analysis is not possible.
- ✓ **Data Recording Tools**: For documenting all observations and results.
- ✓ Obtain approval from your institution's animal care and use committee.

Methodology

Pre-Surgical Preparations

- Ethical Review: Ensure all procedures have IACUC approval.
- Training and Setup: Confirm all participants are trained in the handling and care of laboratory mice and the specific surgical techniques to be used.
- Animal Preparation: Fast the mice for a few hours before the surgery to reduce risks during anesthesia.

Surgical Procedure

- Anesthesia: Administer isoflurane via an inhalation chamber to induce anesthesia.
- Surgical Site Preparation: Shave and disinfect the surgical area.
- Make a small incision in the lower abdomen to access the ovaries.

1. Isolate and remove the ovaries, taking care to litigate any blood vessels to prevent hemorrhage.
2. Suture the incision.
3. Castration in Males:

- An incision is made just above the scrotum to expose the testes.

- The testes are removed after tying off the spermatic cord to prevent bleeding.
- Suture the incision.

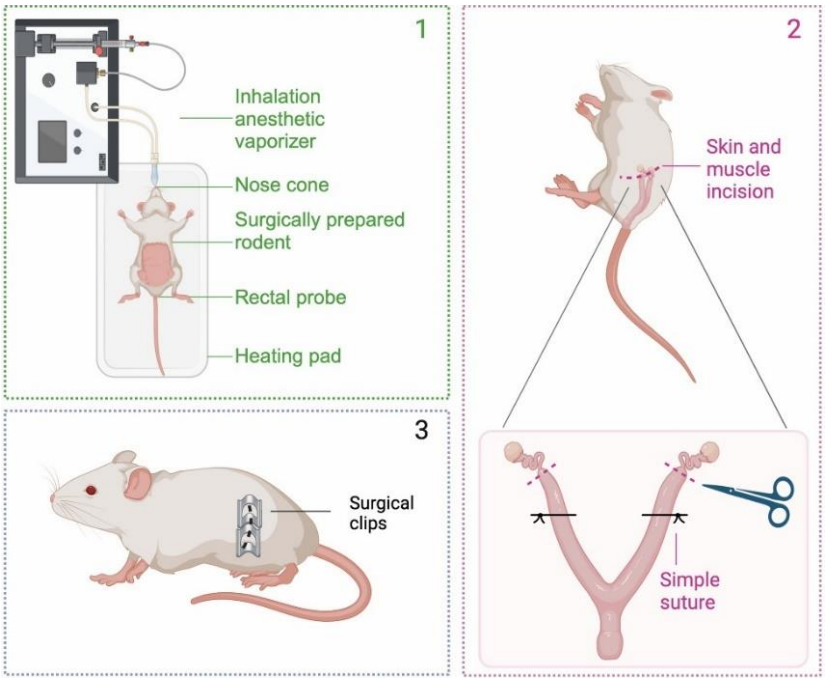

Figure 15:Diagram showing the steps of the ovariectomy procedure.(McLean et al., 2012)

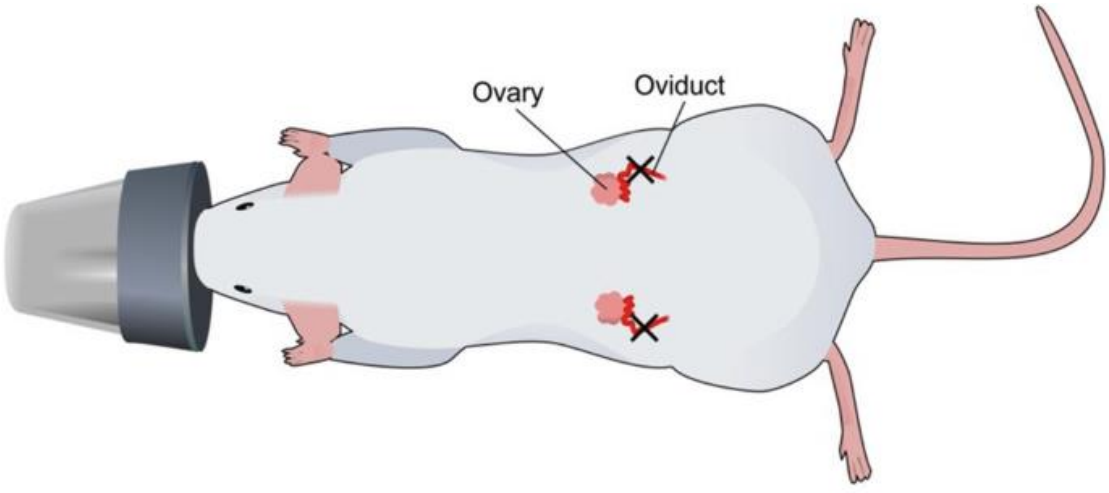

Figure 16:Anatomical Diagram of a Mouse: Ovary and Oviduct Locations in a Laboratory Setting(McLean et al., 2012)

Post-operative Care

- Monitoring: Observe the mice for any signs of distress or infection. Monitor recovery from anesthesia.
- Pain Management: Administer analgesics to manage pain post-surgery.
- Record Keeping: Document the procedure and any observations regarding recovery.

Blood Sampling for Hormonal Analysis

Pre-Operative Sampling:

- Collect baseline blood samples prior to surgery to establish baseline hormone levels for each mouse.
- Use a syringe with a fine needle to collect blood from the tail vein or the saphenous vein.
- Collect approximately 100-200 µL of blood per sample.

Post-Operative Sampling:

- Schedule post-operative blood sampling at various intervals (e.g., 24 hours, 1 week, 1-month post-surgery) to monitor changes in hormone levels due to the removal of gonads.
- Ensure consistent timing for blood collection to reduce variability in hormone levels due to circadian rhythms.

Hormonal Analysis

- Serum Separation: Centrifuge the blood samples to separate the serum.
- Hormone Assays: Perform ELISA (Enzyme-Linked Immunosorbent Assay) following the manufacturer's instructions to quantify hormone levels in the serum.

- Estrogen and Progesterone: Critical for female mice, especially to assess the impact of ovariectomy.
- Testosterone: Important for male mice to evaluate the effects of castration.
- LH (Luteinizing Hormone) and FSH (Follicle-Stimulating Hormone): Assess the feedback mechanisms and overall impact on the hypothalamic-pituitary-gonadal axis.

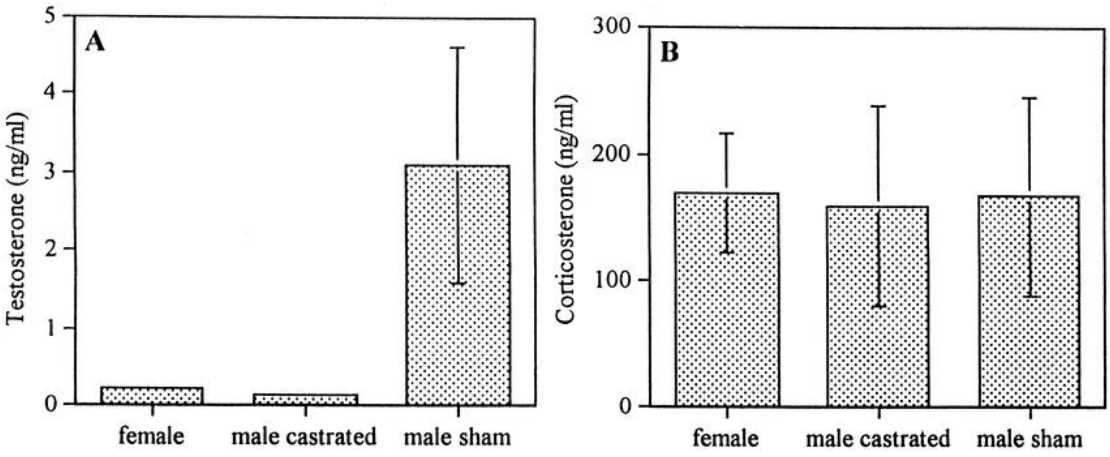

Figure 17:Castration specifically reduces serum testosterone. Male SJL mice were castrated and allowed to recover 1 week before serum was collected.(Bebo et al., 1998)

Data Collection and Analysis

- Physiological Monitoring: Measure changes in weight, feeding behavior, and activity levels.
- Behavioral Studies: Assess changes in social and individual behaviors post-surgery.
- Quantitative Analysis: Compile and analyze the hormone levels at different time points. Use statistical software for analysis to compare pre- and post-operative levels.
- Correlation Studies: Correlate hormonal changes with behavioral and physiological data collected in other parts of the study.

- Discussion: Interpret the results in the context of known biological functions and impacts of gonadal hormones. Discuss any deviations from expected patterns and potential physiological implications.

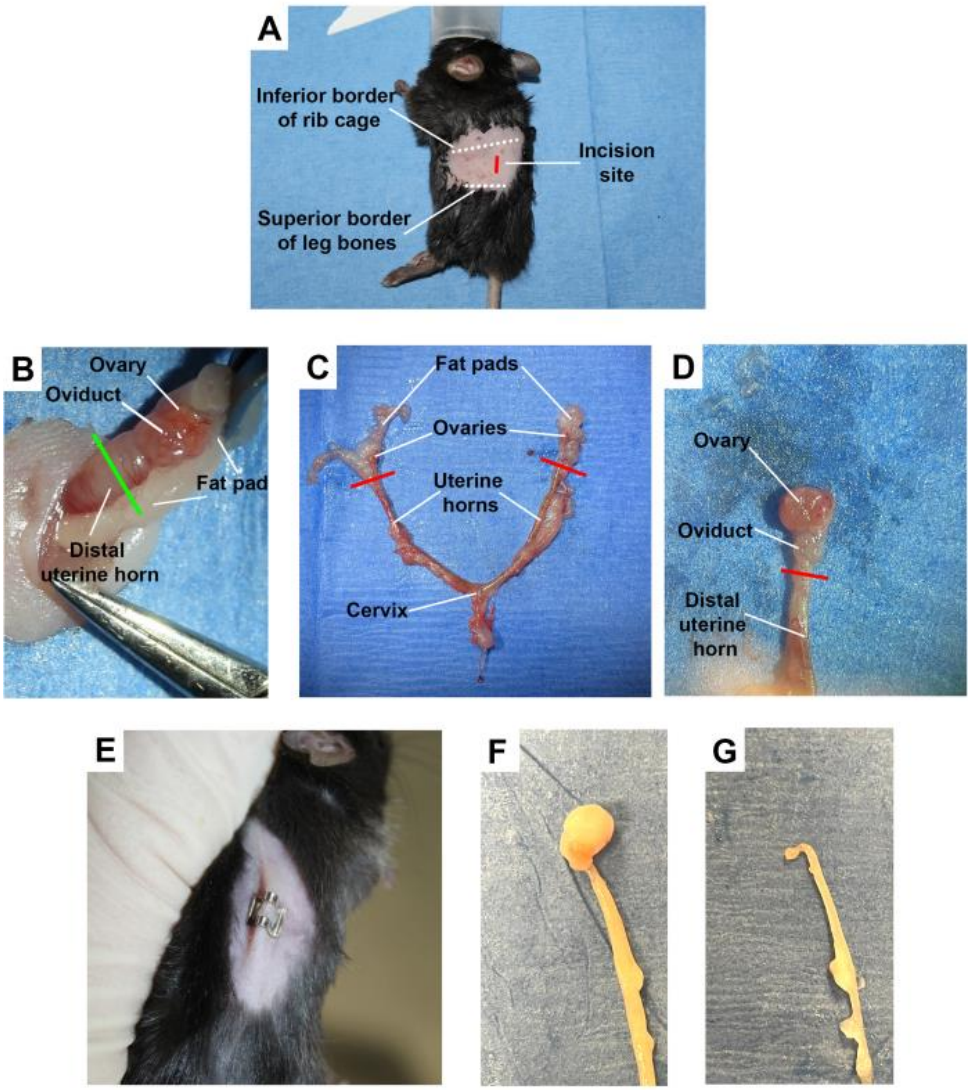

Figure 18:Anatomical Overview of Reproductive and Skeletal Structures in a Laboratory Rodent (Bebo et al., 1998)

Reporting Results

- Discussion: Analyze the data in context with existing literature on the physiological effects of gonadectomy.
- Presentation: Prepare presentations or reports detailing the methodology, findings, and implications of the study.

- Comprehensive Reporting: Include a section on hormonal analysis in your reports and presentations, providing graphs and tables that clearly depict changes over time.
- Peer Review: Encourage students to critique each other's data interpretation and understanding of hormonal regulation.

Safety and Ethical Considerations

- Ensure all procedures minimize animal suffering and distress, adhering to the highest standards of veterinary care and ethical guidelines.

5. Ovarian function

PRACTICAL WORK OVARIAN FUNCTION

Conducting practical work on ovarian function involves understanding various aspects of ovarian physiology, including hormone production, follicle development, and ovulation. Below is a protocol that can be used in a laboratory setting to study ovarian function.

Objective

To study and understand the functioning of the ovaries, focusing on aspects such as hormone secretion, follicular development, and the ovulation process.

Materials Needed

- ✓ Laboratory animals (e.g., rats or mice)
- ✓ Dissection kit
- ✓ Microscope with camera
- ✓ Hormone assay kits (for estrogen, progesterone, FSH, LH)
- ✓ Blood collection equipment
- ✓ Glass slides and coverslips
- ✓ Histological stains
- ✓ Anesthesia and euthanasia equipment
- ✓ Protective gear (gloves, lab coats, eye protection)

Methodology

Step 1: Animal Selection and Care

- Select female animals of reproductive age.
- Ensure they are healthy and have been housed in controlled conditions (light, temperature).
- Follow ethical guidelines for the care and use of laboratory animals.

Step 2: Blood Sampling and Hormone Assay

- Collect blood samples at different stages of the estrous cycle.
- Use hormone assay kits to measure levels of estrogen, progesterone, FSH, and LH.

Step 3: Dissection and Ovary Extraction

- Euthanize the animals according to ethical guidelines.
- Dissect to extract the ovaries, being careful not to damage them.
- Record any observable characteristics (size, color, presence of visible follicles or corpus luteum).

Step 4: Histological Preparation

- Fix the ovaries in a suitable fixative (like formalin).
- Dehydrate, clear, and embed the tissue in paraffin.
- Section the embedded tissue using a microtome.
- Stain the sections with appropriate histological stains for ovarian tissue (e.g., H&E stain).

Step 5: Data Analysis and Interpretation

- Analyze the hormone assay results in conjunction with the histological findings.
- Determine the correlation between hormone levels and stages of follicle development and ovulation.

Step 6: Report Writing

- Compile a comprehensive report detailing the methodology, findings, and interpretations.
- Discuss the role of ovarian hormones in the reproductive cycle and their physiological significance.

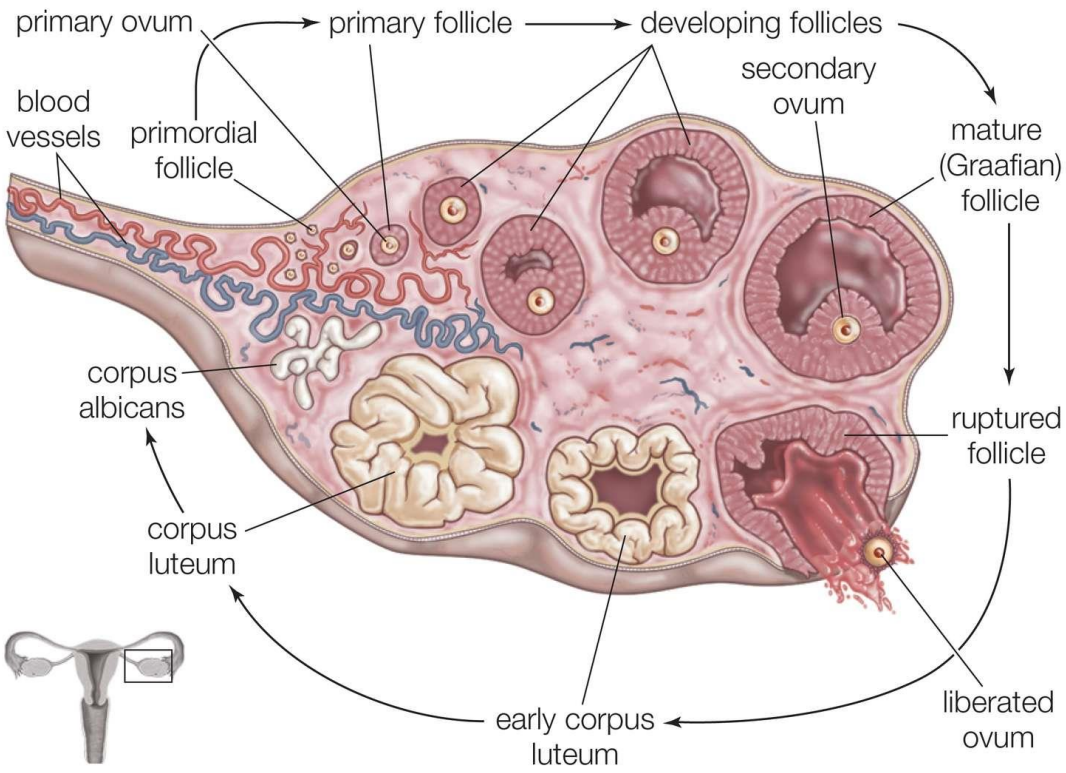

Figure 19: The steps of ovulation, beginning with a dormant primordial follicle that grows and matures and is eventually released from the ovary into the fallopian tube.(Bebo et al., 1998; Ovary | Animal & Human | Britannica*, n.d.)*

Ethical Considerations

- Ensure all animal experiments are conducted in accordance with institutional and national ethical guidelines.
- Minimize animal suffering and use the minimum number of animals necessary for meaningful results.

Conclusion

This practical work will provide insight into the complex processes governing ovarian function, including hormonal regulation, follicular development, and ovulation. It's essential for understanding female reproductive health and can have applications in fields like reproductive medicine and endocrinology.

