PRACTICAL MANUAL

OF

VETERINARY PHYSIOLOGY

(B.V.Sc. & A.H. FIRST PROFESSIONAL YEAR-2023-24)

Volume II (Unit III-IV)

PREPARED BY:

NAME :----------

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CERTIFICATE

This is to be certify that ...…..……..

Mr./Ms. ....…….……..……

Roll No. …… of First Year B.V.Sc. & A.H. has successfully completed all practical's in Veterinary Physiology during first year of the academic year ……...

Date :

Place :

Signature
(Head of Department)

Signature
(Course Teacher)

FOREWORD

I am very happy to go through the Practical Manual entitled "Veterinary Physiology" Department of Veterinary Physiology, MJF College of Veterinary & Animal Science, Chomu, Jaipur (Rajasthan). The Manual covers the practical syllabus of undergraduate course (Veterinary Physiology, Volume II) Prescribed by Veterinary Council of India (New VCl 2016) for B.V.Sc. & A.H. programme.

The Manual is a good attempt and is based on cumulative experience of teaching undergraduate courses. The language used in the manual is simple and lucid. The outline and description of practical exercises covering objectives, materials required, procedures and observations to be taken have been nicely presented which would be helpful in conducting practicals more effectively.

I hope this manual will make its own place in the library of Agricultural Universities, Veterinary and Animal Science Colleges and various Livestock Institutions in near future.

I congratulate the authors for the efforts put in bringing out this practical manual.

Dean
(MJF College of Veterinary &
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ACKNOWLEDGEMENT

Ever since the introduction of new course for professional B.V.Sc. & A.H. degree programme under Veterinary Council of India pattern in Veterinary Colleges/Universities in the country, there was a dire need to have a practical manual on Veterinary Physiology subject which covers the practical syllabus of undergraduate degree course. These new course was not dependently developed in most of the Veterinary College/University before the introduction of Veterinary Council of India programme. The present manual covers the practical with objectives, material required, procedure, steps to follow precautions to be taken, observations to be recorded and exercise to be done by the students. We hope that users will find the manual immensely useful.

We look forward to receiving the valuable suggestions of readers for improvement of this manual

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UNIT- 3

Practical No.1 **Date:**

URINE ANALYSIS

Introduction:

Urine is the chief excretory product of the animal body. It is formed in the kidney by filtration of plasma and stored in the urinary bladder. It is eliminated periodically through urethra. A majority of waste products are eliminated through this product. Normally much variation is observed in the chemical and physical composition of urine. Presence of certain normal constituents in abnormal quantity and the presence of certain abnormal constituents in the urine reflect certain disease conditions and these are of importance in diagnosing the clinical conditions in the animals. Therefore a very thorough examination of urine forms one of the most important tools of all veterinary diagnostic procedure. Hence the urine examination is to be conducted on a selective basis and every test carried out in relation to the clinical signs must be under the close supervision of the veterinarian. Many normal as well as pathological mechanisms may influence the physical and chemical composition of the urine sample.

Purpose:

Urine analysis can be performed for several reasons like:

1. General evaluation of health.
2. Diagnosis of metabolic or systemic diseases that affect kidney function.
3. Diagnosis of endocrine disorders. 24 hrs urine studies are often ordered for these tests.
4. Diagnosis of diseases or disorders of the kidneys or urinary tract.
5. Monitoring of patients with diabetes.
6. Testing for pregnancy.
7. Screening for drug abuse.

When a sample of urine is submitted to a clinical laboratory, the following examinations are done:

1. Physical examination
2. Chemical examination
3. Microscopic examination

Collection and Preservation of Urine sample:

Containers used for urine sample must be clean and dry before collecting the urine. It is necessary to clean the vulva or the prepuce of the animal to keep away contamination of urine sample. The parts must be dried with a clean dry piece of cotton cloth. Urethral catheter can also be used to collect the urine directly from urinary bladder. Catheterization should be done very carefully by trained person to avoid any

damage to the soft tissue and to avoid subsequent complications. A properly sterilized catheter must be used. In small animals like dog and cat urine can be obtained by a bladder puncture.

For quantitative analysis, attempts must be made to collect 24 hours urine sample. In collected urine a preservative is required to be added. The urine after collection is kept in a wide mouthed bottle in the refrigerator.

Methods of preserving urine sample:

Urine provides an excellent culture medium for a variety of micro-organisms. Therefore, its preservation is important if the analysis cannot be carried out immediately after the collection of fresh urine samples. This is particularly followed in case of 24 hours urine sample.

Importance of addition of preservatives to urine:

- Conversion of urea to ammonium carbonate by urea splitting organisms is prevented by addition of preservative to urine.
- Destruction of glucose by yeast or bacteria is prevented.
- Degradation of organized sediments, pus cells, blood cells and epithelial casts is prevented by the preservatives.
- A false positive albumin test due to presence of bacteria proteins is prevented.

Methods for preservation of urine samples:

I. **General Methods**:

a. **Refrigeration**: As far as possible, freshly collected urine samples must be tested within 30 minutes of collection or the sample should be at refrigerated temperature with/without addition of preservatives. The refrigerated samples must be warmed to room temperature before conducting any test.

b. **Addition of Toluene as a preservative**: When it is not possible to refrigerate the urine sample, a small amount of toluene just enough to form a layer on the surface urine sample should be added. The urine is pipetted from beneath the toluene layer whenever required for analysis. Toluene does not interfere with the urine tests.

II. **Specific Methods**:

a. **Phenol or Cresol**: This is an excellent preservative and is often used in specimens forwarded for distant laboratories. One drop is added for 30 ml of urine.

b. **Thymol**: Add a small crystal (5mm diameter for 90- 120 ml of urine. An excess of thymol added may give a false positive albumin test and may interfere with the test for bile.

c. **Formalin (40%)**: One drop of formalin is required for 30 ml of urine. This preserves the urinary sediments well, but it prevents Obermeyer test for indicant, reduces the alkaline copper solution and may give precipitate with urea that interferes with microscopic examination.

d. **Chloroform**: Few drops are added at a time until some of them remain in the bottom after mixing gently. It reduces the alkaline copper solution and is not a very good preservative.

Characteristics of normal urine on urinalysis

Character	All Species
Specific Gravity	1.001 - 1.080
Color	Yellow to Brownish
Transparency	Clear to Hazy
Ph	5.0 - 9.0
Glucose	Negative
Ketones	Negative
Protein	Negative to Trace
Blood	Negative
Bilirubin	Negative
Urobilinogen	Negative - 0.1
RBC/HPF	0 - 5
WBC/HPF	0 - 3
Epithelial/LPF	0
Casts	None
Crystals	None

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Practical No. 2 **Date:**

PHYSICAL EXAMINATION OF URINE

The gross or physical examination of urine includes the observations and measurements of the following characters of the urine sample,

1. Colour 2.Appearance 3.Odour 4.Volume 5. Specific gravity

1. Colour:

Normally, urine is some shade of yellow colour and may range from straw, a very pale yellow, to amber. The colour of the urine varies with the volume and concentration of the urine voided. In polyuria the urine is pale yellow or colorless, while in oliguria it is dark yellow or dark amber in colour. Acid urine is usually darker than alkaline urine. Blue or green urine is usually the result of ingested dyes or drugs. Pathologically, urine may be of any color.

Procedure:

1. Thoroughly mix the urine in the collection container by inversion (be sure the lid is on tight) and
2. Record the colour of the urine.

Urine Colours	Causes
Normal: Straw, very pale yellow, amber	Presence of the normal metabolic end products/ pigments such as urochrome, urobilin and uroerythrin in the urine.
Dark yellow colour	If urine is concentrated, the amount of urochromes per volume increased and urine appears darker than normal. During fever and jaundice the colour of urine is dark yellow.
Extreme pale colour	If urine is diluted, the amount of urochromes per volume decreased and urine appears paler than normal. This happens during diabetes, ADH deficiency and iron deficiency.
Orange colour	Associated with the presence of drug (Senna, Santonin, Rhubarb), dye or food metabolites (Carotene).

Green colour	This colour indicates increased indican. Riboflavin and acriflavin also imparts this colour. Blue green colour is due to presence of Methylene blue.
Red colour	This colour indicates abnormal concentration of uroerythrin (normal pigment), abnormal pigments (hemoglobin, myoglobin, RBCs and porphyrins) and medicines (mercurochrome, pyridium, phenothiazene, phenolpththalein), blood, aniline dyes (from sweets), beet root.
Brown/ Black colour	Due to presence of penolic drugs, porphyrins, melanin pigments. Also observed in normal horses due to oxidation of pyrocatechin upon standing, in azoturia due to myoglobin in urine.
Milky colour	Due to excess of phosphates, urates and presence of chyles, fat globules and rupture of lymphatics or thoracic duct.

2. Appearance:

Appearance may be recorded as clear, flocculent or cloudy. Normally, freshly voided urine is clear and transparent to very slightly cloudy. In horse urine is normally quite thick and cloudy due to the presence of calcium crystals and mucus. Normal urine from other species of animals although clear, may become cloudy as it cools and precipitation of crystals. Sometimes it may be excessively cloudy or turbid. Excess turbidity results from the presence of suspended particles (crystals or pus cells or bacteria, RBC, mucus, epithelial cells) in the urine.

Procedure:

1. Mix the sample well.
2. Note if it is clear or turbid.

Turbidity may be reported as slight, moderate or excessive.

Determination of cause of turbidity:

The cause of the turbidity can be determined by centrifuging a portion of the urine. After centrifugation if the cloud settles to the bottom leaving a clear supernatant, the turbidity is caused by cells or crystals. If the urine remains cloudy after centrifugation, bacteria are present. The cause can usually be determined based on the results of the microscopic urine sediment examination.

Causes of Turbid Urines

Amorphous Phosphates	Normal constituents in alkaline or neutral urines; dissolve upon addition of a dilute acid (e.g. acetic). If combined with carbonates, gas will be evolved upon the addition of the acid.
Amorphous Urates	A normal constituent in acid urine; often appear as yellow crystals or may be pink (brick dust deposit) due to increased uroerythrin. They dissolve upon warming to 40^0C.
Bacteria	Usually seen as a uniform cloud when an excess of bacteria is present; cannot be removed by ordinary filtration or centrifugation; seen in microscopic examination.
Blood/RBC	May give a reddish or brown, smoky appearance to the urine; recognized by seeing red cells upon microscopic examination or chemical tests for hemoglobin.
Colloidal Particles	Cannot be cleared from urine by filtration or centrifugation; are not visible in microscopic examination and not removed by ether. Their cause is unknown.
Fat Globules	Will usually give a milky appearance to urine; may be opalescent; are seen in microscopic examination and removed by ether.
Mucus and Epithelial Cells	Upon cooling and standing, a faint cloud ("nubecula") of mucus, leukocytes and epithelial cells may settle to the bottom. In urine of high specific gravity it may float near the middle. The nubecula of normal urine is probably due to nucleoprotein (phosphoprotein) and not a mucin or mucoid (glycoprotein).
Pus cells/ WBC	May resemble amorphous phosphates to the naked eye. Microscopic examination will reveal that the cloud is due to leukocytes.

3. Odour:

Not many people enjoy smelling the urine they analyze, but one should note very strong odors. Identification of strong odour of urine is important because the odour or smell of urine can give clues about the origin of diseases.

Procedure:

1. Open the lid of the collection container and
2. Observe the smell of the urine

Urine Odour and Their Causes

Normal	
Faintly Aromatic or Urinoid	Presence of volatile organic acids or a substance called "Urinoid" in the urine specimens.
Abnormal	
Ammoniacal	Normal urine on standing or retention within urinary bladder gives ammoniacalodour due to decomposition of urea into ammonia by bacterial action. It may also be related to some bacterial infections.
Foul or Putrid	Foul-smelling urine is a common symptom of urinary-tract infection. Decomposition of urine containing pus will give the odour of rotten eggs (H_2S).
Fecal	Due to contamination with feces or E. coli; often related to bladder-GI tract fistula
Fruity/ sweetish	Usually due to the presence of acetone (ketone bodies) in the urine during diabetic ketosis, starvation or dieting.
Effects of drugs and diet	Many ingested substances will give the urine a distinct odour. Example: ingestion of asparagus and drugs like turpentine oil, menthol etc. giving urine its characteristic odour.

4. Volume:

The volume of urine excreted is taken for the total volume excreted within 24 hours. The volume of urine excreted varies with the substance to be excreted, temperature and exercise. Volume of urine voided by domestic animals and human being is given below.

Species	Urine Volume(ml/ KBW/ day)
Cattle	17-45
Horse	3-18

Sheep	10-40
Goat	10-40
Cat	10-20
Dog	20-100
Swine	5-30
Man	8.6-28.6

Procedure:

1. Collect all the urine voided in 24 hours.
2. Measure the volume of urine using a measuring cylinder.

Some conditions and terms related to volume of urine

Polyuria: - An abnormal increase in volume of urine voided per 24 hours. Urine volume will be increased in diabetes mellitus, diabetes insipidus, diseases of nervous system etc. Some drugs like calomel, acetates, salycylates etc. also can increase the urine volume.

Oliguria: - An abnormal decrease in urine volume is called Oliguria. Volume of urine decreases in acute nephritis, fever, diarrhea, vomiting, diseases of heart, hot weather etc.

Anurea: - Total stoppage of urinary excretion for 12- hours or excretion of less than 100 ml of urine per 24-hours. This is observed in case of acute renal failure, cardiac failure, or surgical shock.

Nocturea:-An abnormal increase in night urine. If an adult voids more than 500ml of urine in the night the condition is known as nocturea.

Diuretics: - Diuretics are substances that stimulate the formation of large volumes urine. Tea, coffee and alcoholic drinks have diuretic effects. People with chronic high blood pressure (hypertension) often take diuretics to control their blood pressure. Diuretics reduce Na reabsorption from the lumen of the nephron. Water reabsorption is also reduced. Therefore, Na and water are lost in the urine, which increases urine flow. The decreased reabsorption of Na and water from the nephron reduces blood volume, thereby reducing blood pressure. Frusemide, acetozolamide, glucose, mannitol etc. are some commonly used diuretics.

5. Specific gravity:

Specific gravity of urine varies with the relative proportion of dissolved matter and water. Urine specific gravity is a measurement of the density of urine compared to pure water. Water has a specific gravity of 1.000. Urine will always have a value greater than 1.000 depending upon the amount of dissolved substances (salts, minerals, etc.) that may be present. Very dilute urine has a low specific gravity value and very concentrated urine has a high value. Specific gravity measures the ability of the kidneys to concentrate or dilute urine depending on the fluctuating conditions. Usual range for man is 1.003 - 1.060 (Average range 1.010 - 1.025)

Normal values of specific gravity of urine in domestic animals and man:

Species	Specific Gravity
Bovine	1.025-1.045
Ovine	1.015-1.045
Caprine	1.015-1.045
Porcine	1.010-1.030
Equine	1.020-1.045
Canine	1.015-1.045
Feline	1.020-1.045
Man	1.002-1.040

6. Total Solids:

Total solids in urine are derived by multiplying last two figures of specific gravity by a constant 25 (Long's coefficient). This gives gm of solids per litre of urine.

7. Osmolality:

It is the number of osmomoles of a solute per Kg of solvent (water) and is directly related to the depression of the freezing point of an aqueous solution. A change in Osmolality of urine may not be reflected in the specific gravity.

8. Freezing Point:

It is a measure of salt concentration of the urine and is not materially influenced by large molecular substances such as proteins. Higher the water content, the freezing point will be closer to zero.

Species	Freezing Point Values
Horse	-177^0 to -20^0C
Goat	-157^0 to -36.3^0C
Cat	-5^0C

Methods of determination of specific gravity:

Specific gravity can be determined by either of the two methods,

1. Refractometer method.

2. Urinometer method.

Refractometer Method:

The refractometer measures the refractive index of the total soluble solids in the urine which parallels the specific gravity. When a beam of light passes through one substance into another, the beam is refracted so that it travels in another direction. The extent to which the beam is refracted depends on the concentration of the total soluble solids.

Advantages:

1. Only a few drops of urine are required.
2. The method is rapid and easy to perform

Urinometer Method:

Principle: Urinometer is a weighted, bulb shaped device that has a specific gravity scale on its stem.It is a hydrometer and is based on the principle that dissolved substances will cause a body to float (buoyancy).

Materials required:

i. Urine sample,
ii. Urinometer cylinder,
iii. Thermometer.

Procedure:

1. If the urine sample is refrigerated, allow it or warm it to reach the room temperature.
2. Mix the urine sample well and pour it into the urinometer cylinder (25 ml capacity) without producing bubbles.
3. Keep the cylinder on a flat surface.
4. Grasp the stem of the urinometer at the top and insert it into the urine in the urinometer cylinder slowly.
5. Rotate the urinometer to prevent it from touching the sides of the cylinder.
6. Read the specific gravity as soon as the urinometer comes to rest and floats freely without touching the sides or bottom of the urinometer cylinder.
7. Take the reading from the graduation given on the stem of the urinometer at the interface of the air and urine (lower meniscus).
8. Record the reading in decimals. E.g. 1.030.

Precautions:

1. Urinometer and urinometer cylinder should be clean and dry.
2. While filling the urinometer cylinder take care to leave sufficient space at the top of the cylinder so that urine does not overflow when the urinometer is floated.
3. While taking the reading urinometer should not touch the bottom or sides of the cylinder.
4. Take the reading of the lower meniscus at the interface of the air and urine.
5. Apply temperature correction for accurate results.

Temperature correction:

The urinometers are calibrated to show the accurate specific gravity at a particular temperature (say 20 °c). This temperature will be printed on the stem of the urinometers. Temperature correction is recommended when the temperature of urine is significantly different from the temperature calibrated for the urinometer. For very precise and accurate measurements of specific gravity, corrections should be made +/- .001 for each 3□ C above or below 20□ C. Add .001 to the observed specific gravity reading if the temperature of the urine is 3□ C above 20□ C, subtract .001 if below 20□ C.)

1. Causes for low specific gravity of urine:

Physiological: Excessive water intake, estrus, administration of intra venous fluids, diuretics, corticosteroids etc.

Pathological: Diabetes insipid us due to deficiency of ADH, chronic renal failure, acute nephritis.

2. Causes for high specific gravity of urine:

Physiological: Decreased water intake, high environmental temperatures, or excessive hyperventilation (panting)

Pathological: Excessive water loss due to vomiting, diarrhea, fever, or exudation associated with burns, Diabetes mellitus, adrenal abnormalities, or shock

Observation/Result:

UNIT-3

Practical No.3 **Date:**

CHEMICAL EXAMINATION OF URINE

The chemical examination of urine refers to the analysis of urine for,

1. Measuring the pH or reaction of the urine and to detect the presence of 2. Glucose 3. Protein 4. Ketone bodies 5. Bile salts 6. Blood in the urine.

The chemical examination can be qualitative or quantitative or both. A qualitative analysis determines the presence or absence of a substance in a solution (urine). A quantitative analysis measures the quantity of the substance present in the solution. Normal urine does not contain glucose, proteins, ketone bodies, bile salts and Blood. If the urine contains these substances in appreciable quantities it may indicate some states of disease or body dysfunction. It may serve as a useful indicator of health or disease, especially in the areas of metabolic and renal disorders.

Abnormal Constituent	**Associated Causes**
Protein (albumin)	Albumin is normally too large to pass through glomerulus. Indicates abnormal increased permeability of the glomerulus membrane. ***Non-pathological causes*** *are: pregnancy, physical exertion, increased protein consumption.* ***Pathological causes*** *are: glomerulonephritis bacterial toxins, chemical poisons.*
Glucose	Glycosuria is the condition of glucose in urine. Normally the filtered glucose is reabsorbed by the renal tubules and returned to the blood by carrier molecules. If blood glucose levels exceed renal threshold levels, the untransported glucose will spill over into the urine. *cause: diabetes mellitus*
Ketones	Ketone bodies such as acetoacetic acid, beta-hydroxybutyric acid, and acetone can appear in urine in small amounts. These intermediate by-products are associated with the breakdown of fat. *Causes: diabetes mellitus, starvation, diarrhea*
Bilirubin	Bilirubin comes from the breakdown of hemoglobin in red blood cells.

	The globin portion of hemoglobin is split off and the heme group of hemoglobin is converted into the pigment bilirubin. Bilirubin is secreted in blood and carried to the liver where it is conjugated with glucoronic acid. Some is secreted in blood and some is excreted in the bile as bile pigments into the small intestines. *Causes: liver disorders, cirrhosis, hepatitis, obstruction of bile duct*
Urobilinogen	Bile pigment derived from breakdown of hemoglobin. The majority of this substance is excreted in the stool, but small amounts are reabsorbed into the blood from the intestines and then excreted into the urine. *Causes: hemolytic anaemias, liver diseases*
Hemoglobin	Hemoglobinuria is the presence of hemoglobin in the urine. *Causes: hemolytic anemia, blood transfusion reactions, massive bums, renal disease*
Red blood cells	Hematuria is the presence of intact erythrocytes. *Causes: kidney stones, tumors, glomerulonephritis, physical trauma*
White blood cells	The presence of leukocytes in urine is referred to as pyuria (pus in the urine). *Causes: urinary tract infection*
Nitrite	Presence of bacteria. *Causes: urinary tract infection*

1. Determination of pH/ Reaction of urine:

In the human beings freshly voided normal urine is slightly acidic in reaction. The pH of the urine can vary from 4.5 - 8.0. Average pH is 6.0.

Methods:

The pH or reaction of the urine sample can be determined by either of the two methods,

i. Litmus paper method

ii. pH paper method.

i. Litmus paper method:

Blue and red litmus papers can be used to determine the pH or reaction of the urine sample qualitatively.

Procedure:

- Dip the blue litmus paper into the urine. If it turns red it indicates that the urine is acidic in reaction.
- Dip the red litmus paper into the urine. If it turns blue it indicates that the urine is alkaline in reaction.

- If both the blue and red litmus papers does not change colour after dipping into the urine it indicates that the urine is neutral in reaction.
- If both blue and red litmus papers change colour after dipping into the urine it indicates that the urine is amphoteric in reaction.

ii. pH paper method:

Wide range (2.5-10) pH paper strips can be used to determine the pH or reaction of the urine sample quantitatively.

Principle:

The test is based on the double indicator (methyl red/bromothymol blue) principle that gives a broad range of colors covering the entire urinary pH range. Colors range from orange through yellow and green to blue.

Procedure:

- Detach one strip of pH paper from the packet.
- With a clean glass rod, transfer a drop of urine to the surface of the pH paper strip and spread evenly by stroking or leave a small drop on the paper.
- Leave the strip undisturbed for one minute for the colour to develop.
- After 1 minute match the colour developed on the strip with the standard colour chart given on the top of the packet of pH paper strips.
- It will give the approximate value of the pH of the urine sample.
- For accurate measurement of pH of urine pH meter can be used.

Observation/Result:

Normal reaction and pH of urine in domestic animals and man:

Species	Reaction of urine	pH range
Horse	Alkaline	7.0-8.0
Cattle	Alkaline	7.4-8.4
Sheep& Goat	Alkaline	7.4-8.4
Pig	Acid or Alkaline	5.0-8.0
Dog	Acid	5.0-7.0
Cat	Acid	5.0-7.0
Man	Usually Acid	4.5-8.0

2. Qualitative determination of glucose in the urine

Normally sugars are absent in the urine. Even though the sugars are filtered into the glomerular filtrate from the plasma they are completely reabsorbed in the tubules (PCT) of the kidney. But sugars are present in the urine under some pathological conditions like diabetes mellitus. The term melituria refers to the presence of an abnormal amount of any sugar in the urine. Glucose is the most common sugar in the urine and its presence is termed glycosuria. Other sugars occasionally found in the urine are laevulose (laevulosuria), lactose (lactosuria), Galactose (Galactosuria) and Pentose (Pentosuria).

Methods:

The presence of glucose in the urine can be determined qualitatively by either of the two methods,

i. Benedict's test
ii. Fehling's Test.

Principle:

Both the tests are based on the principle that the reducing sugars if present in the urine will readily reduce the alkaline copper sulfate in the reagent solutions (Bendicts qualitative reagent, Fehling's Solution "A", and Fehling's Solution "B") to cuprous oxide. The cuprous oxide will form insoluble green, yellow or red precipitates depending on the quantity of glucose present.

Materials required:

Urine sample, Test tube, Test tube holder, Test tube rack, Pipettes, Hot water bath or Bunsen burner

Reagents required:

Benedict's qualitative reagent, Fehling Solution "A", and Fehling Solution "B"

i. Benedict's test:

Procedure:

- Pipette out 5ml of Benedict's qualitative reagent to a test tube.
- Pipette out 0. 5ml of urine and add it to the test tube containing Benedicts qualitative reagent.
- Heat the test tube vigorously for 5 minutes or boil it in a boiling water bath for 2 minutes.
- Allow to cool.
- After cooling observe and record the colour of the insoluble precipitates developed.

Precautions:

1. Do not hasten cooling by immersing in cold water.
2. If a large number of tests are to be conducted, the tubes may be placed in a boiling water bath or a beaker of boiling water.

Interpretation:

1. If glucose is absent, the solution will either remain perfectly clear, or will show a very faint turbidity due to precipitated urates.
2. If glucose is present, the entire solution will be filled with a bulky precipitate which may be greenish yellow, yellow, orange, or brick red in colour depending on the amount of glucose present.

Fehling's Test:

Procedure:

- Pipette out 1ml of Fehling's solution "A" into a test tube.
- Pipette out 1ml of Fehling's solution "B" into the same test tube.
- Add 6 ml of distilled water to the same test tube.

- ❖ Boil the test tube containing the solutions.
- ❖ Pipette out 0.5 ml of urine and add it to the hot mixture drop by drop.
- ❖ Observe and record the colour of the insoluble precipitates developed

Interpretation:

1. If glucose is present a heavy yellow or red precipitate will appear.

2. If glucose is absent, no precipitate will appear in the solution.

Observation/Result

3. Quantitative determination of glucose in urine:

Methods:

The quantity of glucose present in the urine can be determined by Benedict's quantitative test

4. Qualitative determination of protein in the urine

Proteins especially albumin and the globulins are among the most important of the organic constituents occurring in the urine. Normal urine may contain a minute amount of protein that may not be detected by the simple tests. An excretion of large amounts of protein in the urine is termed as proteinurea. Urine containing more albumin foams markedly on shaking and the foams remain for long time. This gives a rough indication of the presence of albumin in the urine.

Methods:

a. Heller's Test
b. Modified heat coagulation Test
c. Sulphosalicylic Test

Principle:

All tests for determination of proteins are based on the principle of coagulation of proteins by heat or precipitation of protein by chemical agents.

Materials required:

Urine sample, Test tube, Test tube holder, Test tube rack, Pipettes, Bunsen burner

a. Heller's Test:

Reagents required:

Concentrated nitric acid,

Procedure:

- Take 2 ml of pure conc. HNO_3 in a clean and dry test tube.
- Add carefully 3 ml of urine from side of the test tube to layer on the acid. Keep for 2-3 minutes

Interpretation:

Appearance of a white ring at the junction of urine and acid indicates presence of protein.

b. Modified Heat Coagulation Test:

Reagents required:

Glacial acetic acid and saturated NaCl solution

Procedure:

- Take 5ml of urine in a clean and dry test tube.
- Add 1 ml of saturated NaCl solution and 5 drops of glacial acetic acid. Then heat to boil.

Interpretation: Appearance of cloudiness indicates albuminuria.

c. Sulphosalicylic Test:

Reagents required:

3% Sulphosalicylic acid

Procedure:

1. Take 1 ml of urine in a clean and dry test tube.
2. Add carefully 3 ml of Sulphosalicylic acid (3%).

Interpretation:

Appearance of turbidity or precipitates indicates presence of protein.

5. Qualitative determination of bile pigments and bile salts in the urine:

Bilirubin is secreted in blood and carried to the liver where it is conjugated with glucuronic acid. In jaundice both bile salts (Taurocholate, glycocholate) and bile pigments (bilirubin) are excreted in detectable amounts.

Determination of Bile Salts:

Hay's Sulphur test for bile salts:

Principle:

The test is based on the property of bile salts to reduce the surface tension of the fluids in which they are placed.

Materials Required:

Urine sample, Refrigerator, Test tube, Test tube holder, Test tube rack, Pipettes, Bunsen burner

Procedure:

- Cool the urine in a refrigerator.
- Take the urine in a test tube (upto 3/4 th level).
- Sprinkle small amount of sulphur powder on the surface of urine.
- If bile salts are present, sulphur powder will sink.
- If bile salts are absent sulphur powder will float.

Determination of Bile Pigments:-

Detection of bilirubin in urine is generally an abnormal finding. Bilirubinuria generally results when conjugated bilirubin levels in blood are elevated as a result of hepatobiliary disease. In some cases of hemolytic anemia, bilirubinuria may be secondary to the hemolysis without any evidence of cholestasis. The renal tubular epithelium is capable of absorbing hemoglobin from the glomerular filtrate and converting it to conjugated bilirubin, which is then excreted in the urine. This will only occur with intravascular hemolysis, when free hemoglobin is filtered by the glomerulus.The presence of bilirubin in the urine is an important finding in the evaluation of liver function.

Methods:

i. Gmelin's test
ii. Iodine test

i. Gmelin's Test:

Principle:

Bilirubin is the most abundant bile pigment. The tests for bilirubin are based on the oxidation of bile pigments by acids to form coloured derivatives like biliverdin (green) bilicyanine (blue) and cholatelin (yellow).

Materials Required:

Urine sample, Test tube, Test tube holder, Test tube rack, Pipettes.

Reagents required:

Concentrated nitric acid

Procedure:

- Place 2 ml of concentrated nitric acid in a test tube.
- Overlay 2ml of urine on the acid.
- Take care to see that the two liquids do not mix.
- If the bile pigments are present various coloured rings (green, blue, violet, red and reddish-yellow) will be seen at the point of contact of the two liquids.

Observations/Result:

ii. Iodine Test:

Principle:

Bilirubin along with iodine oxidized to produce colored compounds of iodine.

Procedure:

- Take few ml of 0.5% alcoholic solution of iodine in a test tube.
- Urine acidified with acetic acid allowed to run down at the side of test tube.
- Observe the foam developed**.**

Interpretation:

If bile pigments are present that gives emerald green colour which diffused into the urine.