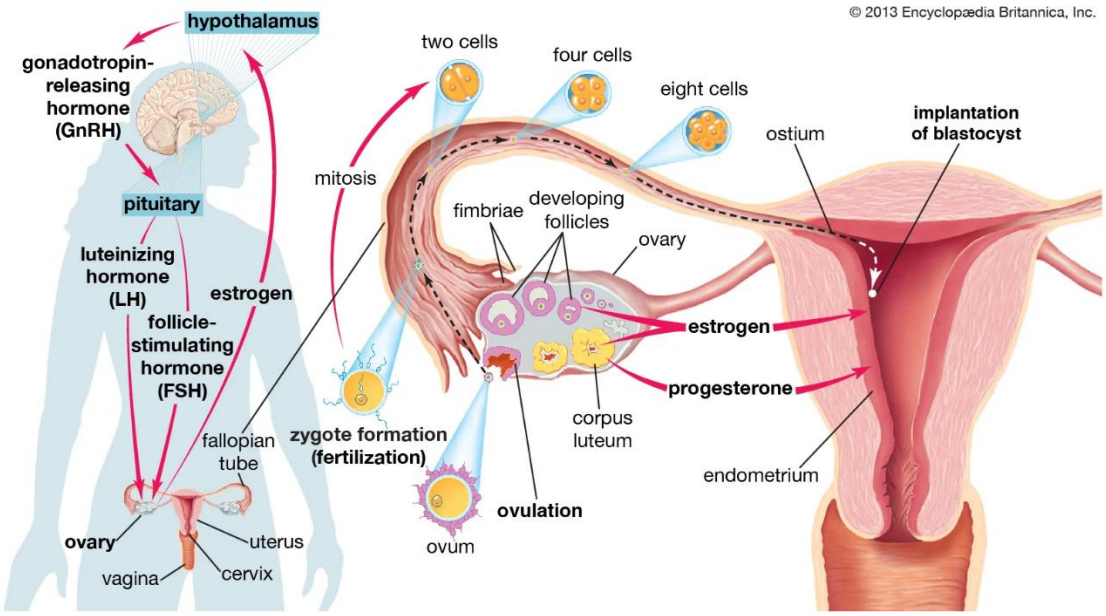

*Figure 20:The ovaries, in addition to producing egg cells (ova), secrete and are acted upon by various hormones in preparation for pregnancy.(*Human Ovaries - Students | Britannica Kids | Homework Help, *n.d.)*

6. Menstrual cycle

PRACTICAL WORK MENSTRUAL CYCLE

Conducting practical work on the menstrual cycle involves studying its various phases and the hormonal changes that occur in women. This type of work typically uses observational and analytical methods, as direct experimentation is not feasible due to ethical considerations. Below is a suggested protocol:

Objective

To understand the physiological and hormonal changes during the menstrual cycle and their impact on various bodily functions and behaviors.

Materials Needed

- ✓ Volunteers: A group of women with regular menstrual cycles
- ✓ Menstrual cycle tracking apps or calendars
- ✓ Hormone assay kits (for estrogen, progesterone, FSH, LH)
- ✓ Questionnaires for symptom tracking (mood, physical changes, etc.)
- ✓ Standardized physical and psychological tests (if applicable)
- ✓ Equipment for collecting biological samples (blood, saliva, urine)
- ✓ Data analysis software

Methodology

step 1: Volunteer Recruitment and Briefing

- Recruit a group of women with regular menstrual cycles.

- Brief them about the study's purpose, procedures, and ethical considerations, ensuring informed consent is obtained.

Step 2: Cycle Tracking and Data Collection

- Ask volunteers to track their menstrual cycles using apps or calendars for at least 1-2 cycles before the study begins.
- Schedule sample collection (like blood, saliva, or urine) at various phases of the cycle (e.g., menstrual, follicular, ovulatory, luteal).

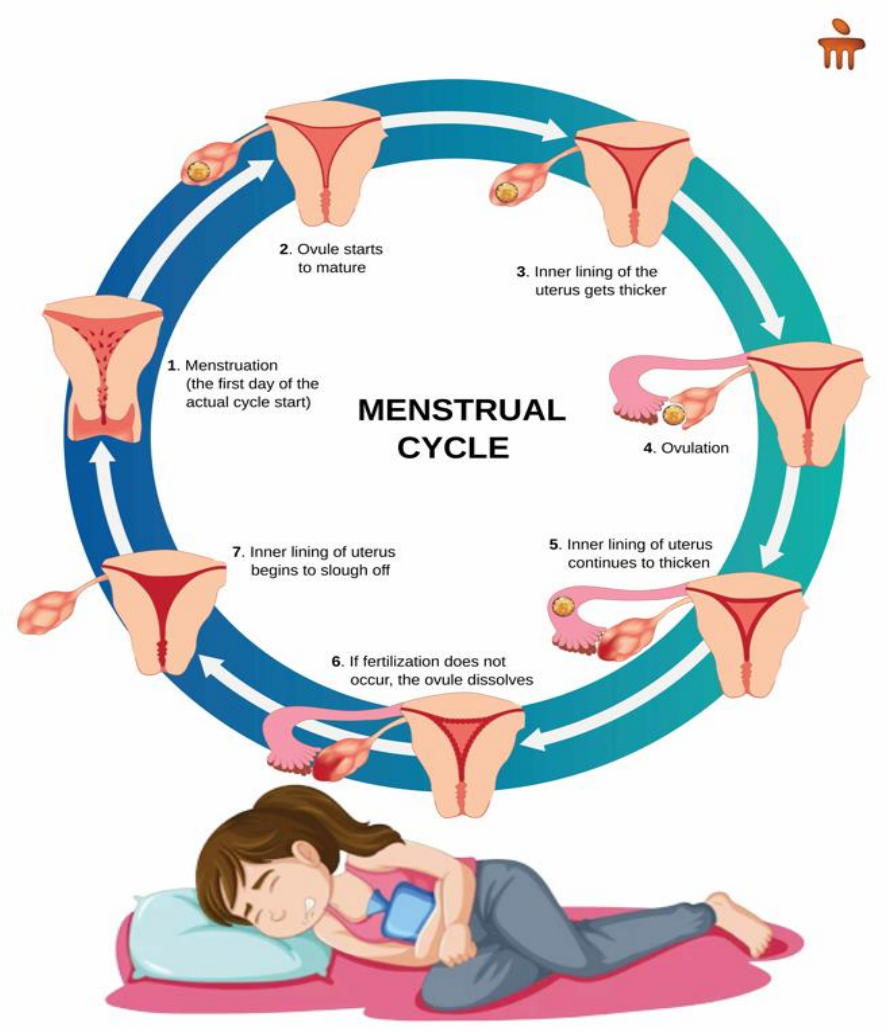

*Figure 21: Understanding Menstrual Cycle: Phases, Hormonal Changes (*Understanding The Menstrual Cycle: It's More Than Just Your Periods*, n.d.)*

Step 3: Hormonal Assays

- Use hormone assay kits to measure levels of estrogen, progesterone, FSH, and LH in collected samples.
- Aim to establish a hormonal profile for each phase of the menstrual cycle.

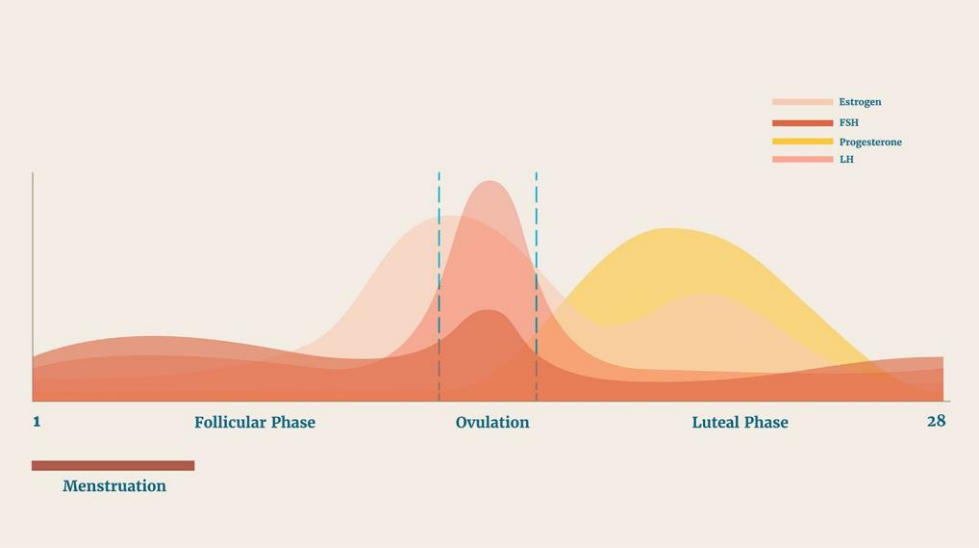

Figure 22:Throughout the phases of menstrual cycle, hormone levels fluctuate, which can affect the emotions.

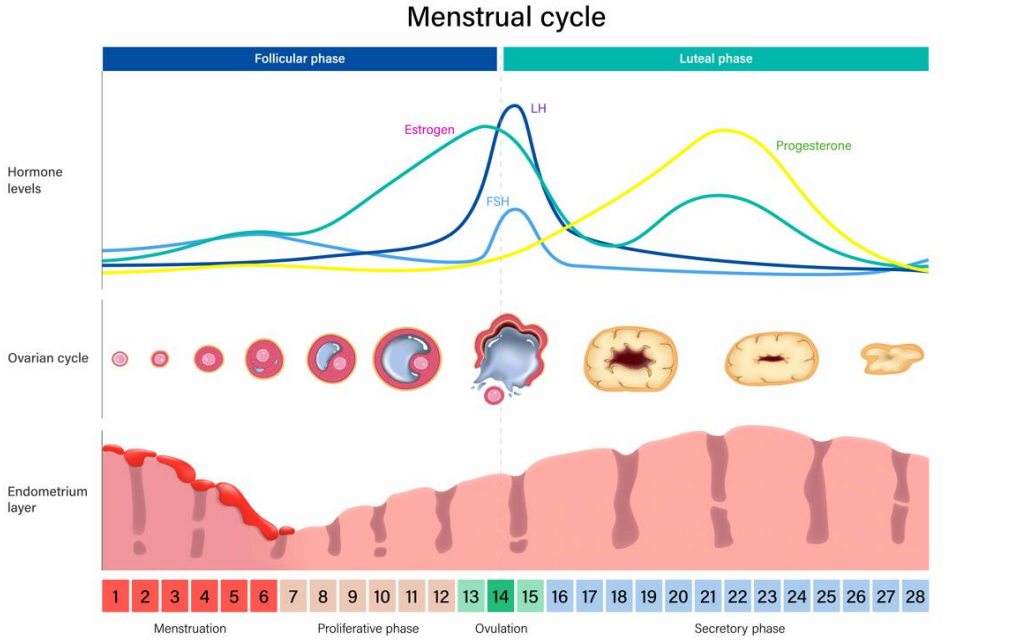

*Figure 23:Menstrual cycle phases 28 days (*Menstrual Cycle - Women's Health Network, *n.d.;* Understanding The Menstrual Cycle: It's More Than Just Your Periods, *n.d.)*

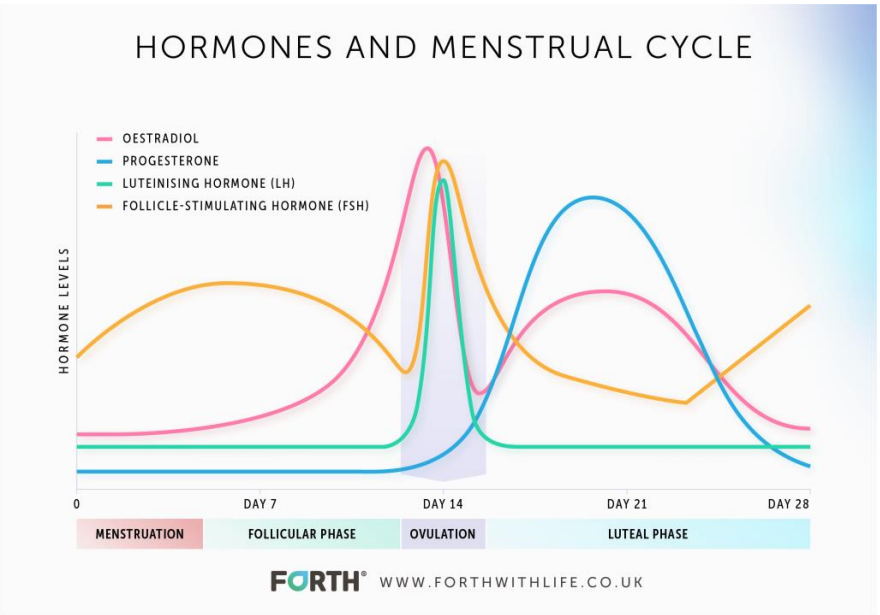

*Figure 24: Hormones and Menstrual Cycle(*Menstrual Cycle | Description, Phases, Hormonal Control, Ovulation, & Menstruation | Britannica*, n.d.)*

Step 4: Symptom and Behavior Logging

- Provide questionnaires for volunteers to log physical symptoms (like cramping, bloating, breast tenderness) and psychological symptoms (mood changes, energy levels).
- Optionally, conduct standardized physical and psychological tests at different cycle phases to assess changes in performance or mood.

Step 5: Data Analysis

- Analyze the hormonal data in conjunction with symptom logs and test results.
- Look for patterns and correlations between hormone levels and reported.
- symptoms or test outcomes

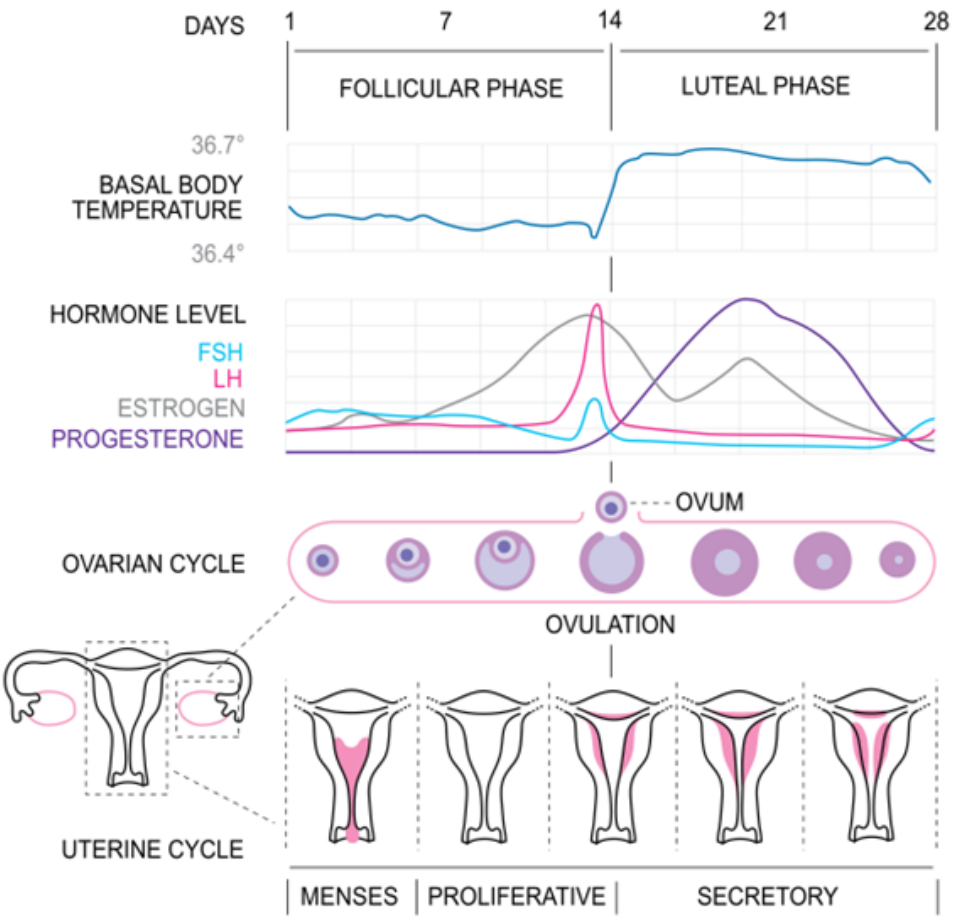

Figure 25: Comprehensive Overview of the Female Reproductive Cycle(Alvergne & Högqvist Tabor, 2018; Menstrual Cycle | Description, Phases, Hormonal Control, Ovulation, & Menstruation | Britannica*, n.d.)*

Step 6: Report Writing

- Compile the findings into a detailed report.
- Discuss the implications of hormonal fluctuations on physical and psychological well-being.
- Highlight any significant patterns or correlations found.

Ethical Considerations

- Ensure the privacy and confidentiality of all participants.

- Make sure participants understand they can withdraw from the study at any time without any consequences.
- Handle all biological samples with care, following proper biohazard disposal protocols.

Conclusion

This practical work will provide valuable insights into the menstrual cycle's effects on women's physical and psychological health. It can be particularly informative for fields such as gynecology, endocrinology, and psychology.

7. Puberty and menopause

PRACTICAL WORK PUBERTY AND MENOPAUSE

Objective

To identify the physiological and molecular changes associated with puberty and menopause. This involves detailed analysis of hormonal profiles, reproductive organ development during puberty, and changes occurring in the reproductive system as the mice approach and enter menopause.

Materials Needed

Mice

- Female mice of a genetically uniform strain, starting pre-puberty (around 3-4 weeks old) for puberty studies.
- For menopause studies, older female mice (approximately 9-12 months old) can be used to simulate peri-menopausal to menopausal stages.

Housing

- Cages with environmental enrichment.
- Controlled environment (temperature, humidity, light-dark cycle).

Nutrition

- Standard laboratory mouse diet and water ad libitum.
- Monitoring and Sampling Equipment:
- Vaginal cytology kit for monitoring estrous cycles.
- Microscope for analyzing vaginal smears.
- Blood collection materials for hormonal assays.
- Scales for body weight measurement.
- Equipment for behavioral assays (activity monitors, video cameras).

Analytical Tools

- ELISA kits or radioimmunoassay (RIA) materials for hormone level measurements (FSH, LH, estrogen, progesterone).
- PCR machines, reagents for gene expression studies.
- Histology equipment for tissue analysis.

Methodology

Physical and Behavioral Observations:

- Females: Look for the vaginal opening and first estrus, which are indicators of puberty. These can be monitored through daily examination and vaginal smear tests.
- Males: Testicular development and the first appearance of sperm in urine can indicate the onset of puberty. Monitor for changes in testis size and the presence of sperm.