6. Qualitative determination of ketone bodies in the urine

Ketone bodies (acetoacetic acid, acetone, and beta hydroxy butyric acid) are substances produced as intermediate compounds during oxidation of fatty acids. In normal conditions these do not appear in the blood or urine. This is because Glucose, the simplest form of sugar, is what the body uses for energy normally. If ketone bodies appear in the blood or urine the conditions are termed as Ketonemia and Ketonuria respectively. Ketonuria indicates deranged energy metabolism such that fat is used in excess of carbohydrate. This can result in production of the ketone bodies in amounts greater than that can be metabolized by peripheral tissue; filtration into urine in excess of tubular reabsorption then results in ketonuria.

i. Test for Acetone:

Method:

Rothera's Test

Principle:

The Rothera's Test for detection of acetone is based on the development of violet colour when acetone reacts with sodium nitroprusside.

Materials required:

Urine sample,Test tube, Test tube rack, Test tube holder, Pipette

Reagents required:

1. Ammonium sulphate
2. 5% aqueous solution of sodium nitroprusside

Procedure:

- ❖ Take 5 ml of urine sample and saturate it with ammonium sulphate powder (1 gm).
- ❖ To it add 2-3 drops of freshly prepared 5% aqueous sodium nitroprusside solution.
- ❖ A reddish purple or potassium permanganate colour develops if ketone bodies present

Note: Ketone bodies (acetoacetic acid**,** acetoneand β-hydroxy butyric acid) result from lipid breakdown and accumulation of acetyl co-A results under conditions of depletion of liver glycogen (starvation, diabetes, vomition for long time, severe diarrhea and high fat & low carbohydrate diet) as a result of low availability of oxaloacetate needed to enter into TCA cycle.

In small domestic animals (dog & cat) the principal cause of ketosis is diabetes mellitus. In ruminants ketosis is frequent due to production of VFA. Ketosis is observed in pregnant ewes carrying twin lambs due to in sufficient supply of diet containing carbohydrates.

7. Qualitative Determination of Blood in Urine.

Normally blood is absent in urine. Under some pathological conditions blood may be present in the urine. The presence of blood in the urine may be classified as hematuria and hemoglobinuria. In hematuria, unruptured red cells and hemoglobin are present in urine. This is due to whole blood passing into the urine because of some lesions in the kidney or urinary tract. In hemoglobinuria only hemoglobin is present in the urine. It is due to hemolysis. Hemolysis may occur in malaria, typhoid, yellow fever, hemolytic jaundice, burns and incompatible blood transfusion. A high number of white blood cells in the urine are usually a symptom of urinary tract infection.

Methods:

1. Benzedine Test

Principle:

This test is based on the Peroxidase-like activity of hemoglobin. Hemoglobin catalytically decomposes hydrogen peroxide and liberates oxygen .This oxygen oxidizes benzidine to a blue or green derivative.

Materials required:

Urine sample, Test tube, Test tube holder, Test tube rack, Pipettes, Water bath, China dish

Reagent:

1. 5% saturated solution of Benzidine in glacial acetic acid.
2. 3 per cent hydrogen peroxide solution.

Procedure:

- ❖ Take 3 ml of 5% saturated solution of Benzidine in a test tube.
- ❖ Add 2 ml of urine and 1 ml of 3 % hydrogen peroxide.

Interpretation:

1. Development of a blue or green colour immediately indicates a positive test.
2. Development of colour after ten minutes is not a positive test. This may be due to the oxidation of Benzidine by atmospheric oxygen.

Observations/Result:

UNIT-3

Practical No.4 **Date:**

ESTIMATION OF CREATININE IN URINE

Principle:

A measured quantity of urine is treated with picric acid and sodium hydroxide. The Creatinine combines with the picric acid to form an orange coloured picramic acid (Jaffe reaction). A known amount of Creatinine is also similarly treated and the colours are compared in suitable colorimeter.

Reagents:

1. Standard Creatinine solution containing 1.0 mg Creatinine in 1ml of 0.1N HCL solution
2. Picric acid solution 1%
3. Sodium hydroxide 10% solution

Equipment/ Glass wares:

1. **Colorimeter**
2. Graduated pipettes
3. Test tubes
4. Wash bottles containing distilled water

Procedure:

- Take three clean test tubes. Mark them with ‘T’ for unknown and ‘S’ for standard and ‘B’ for blank.
- Pipette the following solutions as shown in the table:

Reagents/Solutions	Tube-B	Tube- S	Tube-T
Water	0.1ml	-	-
Urine	-	-	0.1ml
Standard Creatinine	-	0.1ml	-
1% Picric Acid	2ml	2ml	2ml
10% NaOH Soln.	0.15ml	0.15ml	0.15ml

- Mix the contents by shaking gently
- Keep for 15 minutes.
- Dilute the solutions by adding distilled water up to 10ml mark.
- Compare the unknown against the standard in a colorimeter.

Calculations:

mg of Creatinine in 100ml of urine = (OD of unknown / OD of standard) x 1 x 100/0.1

Interpretation:

The adult human urine contains 1 to 1.8 gm of Creatinine/day. In heavy meat diet the content increases. It is entirely a waste product. Exercise increases Creatinine content as result of release from muscle stores. It increases in muscle wasting disease and fever.

UNIT-3

Practical No.5 **Date:**

ESTIMATION OF INORGANIC PHOSPHORUS IN URINE

(Method of Fiske and Subbarow)

Principle:

Phosphate reacts with molybdic acid to form phospho molybdic acid. On treatment with 1-2-4 amino naphtha sulphonic acid the phospho molybdic acid is selectively reduced to produce a deep blue colour (Molybdenum blue) which is probably a mixture of lower oxides of molybdenum. This colour is read in suitable photometer against a standard phosphate solution prepared in the same way.

Reagents:

1. Distilled water
2. Molybdate I reagent
3. 1-2-4 aminonaphtholsulphonic acid
4. Standard phosphate solution containing 0.4 gm phosphorus in 5ml.
5. Given sample urine.

Equipments/ Glass wares:

1. Colorimeter
2. Graduated pipettes
3. Volumetric flasks of 100ml capacity
4. Wash bottle

Procedure:

- Take three clean test tubes and mark them as ‘S’ for standard, ‘T’ for unknown and ‘B’ for blank.
- Pipette the following solutions as shown in the table:

Reagents/Solutions	Tube-B	Tube- S	Tube-T
Std. Phosphate Soln.	-	0.5 ml	-
Urine	-	-	0.2 ml
Molybdate I	1.0 ml	1.0 ml	1.0 ml
ANSA	0.4 ml	0.4 ml	0.4 ml

Dist. Water	6.0 ml	6.0 ml	6.0 ml

- ❖ Make it to 10.0 ml mark by adding distilled water and mix the contents carefully.
- ❖ Read after 5 minutes in colorimeter at 660 to 720 mm.

Calculation:

mg of inorganic phosphates (as phosphorus) in 100 ml of urine =

(OD of unknown / OD of Std.) x 0.04 x (100/0.2)

UNIT-3

Practical No.6 **Date:**

DETERMINATION OF AMMONIA IN URINE

Principle:

Ammonia and ammonium salts can be readily detected in very minute quantity by addition of Nessler's solution. It is a 0.09 mol/L solution of potassium tetraiodomercurate ($K_2[HgI_4]$). A yellow coloration indicates the presence of ammonia. The sensitivity of the spot test is about 0.3 µg of ammonia in 2 µl.

$$NH_4^+ + 2(HgI_4)^- + 4\ OH^- \rightarrow HgO.\ Hg\ (NH_2)\ I + 7\ I^- + 3\ H_2O$$

Nessler's Solution:

It is prepared by adding hot concentrated solution of mercuric chloride to concentrated solution of potassium iodide until the precipitate of mercuric chloride stops dissolving. The liquid is filtered and cooled down.

Other names of Nessler's solution are:

1. Mercuric potassium iodide
2. Channing's solution
3. Potassium mercuric iodide
4. Potassium tetraiodomercurate (II)

Procedure:

- Take small quantity of urine in a clean test tube.
- To it add few drops of Nessler's reagent and mix properly.
- A yellow coloration indicates presence of ammonia in traces.
- But at higher concentration of ammonia, there is formation of brown precipitate.

UNIT-3

Practical No. 7 **Date:**

BIO-ASSAY OF TROPIC HORMONES BY RADIO-ACTIVE TECHNIQUE (RIA)

Atom:

An atom has a central nucleus composed of protons and neutrons surrounded by a cloud of electrons. The energy level of electrons can be defined by the laws of quantum mechanics. Protons carry a mass of 1 and charge of +1; neutrons carry a mass of 1 but no charge and electrons have insignificant mass but carry a charge of -1. In a neutral atom the no. of protons is equal to the no. of electrons.

Ions:

These are atoms, to/from which one or more electrons have been added or removed.

Atomic Number:

Atomic Number is the number of protons present in nucleus. Atomic number determines the chemical properties of an atom.

Atomic mass:

The total number of neutrons and protons present in an atom.

Isotopes:

Atoms with constant nuclear charge i.e. constant number of protons, but with variable mass, ie, numbers of neutrons are called as isotopes. Different isotopes of an atom react alike and having very nearly the same chemical properties.Specific symbols used to depict the mass and charge of an atom. For any atom, the superscript value denotes the nuclear mass and subscript value denotes atomic number. These can also be written by omitting subscript value.

Radio-Isotopes:

There are a variety of nuclear transformation mechanisms by which unstable isotopes achieve a stable state. Many of them processes result in omission of specific particles. Such unstable-isotopes emitting particles are called as radio isotopes or

radioactive form of an element. Any radio-isotopes decay by one or at a few nuclear transformation mechanisms. The characteristics of different emitted particles are sufficiently different from one another that the procedure for detection of different particles is also different. The great majority of biochemical application of isotopes involve only two types of radioactive decay, i.e., β-emission or γ-emission.

Measurement of Radioactive Decay:

For biochemical analysis mostly the radioisotopes that emit β or γ particles are used frequently. A number of different techniques have been developed for detecting the rates and energies with which these particles are released during radioactive decay. Two of them are liquid and solid scintillation counting and radio-autography using photographic and phosphor-storage technique.

Scintillation Counting:

Scintillation counting is used to detect both β and γ particles. Emitted particle causes a series of bright light flashes that are detected by a photocell of recorded as count for interpretation.

Solid scintillation counting is preferred for the measurement of γ particles. The atoms of sodium iodide present in detection crystal optimize the absorption of γ particles.

Liquid scintillation counting is preferred for the measurement of β-particles.

Liquid Scintillation Counting:

Light emitting process is the base of scintillation counting and the emission of light occurs as a result of a radioactive samples placed in a scintillation cock-tail containing an excitable solvent of one or more fluorescent compound called ‘Fluors’. Emitted β particle come in contact with solvent molecules (s) and transfer some of their energy to those molecules. This produce an excited solvent molecule (s*) and a β particle containing less energy.

$$\beta^- + S \rightarrow S^* + (\beta^- - E)$$

The energy required to excite the solvent molecule is very small, so the residual β-particle may excite many solvent molecule before its energy level depleted. The number of excited solvent molecule is proportional to the energy level of β- particle.

Excited solvent molecule transfer their energy to other solvent molecule and then to a fluor molecule (F_1*). Then F_1*emits a photon and decays to its normal state(F_1).

$$\mathbf{S^* + F_1 \rightarrow F_1^* + S}$$

$$\mathbf{F_1^* \rightarrow F_1 + hv_1 \ (photon)}$$

A secondary fluor may be used in some scintillation cock-tail which absorbs the photon emitted by the primary fluor. The excited secondary fluor then emits a new photon at a wavelength more favourable for detection of experimental materials in the scintillation cocktail or more favourable to the phototubes of scintillation counter. Now-a-days modern scintillation counters no longer require the uses of a second fluor.

The photons emitted by fluors are detected by a phototube-photomultiplier system. The life time of β particle passages, solvent excitation and photon emission from fluors is compared with the responses time of photomultiplier tube system. So all the events from a single β emission are scored as single '+' by photomultiplier system. The overall energy of the photons from a single β emission is often quite small. The photomultiplier tube used to detect such low energy events must be very sensitive. This higher sensitiveness also detects 'photomultiplier noise' or 'spurious counts' unrelated to β emission.

This noise is greatly reduced by a coincident circuit that contains 2 photomultiplier tubes which simultaneously detect a light flash to yield a count on scale. Thus most noises that occur in a single tube are screened out. Sometimes coincident circuit cannot detect low energy β emission. This prevents scintillation counter from achieving 100% efficacy.

Measurement of radioactive decay can also be affected by various components present in or added to the scintillation cock-tail. These components can cause quenching. Quenching means decrease in efficiency of the scintillation process. Scintillation counting provides data in "counts per minute" (cpm). Quenching dictates

that the “counts per minute detected” is less than the actual decay rates or “disintegration per minute” (dpm). Almost every sample encountered experimentally is quenched to some extent.

Example:

O_2 picked up by the scintillation fluid from contact with air serves as a quencher. So everybody should count an additional sample containing a standard of known decay rate (dpm) along with experimental sample vial. Comparison of the observed counts per minute (cpm) with the known disintegration rate per minute, determines the counting efficiency of the scintillation system.

$$\% \text{ counting efficiency} = X \frac{cpm}{dpm} 100\%$$

Quenching can occur in three ways:

Colour quenching:

The colour of sample absorbs the photon emitted from flours before they detected by photomultiplier tube. It can be overcome by refining the sample before adding the scintillation fluid

Point quenching:

Sample is not solubilized in scintillation fluid and β-particles absorbed at their origin before they contact the solvent. It can be overcome by adding solubilizer or detergents.

Chemical quenching:

This occurs when various compounds present in scintillation vial decrease the efficiency by interacting with excited solvent or fluor molecules so as to dissipate the energy without yielding photons. It cannot be avoided.

β- counter

Solid Scintillation counting of β-Emissions:

β-radioactivity can be measured by scintillation of solid samples. Liquid samples containing the radioactive material to be counted are placed in small sample cups coated on the bottom with a solid matrix containing Yttrium (Y, atomic no:39). Here yttrium acts as scintillant. The sample is dried and can be counted in liquid scintillation counter with some changes in counting-windows and coincidence circuit. The volume of liquid sample should be restricted to 200µl and the radioactive material should not be volatile.

UNIT-3

Practical No. 8 **Date:**

Serial No: 1

RADIO-IMMUNO-ASSAY (RIA) OF HORMONES

Principles:

The principle of Radio-Immuno-Assay (RIA) is based on the competition between labeled (radioactive) and unlabeled antigen/hormone for fixed binding sites on the antibody molecules. As a result of this competition, the amount of radioactive antigen/hormone that can bind to the antibody is related inversely to the concentration of unlabelled antigen/hormone (standard or unknown) present in the reaction.

This may be represented as follows:

Ag AgAb - (1)

(free antigen/hormone)

+ Ab.....

Ag* Ag*Ab - (2)

(tagged antigen/hormone)

(1 & 2) corresponding antigen-antibody complexes

The aforesaid reaction is carried out by incubating fixed quantity of antibody (Ab) with a mixture containing a fixed quantity of labeled antigen/ hormone (Ag*) and varying concentration of unlabeled hormone/antigen (Ag). Free antigen/hormone (Ag and Ag* are separated from the antibody bound

antigen/ hormone (Ag Ab and Ag*Ab) and the radioactivity of either the bound or free fraction is counted. A standard curve relating the antigen/hormone concentration and bound radioactivity is plotted. The antigen/hormone concentration of an unknown sample is determined from this standard curve

Procedure:

The essential requirements of RIA are:-

1. Standard: Sample of pure antigen/hormone of unknown potency
2. Radio-labeled antigen/ hormone
3. Specific antibody
4. Method for separating the antigen/hormone - antibody complex from the free antigen/hormone.