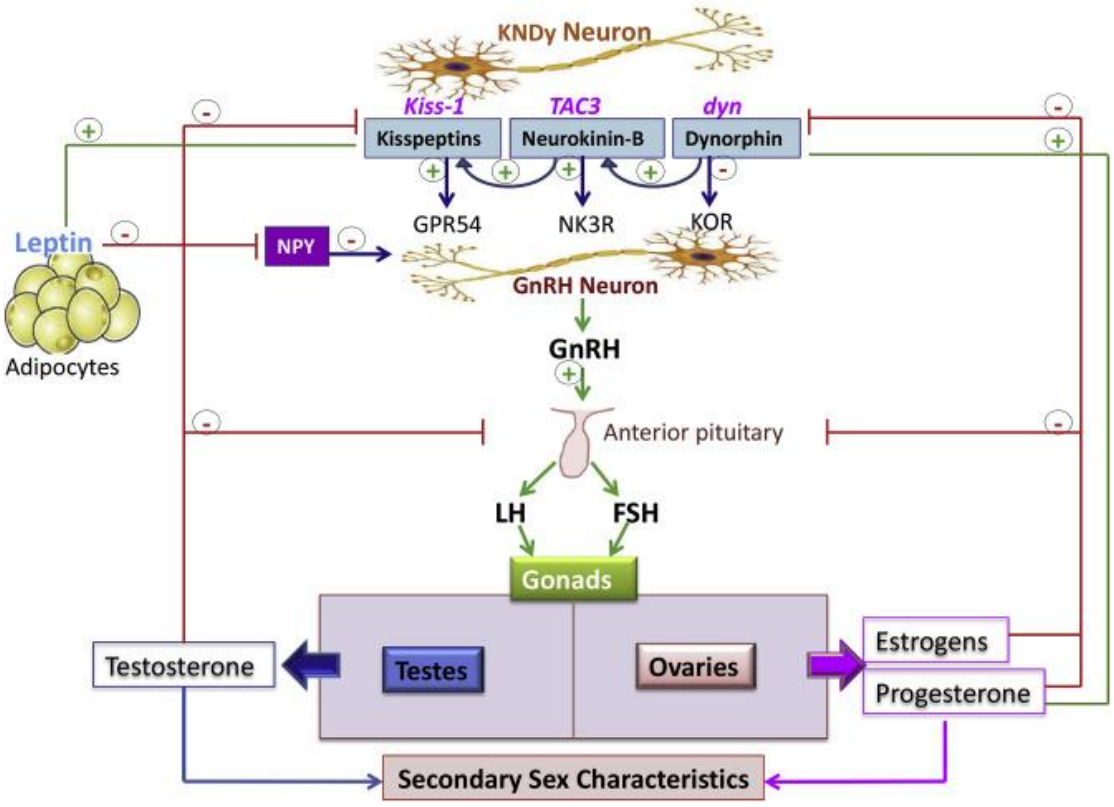

Figure 26:The release of FSH and LH is pulsatile in nature consequent to the pulsatile release of GnRH.(GnRH Analogs - Indications & Use, *n.d.)*

- Hormonal Assays: Measure levels of sex hormones such as estrogen, testosterone, and luteinizing hormone (LH) to confirm the onset of puberty. These can be measured through blood samples.

- Environmental and Genetic Factors: Study the effects of environmental factors (like diet and light exposure) and genetic modifications on the timing of puberty.

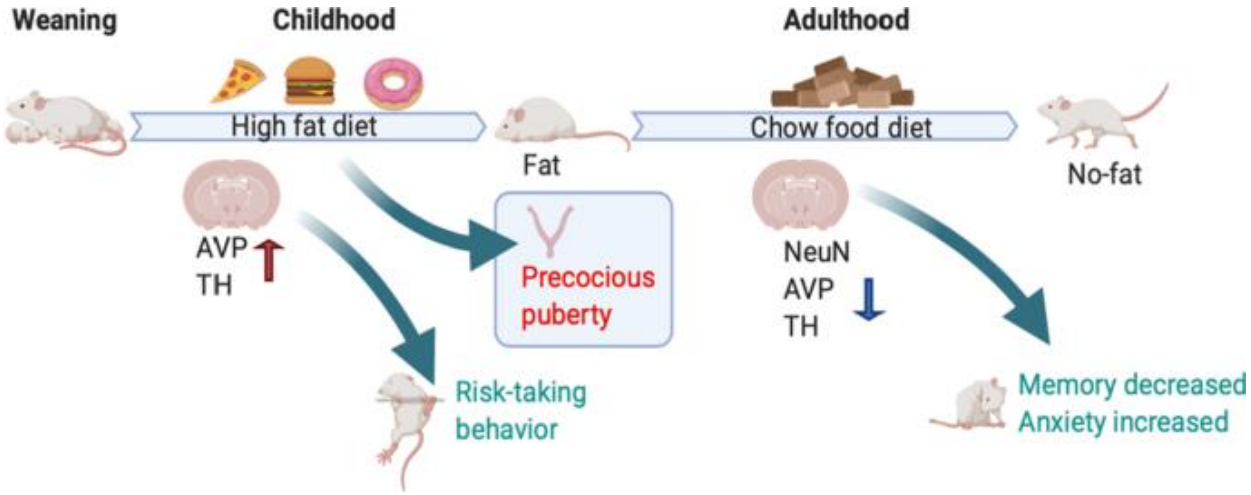

Figure 27:Impact of Dietary Fat on Developmental Stages and Behavioral Outcomes in Mice(Sullivan et al., 2012)

For Menopause (or its murine equivalent, Reproductive Senescence)

- Selection of Mice: Older female mice are required for studying menopause, as mice do not experience menopause in the same way humans do. Instead, they go through reproductive senescence, a gradual decline in fertility.
- Monitoring of Estrous Cycle: Regular monitoring of the estrous cycle through vaginal cytology can help identify changes in cycle regularity, length, and eventual cessation, indicative of reproductive senescence.
- Hormonal Assays: Assess levels of reproductive hormones such as estrogen, follicle-stimulating hormone (FSH), and LH, which change with age and can indicate the onset of reproductive senescence.

- Ovarian Histology: Examine the morphology of the ovaries to observe changes in follicle number and quality, which decline with age.
- Lifespan and Aging Studies: Since menopause is associated with aging, studying the overall health, lifespan, and age-related diseases in mice can provide insights into the effects of reproductive aging.
- Genetic and Environmental Influences: Investigate how genetic modifications or environmental factors (diet, exercise, environmental toxins) impact the timing and progression of reproductive senescence.

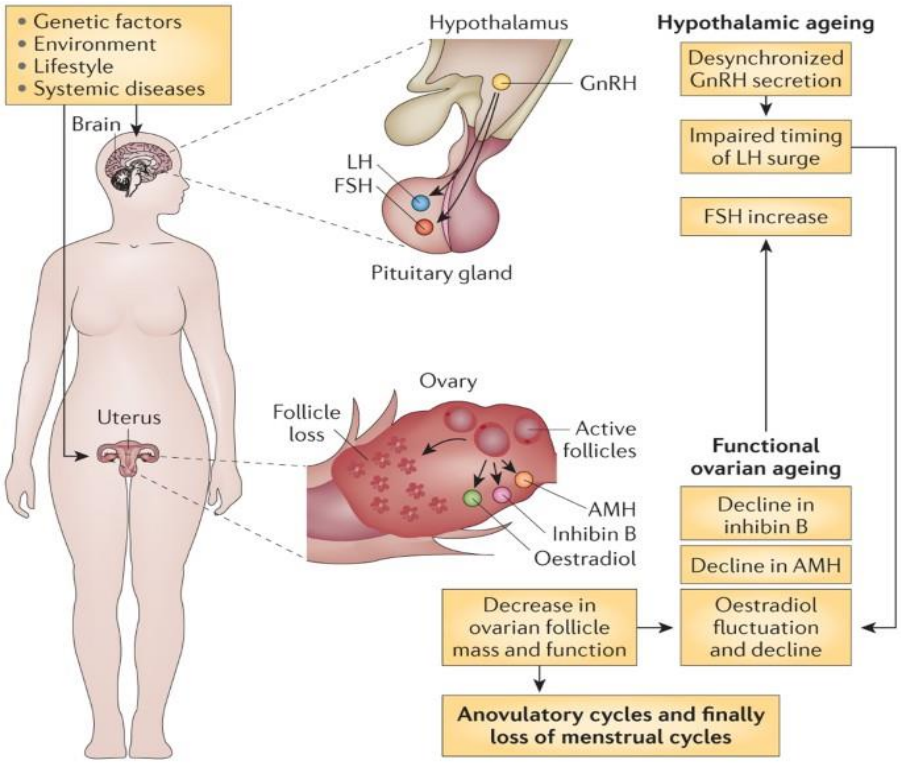

Figure 28:Mechanisms of Reproductive Aging: Hypothalamic-Pituitary-Ovarian Axis and Hormonal Dynamics(Mikkonen et al., 2023)

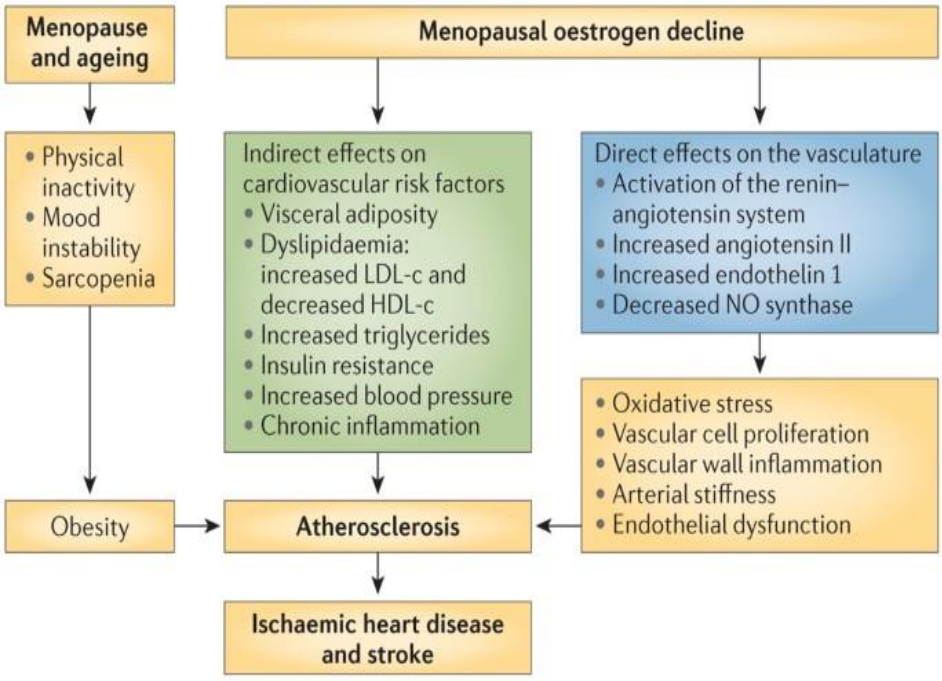

Figure 29:Pathways of Cardiovascular Risk in Menopause: The Role of Estrogen Decline(Mikkonen et al., 2023)

Ethical Considerations

- Ensure all experimental protocols comply with institutional and national ethical guidelines for the care and use of laboratory animals.
- Minimize animal distress through proper handling, housing, and enrichment.
- Use the minimum number of animals necessary to achieve statistical significance.

Data Analysis

- Statistical analysis of the collected data will be crucial to understand the onset and progression of puberty and reproductive senescence.
- Use appropriate controls to distinguish between normal aging processes and experimentally induced changes.

- This general protocol can be adjusted based on specific research questions and objectives. It's important to stay updated with the latest literature and methodologies, as the field continuously evolves.

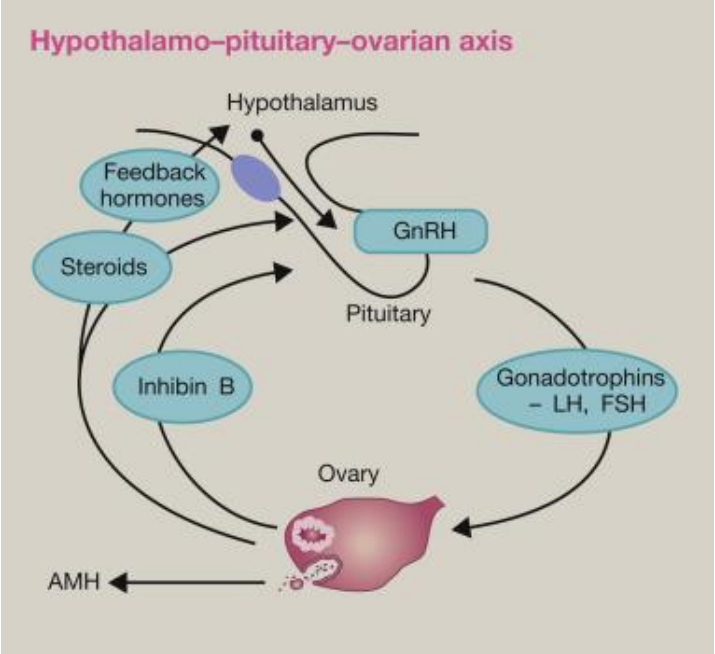

Figure 30: Diagrammatic representation of the hypothalamo–pituitary–ovarian relationships. (Mikkonen et al., 2023)

8. Physiological modification of pregnancy

PRACTICAL WORK PHYSIOLOGICAL MODIFICATION OF PREGNANCY

Objective

To explore and understand the physiological changes that occur during pregnancy in mice, including hormonal, cardiovascular, renal, and metabolic modifications.

Materials Needed

- ✓ **Breeding Mice: Suitable strains for breeding and pregnancy studies.**
- ✓ **Animal Housing:** Cages, bedding, and controlled environment facilities. Monitoring Equipment: Scales for weighing, ultrasound for pregnancy confirmation and monitoring and Equipment to monitor vital signs (e.g., heart rate monitors)
- ✓ **Data Recording Tools:** Charts, software, or lab notebooks for documenting observations.
- ✓ **Standard Lab Supplies:** Gloves, disinfectants, syringes for possible blood sampling**.**

Methodology

Step 1: Preparation and Ethical Considerations

- Preparation of Mice: Select healthy female mice and appropriate male mice for breeding. Confirm pregnancy through physical signs or ultrasound.

Step 2 : Monitoring Gestational Development

- Daily Observations: Monitor and record weight gain, food and water intake, and general behavior.
- Ultrasound Monitoring: Periodically check fetal development and count using ultrasound equipment.

Step 3 : Study of Physiological Changes

- Cardiovascular Monitoring: Measure heart rate and blood pressure at various stages of gestation to observe changes.
- Blood Sampling: (if approved) Collect blood samples to measure hormonal levels (e.g., progesterone, estrogen) and other physiological markers like glucose levels.
- Renal Function: Observe changes in urine output and composition, indicating renal adaptation during pregnancy.

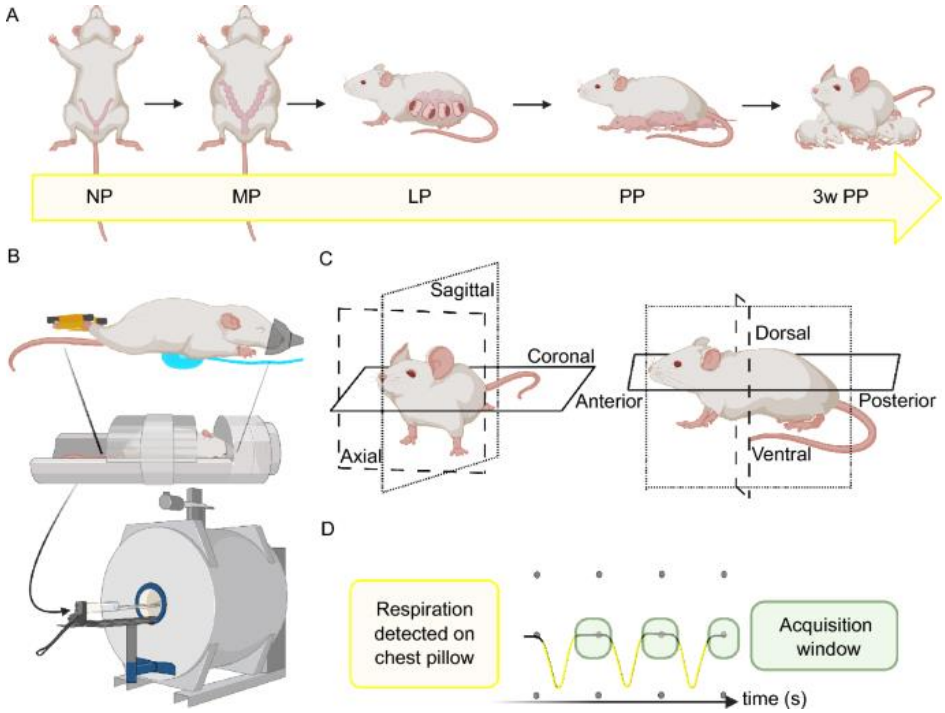

Figure 31:Pregnancy-induced remodeling of the murine reproductive tract.(Suarez et al., 2024)

Step 4: Labor and Delivery Observation

- Preparation: As the expected delivery date approaches, increase observation frequency to capture the onset of labor.
- Labor Monitoring: Document the labor stages, duration, and any complications. Ensure minimal stress and humane handling.
- Postpartum Recovery: Monitor the mother for postpartum health and the initiation of nursing.

Step 5: Data Analysis

- Group Discussions: Analyze the collected data in group settings to identify patterns and discuss physiological implications.
- Comparative Analysis: Compare observed data with established literature to understand deviations or confirmations.

Step 6: Reporting and Documentation

- Report Writing: Students should prepare a detailed report outlining their methodology, observations, data analysis, and conclusions.
- Presentation: Encourage students to present their findings to the class, fostering a deeper understanding through peer learning.

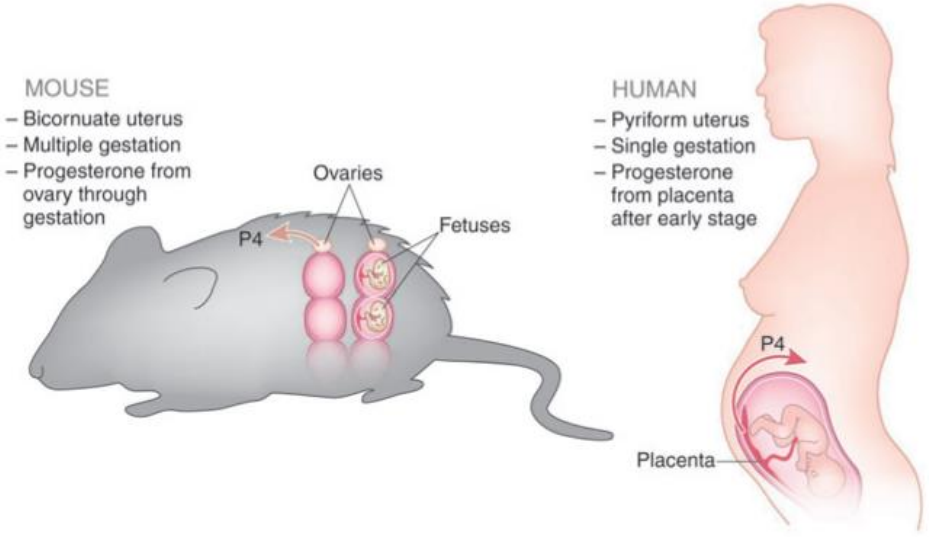

Figure 32:. Comparison of human and mouse pregnancy. Fundamental differences in the anatomy and physiology between these species(Ratajczak et al., 2010)

Follow-up Care

Animal Care: Post-study, ensure all animals receive appropriate care and monitoring, respecting ethical guidelines for post-research animal handling.

Conclusion

This session aims to provide students with a comprehensive understanding of pregnancy in a mammalian model, emphasizing real-world research techniques and ethical considerations. By the end of the session, students should be able to link theoretical knowledge with practical observations and understand the complexities involved in physiological studies during pregnancy.

9. Dissection of Pregnant Mice

PRACTICAL WORK DISSECTION OF PREGNANT MICE

Objective

To provide students with hands-on experience in understanding the anatomical and physiological changes during mouse pregnancy, focusing on the development of the reproductive system and the impact on other organ systems.

Materials Needed

- ✓ **Breeding Mice Specimens:** Ethically sourced pregnant mouse specimens at various gestation stages.
- ✓ **Dissection Tools:** Scalpels, scissors, forceps, pins, dissection trays, and magnifying glasses or dissecting microscopes.
- ✓ **Protective Equipment:** Gloves, lab coats, goggles.
- ✓ **Documentation Tools:** Cameras for photographing dissection stages, notebooks for observations, diagrams for reference.
- ✓ **Disposal Supplies:** Biohazard containers for proper disposal of biological waste

Methodology

Preparation and Ethical Considerations

- Ethical Training: Brief all participants on the ethical aspects and the importance of respectful handling of animal specimens.
- Background Lecture: Provide a lecture on mouse reproductive anatomy, the stages of pregnancy, and expected physiological changes.
- Safety Briefing: Review safety procedures, including the proper use of dissection tools and disposal of biological materials.

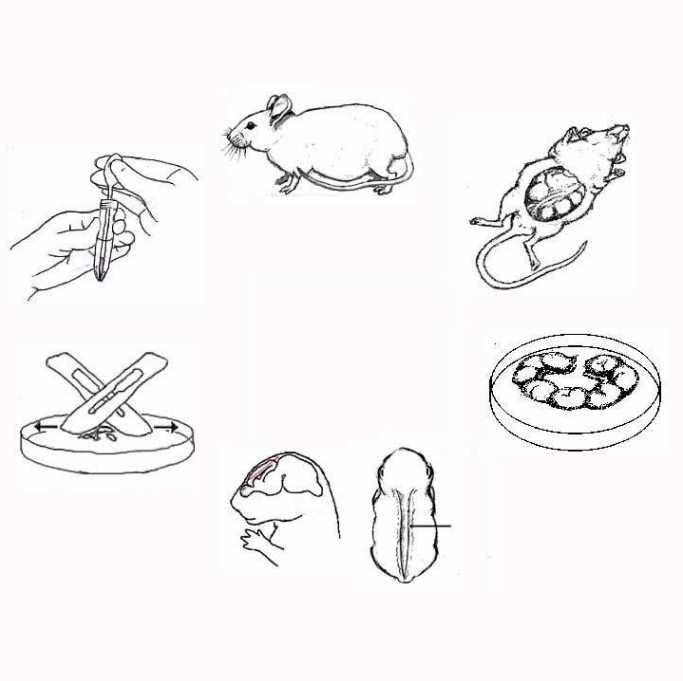

*Figure 33:Illustrative Guide to Mouse Dissection and Anatomical Study(*Vascular Microdissection, Perfusion, and Excision of the Murine Arterial Tree for Use in Atherogenic Disease Investigations*, n.d.)*

Step 1: External Examination

- Initial Observation: Record external features and any signs indicating the stage of pregnancy.
- Body Measurements: Measure and record body weight and size.

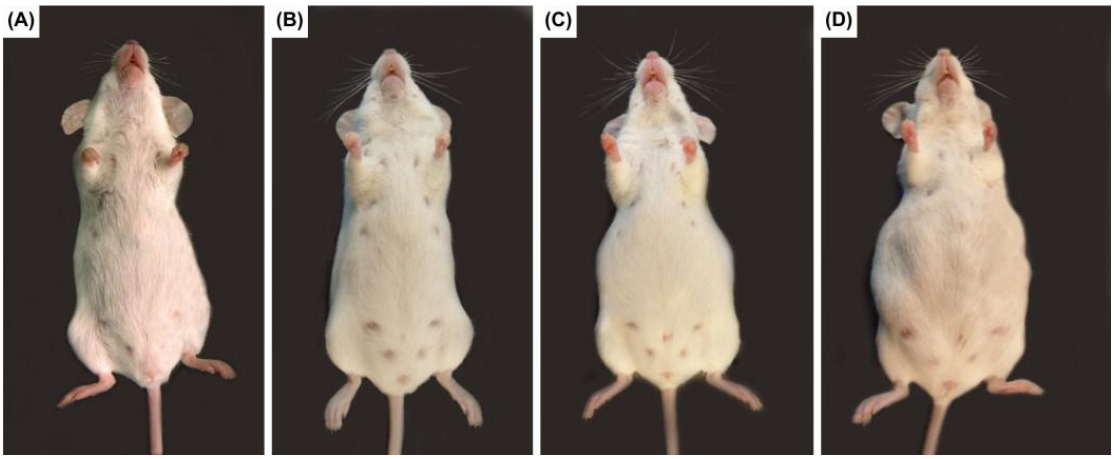

Figure 34:Physical changes in body appearance during a mouse pregnancy—ventral view(Suarez et al., 2024)

Step 2: Opening the Specimen

- Ventral Incision: Make a careful midline incision from just below the chin to the pelvic area, avoiding deep cuts to protect internal organs.
- Skin and Muscle Reflection: Reflect the skin and abdominal muscles to expose the abdominal cavity.

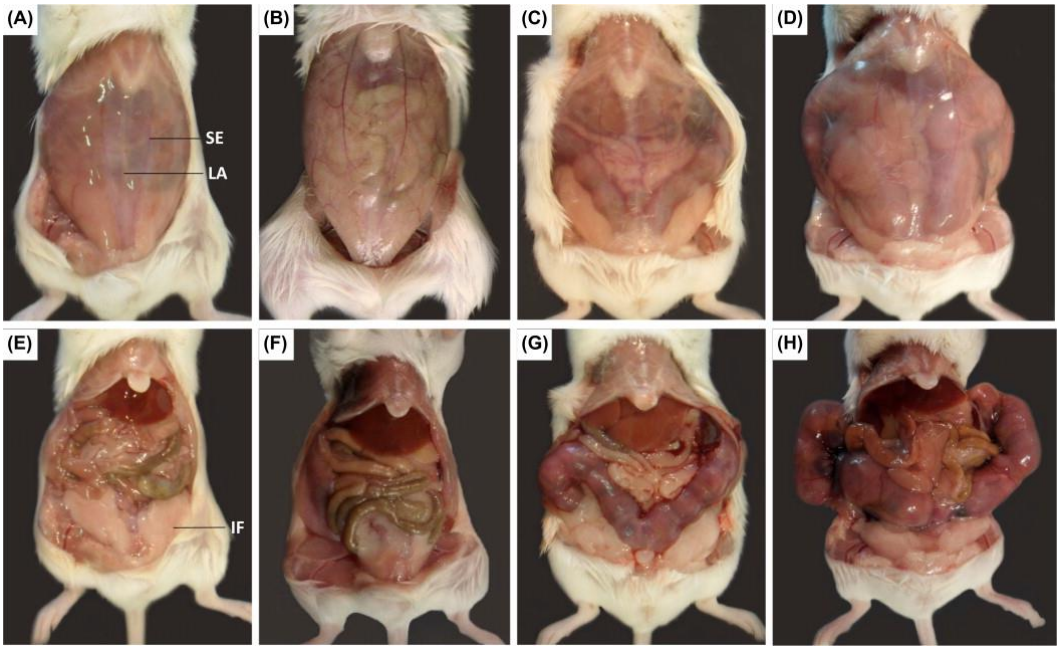

Figure 35:The pregnant mouse abdomen, gestational days 5.5–18.5—ventral view: abdominal wall intact (upper row: A–D) and abdominal wall opened (lower row: E–H).(Suarez et al., 2024)

Step 3: Reproductive System Examination

- Uterus Identification: Locate and gently expose the uterus. Note the size and number of embryonic chambers.
- Fetus Observation: Count and examine the fetuses, noting their development stage.

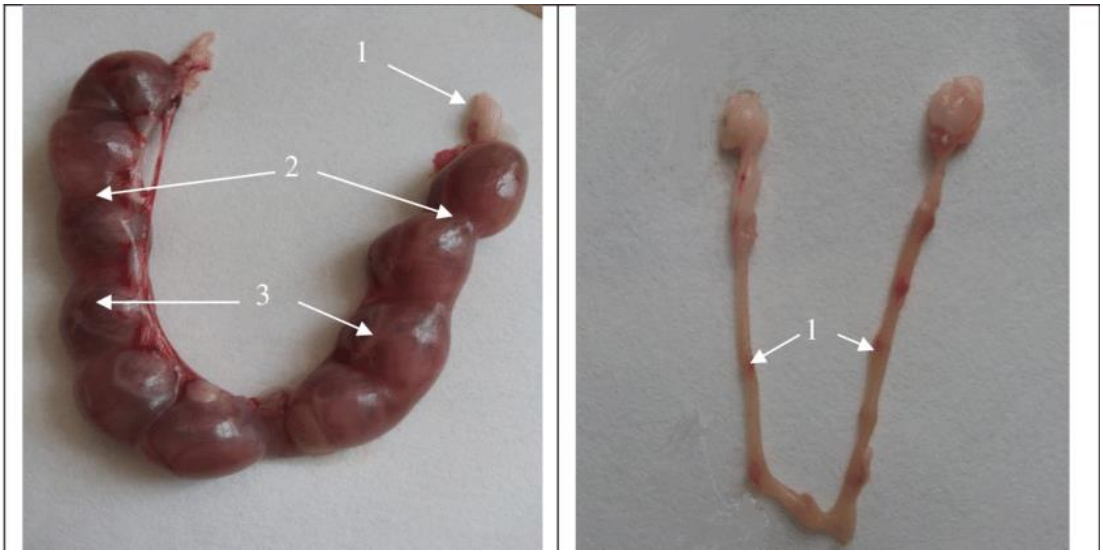

Figure 36:Reproductive system of pregnant mouse treated with OTA (4mg/kg) showing: resorption of embryo in uterine horns (1). Figure 10. Reproductive system of control pregnant mouse showing: ovary (1), uterine horns (2), embryos (3) .(Al-Timimi & Gali, 2018)

- Placenta Examination: Discuss the function and structure of the placenta, observing its attachment to the uterus and fetal membranes.

Step 4: Examination of Other Organ Systems

- Digestive System: Identify changes in the gastrointestinal tract positioning and size due to pregnancy.
- Respiratory System: Observe lung positioning and any observable impact of the enlarged uterus.

- Cardiovascular System: Note any visible changes in blood vessel size or configuration, especially those supplying the reproductive organs.

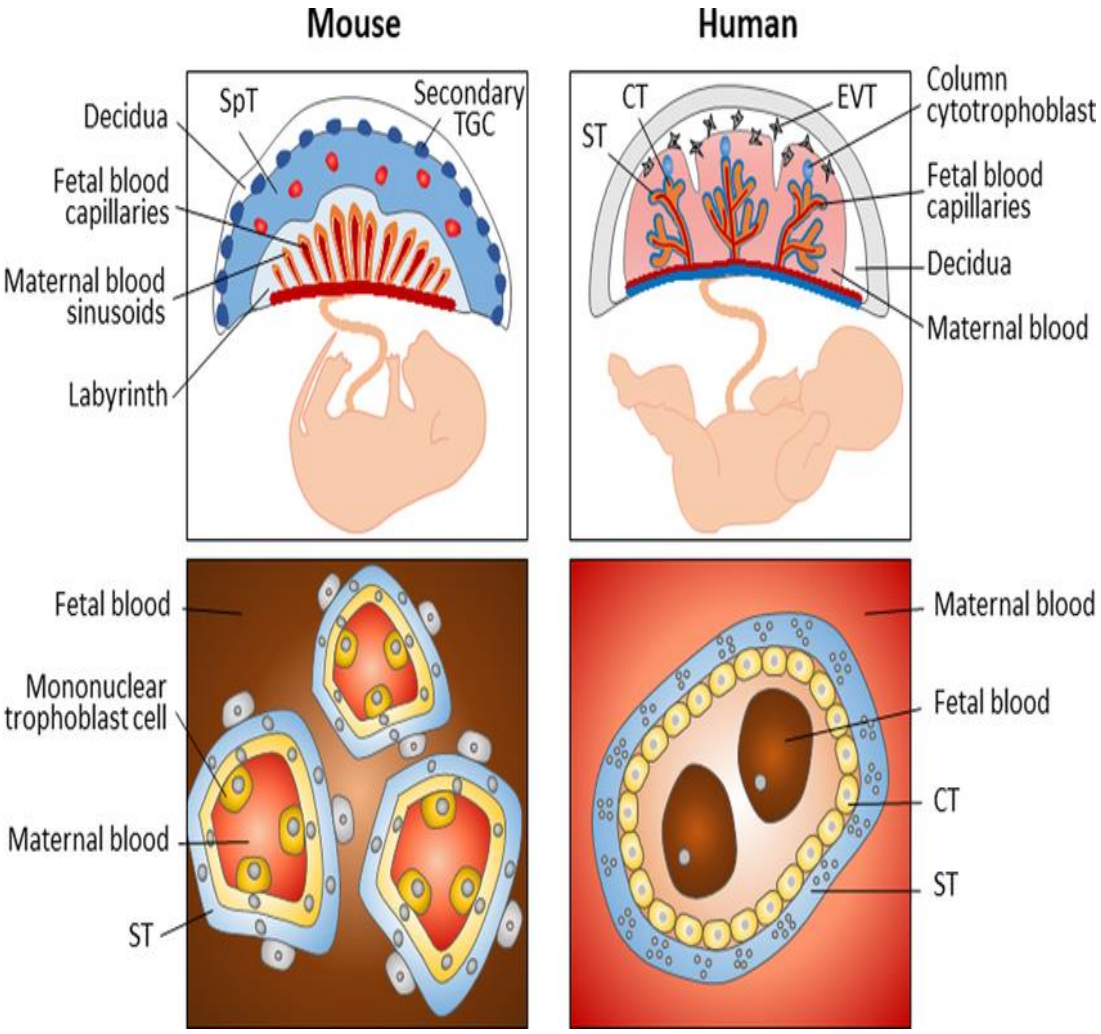

Figure 37:Structure of human and mouse mature placenta. Structure of the mouse placenta. The inset details the fetal-maternal interface in the labyrinth. Structure of the human placenta(Shibata et al., 2020)

Step 5: Mammary Gland Observation

- Gland Identification: Locate the mammary glands and discuss their enlarged state and role during and post-pregnancy.
- Microscopic Examination: Examine gland tissue under a microscope to identify changes at the cellular level.

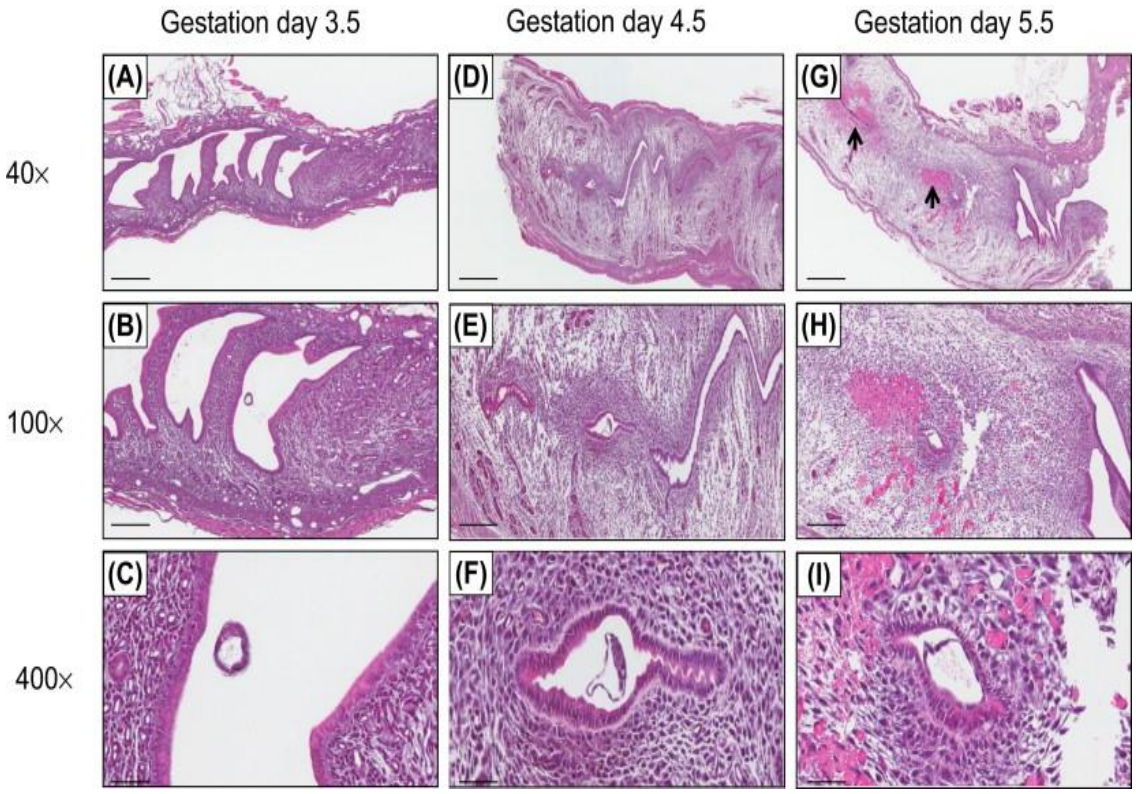

Figure 38:. Hematoxylin and eosin–stained longitudinal sections of gd3.5–5.5 implantation sites.(Waterhouse et al., 2014)

Step 6: Documentation and Discussion

- Record Findings: Students should document their observations with sketches or photographs.
- Group Discussion: Engage in a discussion about the physiological significance of the observed changes and their impact on the health and behavior of the mouse.

Step 7: Cleanup and Ethical Considerations

- Proper Disposal: Ensure all tissues and biological materials are disposed of according to biohazard protocols.
- Tool Cleaning: Clean and sterilize all dissection tools and trays.

- Debrief: Discuss the dissection experience and any emotional impacts, reinforcing the importance of ethical considerations in scientific research.

Conclusion

This dissection lab provides a comprehensive exploration of pregnancy in mice, offering students practical insights into mammalian reproductive physiology and the associated changes in other organ systems. This protocol not only fosters a deeper understanding of biological concepts but also cultivates respect for ethical practices in biological research.

10. Physiology of Parturition

PRACTICAL WORK PHYSIOLOGY OF PARTURITION

Objective

To understand the physiological and hormonal changes during parturition in mice, and to observe the stages of labor and delivery in a controlled laboratory setting.