1. Standard:

Standard hormone/antigen plays a crucial role in RIA because unknown sample is quantified by comparing its response to that of the standard. Hence it is essential to select an identical antigen/hormone as standard to that of unknown.

2. Radio-labeled antigen/hormone:

This reagent of RIA has many synonyms such as tracer, labeled antigen/hormone, tagged antigen/hormone etc. It is highly purified analyte to which a radioactive atom has been attached. The radioactive atom commonly used to label antigen/hormone are tritium (^{3}H), cobalt 60 (^{60}Co) ^{24}Na, ^{22}Na, ^{121}I, ^{125}I and ^{131}Ietc.

It is to be noted that the labelled antigen/hormone must be identical to the antigen/hormone being measured in terms of its affinity towards the antibody.

3. **Specific antibody:**

Antibodies belong to the class of serum proteins known as immunoglobulins. Antibodies that bind strongly to the antigen/hormone are naturally having a strong affinity for the antigen and such antibodies result in highly sensitive RIAs

4. **Separation procedure:**

Separation procedure of RIA comes after antigen/ hormone and antibody reaction. By this procedure antigen-antibody complex is separated from free antigen/hormone so that the radioactivity of either fraction can be counted. The separation technique may be classified into three general categories.

a) Absorption method:

Here free antigen/hormone is adsorbed onto adsorbent like dextran charcoal.

b) Precipitation method:

Here only bound fraction is separated as precipitation of antigen-antibody complex may be of two types:

1) Non-specific precipitation

2) Specific precipitation

Non-specific precipitation of antigen-antibody complex is done by reagents like ammonium sulfate, alcohol or poly-ethylene glycol etc.

In the specific precipitation methods a sound antibody directed against the primary antigen-antibody complex.

c) Solid phase method:

This method consists of the primary or second antibody either bound to be solids like cellulose, sephadex, plastic or polysterene tubes, beads etc. In the separation step, the solid phase containing the bound fraction is separated and counted.

The reagents for RIA are supplied by the manufacturer in kit form and the user has to reconstitute these reagents as described in the assay protocol.

UNIT-3

Practical No. 9 **Date:**

Serial No.- 2

ESTIMATION OF HORMONES BY ELISA TECHNIQUE

Immunoassay are rapidly replacing many other methods used to detect or quantitate substrates with important biological or pharmacological properties.

ELISA (Enzyme Linked Immuno-Sorvent Assay) is based on the same principle as that of RIA. In ELISA, a suitable enzyme is used in place of radio-isotops for labeling/conjugating antigen/hormone are –

1. Alkaline phosphatase from calf-intestine
2. Horseradish peroxidase &
3. E. Coli β-galactosidase

ELISA having some advantages (compared to RIA)

1. ELISA have much longer shelf lives, whether in RIA, the commonly used isotops (I^{25I} and I^{31I}) have short half- life.
2. Performance of RIA requires special precautions because of the health hazards passed by radioactive isotops, and the regulation restricting their handling are becoming more. In contrast, enzyme labeled materials are not hazardous
3. Instruments used in RIA are costly

One limitation of ELISA, there is relative lack of control of enzyme labeling reactions as compared with radio-labelling procedures.

EIA &RIA, nearly the same sensitivity.

UNIT-4

Practical No. 1 **Date:**

PHASES OF OESTRUS CYCLE IN DIFFERENT SPECIES

All mammalian females exhibit the sign of estrous, which could be advantageously used for successive breeding. Unlike the pubertal male, which is always ready for mating, the pubertal female exhibits rather short periods of sexual readiness cyclically at species characteristic intervals. The time at which a female animal is receptive to the sexual advances of the male and allows mating is called the estrus (means made desire). The behavioral manifestation is associated with cyclical changes in the structure and function of the reproductive organs. The time interval between two successive estrus period is called the estrous cycle. The length of estrus and the estrus cycle is vary among species.

Cow:

The length of oestrus cycle is about 21 days and estrus lasts about 14 to 20 hrs in cows. The behavioral changes in estrus cows are short in duration (12 to 22 hrs). During the restricted 12 to 22 hrs period of estrus the female bellows, attempts to mount other cows or bulls, but will stand to be mounted by bulls or other cows. The heat period in heifer is shorter than the mature cow. Appetite declines in estrus cows and milk production may drop.

A bull upon approaching the estrus cow muzzles the external genitalia and rear quarters for several minutes and then reacts by standing rigidly with the head extended and the upper lip raised.

The signs of estrus include mucus discharge, vulvar edema, vocalization, increased activity, decreased milk production and the mounting of other cows. Cervical mucus is less viscous on the day of estrus and hangs as a string of clear mucous from the vulva. During heat the cervix is dilated. Most cows and heifers show bleeding on the 2^{nd} or 3^{rd} day after estrus.

During luteal phase cervical mucus is thick and tenacious, the cervical canal is tightly closed and the myometrium relaxes. Cattle are unique among farm animals in

that they ovulate 10 to 12 hrs after the end of estrus or on the average 30 hours after the onset of estrus.

Water Buffalo:

Buffaloes calving in summer or fall resume ovarian cyclicity earlier than those in winter or spring. Probably decreasing day length and cooler ambient temperatures favour the cyclicity. During summer when ambient temperature and photoperiod are at their maximum, prolactin level is at highest and plasma progesterone level is at lowest. High ambient temperatures may contribute to this seasonality by depressing male libido.

The length of oestrus cycle is about 23 days and estrus lasts about 12 to 30 hours (1-1.5 days). Signs of estrus are less intense than in cattle. Acceptance of the male is the most reliable sign of estrus in the buffalo. Less than a third of buffaloes in estrus are detected by homosexual behaviour. A discharge of clear mucus from the vulva, restlessness, increased frequency of urination, vocalization and a drop in milk production are not reliable signs of estrus. Maximum ferning of mucous occurs during estrus. Estrus commences towards late evening with peak sexual activity between 6 p.m. and 6 a.m. Mating continue until late morning in the river buffalo, but usually cease during day light hours in case of swamp buffalo.

Mare:

The oestrus cycle of mare averages one or two days longer than that of cow. Mare has a much longer estrus period (4-7 days). The corpus luteum of the mare is active for about 13 or 14 days of the 22 (18-24) days cycle. The period of estrus or sexual receptivity is 1 to 2 days longer during early part of the breeding season, but about 1 day shorter in the lactating mare. Ovulation occurs during the last half of oestrous. Ovulation usually occurs last 2 days of estrus.

The usual criterion for estrus is whether the mare will stand for breeding by the stallion. Since courtship behaviour in horses is violent, protective measures are ordinarily taken and the actual time of first standing is hard to determine. This may account for the variability in the reported length of oestrous in mares.

During estrus the vulva becomes large and swollen; the labial folds are loose and readily open on examination. The vulva becomes scarlet or orange, wet, glossy and covered with a film of transparent mucous. The vaginal mucosa is highly vascular and thin watery mucous may accumulate in the vagina. During estrus the cervix dilates enough to permit two to four fingers and during diestrus only one finger can be inserted.

During estrus the mare assumes a stance characteristic of urination. The tail is raised, urine is expelled in small amounts and the clitoris is exposed by prolonged rhythmic contractions.

Sheep and Goats:

The length of normal oestrus cycle is 16 days for sheep and 20 days for goats. Estrus lasts 24 to 36 hours (1-2 days) in the ewe and 24 to 48 hrs (1-3 days) in the doe. Wool breeds have longer estrus period than meat breeds. Angora goats have a shorter duration of estrus (22 hrs) than the dairy breeds.

Signs of estrus are more conspicuous in does than in ewes. A doe in estrus is restless, bleats frequently and wags her tail constantly and rapidly, she may have a reduced appetite and decreased milk secretion. Estrus in ewe is inconspicuous and is not evident in the absence of ram. The vulva may be edematous and a mucus discharge from the vagina may be evident in both species. A doe may occasionally exhibit homosexual behaviour, but not the ewe. Without the presence of the male estrus is difficult to detect in ewe and the doe. The introduction of rams to ewes during the transition from the anoestrus season to the breeding season stimulates them to ovulate within 3 to 6 days and oestrus activity occurs 17 to 24 days later. The response of anovular ewes to the ram is due to an androgen dependent pheromone secreted by the sebaceous glands of the ram. That bucks were as effective as Dorset rams may suggest that both species produce similar pheromone. The introduction of a buck into a group of seasonally anoestrous dairy does may not only hasten the onset of breeding season, by several days, but can effectively synchronize them. Most seasonally anoestrus does were detected in oestrus within 6 days after introduction of the buck, and this was followed by ovulation and corpus luteum function. Thus ovulation induced by the “male effect” is more effective in the doe than in the ewe.

Pig:

Oestrus in sow lasts from 40 to 70 hours (1-3 days). Usually the sow seeks out the male when he is within sight, sound or vocal response. There may be muzzling actions and attempt to mount both sows and boar, but more commonly the female assumes a characteristic immobile stance with elevation of ears in response to the bear's vocal response, muzzling and attempts to mount.

The boar will test sows for oestrus by vocalizing, urination, muzzling and attempting to mount and randomly seeks the female with this pattern of courtship. Nasogenital testing is common in the boar. The boar has a corkscrew glans penis that penetrates the female's cervix during ejaculation. Ejaculation lasts 6 to 8 minutes. Pig show the symptom of lardosis.

Dog:

The bitch has only one oestrus period in each reproductive cycle, whether she is mated to fertile or infertile dogs or not mated at all. The inter oestrus interval is highly variable between breeds and among bitches of same breed. After puberty bitches cycle every 4 to 12 months. The average inter oestrus interval is about 7 months. The bitch is in oestrus as long as she accepts the male for mating.

For the bitch proestrus is the beginning of the period of sexual activity. Proestrus is considered to begin when the bitch discharges blood from the vulva. The first day of bloody discharge is generally agreed to represent the first day of proestrus. The blood discharged from the vulva at the time of proestrus is probably of uterine origin and together with secretion from uterine glands is usually first detected and reported as spotting by the bitch's owner. During proestrus the bitch tends to be excitable, restless and may lose her appetite, water intake is usually increased and the bitch tends to urinate frequently.

Bitches become attractive to males during proestrus. Pheromones released in the vaginal secretions and urine stimulates and attracts males. Bitches in proestrus are inclined to roam and usually are followed by a pack of dogs. During proestrus the bitch will not accept the male for mating and many even be aggressive to the male. As the

bitch approaches oestrus, she becomes more acceptive and sexually experienced bitches may even allow mounting by the male.

Proestrus extends from the first day of bloody discharge from the vulva to the first day for acceptance of the male for mounting and lasts on the average 9 days, but may range from 2 to 15 days.

Estrus, the period of sexual receptivity, is reliably determined by the bitch's acceptance of the male for mating. Estrus lasts an average of 10 days, but may range from 3 to 12 days. The estrus bitch adopts a definite stance for mating, deviates and holds the tail to one side and exposes the vulva by arching her back. Bitches in estrus activity seek males for mating. Methyl-p-hydroxy benzoate, which stimulates the mounting mating reaction in the male dog was proposed as a pheromone released from the vagina of the bitch. Due to lengthy proestrus and estrus, the period during which the male remains attached is prolonged.

Interaction between male and female during proestrus consists of frequent urination by both and attention shown towards each other. The male investigates and often licks the ano-genital area of the female. The female may exhibit a bowing posture, but does not allow mounting. Mounting may be discouraged by moving or growling. With the onset of estrus the female lordoses for the male and allows mounting and intromission. Little courtship is involved during full estrus, but during late estrus receptiveness declines.

UNIT-4

Practical No. 2 **Date:**

HEAT DETECTION IN ANIMALS (SIGNS OF ESTRUS)

Relevance:

Heat detection in animals helps for artificial insemination at proper time for achieving optimum conception rate.

Materials Required:

Estrus animals/ herd, teaser male, trained dogs, pedometer, marking devices, etc.

Methods:

1. Use teaser male/ trained dog/ pedometer/ marking device to screen the animals in heat in the herd.

Or

Employ a trained attendant for observation of herd at least twice daily i.e. morning and evening preferably after milking for at least 30 minutes each time to screen the animals.

2. Confirm the animals in heat by visual observation for change in behaviour and external genitalia for at least 30 minutes.
3. Also confirm by pre-rectal palpation of internal genital organs.

External Signs:

- Excitation
- Alertness
- Search for males
- Licking and sniffing of external genital by other animals (homosexual activity also)
- Increased vocalization (Eg. Bellowing)
- Slight raising of tail

- Frequent micturition
- Decreased appetite
- Vulvar lips are edematous
- Vaginal mucous membrane and congested due to increased blood supply.
- Discharge will show spectrum on observation.
- Cervico-vaginal discharge is clear, transport-best sign of heat detection is STANDING HEAT & it is best time for A.I

Internal Signs:

- Os is opened
- Cervix relaxed
- Uterine horns tonic
- Cervical discharge under microscope examination shows fern pattern.

Methods used to detect heat:

(a) Use of teaser bulls

(b) Use of some dyes and levers (in sheep and goat):

Chin ball Marker Method:

This marker device is fixed at brisket region of teaser, lever gets pressed due to mounting of teaser on female and this releases the dye. The dye gets spread over rump of female. Such marked females are considered to be in estrus. It is used in small animals because in large animals there are chances of loss or damage.

(c) Vaginal Electrical Resistance Probes:

Conductivity of vaginal mucus changes with the stage of estrous cycle. The change provides basis for detecting estrus by monitoring the conductivity of vaginal mucus by using vaginal probe and resistance conduction meter. Electrical conductivity of vaginal mucous of estrus animal is at lower side.

(d) By observing estrus fern pattern:

Mucus at estrus on drying gives a fern like appearance on microscopic examination. This fern pattern arises because of high concentration of Na+ crystals in mucus. 3 types of pattern are seen. 1. Typical fern pattern 2. Atypical fern pattern 3. No pattern. This property of cervico vaginal mucus to form fern is used for estrus detection in large animals.

(e) Vaginal Exfoliative Cytology:

The vaginal smears have been stained with a modified Wright–Giemsa stain.