Materials Needed

- ✓ **Species and Strain**: Use a suitable mouse strain known for reproductive studies, like C57BL/6, BALB/c
- ✓ **Animal housing facilities**: Cages, bedding, and environment control systems to mimic natural conditions and ensure animal welfare.
- ✓ **Laboratory equipment:**
  - Video recording equipment to monitor and record labor and delivery.
  - Weighing scales to monitor the weight of pregnant mice.
  - Standard laboratory supplies (gloves, disinfectants, etc.).
  - **Data recording tools**: Sheets or software to record observations and data points.

Ethical Approval

- Obtain approval from your institution's animal care and use committee.

Animal Selection:

- Select healthy, adult female mice and appropriate male mice for breeding.
- Monitor and record the health status and weight of female mice regularly.
- Confirm pregnancy through physical examination and possibly ultrasound if available.

Methodology

Monitoring the Gestation Period

- Record the day when pregnancy is confirmed to accurately predict the parturition date (mice gestation is approximately 19-21 days).
- Observe and record any physiological changes as the pregnancy progresses, such as weight gain and nesting behavior.

Labor and Delivery Observation

- As the expected date of parturition approaches, increase the frequency of observations.
- Utilize video recording to capture the labor process, which can be divided into three stages:
    1. The onset of labor: Characterized by restlessness and nesting behavior.
    2. Active labor: Delivery of pups. Each pup is typically delivered in a separate amniotic sac.
    3. Afterbirth: Delivery of the placenta. Each pup is followed by a placenta; it is crucial to ensure all placentas are expelled to avoid health complications.
- Monitor and assist if necessary, ensuring minimal stress and intervention.

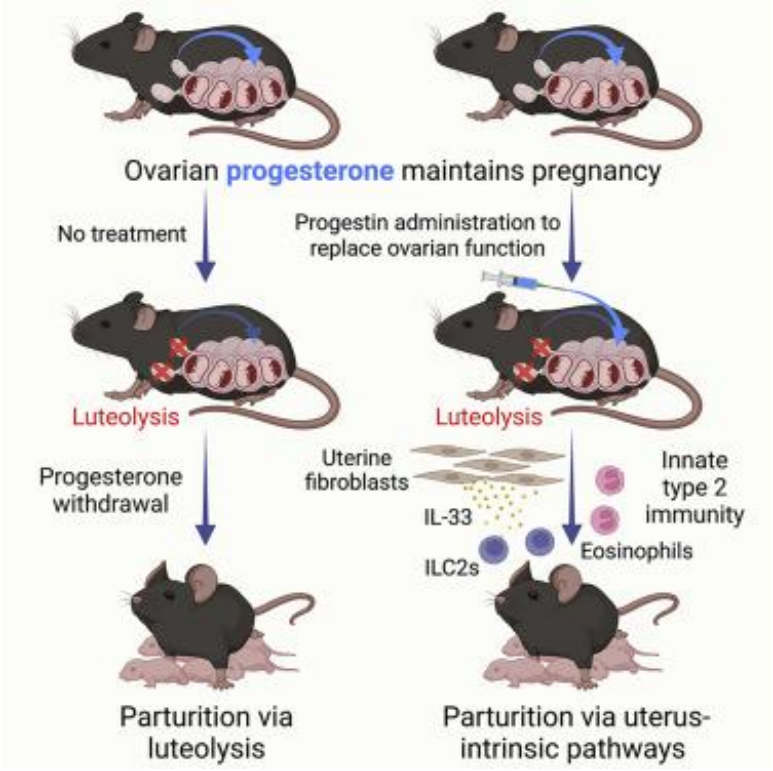

Figure 39:Progesterone Regulation and Immune Pathways in Mouse Parturition(Siewiera et al., 2023)

Post-Partum Observations

- Monitor the mother for any signs of distress or health complications after delivery.
- Ensure that the mother is nurturing the pups properly and that the pups are nursing.
- Record the number of pups born, any stillbirths, and the general health of the newborns.

Data Analysis and Reporting

- Analyze the data collected during the observation period.
- Compare it with existing literature to understand any deviations or confirmations of established patterns.

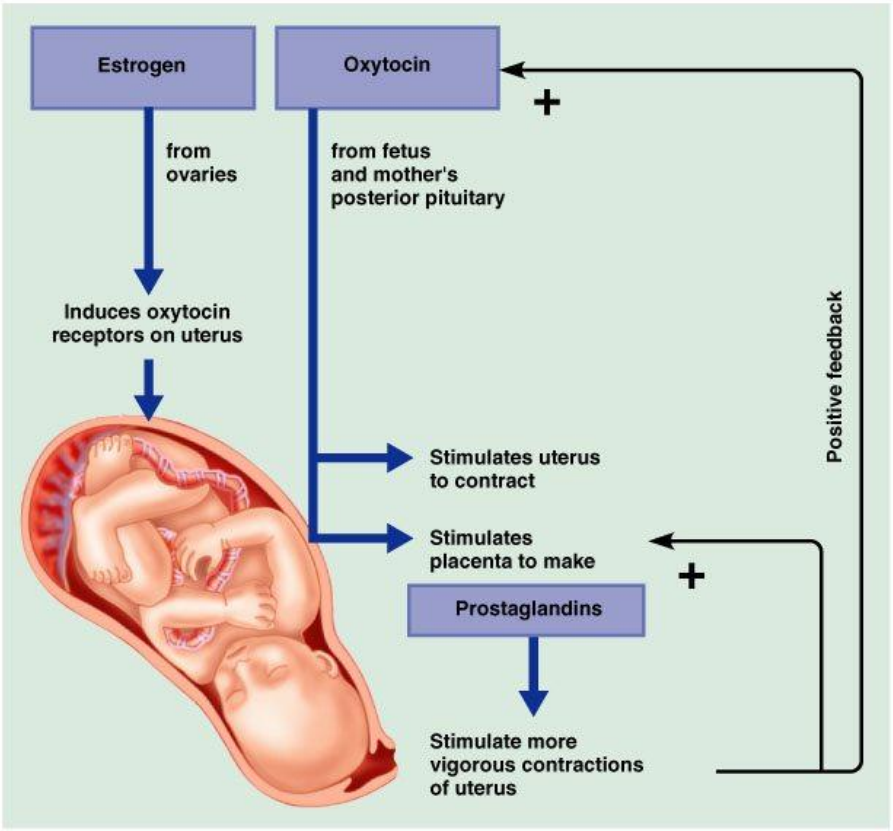

*Figure 40:Hormonal Interactions in Labor Induction: Estrogen, Oxytocin, and Prostaglandins(*What Hormone Causes Uterine Contractions*, n.d.)*

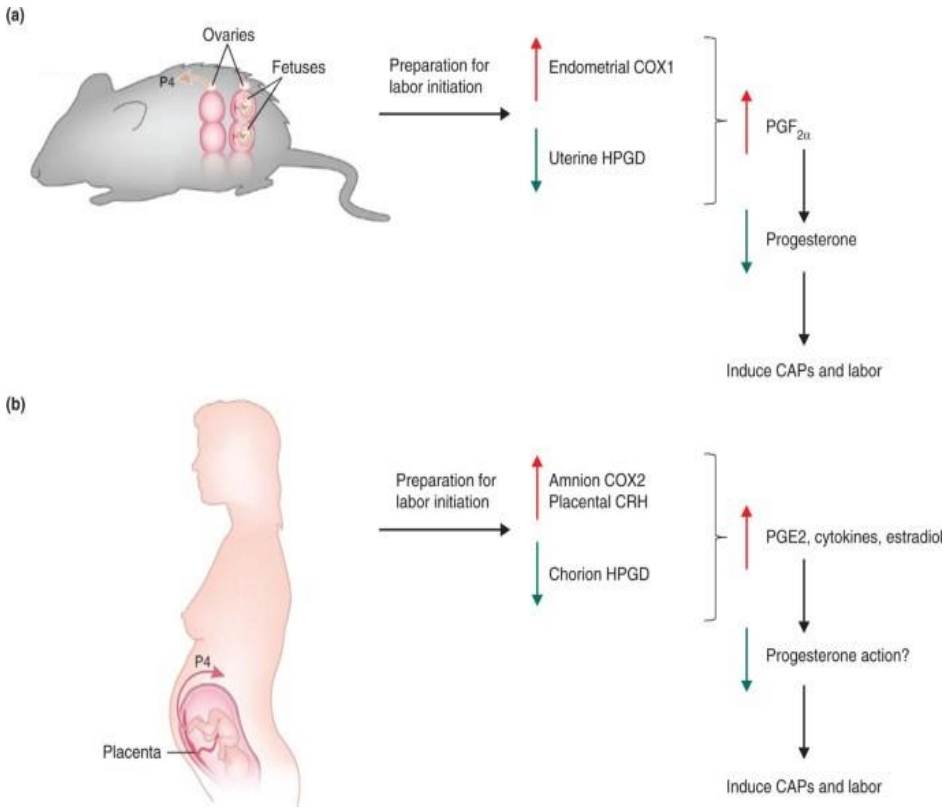

Figure 41:Comparison of mouse and human parturition(Bezold et al., 2013).

Follow-up

- Provide care for the mother and pups post-parturition.
- Plan for the pups' weaning and subsequent housing or use in further studies.

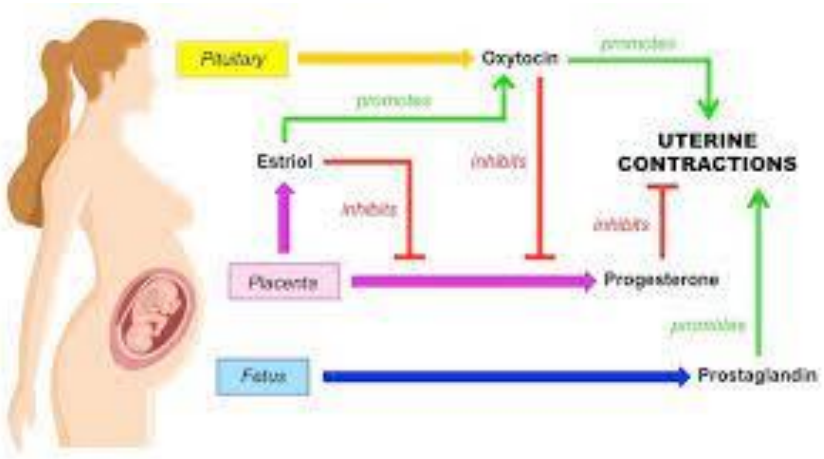

*Figure 42:Regulation of Uterine Contractions: Hormonal Pathways in Labor(*Childbirth: The Role of Hormones in Labor and Delivery - Lesson | Study.Com, *n.d.)*

Notes

Always ensure humane treatment of animals in accordance with ethical guidelines. Be prepared for emergencies or complications and have veterinary support available.

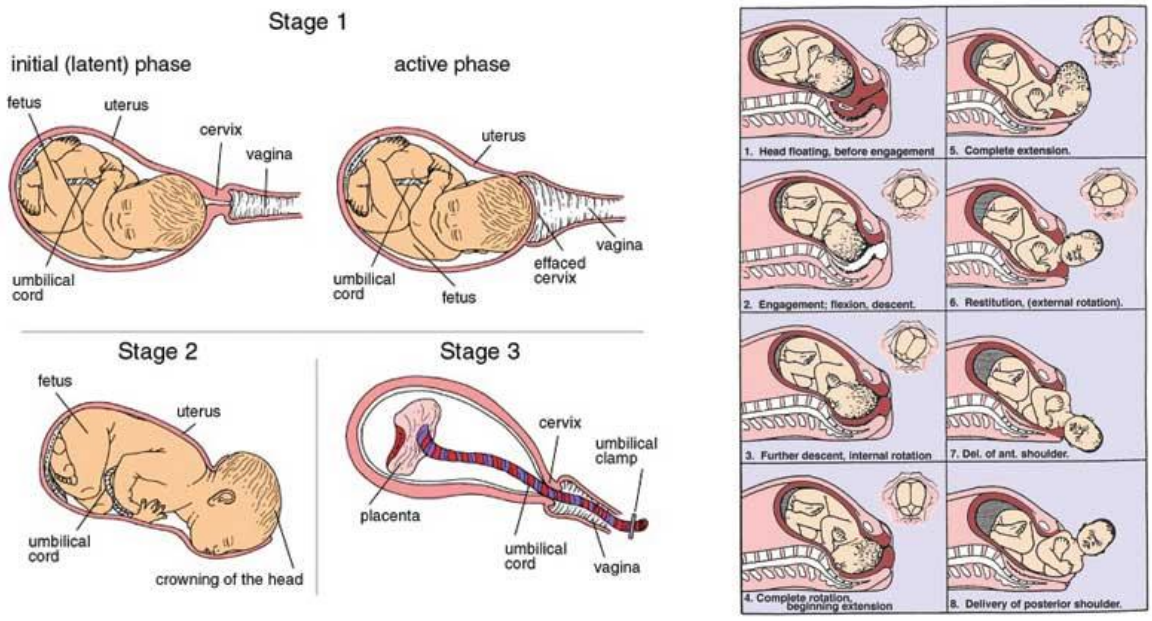

*Figure 43:Stages of Labor: From Latent Phase to Delivery of the Placenta(*Overview of Labor - Stanford Medicine Children's Health*, n.d.)*

11. Physiology of lactation

PRACTICAL WORK PHYSIOLOGY OF LACTATION

Objective

To explore and understand the physiological mechanisms, hormonal regulation, and nutritional aspects of lactation in laboratory animals.

Materials Needed

- ✓ **Lactating Mice**: Select female mice that are currently lactating, having recently given birth.
- ✓ **Microscopes and Slides**: For examining milk samples and possibly mammary gland tissue.
- ✓ **Syringes or Pipettes**: For collecting milk samples.
- ✓ **Scales**: For weighing pups before and after feeding to measure milk intake.
- ✓ **Reference Materials**: Texts and articles on mammalian lactation physiology.
- ✓ **Protective Equipment**: Gloves, lab coats, and goggles for safety.

Methodology

1. Introduction to Lactation Physiology

Lecture: Begin with a lecture on the basics of lactation, including the anatomy of the mammary glands, hormonal influences (prolactin, oxytocin), and the stages of lactation (lactogenesis, galactopoietic, and involution).

Discussion: Discuss the importance of lactation in mammalian reproductive strategies and offspring survival.

2. Observation and Handling

Handling Tutorial: Demonstrate how to handle lactating mice safely and ethically to minimize stress.

Visual Inspection: Observe the mammary gland engorgement and the behavior of the pups during nursing.

4. Milk Collection and Analysis

- *Milk Extraction: Carefully extract milk using a gentle manual expression technique or with a small syringe. This must be done by an experienced handler to avoid harm.*
- Milk Examination:

1. Use slides and microscopes to observe the composition of the milk.
2. Discuss the nutritional content and the presence of antibodies (immunoglobulins).

4. Measuring Milk Production

- Weighing Pups: Weigh pups before and after feeding to estimate milk intake.

- Data Recording: Document the weights and calculate the average intake per pup to assess the adequacy of milk production.

*Figure 44:A laboratory mouse feeds her newborn pups that are about the size of a quarter and weigh less than an ounce.(*Of Mice and Babies: New Mouse Model Links Tra | EurekAlert!*, n.d.)*

4. Hormonal Regulation Experiments

- Hormonal Influence: If applicable, discuss or simulate how the administration of hormones like prolactin or oxytocin affects milk production and ejection.

- Observation: Note changes in behavior or milk yield as a result of hormonal influence

6. Mammary Gland Dissection (Optional)

- Dissection: If permitted, conduct a dissection of euthanized specimens to examine the anatomy of the mammary glands.

- Microscopic Examination: Look at the tissue structure, noting alveoli and milk ducts.

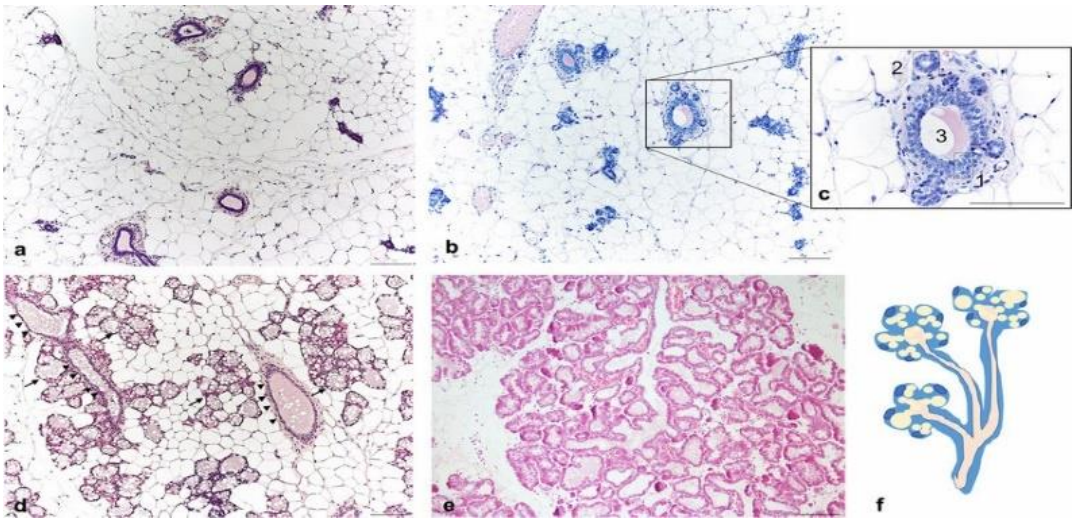

Figure 45:Mammary gland during the lactation cycle.(Colleluori et al., 2021)

7. Discussion on Nutritional Aspects

- Nutrient Requirements: Discuss the nutritional needs of lactating mice and how diet influences milk quality and quantity.

- Feeding Regimen: Review the dietary regimen that supports optimal milk production.

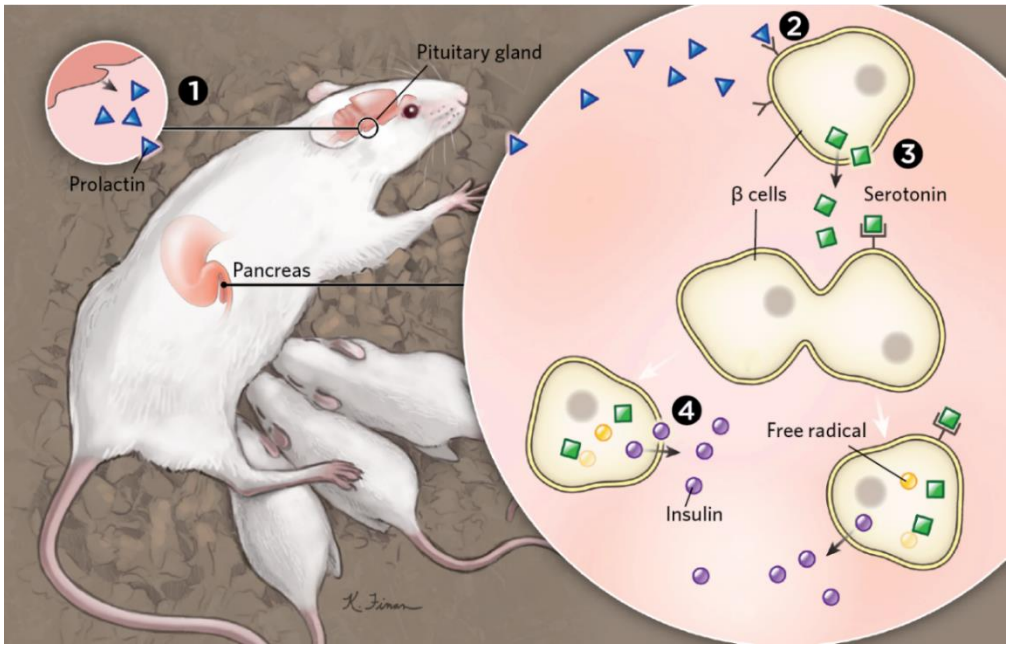

Figure 46:Prolactin and Its Impact on Pancreatic Function and Insulin Regulation in mice.(Stewart et al., 2022)

8. Review and Q&A

- Summary: Review key concepts covered during the practical session.
- Q&A: Allow time for students to ask questions to clarify their understanding of lactation.

9. Cleanup and Ethics

Proper Handling and Care: Emphasize the importance of proper care and ethical treatment of all animals involved in research.

Cleanup: Ensure all materials are cleaned and stored properly, and that all biohazard waste is disposed of according to guidelines.

Conclusion

This practical session aims to provide an in-depth understanding of lactation in mammals through direct observation, experimental interaction, and analytical discussion. It not only highlights the physiological aspects but also integrates ethical considerations essential for handling lactating animals in a research setting.

12. physiology of contraception

PRACTICAL WORK PHYSIOLOGY OF CONTRACEPTIVE

Objective

Studying contraception in laboratory mice involves evaluating the efficacy, safety, and mechanisms of contraceptive methods. This research can be crucial for developing new contraceptive technologies or understanding the biological underpinnings of existing ones. Here's a basic protocol to investigate contraception using mice as a model organism:

Preparatory Steps

Ethical Approval

- Obtain approval from your institution's animal care and use committee.

Animal Selection:

- Select sexually mature male and female mice. Ensure they are healthy and have normal reproductive histories. Genetic background, age, and weight should be considered to minimize variability.

Grouping:

- Randomly assign mice to control and treatment groups. Ensure that there is an adequate number of animals in each group to achieve statistical significance.

Housing Conditions:

- Maintain a controlled environment with appropriate light-dark cycles, temperature, and humidity.

- Provide ad libitum access to food and water.

Contraceptive Administration:

- Observe and record the estrous cycle of female mice using vaginal cytology to ensure they are cycling normally before starting treatments.

Contraceptive Method Application:

- ✓ Hormonal Methods Administer through injections, oral gavage, or implanted pellets, depending on the formulation of the contraceptive.
- ✓ Non-hormonal Methods: Apply as per the method's design, which could include injections of immune-contraceptives or the use of devices if applicable to mice.
- ✓ Gene Editing or Knockouts for Contraception Research: Perform according to established protocols for genetic modification.

Figure 47:Overview of Contraceptive Methods: A Visual Guide(*Contraceptive Methods for Pregnancy by Athena Womensclinic - Issuu*, n.d.)

1. PHYSICAL BARRIERS

- Intrauterine Devices (IUDs): Although more commonly used in humans, miniature versions could be developed for mice to study the mechanism of action, primarily how they prevent sperm from fertilizing the egg.
- Vaginal Rings: Designed to release non-hormonal spermicides or block sperm entry, adapted for mice to assess efficacy and safety.

2. SPERMICIDES

- Nonoxynol-9: A commonly used spermicide in humans that disrupts sperm cell membranes. Its efficacy and potential toxic effects can be studied in mice.
- Natural Spermicides: Compounds derived from plants or other natural sources that have spermicidal activity, offering a potentially safer alternative to chemical spermicides.

3. CHEMICAL METHODS

- Buffer Gels: pH-modulating gels that maintain an acidic vaginal environment unfavorable for sperm viability.
- Copper Compounds: Utilized in human IUDs for their spermicidal and ovicidal properties, their effect in a murine model could provide insights into the copper ions' mechanisms.

4. IMMUNOLOGICAL METHODS

- Vaccines Targeting Sperm or Egg Proteins: Immunization against specific proteins involved in fertilization can induce an immune response that prevents sperm from recognizing or penetrating the egg.

- Antibodies Blocking Fertilization: Administration of antibodies that bind to sperm or egg surface proteins, preventing fertilization without affecting hormone levels.

5. MECHANICAL METHODS

- Vaginal Plugs: While not a conventional method in human contraception, studying the efficacy of mechanical barriers at the cervix or vaginal entry in mice could provide insights into barrier contraception's physical aspects.

Dosage and Duration:

- Follow literature or preliminary studies to determine optimal dosages and duration of treatment.

Efficacy Evaluation:

- Mating Trials: after a period of contraceptive administration, allow treated females to mate with untreated males, or vice versa, depending on the study design.
- Monitor for signs of mating (e.g., vaginal plug formation).

Pregnancy Monitoring

- Check females for signs of pregnancy and compare the pregnancy rates between treated and control groups.

- Record litter size and health as indicators of contraceptive efficacy and potential side effects.

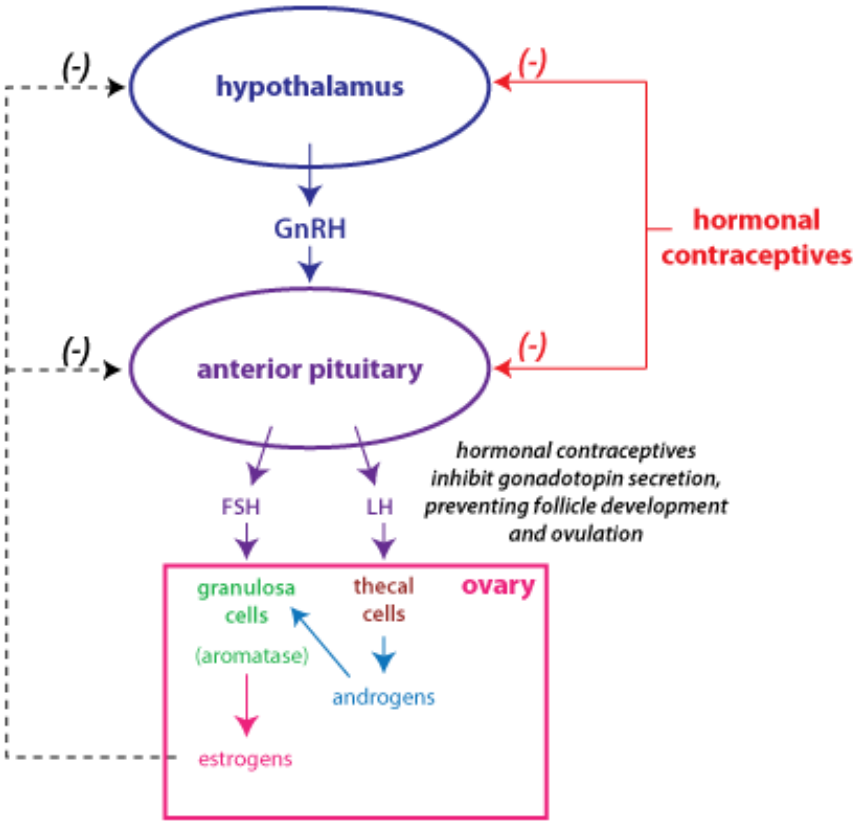

Figure 48:Mechanism of Action of Hormonal Contraceptives on the Hypothalamic-Pituitary-Ovarian Axis(Holtzman & Ackerman, 2019)

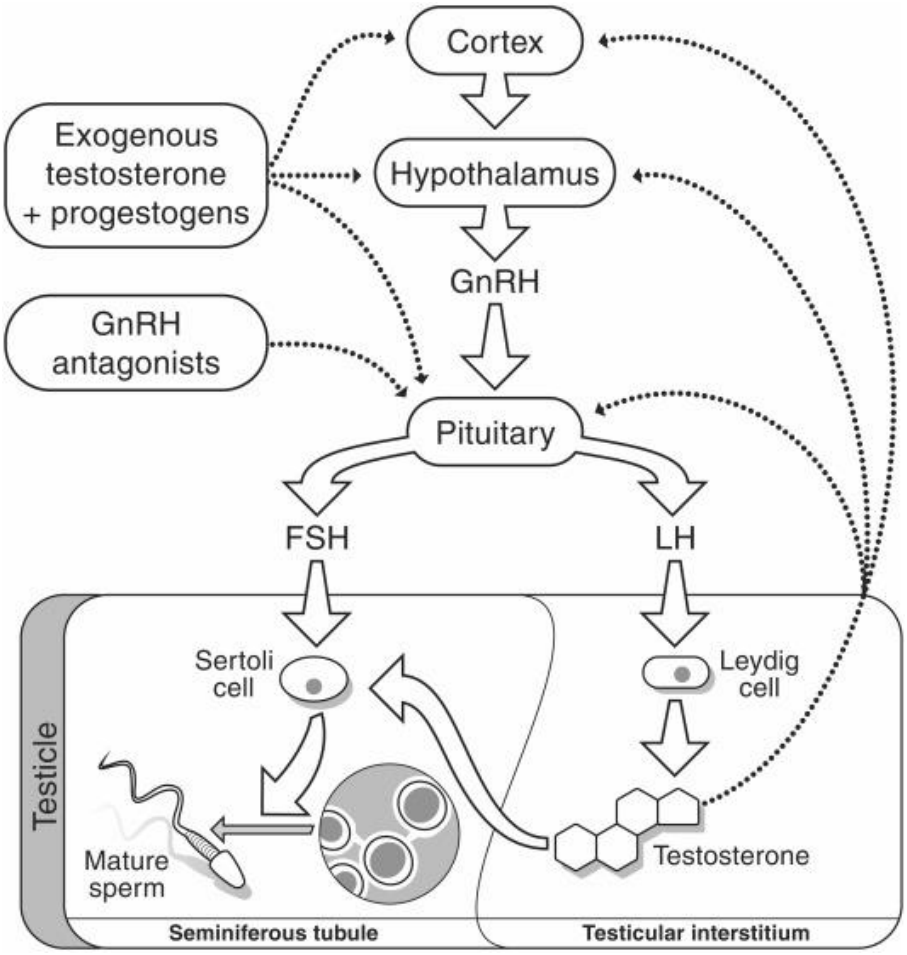

Figure 49:Effects of Exogenous Hormones and GnRH Antagonists on Male Hormonal Regulation (Kubba et al., 2000)

Safety and Mechanism of Action Studies

Histopathological Examination

- At the end of the study, collect tissues from reproductive organs (and potentially other organs of interest) for histological examination to assess any pathological changes due to contraceptive use.

Hormonal Assays

- Measure serum levels of reproductive hormones (e.g., estrogen, progesterone, testosterone) to evaluate the impact of contraceptive methods on endocrine functions.

Data Collection and Analysis:

- Collect and systematically record all observations and measurements.
- Use appropriate statistical methods to analyze the data, focusing on the efficacy in preventing pregnancy, impact on reproductive health, and any side effects observed.

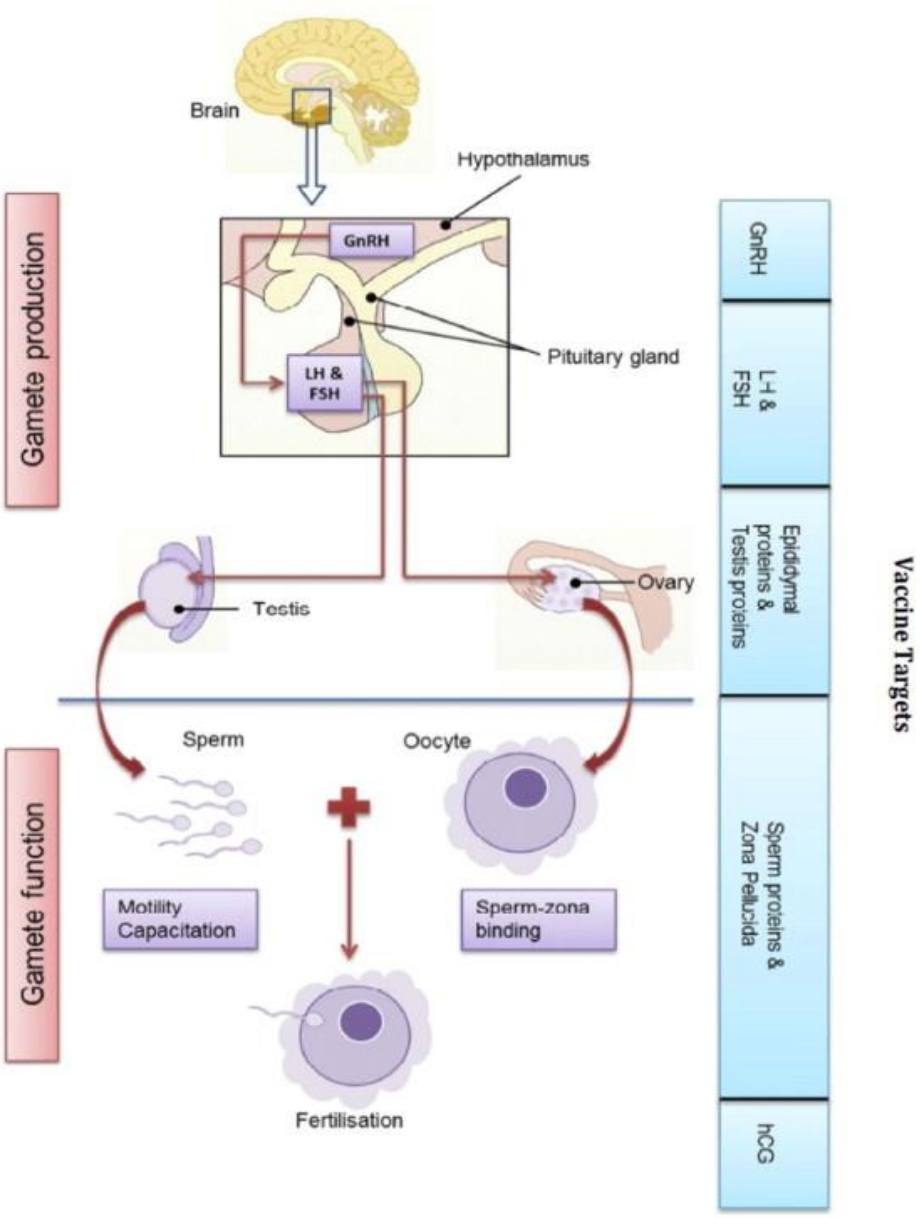

Figure 50:Overview of Hormonal Control in Human Reproduction and Potential Vaccine Targets`

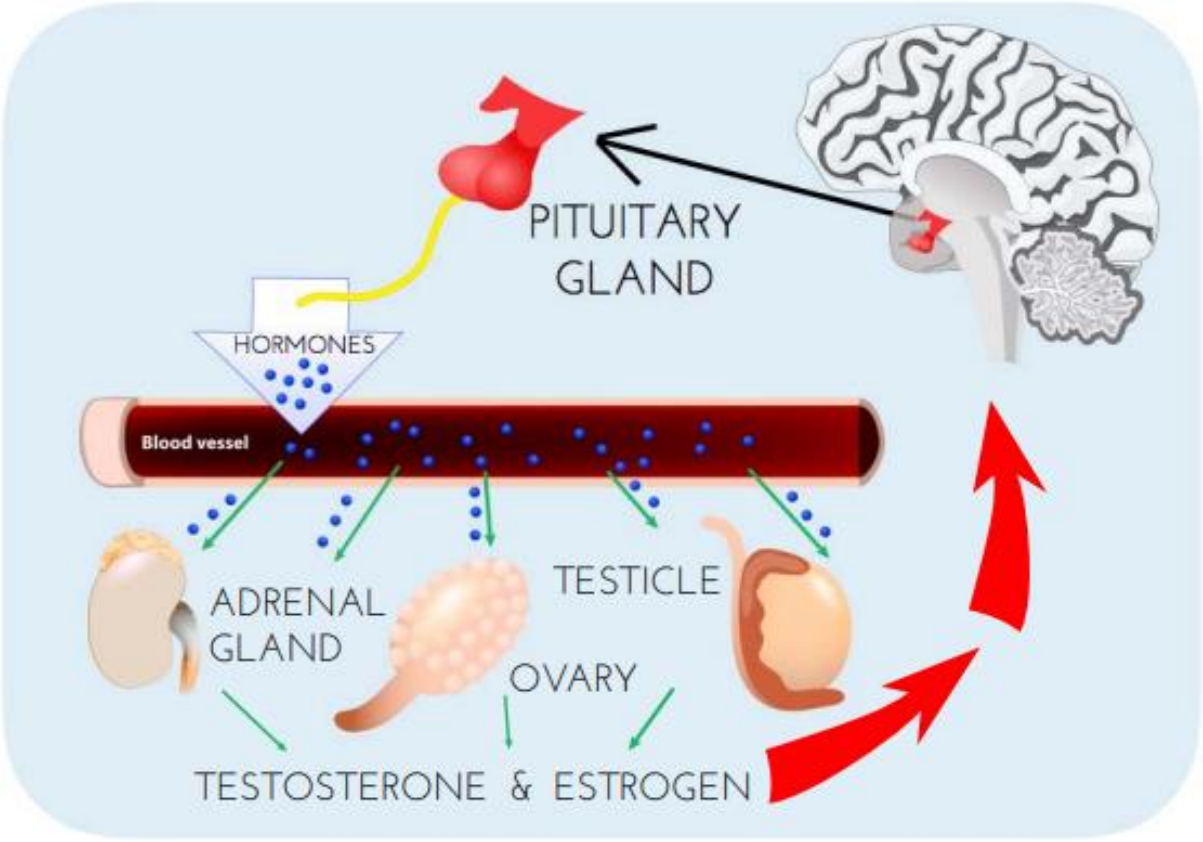

Figure 51:Hormonal Communication Pathways: Brain, Pituitary, and Reproductive Glands (Molnar & Gair, 2015)

Ethical Considerations:

- Ensure all procedures minimize stress and discomfort to the animals.
- Regularly monitor animal health and well-being throughout the study.
- Follow ethical guidelines for euthanasia for tissue collection or if necessary due to adverse effects.