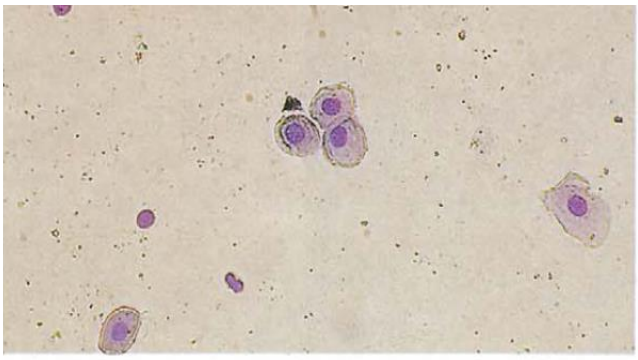
a) Anoestrus: Parabasal epithelial cells and small intermediate epithelial cells.
b) Pro-estrus: Small intermediate epithelial cells, large intermediate epithelial cells and erythrocytes. Polymorph nuclear leucocytes are also found in low numbers during this stage of the cycle (but are not demonstrated her).
c) Early estrus: Large intermediate epithelial cells, anuclear epithelial cells and erythrocytes. Polymorph nuclear leucocytes are generally absent during this stage of the cycle.
d) Estrus: Anuclear epithelial cells, large intermediate epithelial cells and erythrocytes. The percentage of anuclear cells is high.
e) Met-estrus: Small intermediate epithelial cells and large numbers of polymorphonuclear leucocytes. During early met-estrus large intermediate epithelial cells may be present, and later numbers of para-basal epithelial cells increase. There I often a large amount of background debris.
f) Late met-estrus (higher magnification than a–d): Parabasal epithelial cells and small vacuolated intermediate epithelial cells are typical of this stage of the cycle but may also be found during anoestrus and more rarely during pro-oestrus.

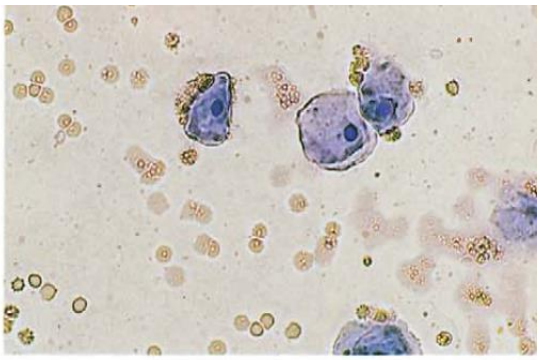
Standing Heat:

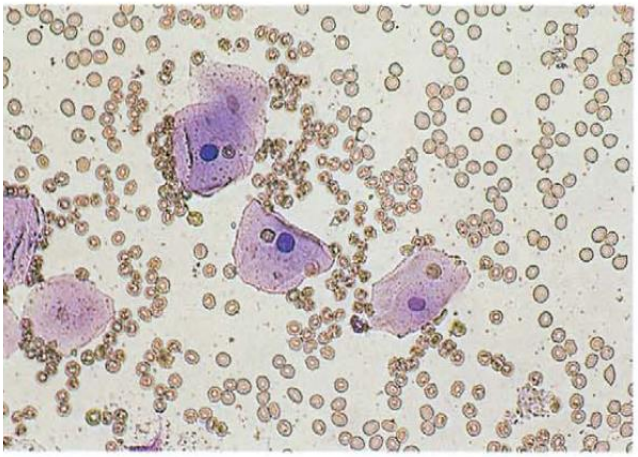
Cow in estrus is usually restless and often remains standing rather than lying down. Tail may be raised or there is often long string of clear mucus hanging from vulva or present on tail or buttock. Cow urinates frequently and has an alert, interested even inquisitive attitude. Vulva is flaccid, edematous and released. Cow will attempt to mount other cows and will stand to be mounted by other animals.

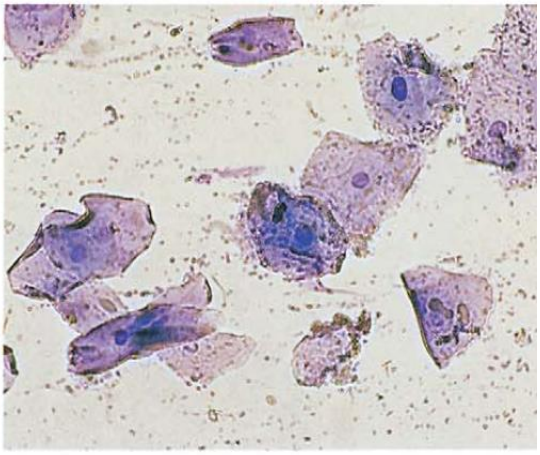
Lardosis (Copulatory Reflex):

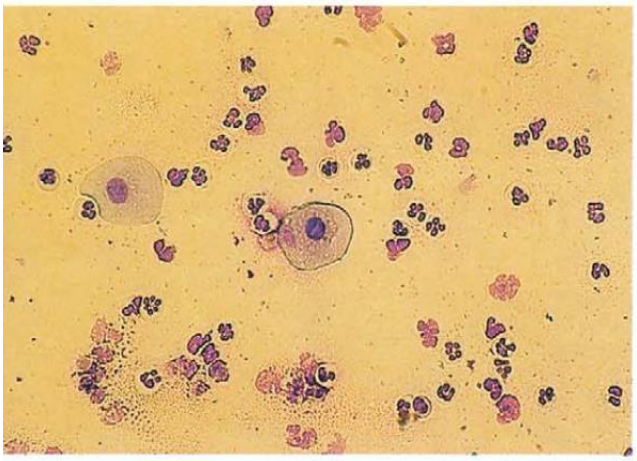
It is found in rat, cat, guinea pigs, pigs etc. and is found as a result of mating behaviour. The female adopts mating posture in response to male, she lowers hers front quarter with hind legs extending resulting in lardosis. It is arching of back in cow in estrus.

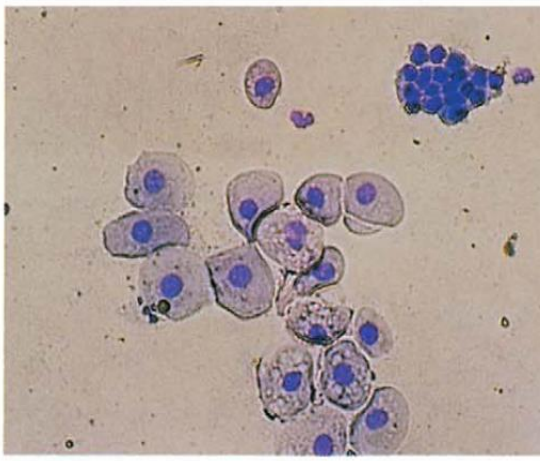












Photomicrographs of exfoliative vaginal cells during various stages of the reproductive cycle.

OBSERVATIONS: **Name of species _____**

1. Behavioral signs of heat

i. Restlessness	Yes/ No
ii. Grouping tendency	Yes/ No
iii. Allows mounting by other animals	Yes/ No
iv. Discharge of cervical mucus	Yes/ No
v. Swelling and congestion of vulva	Yes/ No
vi. Increased physical activity	Yes/ No
vii. Mounting other animals	Yes/ No
viii. Bellowing	Yes / No
ix. Consistency of cervical mucus	Thin/ Thick

2. Change in the genital organs

1. Ovaries (not to be palpated during estrus except by an expert)

	Right	Left
i. Follicle	Present/ absent	Present/ absent
ii. Corpus luteum	Present/ absent	Present/ absent

2. Oviducts — Normal/ Inflamed — Normal/Inflamed

3. Uterus — i. Coiled/ Erect

ii. Turgid/ Flaccid

4. Cervix — Open/Close

5. Vaginal Mucosa — Pink/Pale

6. Vulva — Relaxed & edematous/ Non-relaxed & Non edematous

7. Cervical mucus

i. Amount	Copious/ Scanty
ii. Consistency	Thick/ Thin

RESULTS:

Animal(s) is in heat / not in heat

PRECAUTIONS:

1. In buffaloes, observe the signs of heat during early morning and late evening.
2. Observe the animals under disturbance free conditions.
3. Use well trained observer detection of heat signs.

REMARKS:

UNIT-4

Practical No. 3 **Date:**

EXAMINATION OF CERVICO-VAGINAL MUCUS SAMPLES

Relevance:

Collection and examination of cervico-vaginal mucus sample would help in the assessment of physio-pathological condition of female genital organs like estrus and infectious diseases in general and campylobacteriosis and trichomoniasis etc. in particular.

Materials:

Sterile swabs, pipettes, catheters, vaginal speculum, disinfectant syringe and rubber connector etc.

Methods:

1. Collect vaginal mucus on a clean dry glass slide for examination of fern pattern.
2. Collect the samples using sterile swab/ catheter through vaginal speculum for general bacterial examination.
3. Collect vaginal mucus by tampon prepared locally with gauze pad of an absorbing material like surgical sponge. Leave sterilized tampon in the anterior vagina for 30 minutes, then withdraw and collect the mucus. Process the sample for vaginal mucus agglutination test for campylobacteriosis.
4. Collect muco-purulent discharge from vagina with pipette and syringe for microscope demonstration of *Trichomonas fetus*.

Observations: Record the following observations for the mucus sample

1. Physical examination

   i. Colour _____

   ii. Consistency Thick/ Thin

iii. Nature Serious/ Mucous/ Muco- purulent

iv. Presence of blood Yes/No

2. Microscopic examination:

i. Fern pattern if present Present/ absent

ii. R.B.C Present/ absent

iii. Protozoa Present/ absent

Fig: Fern pattern

3. Bacteriological examination:

Precautions:

1. Take aseptic measures in the entire process.
2. Take samples at appropriate time for specific diseases.
3. Protect the sample from being spoiled.
4. Always use sterilized equipments.

Results:

Remarks:

UNIT-4

Practical No. 4 **Date:**

EXAMINATION AND EVALUATION OF SEMEN

What is semen?

Semen is a suspension of spermatozoa in seminal fluid. The sperm are produced in the testes and stored in the epididymis whereas seminal plasma is contributed by the secretory fluids produced by accessory sex glands such as prostrate, seminal vesicles and Cowper's gland.

The fertility level of an ejaculate is the ultimate evaluation which is possible only by inseminating females and subsequently confirming their pregnancy diagnosis. Although the laboratory tests of semen evaluation are not actual measures of fertility however, the average conception rate of all ejaculates that score high on the laboratory tests will be higher than the average fertility rate of those that score low.

The following tests are generally conducted to examine/evaluate the semen:

A. Macroscopic/ Gross Examination
    1. Volume.
    2. Colour
    3. Consistency/ Density
    4. Specific gravity
B. Microscopic Examination
    1. Mass activity.
    2. Initial/ Progressive Motility
    3. Total sperm count or sperm concentration
    4. Live and dead count
    5. Morphological sperm abnormalities

C. Chemical tests

1. Fructolysis
2. Respiration coefficient
3. Methylene Blue Reduction Time (MBRT)
4. Hydrogen ion concentration
5. Catalase test

D. Bacteriological tests

1.1 Macroscopic/ Gross Examination:

1.1.1 **Volume**

The volume of an ejaculate is measured immediately following semen collection through the graduated collection tubes. The volume of ejaculate is of prime importance in artificial insemination as high volume will lead to more extended volume which can be used to inseminate large population. The volume is determined not only for use in processing but also to establish a pattern for the individual male. Deviations from this pattern, particularly downward trends in volume, indicate a problem. The problem may be due to health factors or it could be an indication which makes us think to change the collection procedures for that particular bull.

1.1.2 Colour

The normal colour of bull semen is creamy or milky white whereas in case of buffalo bulls it is chalky white and in bucks it is thick creamy or lemon coloured. The lemon colour in the semen of some individuals is due to riboflavin pigment. Any deviation from normal colour is mostly due to some pathological condition in the genital tract. Abnormal colours like reddish, brownish or blackish indicate bleeding of capillaries on the surface of glands or in seminal vesicles or deep parts of reproductive tract respectively, giving the semen respective colours. Haemospermia is generally the cause of brownish semen which may be due to tumor or cold abscess or seminal vesicles or other parts of reproductive tract pressing it and causing breakage of capillaries. All contaminated semen should be discarded.

1.1.3 Consistency/ Density

Consistency or density of semen should normally be thick opaque but variations are also noted ranging from watery to thick creamy and opaque.

Consistency	Grading of semen
Thick creamy	Excellent
Thin creamy	Very Good
Thick milky	Good
Thin milky	Fair
Watery	Extremely poor

The consistency depends upon the frequency of semen collection, body size and age of animal nutrition, exercise, teasing etc.

1.1.4 Specific gravity

The average specific gravity of whole semen is 1.035 in bull and 1.011 in dog with some fluctuations in the ratio between sperm and seminal plasma. The seminal plasma is so much lighter than the spermatozoa in bull that in practice the specific gravity of semen is often found to be directly proportional to sperm concentration.

In bull semen, low specific gravity is usually associated with low sperm concentration whereas high values accompany good density and good quality.

1.2 Microscopic Examination

1.2.1 Mass activity

This is a routine method of semen examination in which immediately after collection, a drop of neat semen is spread uniformly over a clean and dry glass slide maintained at 37^0C. It is then observed under the low power (10X) and semen is rated/ graded on the basis of eddies/ waves or swirling movement of sperm in groups.

Interpretation

0 =No motility of sperm

+ = No wave motions are observed and only about 20% of sperm have progressive motility

++ =Swirls are absent. About 40% sperm have progressive movement

+++ =The swirls are present but these are slow and scattered. About 60% sperm shows progressive motility.

++++ = The swirls are comparatively quick and waves are observed to move towards the extremities. About 80% sperm have progressive motility.

+++++ = The swirls are very quick and it is very difficult to trace when the eddies are formed and when they are disintegrated. Swirls are created with mass movement of the sperm. 80 to 100% sperm have progressive movement.

As a rule a semen sample of having mass activity **+++or more** is only recommended for artificial insemination or deep freezing.

UNIT-4

Practical No. 5 **Date:**

COLLECTION OF SEMEN FOR EXAMINATION

Mounts and teasing procedure:

Live mounts, such as a teaser female, another male or a castrated male have proven to be the most successful techniques for routine semen collection. Boars can be trained to mount dummies equally well. An estrogen treated female can provide added incentive during the training period. Dummies may be constructed to hold artificial vagina. Dummies have an advantage over live mounts to provide stability and over teaser females in permitting disease control.

Sexual stimulation prior to semen collection from bulls increases the number of sperm cells obtained by as much as 100%. False mounting a bull several times and/or intensive teasing for 5 to 10 minutes without false mounting is effective. Fluids from the accessory sex glands secreted during this preparatory period may flush out contaminating material from the urethra.

Frequency of semen collection:

Collect semen twice a day, two days per week to harvest more sperm to freeze at one time.

Sheep:

Rams are ejaculated many times a day for several weeks before severely depleting epididymal reserves of sperm. This is due to small epididymal reserves. Rams often mate or are ejaculated many times per day during the breeding season. Bucks ejaculate less frequently than rams.

Boar:

Males expel large numbers of sperm in each ejaculate and deplete their epididymal reserves more quickly. Regular semen collections not more than every other day are recommended.

Horse:

Stallion expels large number of sperms per ejaculate and depletes their epididymal reserve quickly. Regular semen collections not more than every other day are recommended.

The best procedure for collecting semen is with the artificial vagina. It provides suitable temperature, pressure and lubrication to evoke ejaculation and a calibrated tube is attached to collect the semen. Semen can be collected by use of Breeder's-Bag made up of latex.

The best procedure for collecting semen is with the artificial vagina. It provides suitable temperature, pressure and lubrication to evoke ejaculation and a calibrated tube is attached to collect the semen.