Reporting

- Compile the findings into a detailed report or publication, outlining the methodology, results, conclusions, and implications for human contraception.
- This protocol provides a foundational framework for contraception research in mice and can be adapted or expanded based on specific research questions, the type of contraceptive being tested, or novel hypotheses about reproductive biology.

13. Studying Menopause Using Mouse Models

PRACTICAL WORK STUDYING MENOPAUSE USING MOUSE MODELS

Objective

To explore the physiological and hormonal changes associated with menopause using aged female mice, focusing on understanding the mechanisms and potential interventions.

Preparatory Steps

Ethical Approval

- Obtain approval from your institution's animal care and use committee.

Materials Needed

- ✓ Aged Female Mice: Preferably mice that are genetically modified or aged naturally to exhibit menopausal symptoms.
- ✓ Control Group: Young female mice or those not exhibiting menopausal signs.
- ✓ Laboratory Equipment: Cages, bedding, standard feeding supplies.
- ✓ Blood Collection Supplies: For hormone assays.

- ✓ Hormone Assay Kits: To measure levels of estrogen, progesterone, and other relevant hormones.
- ✓ Bone Density Scanners: To analyze bone density changes.
- ✓ Behavioral Assessment Tools: To evaluate changes in activity, anxiety, and cognitive function.
- ✓ Data Recording Tools: Notebooks or digital devices for recording observations.

Methodology

1. Introduction to Menopause in Mice

- Lecture: Begin with a lecture explaining menopause, its symptoms, and its impact on health.
- Discussion: Discuss how mice are used as models to study human menopause, including the limitations and benefits.

2. Animal Preparation and Care

- Animal Selection: Choose aged female mice that exhibit natural decline in reproductive function.
- Control Group Setup: Prepare a control group for comparative analysis.
- Housing and Handling: Ensure proper care and minimal stress during handling.

3. Hormonal and Physiological Assessments

- Blood Sampling: Collect blood samples from both aged and control mice at regular intervals to measure hormonal levels.

- Bone Density Analysis: Perform scans to assess bone density and compare changes between aged and control mice.

4. Behavioral Studies

- Cognitive and Physical Tests: Conduct tests to assess changes in cognitive abilities and physical activity.
- Anxiety and Mood Assessments: Use established tests like the elevated plus maze to evaluate anxiety levels.

5. Data Collection and Analysis

- Record Keeping: Document all experimental data meticulously.
- Statistical Analysis: Analyze data using appropriate statistical methods to compare aged mice and controls.

6. Review and Discussion

- Data Review Session: Review collected data and initial findings with participants.
- Group Discussion: Encourage discussion about the implications of the findings and potential interventions for menopause-related issues.

7. Conclusion and Further Research

- Summarize Key Findings: Summarize the main outcomes of the lab work.
- Propose Further Studies: Suggest how these findings could lead to further research, particularly in developing treatments or preventative measures for menopause.

Cleanup and Ethical Considerations

- Proper Handling and Care: Stress the importance of ethical treatment and proper care of the animals throughout the experiment.
- Cleanup: Ensure all equipment is cleaned and stored properly; ensure proper disposal of all biological waste.

Conclusion

This lab session provides a comprehensive approach to studying menopausal changes using mouse models, bridging basic research with potential clinical applications. It offers a practical understanding of the biological and physiological changes during menopause, fostering a deeper understanding of women's health issues.

14. Production of Congenital Malformations in Mice

PROTOCOL FOR EXPERIMENTAL PRODUCTION OF CONGENITAL MALFORMATIONS IN MICEMODELS

Objective

To examine the effects of specific teratogens on embryonic development in mice, identifying the stages of development most susceptible to these agents

Materials Needed

- ✓ **Pregnant mice:** Timed pregnancies for accurate developmental stage exposure.
- ✓ **Teratogens**: Selected based on known effects (e.g., retinoic acid, thalidomide).
- ✓ **Control agent**: Typically, saline solution.
- ✓ **Injection apparatus:** Syringes, needles for precise dosage administration.
- ✓ **Housing units**: Separate cages for control and experimental groups.
- ✓ **Personal Protective Equipment (PPE):** Gloves, masks, goggles, lab coats.
- ✓ **Ultrasound machine**: For in vivo examination of fetal development.

- ✓ **Digital scale and calipers**: For measuring maternal weight and offspring size.
- ✓ **Documentation tools**: Lab notebooks, digital recording tools, cameras.
- ✓ **Dissection kit**: For post-mortem examination.
- ✓ **Histology supplies:** Fixatives, slides, microtome for tissue processing.

Methodology

Step 1: Preparation and Ethical Considerations

- Ethical Approval: Secure approval from your institution's animal care committee.
- Training: Ensure all participants are trained in ethical animal handling and experimental procedures.

Step 2: Animal Grouping and Teratogen Preparation

- Animal Grouping: Assign pregnant mice to groups based on the teratogen they will be exposed to, including a control group receiving a placebo.
- Teratogen Preparation: Prepare solutions of teratogens and the control substance under sterile conditions.

Step 3: Teratogen Administration

- Dosing: Administer the teratogen or control substance at a specific gestational day to ensure exposure during critical periods of embryonic development. Administration routes may vary (e.g., subcutaneous, oral).
- Recording: Document the dose, time, and method of administration for each animal.

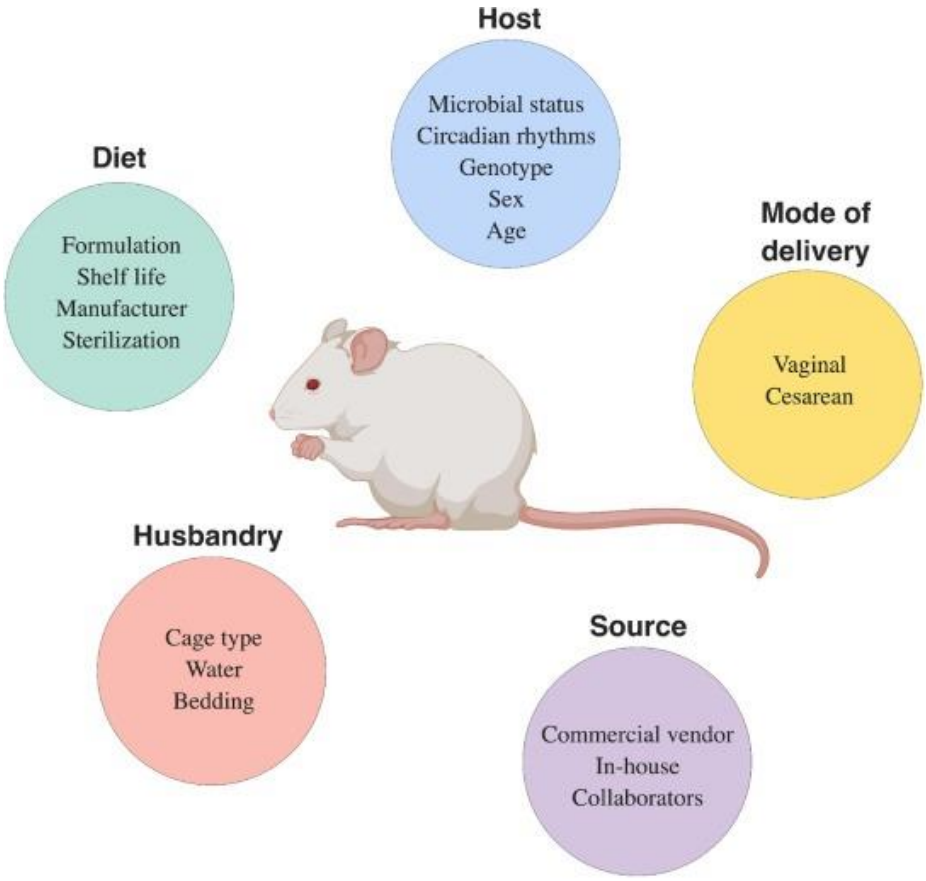

Figure 52:Factors Influencing Laboratory Mouse Models: Diet, Husbandry, Host Characteristics, and Source(Weiskirchen et al., 2020)

Step 4: Monitoring and Maintenance

- Health Monitoring: Regularly check the health of the pregnant mice, observing any adverse reactions.
- Environmental Conditions: Maintain stable conditions in the animal facility regarding temperature, humidity, and light.

Step 5: Developmental Monitoring

- Ultrasound Scanning: Perform periodic ultrasound scans to monitor fetal development and detect any abnormalities in utero.

- Data Recording: Log developmental stages and any detected abnormalities.

Step 6: Birth and Postnatal Care

- Observation: Monitor the birth process, recording any anomalies in the delivery and condition of the neonates.
- Postnatal Assessments: Examine and record any visible congenital abnormalities in the offspring.

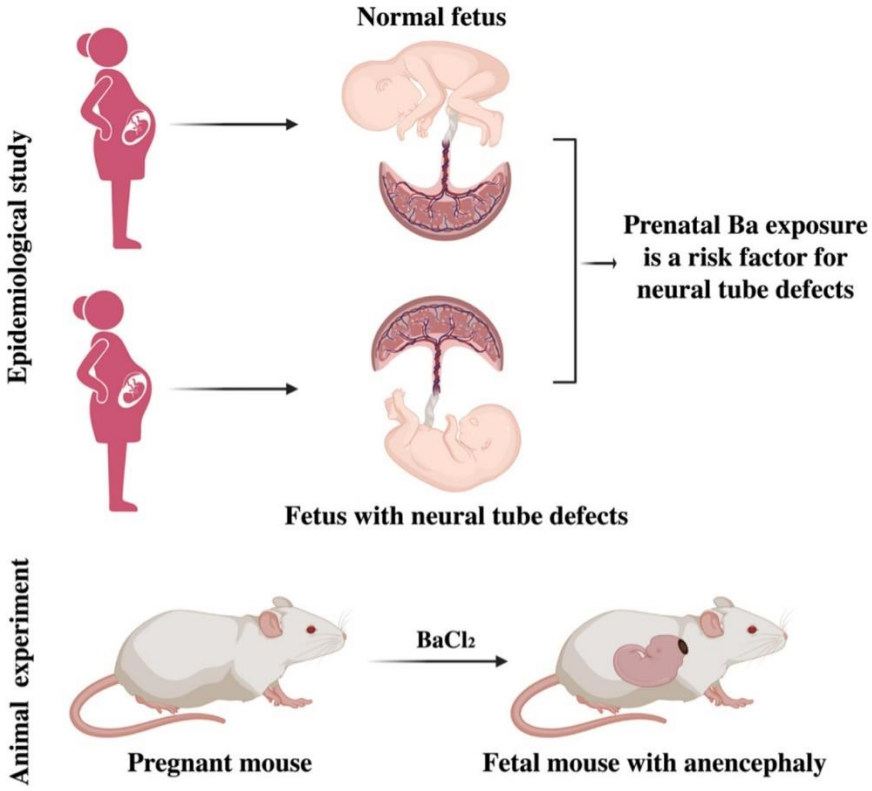

Figure 53:Impact of Prenatal Barium Chloride Exposure on Neural Tube Development in Human and Mouse Models(Wang et al., 2021)

Step 7: Post-mortem and Tissue Analysis

- Necropsy: Conduct detailed necropsies on any deceased offspring or sacrificed neonates for ethical reasons.

- Histological Analysis: Prepare tissue samples for microscopic examination to identify cellular-level malformations.

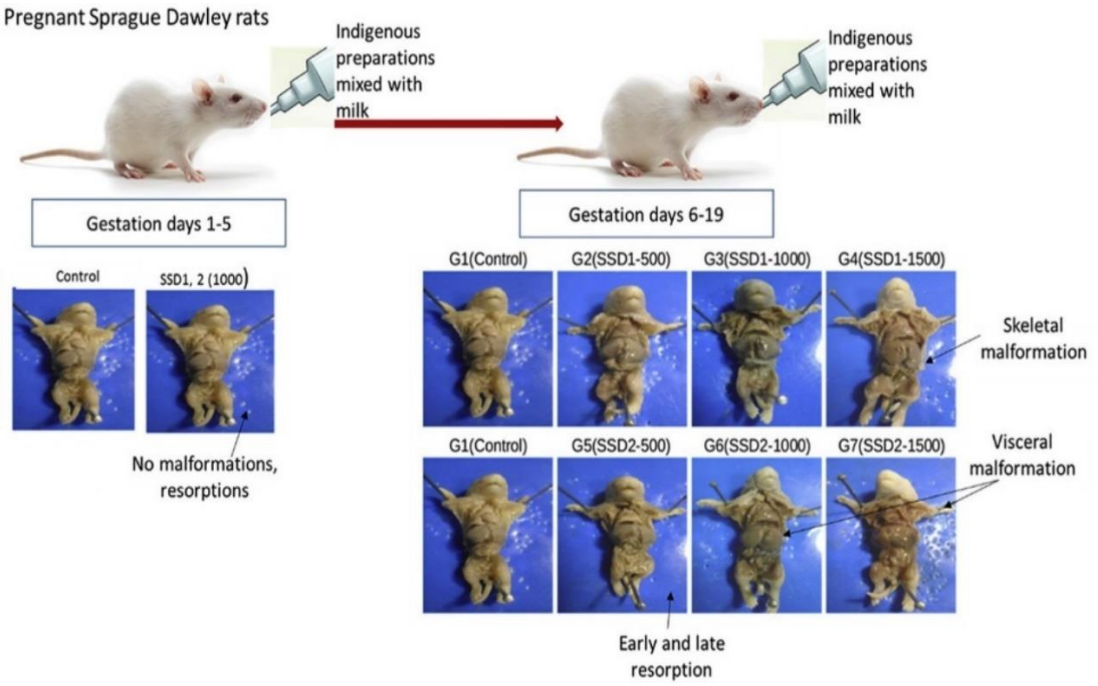

Figure 54:Effects of Indigenous Medicinal Preparations on Fetal Development in Pregnant Sprague Dawley Rats(Bandyopadhyay Neogi et al., 2021)

Step 8: Data Analysis

- Statistical Analysis: Use appropriate statistical methods to analyze the data collected, comparing the incidence of malformations across different groups.
- Interpretation: Discuss the implications of the findings in relation to the timing and dosage of teratogen exposure.

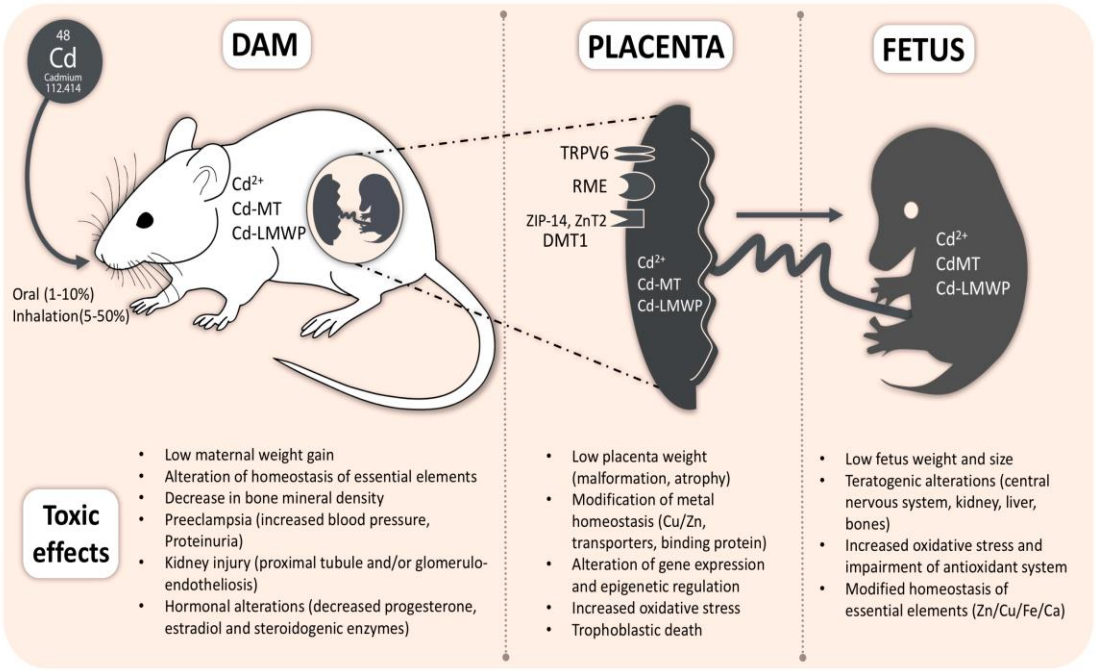

Figure 55:Pathways and Toxic Effects of Cadmium Exposure in Maternal-Fetal Transfer in Mice(Liu et al., 2022)

Step 9: Reporting and Presentation

- Report Preparation: Compile detailed reports on the methodology, findings, and statistical analyses.
- Presentation: Present the findings in a formal setting, such as a class seminar or scientific conference.

Step 10: Debrief and Ethical Review

- Debrief: Hold a session to discuss the ethical aspects of the study and its implications for both science and animal welfare.
- Review: Evaluate the experimental procedures for any potential improvements in future studies.

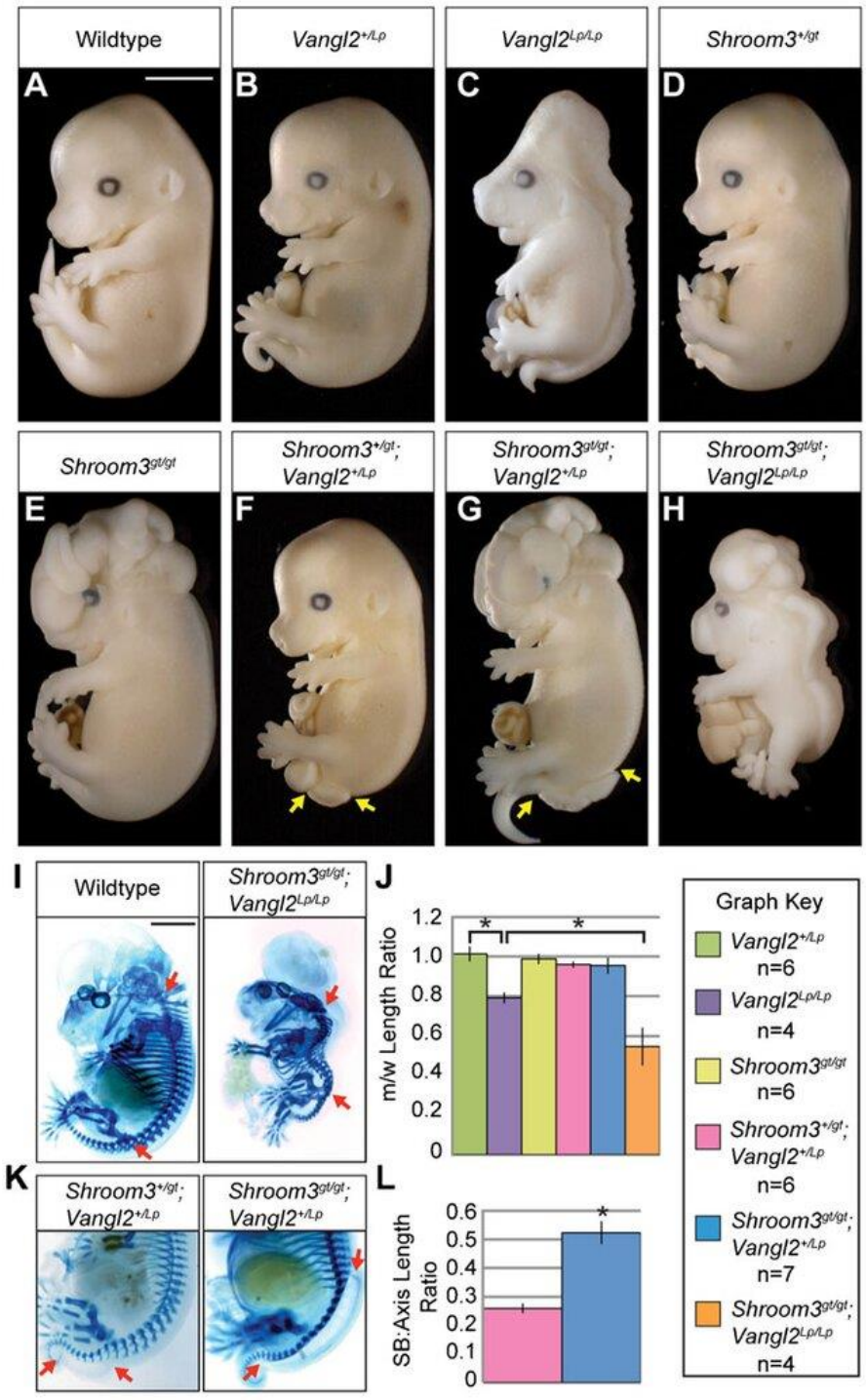

Figure 56:Genotypic Variations and Their Phenotypic Manifestations in Mouse Embryos: The Impact of Shroom3 and Vangl2 Mutations(McGreevy et al., 2015)

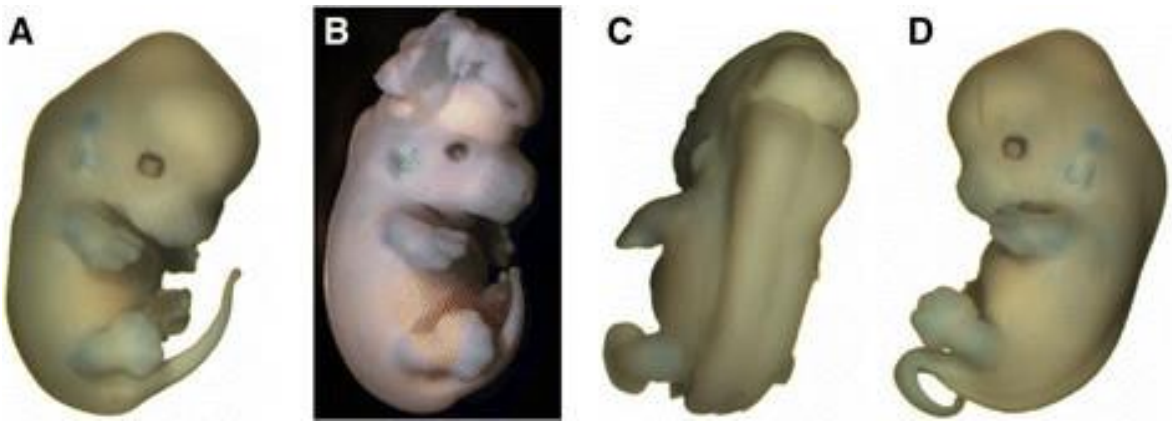

Figure 57:Examples of congenital defects in Apob and Lp mutant mice. A. Normal embryo, B. Apob – exencephaly, C. Lp – craniorachischosis, and D. Lp – loop-tail.(McGreevy et al., 2015)

Conclusion:

This protocol aims to provide a comprehensive framework for understanding the teratological effects of chemicals on mammalian development, equipping students with the skills and knowledge to conduct responsible biological research.

Conclusion

The various laboratory work sessions designed to explore physiological processes in laboratory animals, such as parturition, lactation, and surgical interventions like castration, collectively provide a profound understanding of mammalian biology. These exercises are crucial for students and researchers alike to gain hands-on experience and insights into complex biological systems. Here's a general conclusion that encapsulates these lab works along with a focus on hormonal regulation:

General Conclusion: Laboratory Work Sessions and Hormonal Regulation in Mammals

- ✓ Comprehensive Learning: The lab sessions cover a range of vital physiological processes, from the reproductive events of parturition and lactation to the surgical technique of castration. Each session is designed not only to teach specific skills and knowledge but also to provide practical experience in handling and manipulating biological systems.
- ✓ Understanding Hormonal Regulation: At the core of these physiological processes is hormonal regulation, which plays a pivotal role in directing and modulating the body's activities. Hormones such as prolactin, oxytocin, estrogen, and testosterone are crucial in driving events like pregnancy, lactation, and reproductive development. The lab sessions

help illuminate how these hormones orchestrate complex biological functions, ensuring survival and reproduction.

- ✓ Ethical and Practical Skills Development: Through these practical sessions, participants learn not just about the biological aspects but also develop essential skills in ethical reasoning, surgical techniques, and animal handling. These skills are critical, particularly in ensuring that all procedures adhere to ethical standards that prioritize animal welfare and scientific integrity.
- ✓ Integration of Theory and Practice: The laboratory work sessions provide a unique opportunity to integrate theoretical knowledge from textbooks with practical, real-world applications. This integration is crucial for a deep understanding of subjects like physiology, endocrinology, and anatomy, allowing students and researchers to apply their knowledge in both academic and applied research settings.
- ✓ Preparation for Advanced Studies and Research: For those intending to progress in fields such as veterinary medicine, biomedical research, or zoology, these sessions are invaluable. They provide a foundation upon which further specialized study can be built, such as in reproductive technologies, endocrine therapies, and conservation biology.
- ✓ Contribution to Science and Medicine: Beyond educational value, the insights gained from these lab works contribute to the broader fields of science and medicine. Understanding hormonal regulation and physiological processes in animals' aids in the development of medical

treatments, fertility therapies, and can even contribute to conservation efforts by understanding animal breeding patterns and health.

- ✓ Future Directions and Improvements: Continual improvement of these lab sessions is necessary to keep pace with advances in scientific techniques and ethical standards. Incorporating new technologies, such as more refined surgical tools, imaging technologies, and computer simulations, can enhance the learning experience and reduce the need for animal use.

In conclusion, these laboratory sessions are not only fundamental for learning about mammalian physiology and hormonal regulation but also serve as a steppingstone for further scientific inquiry and ethical practice. They prepare participants to tackle complex biological questions and ethical dilemmas in their future careers, ensuring that the next generation of scientists and clinicians is well-equipped to advance our understanding of life sciences.

Final Words from Dr. Fatima Benchikh

As we conclude this Practical Manual of Hormonal Regulation, tailored specifically for third-year Animal Biology and Physiology students, I hope it has provided you with a comprehensive understanding of the intricate hormonal mechanisms that drive physiological processes across various species. This manual is not just a compilation of theoretical knowledge, but a practical guide designed to bridge your classroom learning with real-world applications in biology and physiology.

Throughout your journey in this course, you have encountered detailed explorations of hormonal pathways, their interactions, and their impacts on animal health and behavior. It is my sincere hope that these insights ignite a passion for further research and inspire a deeper curiosity about the natural world.

As you move forward in your academic career, remember that the true value of this knowledge lies in its application—whether in research, further study, or eventual professional practice. May this manual serve as a foundational tool that you refer to as you develop as a scientist and as a thinker.

Thank you for your diligence and enthusiasm. I am confident that the skills and understanding you have gained here will serve you well in your future endeavors. Best wishes for a successful completion of your studies and a bright future in the field of biological sciences.

References

Ajayi, A. F., & Akhigbe, R. E. (2020). Staging of the estrous cycle and induction of estrus in experimental rodents: an update. *Fertility Research and Practice 2020 6:1*, *6*(1), 1–15. https://doi.org/10.1186/S40738-020-00074-3

Al-Timimi, Z. K., & Gali, M. A. H. (2018). Effect of Ochratoxin-A on mouse embryos. *Baghdad Science Journal*, *15*(1), 1–8. https://doi.org/10.21123/BSJ.2018.15.1.0001

Alvergne, A., & Högqvist Tabor, V. (2018). Is female health cyclical? Evolutionary perspectives on menstruation. *Trends in Ecology & Evolution*, *33*(6), 399–414. https://doi.org/10.1016/j.tree.2018.03.006

Balls, M., & Combes, R. D. (2016). Animal experimentation: The statistics speak for themselves. *ATLA Alternatives to Laboratory Animals*, *44*(6), 511–513. https://doi.org/10.1177/026119291604400605

Bandyopadhyay Neogi, S., Roy, D. K., Sachdeva, A. K., Sharma, R., Gupta, R., & Ganguli, A. (2021). Evidence of prenatal toxicity of herbal based indigenous formulations for sex selection in rat models. *Journal of Traditional and Complementary Medicine*, *11*(1), 9–15. https://doi.org/10.1016/J.JTCME.2019.09.005

Bebo, B. F., Zelinka-Vincent, E., Adamus, G., Amundson, D., Vandenbark, A. A., & Offner, H. (1998). Gonadal hormones influence the immune response to PLP 139-151 and the clinical course of relapsing experimental autoimmmune encephalomyelitis. *Journal of Neuroimmunology*, *84*(2), 122–130. https://doi.org/10.1016/S0165-5728(97)00214-2

Because in laboratory safety comes first - Personal Protective Laboratory Equipment poster. (n.d.). Retrieved July 12, 2024, from https://www.bionity.com/en/whitepapers/1126613/because-in-laboratory-safety-comes-first.html

Bezold, K. Y., Karjalainen, M. K., Hallman, M., Teramo, K., & Muglia, L. J. (2013). The genomics of preterm birth: from animal models to human studies. *Genome Medicine 2013 5:4*, *5*(4), 1–11. https://doi.org/10.1186/GM438

Childbirth: The Role of Hormones in Labor and Delivery - Lesson | Study.com. (n.d.). Retrieved July 12, 2024, from https://study.com/academy/lesson/childbirth-the-role-of-hormones-in-labor-and-delivery.html

Colleluori, G., Perugini, J., Barbatelli, G., & Cinti, S. (2021). Mammary gland adipocytes in lactation cycle, obesity and breast cancer. *Reviews in Endocrine and Metabolic Disorders*, *22*(2), 241–255. https://doi.org/10.1007/S11154-021-09633-5

Contraceptive Methods for Pregnancy by athena womensclinic - Issuu. (n.d.). Retrieved July 12, 2024, from https://issuu.com/athenawomensclinic/docs/contraceptive_methods_for_pregnancy.pptx

dissection tools - Google Search. (n.d.). Retrieved July 12, 2024, from website

GnRH Analogs - Indications & Use. (n.d.). Retrieved July 12, 2024, from https://www.invitra.com/en/gnrh-analogues/

Holtzman, B., & Ackerman, K. E. (2019). Hypothalamic–pituitary–gonadal axis in women's sport: injuries, manipulations, and aberrations. *Current Opinion in Endocrine and Metabolic Research*, *9*, 78–85. https://doi.org/10.1016/J.COEMR.2019.08.003

human ovaries - Students | Britannica Kids | Homework Help. (n.d.). Retrieved July 12, 2024, from https://kids.britannica.com/students/assembly/view/19665

Kubba, A., Guillebaud, J., Anderson, R. A., & MacGregor, E. A. (2000). Contraception. *The Lancet*, *356*(9245), 1913–1919. https://doi.org/10.1016/S0140-6736(00)03269-4

Laboratory animals used in research. (n.d.). Retrieved July 12, 2024, from https://norecopa.no/species/laboratory-animals/

Liu, W. B., Zhu, H. L., Xiong, Y. W., Lv, J., Huang, Y. C., & Wang, H. (2022). Environmental cadmium exposure during gestation impairs fetal brain and cognitive function of adult offspring via reducing placenta-derived E2 level. *Chemosphere*, *307*, 135668. https://doi.org/10.1016/J.CHEMOSPHERE.2022.135668

McGreevy, E. M., Vijayraghavan, D., Davidson, L. A., & Hildebrand, J. D. (2015). Shroom3 functions downstream of planar cell polarity to regulate myosin II distribution and cellular organization during neural tube closure. *Biology Open*, *4*(2), 186–196. https://doi.org/10.1242/BIO.20149589

McLean, A. C., Valenzuela, N., Fai, S., & Bennett, S. A. L. (2012). Performing vaginal lavage, crystal violet staining, and vaginal cytological evaluation for mouse estrous cycle staging identification. *Journal of Visualized Experiments*, *67*. https://doi.org/10.3791/4389

Menstrual cycle | Description, Phases, Hormonal Control, Ovulation, & Menstruation | Britannica. (n.d.). Retrieved July 12, 2024, from https://www.britannica.com/science/menstrual-cycle

Menstrual Cycle - Women's Health Network. (n.d.). Retrieved July 12, 2024, from https://www.womenshealthnetwork.com/pms-and-menstruation/your-menstrual-cycle-the-basics/

Mikkonen, R. S., Ihalainen, J. K., Davis-Wilson, H. C., & Hackney, A. C. (2023). The Hypothalamic–Pituitary–Ovarian Axis, Menstrual and Oral Contraceptive Cycles: Regulation and Function in the Context of Exercise and Sport. *Sex Hormones, Exercise and Women: Scientific and Clinical Aspects, Second Edition*, 1–24. https://doi.org/10.1007/978-3-031-21881-1_1

Molnar, C., & Gair, J. (2015). *24.4. Hormonal Control of Human Reproduction*. BCcampus.

Mouse male reproductive system, illustration - Stock Image - C047/4178 - Science Photo Library. (n.d.). Retrieved July 12, 2024, from https://www.sciencephoto.com/media/1057775/view/mouse-male-reproductive-system-illustration

Of mice and babies: New mouse model links tra | EurekAlert! (n.d.). Retrieved July 12, 2024, from https://www.eurekalert.org/news-releases/706180

Ovary | Animal & Human | Britannica. (n.d.). Retrieved July 12, 2024, from https://www.britannica.com/science/ovary-animal-and-human

Overview of Labor - Stanford Medicine Children's Health. (n.d.). Retrieved July 12, 2024, from https://www.stanfordchildrens.org/en/topic/default?id=overview-of-labor-90-P02896

Protocols for field and laboratory rodent studies. (n.d.). Retrieved July 12, 2024, from https://www.researchgate.net/publication/337137837_Protocols_for_field_and_laboratory_rodent_studies/figures?lo=1&utm_source=google&utm_medium=organic

Ratajczak, C. K., Fay, J. C., & Muglia, L. J. (2010). Preventing preterm birth: The past limitations and new potential of animal models. *DMM Disease Models and Mechanisms*, *3*(7–8), 407–414. https://doi.org/10.1242/DMM.001701

Shibata, S., Kobayashi, E. H., Kobayashi, N., Oike, A., Okae, H., & Arima, T. (2020). Unique features and emerging in vitro models of human placental development. *Reproductive Medicine and Biology*, *19*(4), 301–313. https://doi.org/10.1002/RMB2.12347

Siewiera, J., McIntyre, T. I., Cautivo, K. M., Mahiddine, K., Rideaux, D., Molofsky, A. B., & Erlebacher, A. (2023). Circumvention of luteolysis reveals parturition pathways in mice dependent upon innate type 2 immunity. *Immunity*, *56*(3), 606-619.e7. https://doi.org/10.1016/J.IMMUNI.2023.01.005

Stewart, T., Bode, K., Kissler, S., & Yi, P. (2022). Bioluminescent Monitoring of Graft Survival in an Adoptive Transfer Model of Autoimmune Diabetes in Mice. *Journal of Visualized Experiments*, *2022*(189). https://doi.org/10.3791/64836

Suarez, A. C., Gimenez, C. J., Russell, S. R., Wang, M., Munson, J. M., Myers, K. M., Miller, K. S., Abramowitch, S. D., & De Vita, R. (2024). Pregnancy-induced remodeling of the murine reproductive tract: a longitudinal in vivo magnetic resonance imaging study. *Scientific Reports 2024 14:1*, *14*(1), 1–19. https://doi.org/10.1038/s41598-023-50437-1

Sullivan, E. L., Nousen, E. K., Chamlou, K. A., & Grove, K. L. (2012). The impact of maternal high-fat diet consumption on neural development and behavior of offspring. *International Journal of Obesity Supplements 2012 2:2*, *2*(2), S7–S13. https://doi.org/10.1038/ijosup.2012.15

Understanding The Menstrual Cycle: It's More Than Just Your Periods. (n.d.). Retrieved July 12, 2024, from https://www.youthkiawaaz.com/2020/05/understanding-menstrual-cycle-its-more-than-just-your-periods/

Vascular microdissection, perfusion, and excision of the murine arterial tree for use in atherogenic disease investigations. (n.d.). Retrieved July 12, 2024, from https://www.researchgate.net/publication/9078769_Vascular_microdissection_perfusion_and_excision_of_the_murine_arterial_tree_for_use_in_atherogenic_disease_investigations/figures?lo=1

Wang, C., Pi, X., Chen, Y., Wang, D., Yin, S., Jin, L., Li, Z., Ren, A., Wang, L., & Yin, C. (2021). Prenatal exposure to barium and the occurrence of neural tube defects in offspring. *Science of The Total Environment*, *764*, 144245. https://doi.org/10.1016/J.SCITOTENV.2020.144245

Waterhouse, D. N., Sutherland, B. W., Santos, N. Dos, Masin, D., Osooly, M., Strutt, D., Ostlund, C., Anantha, M., Harasym, N., Manisali, I., Wehbe, M., Bally, M. B., & Webb, M. S. (2014). Irinophore C™, a lipid nanoparticle formulation of irinotecan, abrogates the gastrointestinal effects of irinotecan in a rat model of clinical toxicities. *Investigational New Drugs*, *32*(6), 1071–1082. https://doi.org/10.1007/S10637-014-0138-X

Weiskirchen, S., Weiper, K., Tolba, R. H., & Weiskirchen, R. (2020). All You Can Feed: Some Comments on Production of Mouse Diets Used in Biomedical Research with Special Emphasis on Non-Alcoholic Fatty Liver Disease Research. *Nutrients 2020, Vol. 12, Page 163*, *12*(1), 163. https://doi.org/10.3390/NU12010163

What Hormone Causes Uterine Contractions. (n.d.). Retrieved July 12, 2024, from https://hotcore.info/babki/what-hormone-causes-uterine-contractions.htm