Different parts of artificial vagina (Large animals):

Artificial Vagina (AV) is a unit of instruments designed for collection of semen from male animals. The unit provides suitable temperature, pressure and lubrication simulating natural conditions for ejaculation. The AV for bulls consists of the following parts.

a) Hard rubber outer cylinder.

b) Thin rubber (Latex) inner liner

c) Rubber (Latex) collecting cone

d) Insulating bag

f) Rubber bands.

a) Hard rubber outer cylinder:

It is the cylindrical outer rubber casing of the AV. Its length may vary from 12 inches to 18 inches depending on the age and size of the bulls. The ends are raised to form notch for fixation of the inner liner properly. Its outer and inner diameters measure

7.5 and 6.5 cm respectively. There is a combined valve for water and air at 1/3rd of its length from one end. The two valves are provided for filling the casing or jacket (formed by the outer cylinder and inner liner) of the AV with hot water and air.

b) Thin rubber inner liner:

It is a cylindrical sheath made of rubber/latex and is introduced into the outer cylinder to form a jacket to be filled with water and air. The inner surface when lubricated provides smooth friction to the wall of the bull's penis at the time of intromission. It's length is 22 inches while the uniform breadth measures 3.5 inches. It's ends are rolled.

c) Rubber collecting cone:

It is made of thin rubber/latex sheath in the form of a cone. It measures 10 inches in length. The broader and shorter breadths measure 3.5 and 0.5 inches respectively. It directs the ejaculated semen to the collection tube. The ends are rolled to facilitate better fixation to the outer cylinder in one end and to the collection tube in the other.

d) Graduated glass collection tube:

It is a glass tube graduated nearest to 0.1 ml. Volume of semen can be measured and recorded directly from the tube immediately after collection. Moreover, the colour of the semen can be noted by direct observation of ejaculate.

e) Insulating bag:

It is made of rubber and silk and is used to maintain the temperature of the collecting cone and the collection tube to reduce the possibility of cold shock and to prevent breakage of the collection tube. The insulating bag is applied over the collecting cone and the collection tube and tied over the fixed end of the outer cylinder. The insulating bag measures 14 inches in length.

f) Rubber bands:

Rubber bands are used to held thin rubber inner liner tightly over the hard rubber outer cylinder.

Different parts of artificial vagina for buck:

Artificial vagina for buck is similar to that used for bull except that it is smaller in size. The parts are as follows:

a) Hard rubber outer cylinder (15 x 15 cm)

b) Thin rubber (Latex) inner liner (25 x 3.75 cm)

c) Rubber (Latex) collecting cone (18 cm)

d) Glass graduated collection tube/cup (5 ml capacity, graduated nearest to 0.1ml)

e) Insulating bag (25 cm)

f) Rubber bands

Procedure of preparation of AV:

- Thin rubber inner liner is put into the hard rubber outer cylinder.
- Both the ends of the inner rubber liner are averted over both ends of the hard rubber outer cylinder forming a jacket to be filled with water and air.
- The inner rubber liner is tightly held in place by the rubber bands.
- Place the AV over an AV stand. Remove the valve. Pour hot water at 60°C through the valve into the jacket with the help of a funnel. Allow to stand for 5 minutes. Then pour it out. This is done to pre-warm the AV.
- Pour 400 to 500 ml of hot water at 47°C (45°-50°C) through the water valve of the AV using a funnel and fill only 2/3rd (up to the valve) of the AV.
- Fix the valve in the AV.
- Make the air valve loose and blow air through the air valve into the jacket with the help of an air blower or by mouth. Inflation should be done in such a way that the inner liner bulges out, forms a cushion and thus closes the opening of the AV.
- The base of the rubber collecting cone is fitted to the end of the hard rubber outer cylinder which is nearer to the valve.

- A glass graduated collection tube is fitted at the apex end of the rubber collecting cone.
- The incubated insulating bag is applied over the collecting cone and the collection tube and tied over the end of the hard rubber outer cylinder.
- The free end of the AV is lubricated with sterilized white Vaseline using a glass lubricating rod. The lubrication should not be more than 1/3rd of the length of AV.
- Check up the internal temperature of the AV by using a breeder's thermometer. Final internal temperature should be 42°C (40°-45°C). It may be readjusted if required.

The temperature of the AV is more important than the pressure it exerts on the penis. Water is used to control both, but final pressure may be adjusted by pumping in air. Temperature inside AV is near 45^{0}C, but 38^{0}C to 55^{0}C has been employed. Place assembled AV in an incubator at 45^{0}C or slightly higher temperature. Semen collection tube should be maintained near body temperature to prevent sperm damage due to overheating or cold shock.

Ram:

A.V. temperature and technique is similar to cattle. Forward thrust of the ram is less vigorous but rapid. Semen collector must coordinate movements swiftly with those of ram. AV may be attached to a dummy.

Boar:

Pressure is especially important for collecting semen. The boar ejaculates when curled tip of penis is firmly engaged in the sow's cervix, the AV or the operator's hand. When using the AV or the gloved hand pressure is exerted on the coiled distal end of the penis throughout ejaculation. Once ejaculation has started the boar remains quite for the few minutes required for ejaculation. Ejaculation consists of three fractions. **(1)** Pre-sperm fraction. **(2)** The sperm rich fraction and **(3)** Post sperm fraction, which contains mostly seminal fluids with gelatinous pellet like material from Cowper's glands. This material tends to seal the cervix of the sow during mating preventing loss of semen. Pre-sperm fraction is discarded before sperm rich fraction is collected or gel can be filtered.

Horse:

Prior to ejaculation wash the penis with warm soapy water and rinse with clean water to remove smegma and other debris on the surface of the penis. The mare should be hobbled. AV is larger than that for other animals in order to accommodate the stallion's erect vascular penis.

Collection of semen:

There are various methods of collection of semen from bull, buffalo bull, buck and ram. The most common hygienic method yielding maximum sperm output with better semen quality is the artificial vagina method. In electro-ejaculation method, the volume obtained is high but the sperm concentration is low.

Materials required:

1. Assembled AV 2. Breeder's thermometer 3. Funnel 4. Hot water
5. AV stand 6. Vaseline 7. Glass lubricating rod 8. Air blower

Procedure:

- Clean the bull properly to avoid contamination.
- Place another bull (teaser) in a semen collection crate. The hind quarter of the teaser bull is to be cleaned properly. This bull is used as a mount or teaser.
- Allow the bull to give false mount 2 to 3 times on the teaser. This is done to make the bull sexually excited fully and thus to get better quality semen.
- Allow the bull to mount on the teaser while holding AV by the side of the teaser in a slanting position (approximately 45°C) with the horizontal plane of teaser.
- Guide the penis of the bull into the AV as soon as the bull mounts by grasping the sheath of the penis with the left hand just behind the preputial orifice.
- Allow the bull to give thrust into AV.
- Take away the AV after thrust and ejaculation and allow the bull to dismount.
- Hold the AV vertically and let the semen to come into the collection tube.

- Release the air and pour out a portion of water from the AV to facilitate drainage of semen into the collection tube.
- Remove the insulating bag from the AV and detach the collection tube from the rubber-collecting cone. Write bull number on the collection tube.
- Protect the collected semen against external shock i.e. heat, cold, light, dust, water etc.
- Keep the collection tube containing semen in a water bath at 30°C till the semen is examined and used.

UNIT-4

Practical No. 6 **Date:**

ESTIMATION OF INITIAL MOTILITY/PROGRESSIVE MOTILITY/INDIVIDUAL MOTILITY OF SPERM

Initial motility or individual motility of sperm refers to the percentage of progressively motile sperm present in semen sample.

Materials required:

1. Compound microscope
2. Bio-therm /spirit lamp
3. Micro-slide
4. Cover slip
5. Pasteur pipette
6. Semen sample

Procedure:

1. Take a clean, dry, pre warmed (at 37^0C) microslide. The slide is maintained at 37^0C using a bio-therm/spirit lamp.
2. Place a drop of semen (preserved) on the micro slide with the help of a Pasteur pipette. The fresh semen is to be diluted @ 1:100 in sodium citrate buffer (37^0C) before examination.
3. Put a cover slip on the drop
4. Examine the drop under high power magnification (400X)
5. Observe various types of movements of sperm at least in 5-6 fields in the center and periphery.
6. Determine the percentage of progressively motile sperm.

The rating of initial motility by usual examination is subjective and can only be done accurately by an experienced technician. The beginners can follow the following procedure:

a) Examine several fields in center and periphery of the semen drop.

b) Count the numbers of sperm other than progressively motile sperm i.e. non-motile, circulating motile, backward motile and undulating motile sperm in 5 fields.

c) Pass the slide over flame

d) Count the total number of sperm in 5 fields.

Calculation:

Calculate the Percent progressively motile sperm using the following formula:

% of progressively motile sperm = A-B/ A x 100

A: Total number of sperm in 5 fields.

B: Number of sperm other than progressively motile sperm in 5 fields.

The percentage of progressive motility of a semen ejaculate ranges from 0-80% and expressed as follows:

1 = 20%

2 = 40%

3 = 60%

4 = 80%

5 = more than 80% progressive motile sperm

UNIT-4

Practical No. 7 **Date:**

DETERMINATION OF SPERM CONCENTRATION

Materials required:

1) Compound microscope
2) Cover slip
3) Number's counting chamber
4) RBC pipette
5) Diluting fluid
6) Watch glass

Composition of diluting fluid:

Eosin - Y (water soluble)	-	0.05gm
Sodium chloride	-	1.00 gm
Formaldehyde	-	1ml
Dist. Water	-	100ml

Procedure:

1. Mix the semen sample thoroughly. Then draw semen up to 0.5 marks into a clean standard RBC pipette and clean the end of the pipette with the help of soft tissue
2. Immediately draw the diluting fluid up to 101 marks of the pipette and roll the pipette between the palms of hands for 2-3 minutes to ensure thorough mixing of semen with diluting fluid.
3. Discard a few drops of fluid from the pipette
4. Place a cover slip over the ruled area

5. Charge the counting chamber of haemocytometer with the contents of RBC pipette (semen + diluting fluid). Avoid overflow.

6. Wait for 2 minutes allowing the spermatozoa to settle down. Examine the charged areas of Neubauer's counting chamber under low magnification (10x) till the central square is focused.

7. Count the number of spermatozoa present in 5 small squares of the central large square under high power (40X) magnification. Five small squares i.e. left top, right top, left bottom, right bottom and Central Square. Total up the nos. of spermatozoa counted in the 5 small squares.

Calculation:

- The number of cells in 400 small squares i.e. Let the total number of cells counted in the 5 medium squares (80 small squares) is "X".
- 1 sq.mm area and 1/10mm depth or 1/10cu.mm. volume of diluted semen =X/80 x 400 sperms
- Therefore, 1cu.mm. of diluted blood contains (X/80) x400x10 sperms
- Since semen is diluted 200 times (i.e., 0.5 parts in 101-1=100 parts or 1.0 part in 200 parts), 1cu.mm. of undiluted semen contains = (X/80) x 400 x 10 x 200 R.B.C.= X x 10,000 sperms

The column of diluting fluid which occupies stem (from tip of the pipette to mark 1) does not enter the mixture. Therefore, 0.5 is added to 100 and not to 101 and hence the dilution is 1 in 200.

Normal values in different animal species:

Bull: 800 - 2000 x 10^6/ ml

Ram: 2000 - 3000 x 10^6/ ml

Boar: 200 - 300 x 10^6/ ml

Stallion: 150 - 300 x 10^6/ ml

Cock: 3000 - 7000 x 10^6/ ml

UNIT-4

Practical No. 8 **Date:**

DETERMINATION OF PERCENTAGE OF LIVE & DEAD SPERM

Principle:

Eosin-nigrosin stain has been utilized for counting live and dead sperm in semen. Cell membrane of dead sperm is permeable to eosin. The dead sperm take eosin (pink) while live sperm do not take any colour. Nigrosin provides a dark background colour. Partially stained sperm are considered as dead.

Reagents:

1. 5% Eosin, i.e. 5gm of Eosin dissolved in 100 ml of 2.9% sodium citrate solution
2. 10% Nigrosine i.e. 10gm Nigrosin dissolved in 100 ml of 2.9% sodium citrate solution. Mix the solution (1) and (2) in the proportion of 1:4

Procedure:

1. Take 1 drop of semen in a watch glass and then add 4-5 drops of mixed solution of Eosin and Nigrosin wait for a few seconds and mix it by blowing slowly with a pasteur pipette. Slide should be previously.
2. Prepare a smear on a slide pre-warmed to $37^{O}C$ from above preparation.
3. Dry the smear instantly on warm plate or biotherm
4. Place a drop of cedar wood oil over the smear and examine under oil immersion objectives of microscope.
5. Count unstained sperm as live sperm and stained/partially (pink) stained sperm as dead sperm.

6. Count at least 200 sperms from the different fields of the smear and determine the percentage of live sperm. If the slide is to be examined later, the smear should be mounted under a cover slip using DPX mount.

Observations:

No. of field	No. of live sperm	No. of dead sperm	Total number	Percentage of live sperm

UNIT-4

Practical No. 9 **Date:**

DETERMINATION OF ABNORMAL SPERM COUNT

An important part of any breeding soundness exam is an evaluation of sperm morphology. In the most fundamental case, the size and shape of the head, mid-piece and tail are examined. Additional information can be gained by evaluating integrity of the acrosome and sperm membranes.

Sperm from different species vary in size and shape. Bull and human sperm, for example, have paddle-shaped heads, rodent sperm have hook-shaped heads, and the heads of chicken sperm are spindle-shaped and almost difficult to distinguish from the mid-piece. The images shown below of rat, bull and chicken sperm are all at the same magnification.

The bull fertility depends upon morphologically normal spermatozoa present in the semen sample. Abnormal sperm percentage should not exceed 15-20%. Most worker agree that sperm of fertile bull should not have more than 4% head abnormalities, 4-10% mid-piece abnormalities, 5% tail abnormalities and 6% free heads or loose heads.

Types of abnormalities:

The sperm have 3 types of abnormalities such as primary, secondary and tertiary abnormalities.

1. **Primary sperm abnormalities**: are those which originate during development of spermatozoa in seminiferous tubules due disorder of germinal epithelium. Defects include:
    - i) Micro head
    - ii) Mega head
    - iii) Elongated narrow head
    - iv) Pyriform head

v) Double head

vi) Double mid-piece and tail

vii) Swollen mid-piece

viii) Abaxial attachment of middle piece

ix) Tightly coiled mid-piece and tail

2. **Secondary sperm abnormalities**: are due to changes taking place during storage in epididymis or beyond that. It includes:

i) Detached head, mid-piece and tail

ii) Proximal or distal protoplasmic droplets

iii) Bent tail

iv) Detached and loosen galea capitis.

3. **Tertiary sperm abnormalities**: are due to faulty handling which causes damage to spermatozoa during or after ejaculation such as cold-shock, heat shock, osmotic effect, toxicity of stain or changes in pH

i) Acrosomal abnormality

ii) Coiled tail

iii) Bent tail

Some sperm abnormalities :-

1. Pyriform and tapered heads: Pyriform head defect is the most common abnormality of the head shape. The pyriform head is distinctly pear shaped, the acrosomal region is full and rounded and the post acrosomal region is narrow. The pyriform defect, however, includes a wide range of aberrations from cells that are slightly pinched in at the post acrosomal region to those that are so severely narrowed that the sperm head looks ‘paddle’ shaped.

2.Tailless sperm (Disintegrated or Decapitated sperm) / Guernsey defect: characterized by virtual absence of intact spermatozoa and originally observed in Guernsey breed from England. The ejaculate contains only free head and tails. More than 50% free head indicates this defect. The sexual behaviour and the clinical examination do not show any abnormality. Sperm concentration may vary.

3. Diadem defect (abnormal nucleus): Just anterior to post acrosomal sheath there appears like a dark necklace of pearls tied around the neck of the sperm. This is not a hereditary defect, associated with lowered fertility and indicates disturbed spermiogenesis. Subsequently there may be recovery. This is due to invagination of nuclear membrane into the nucleus.

4. Knobbed sperm defect: occurs due to cystic malformation of the acrosome, characterized by a localized swelling or bead on the apical ridge. It is a refractile or dark staining area or eccentric thickening at the apex or front part of the head. This acrosomal cap aberration is inherited in Friesian bulls. Here sperm concentration and motility are normal. A very high frequency of cells affected with this abnormality has been associated with sterility and a lower frequency of affected cells with subfertility.

5. Ruffled acrosome: characterized by a wrinkled appearance.

6. Incomplete acrosome: characterized by a piece that is missing along one margin.

Both of these acrosomal abnormalities (Ruffled & Incomplete) may be hereditary and may be involved in subfertility.

7. Detached acrosome (Galea capitis): can occur during epididymal ageing (sexual abstinence) or with improper semen handling procedures. The detachment of acrosome occurs through the shedding of both the outer acrosomal membrane and the overlying plasma membrane.

8.'Dag' defect (strongly coiled, folded or disrupted tail): This defect was first demonstrated in a young Danish Jersey bull whose name was 'Dag'. Ultra-structurally the tail of the spermatozoa is strongly coiled inside the plasma membrane and axial fibers are abnormal, translocated or missing. Whether this defect is hereditary is uncertain. This defect is due to epididymal dysfunction and characterized by low motility, involves 40 to 50% of cells and associated with lowered fertility. This defect involved the principal piece most frequently and the mid piece only occasionally

9. Corkscrew defect: This is due to swelling of mitochondria. There will be residual protoplasmic droplet. Normally this defect is found in older bulls and linked with

testicular degeneration. As frequency of affected sperm increase, the bulls become sub-fertile.

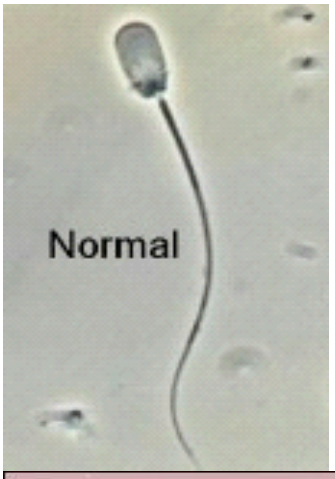
10. Pseudo-droplet: This defect was demonstrated by Blom (1968). A heritable base for this sperm defect is suspected. The defect is located near the center of the mid-piece and appears as rounded or elongated thickenings that contain dense granules on the surface of mitochondrial helix and surrounded by single or several layers of mitochondria of normal appearance.

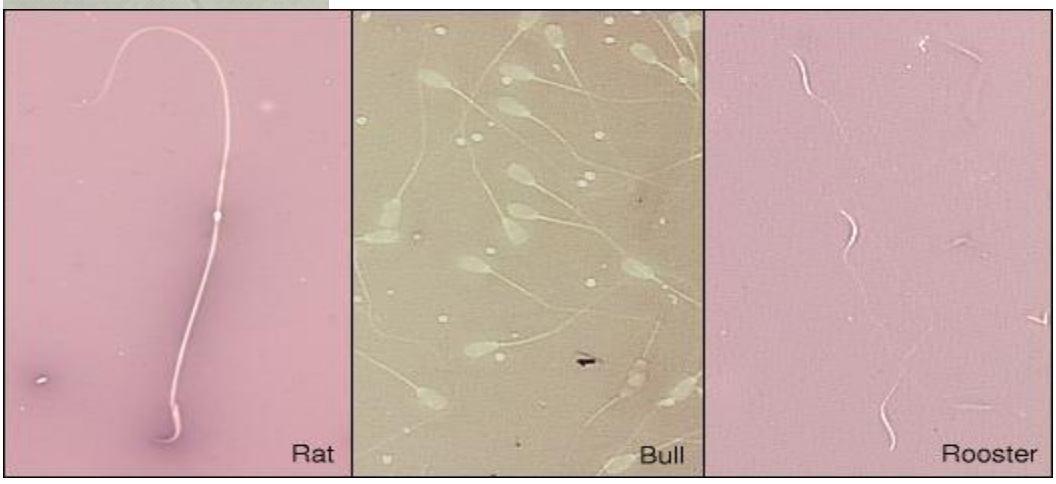
11. Abaxial implantation: Normally the tail is attached to the middle of the head. If the attachment goes to one side then it is known as abaxial implantation. This is more common in boar.

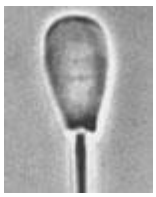
12. Protoplasmic/cytoplasmic droplet: Most epididymal sperm possess a proximal or distal cytoplasmic droplet on the mid piece. These spermatozoa are usually considered normal.

a. Proximal protoplasmic droplet: The droplet is found surrounding the neck or the proximal mid piece region.

b. Distal protoplasmic droplet: This droplet is found surrounding the mid piece just proximal to the annulus.





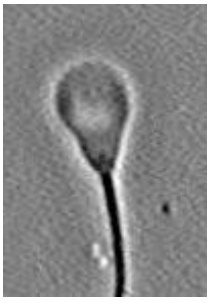


Normal

Decapitated

Macrocephalic

Microcephalic and Stump Tail



Pyriform

Pyriform

Round and Double Tail

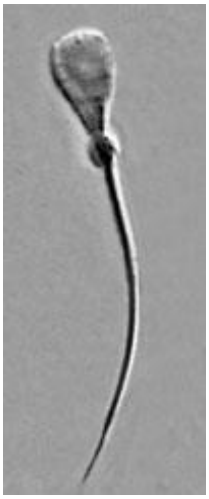
Round head

Ruffled acrosome

Tapered

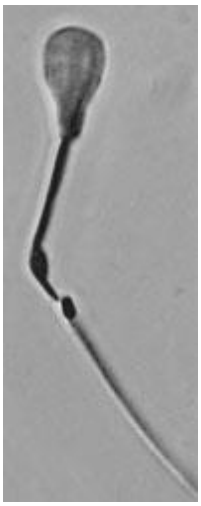
Craters (diadem)

microcephalic



Proximal Cytoplasmic (Protoplasmic) Droplet

Translocating Cytoplasmic Droplets

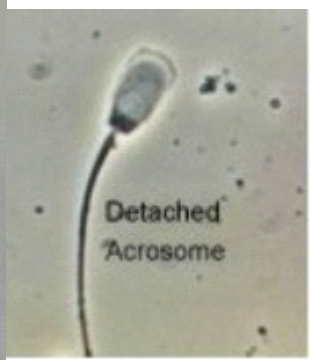


Tail Opening following Droplet Translocation

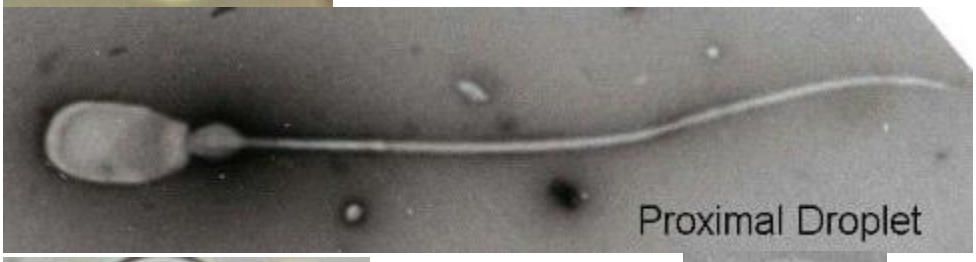
Tail Opening following Droplet Translocation

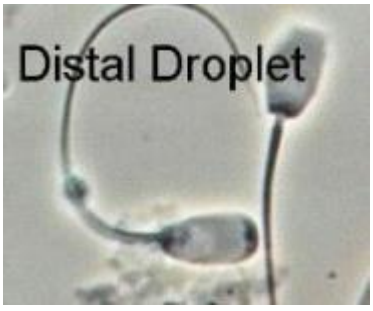

Elongated Head


Pear Shaped Hd


Detached
Acrosome


Coiled Tail


Proximal Droplet


Distal Droplet

Dag defect

UNIT-4

Practical No. 10 **Date:**

PHYSIOLOGICAL MEASUREMENTS OF GROWTH IN VARIOUS SPECIES

Several questions have been developed to analyse and predict growth patterns and relationship during different phases of animal development. In the use of any questions for analysis of growth, one must keep in mind that the growth pattern is not composed of point- to-point changes, but in a continual balance of gain and loss of total animal mass.

During the early phases of growth, increase in mass greatly over weights the loss. The equilibrium between increase and loss of animal mass remains relatively constant during this phase and increase in weight with age is linear. However, as the animal attains maturity the rate of gain and loss show a shift in equilibrium and the growth curve becomes curvilinear.

Even though changes in weight with increased age tend to follow the same pattern, there are variations between species. When the body weight gain is plotted against the time intervals a “S” shaped or sigmoid growth curve is obtained.

According to Brody (1945) growth can be defined as “relatively irreversible time change in magnitude of the measured dimension or function”.

Growth includes three processes

1. An increase in the number of cells i.e. hyperplasia
2. An increase in the size of cells i.e hypertrophy
3. Incorporation of material inside cells.

Body cells in terms of growth can be divided into three types:

1. **Renewing cell population**: Cells are constantly lost or destroyed and are replenished by proliferation of stem cells restricted to a generative zone. Eg. epidermis and derivatives, endometrial and skeletal tissue (in part).

2. **Expanding cell population**: Cell division continues until the adult size of an organ is reached.
   Eg. liver, kidney, glands (endocrine and exocrine) and skeletal tissue (in part).

3. **Static cell population**: Static tissues are expanding systems in which cell division is restricted to the early stages of development, although cellular hypertrophy may occur later.

Growth can be explained under two phases of life

1. Pre-natal growth: It can be defined as the growth process starting from fertilization of ovum till the birth.
2. Post-natal growth: It can be defined as growth after the birth.
   During post-natal growth of an animal two processes take place.

(i) Increase in weight until mature size is reached called growth and
(ii) Changes in body conformation and shape and various functions and faculties come into being called development.

Types of growth:

1. **Compensatory growth**: When part of an organ stops growing or when a part of it is experimentally removed compensatory growth takes place.
   Compensatory growth increases the functional capacity of organs or tissues. Organs capable of compensatory hypertrophy following partial ablation are liver, kidney, endocrine adrenal, ovaries, thyroid) and exocrine (pancreas, salivary glands) glands. Organ which cannot hypertrophy are skeletal muscle, bone, cartilage, skin, limbs, teeth, sense organs and most nervous tissues.

2. **Differential growth**: Different growth centers of the body become active at different times and exhibit different rates of activity. These factors are coordinated and produce pre-determined form characteristics of the species.

Huxley defined the size relationship (allometry or heterogony) between the whole body and its parts mathematically as:

$$Y = bX^k$$

Where Y = Size of an organ

X = Body size

b = fraction of the body size that organ represents

K = co-efficient

When k = 1, growth rate of the organ and body are same i.e. isometric.

Allometric (heterogonic) growth may be positive or negative, depending upon whether the rate of an organ growth is greater (k>1) or less (k<1) than that of the body.

3. **Negative growth**: Negative growth occurs when catabolism is higher than the anabolism. This occurs during old age when cells are gradually lost or not replaced.

Measurement of growth:

1. **Pre-natal**: The size of fetus is judged by the following:

(a). Total fetal length

(b). Crown-rump length

(c). Curved crown-rump length

(d). Fetal weight

Most commonly employed method of measuring growth is by studying an increase in weight during a definite period.

Weight increase related to time can be represented by growth curves given below:

i. **Absolute growth**: It is absolute gain in weight per unit time e.g. grams per day

Average absolute growth rate = $\frac{W_2-W_1}{t_2-t_1}$

Where W_2-W_1 is absolute gain in observed weight difference

t_2-t_1 is corresponding time difference

ii. **Relative growth**: It is percentage increase in weight per unit time. Percentage increase in growth rate is the gain in weight expressed as a function of initial body weight.

Average relative growth rate $= \frac{W_2-W_1}{W_1}$

Percent relative growth rate $= W_2-W_1 / W_1 \times 100$

Where, W_1 is initial weight

W_2 is final weight

iii. Specific growth: In the curve of specific growth logarithm of size/weight is plotted against age.

Post-natal: Since the growth is defined as increase in population size which involves the reproduction of animals whereas increase in body size include all the three processes (hyperplasia, hypertrophy and incorporation of biological material) in the cells. It can be measured by:

iv. Directly weighing the animals on balance

OR

v. Body weight can be estimated by following body measurements.

Body length: The distance from point of shoulder to the point of pin bone.

Body height: Measured by measuring tape vertically from the point of the wither to the ground and recorded in cm/ft.

Girth: The circumference of the body over the flank just in front of the udder.(known by measuring the circumference of chest just behind elbows by means of a measuring tape (in cm)

Body weight: Taken by a weighing balance (in kg/lbs)

There is a relationship between the weight of an animal and its length, girth etc. Some formulas can be used for determining weight of animals when balance is not

available. However, a formula does not give actual weight of an animal; it gives a variable rough estimate of the weight.

Formulae:

i. **Shaffer's formula:** This is the most common formula which can be used for cattle and buffaloes. This formula tends to under estimate weights of very young and very heavy animals

$$\text{Live weight in pounds} = \frac{\text{Length X Girth}^2}{300}$$

ii. Aggarwala's modified Shaeffer's formula:

Formula for Indian cattle is as follows:

$$\text{Live weight in Seers} = \frac{\text{(Girth) (Length)}}{Y}$$

Where y is equal to 9.0 if girth is less than 65″, 8.5 if girth is between 65-80″ and 8.0 if girth is over 80 and Seer is equal to 0.93 Kg

iii. Mullick's Formula for buffaloes:

X = 25.156 (Y) -960.232

X = Estimate of body weight in lbs

Y = Heart girth in inches

iv. Formula of Bhandari and team:

(For dry and non-pregnant buffaloes)

BW in lbs = 2123.73±26.356 (Girth in inches)±21.50 (Length in inches)

(For dry and pregnant buffaloes)

BW in lbs = 1934.48±20.52 (Girth in inches) ±25.90 (Length in inches)

(For lactating buffaloes)

BW in lbs = 2387.60±7.12 (Girth in inches) ±4.55 (Length in inches)

v. Formula for Hariana Cattle:

Body weight (kg) = 3.3 (chest girth in cm) + (posterior girth in cm) +0.7 (Length in cm) - 490

Drawback of time related increase in weight

i. An animal may add to its weight by retention of water or by deposition of fat.

ii. These do not represent an increase in the structural tissues and organs. Such a defect can be partially removed by including size measures such as height, length, heart girth etc.

Example:

If a Holstein cow weighs 1000 pounds at age 1000 days from conception, she gained on the average one pound a day, but there was no day when she actually gained exactly one pound. To cite extreme illustrations, at the end of the first week after conception, she gained only about 0.0001 pound a day; at 5 months after birth, 2 pounds a day; at 1000 days, only one-fourth a day. Thus the concept of average rate, when applied to growth, is an abstraction, and when the average extends over a considerable period of time, as in the example cited, it gives no idea of actual rate at a given age. The shorter the interval of time for which the average is computed, the more nearly dose it approach the true value; and when reduced to an interval, dt, so short that there is no time for the velocity of growth to change, the true growth rate, dw/dt obtained.

Ref. Brody (1968) Bioenergetics and Growth, Hafner Publishing Company, INC,New York.

Drawback of size measures related growth:

i. Size measures crudely represent skeletal growth.

ii. An animal receiving in sufficient energy and protein may not have any muscular growth, but may show an increase in size due to skeletal growth.

UNIT-4

Practical No. 11 **Date:**

MEASUREMENT OF BODY SURFACE AREA OF ANIMAL TO ASSESS HEAT STRESS

INTRODUCTION:

The measurement of body's surface area in form animal helps to assess the heat stress. There is an important role of proportional body's surface area is facilitating heat loss from an animal under hot condition. For this reason, linear measurement is required to be estimated.

METHOD OF MEASUREMENT:

1. **Direct method:**

The hide of a freshly slaughtered animal may be stretched out and its outline is traced on a paper. The area of enclosed space is measured. This area may be measured with PLANIMETER or if squared paper is used, the squares can be counted. If paper of uniform quality is used and kept dry and a very sensitive scale is available (electric balance) the paper is cut out according to shape of animal hide & weigh. By comparison with weight of square meter paper, the area is obtained.

USE OF SERFACE AREA INTEGRATOR:

Surface area of one animal can be measured by use of surface area integrator. This instrument consists of two wheels whose circumferences are known. The wheels are mounted at a fixed distance apart and geared to a revolution counter. One wheel has a rough surface, which rubs chalk from a stick fixed on a holder and deposited on the surface over which it is running. The total mechanism is mounted on a handle, which can push it along.

The area covered is given by the products: **A = cdn**

Where, A=Surface area, c= Circumference of wheel,

d = Distance between the two wheels n= No. of revolution.

The surface area is measured from one side of animal with the animal standing squarely on all its four feet in following manner.

Measurement of surface area of the trunk:

Starting from the base of the tail, with the counter set at zero, the integrator is pushed along the back with the plain wheel running along the central line of the vertebral column.

When the shoulder is reached, the integrator is lifted off and a second run started from the central line under the tail with plain wheel now running along the chalked line made by the marking wheel during the first run.

This process is repeated until there remain only one strip before the mid line of the belly and chest is reached. The central reading is noted down.

The counter is re-set to zero and made to run over the last strip, but since the last strip may not have the same width as same the distance between the two wheels. The count must be reduced proportionally with the ratio of the width of the wheel and the strip.

Again counter is set to zero and run over from midline of center of vertebral column. At the time of measurement of animal's head is turned in opposite direction to remove the wrinkle in the skin and the hand is held in opposite side of dewlap.

Legs:

The forelegs and rear legs are measured with the integrator by placing the feet wide apart. The integrator should be run around the leg up to stifle joint.

The lower part of legs is treated as cylinders. By taking the length from lower mark of the integrator to the top of the hoof with a measuring tape and the average of several measurements of circumference of leg. The surface area of that portion of leg can be calculated as follows.

A = area of the cylindrical region = average circumference × length.

Tail:

Length of tail is measured from base of tail to the tip and average circumference is determined by multiplying length x average circumference.

Head:

The length of head is measured from the top of pole to the end of muzzle and the width is determined from the base of one ear to other.

Then, area (A) = ½ width x length

Ear:

The area of ear is determined by measuring from outside, staring from base to tip and width at the center. The area of area is calculated by treating ear as a rectangle.

So, Area of Ear = length x width

The total surface area of Animal:

$$S = 2(m+f_1+f_2+r+e)+t+h$$

Where, s = total surface area of the animal

m = area of trunk region

r = area of neck region

f_1 = area of fore leg

f_2 = area of hind leg

e = area of ear

h = area of head

t = area of tail

II. **Indirect method**:

In this method, we have to measure the body weight of the animal and the surface area of the animal can be calculated from the following formula.

$$A_{(m^2)} = 0.15\ W(kg)^{0.56}$$

or $$A = W(kg)^{0.74}$$

UNIT-4

Practical No. 12 **Date:**

ENVIRONMENTAL PHYSIOLOGY OF ANIMAL IN RELATION TO CLIMATIC CHANGES

Important terminology related to environmental physiology

1. Climate: It is the long-term average meteorological variables i.e. air temperature, rainfall, relative humidity, sunshine period & solar radiation

2. Climatology: It is the science, which deals with study of climate.

3. Weather It is the short-term fluctuation i.e. day to day fluctuation of climatic variables like temperature rainfall, air velocity etc.

4. Meteorology: It is the science of weather

5. Bio-meteorology: It is the science of which deals with the study of living organisms in relation to environmental parameters.

MEASUREMENT OF HEALTH PARAMETERS OF ANIMALS

(BODY TEMPERATURE, PULSE RATE, RESPIRATION RATE & HEART RATE)

The term “climate” is derived from the Greek word “*Klima*” meaning inclination to the sun’s influence. In the present context, climate refers to the long term average (some 30 days) meteorological conditions in a given region. It can be divided into macro climate and micro climate. Macro climate refers to the climatic condition of a large area as distinguished from that of a small area, while micro climate can be defined as the climatic condition directly surrounding the animal.

Climatology is a branch of science which deals with the study of climate. An animal is intimately and in separately associated with its physiological and chemical environment from conception until death. The meteorological elements – air temperature, air humidity, wind and solar radiation - act on the surface of the animal,

thereby initiate behavioral and physiological changes in the animal in order to maintain homeostatic mechanisms.

Diurnal variation:

Diurnal variation means fluctuation of different physiological parameters like body temperature, pulse, respiration rate etc. in relation to the environmental changes during the day (24 hours). An index of deep body temperature is mostly easily obtained in animals by insertion of a thermometer to a constant depth in each animal. Domestic mammals exhibit a diurnal rhythm of body temperature. In general, there is a minimum in the early morning and maximum in the late afternoon.

Normal physiological parameters of animals:

Species	Rectal temperature	Pulse rate	Respiration rate	Heart rate
Cattle	38.5^0C ($38\text{-}39.3^0$C)	50-60/min	30/min	60-70/min
Goat	39.1^0C ($38.5\text{-}39.7^0$C)	50-60/min	19/min (20-25/min)	90/min (70-135/min)
Swine	39.2^0C ($38.7\text{-}39.8^0$C)	50-70/min	13/min	55-86/min
Horse	38^0C ($37.2\text{-}38.2^0$C)	30-40/min	12/min	44/min (23-70/min)

Exercise:

Record of body temperature, pulse rate and respiration rate of different animals.

UNIT-4

Practical No. 13 **Date:**

BASIC EQUIPMENTS TO MEASURE DIFFERENT CLIMATIC PARAMETERS

Serial No. 1: (a) MEASUREMENT OF AMBIENT TEMPERATURE

Ambient temperature varies during 24 hours of the day. It is colder at night and warmer at daytime. In the earth, temperature is always highest near the equator and where there is no cloud so that the heat from the sun can easily reach the ground. They are lowest far from the equator and where there is no cloud so that heat can escape easily into space. The temperature also depends on how shiny the earth surface is, so they reflect solar radiation.

Maximum and Minimum thermometer:

The diurnal (daily) variation in temperature may be about 10^0C (18^0F) and this can be measured with the help of minimum and maximum thermometers. The liquid in each tube of the thermometer moves an indicator, which says, at the highest or lowest temperature reached over 24 hours of the day.

Thermoelectric thermometry (Thermocouple):

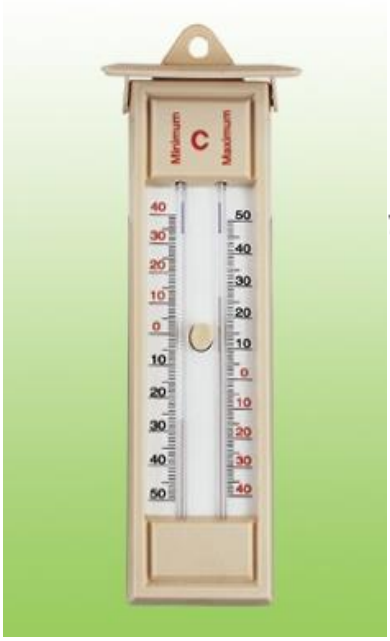
This class of instrument utilizes the phenomenon that, when electric current flows continuously in a close circuit of two dissimilar metals (thermos-junctions), when the junctions are maintained at different temperatures. The current generated are a function of the temperature difference between two junctions and are connected to a potentiometer to measure the voltage. A great variety of metals are available with which thermos-junctions of thermocouple can be prepared. For measurement of the biological and meteorological range, copper and constantan (a copper-nickel alloy) thermocouples are used. With calibrated wires and one junction immersed in an ice bath, temperature is read directly from the potentiometer scale.

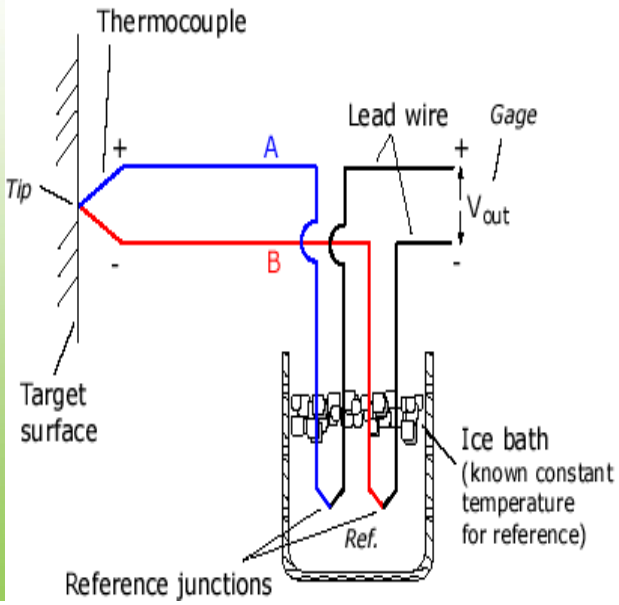
The electric characteristic of thermocouples makes them ideal for use with recording instrument. A further advantage is that the junction can be made small for

affixing to the skin of animals or other surfaces or for insertion into the rectum or other body cavities for measuring deep body temperature.

Thermistor:

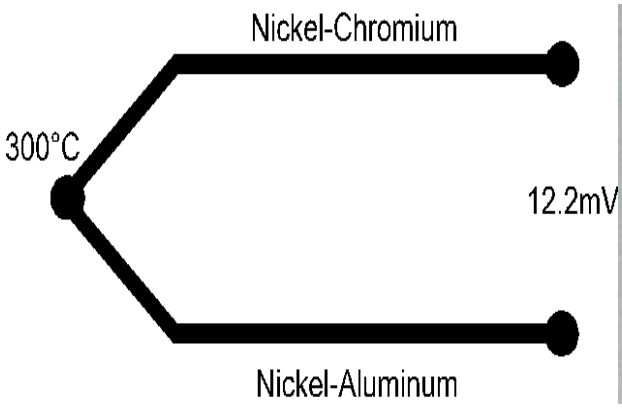
Thermistor thermometer is a very useful device to measure ambient temperature. Certain semiconductors exhibit a pronounced change in electrical resistance in response to slight change in temperature. Measurement of this change in resistance provides a measurement of temperature at the thermistor. Battery powered thermistor thermometer is commonly used portable instrument well suited to bioclimatology. Thermistor beads can be placed in hypodermic needles for sub-dermal thermometry.

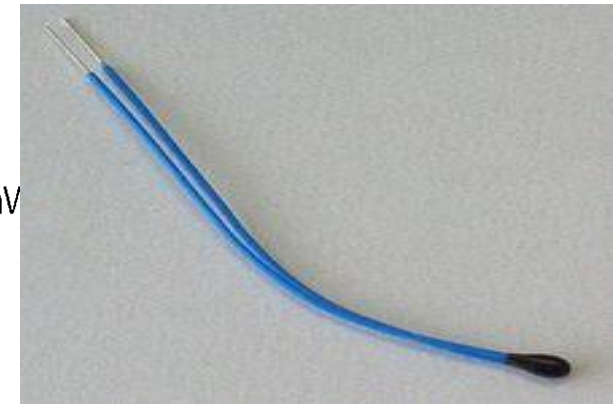




MAX-MIN THERMOMETER

THERMOCOUPLE





THERMISTOR

UNIT-4

Practical No. 13 **Date:**

BASIC EQUIPMENTS TO MEASURE DIFFERENT CLIMATIC PARAMETERS

Serial No. 2: (b) MEASUREMENT OF ATMOSPHERIC PRESSURE

Air pressure is the force exerted by the weight of a column of air above a particular location. Air pressure is not the same everywhere. If the air is cold, it shrinks and pushing down to create a higher pressure on earth. If the air is warm, it rises and so there is lower pressure on the earth.

A **barometer** is a scientific instrument used in meteorology to measure atmospheric pressure. It can measure the pressure exerted by the atmosphere by using water, air or mercury. Pressure tendency can forecast short term changes in the weather. Numerous measurements of air pressure are used within surface weather analysis to help find surface troughs, high pressure systems, and frontal boundaries.

1. **Fortin's Barometer**:

Fortin's barometer consists of a uniform glass tube of 1 meter length and closed at one end. The glass tube is completely filled over a trough containing mercury. The upper part of the trough is of glass at the bottom of which there is leather bag placed on a screw B by rotating the screw the mercury level in the trough can be raised or lowered. The glass tube is covered by a metal tube. The metal tube has a glass portion through which the level of mercury in the tube can be seen from outside. There are two scales in cm and in inches attached by the side of the glass portion of the metal tube. There is a vernier scale in between the two scales, which can be raised or lower by the rack and pinion arrangement P, the zero of both the scales starts from the index 'I' made of ivory and placed just over the mercury surface in the trough. A thermometer is also fixed to the body of the barometer for the record of temperature.

By operating the screw B, the mercury surface in the trough is allowed to touch the index I. The vernier V is then adjusted and the height of the mercury column 'h' in the tube is measured.

If ‘p’ is the density of mercury and ‘g’ the acceleration due to gravity, then,

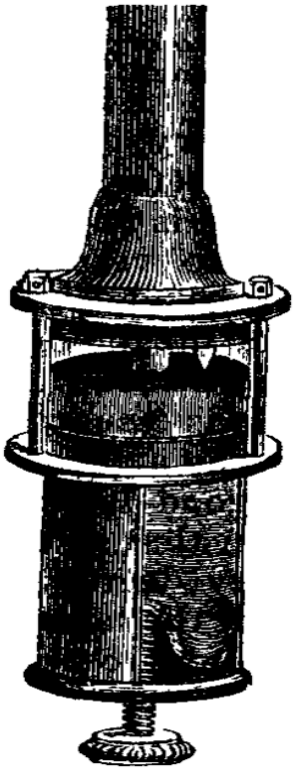
Atmospheric pressure = hpg.

2. Aneroid Barometer:

It consists of an evacuated metallic box closed by a corrugated diaphragm at one side. The lid is connected to a spring (B), which is connected to a lever. The lever in turn is connected to another spring through a string passing over a drum (D). To the center of the drum a pointer is attached. The pointer moves over a graduated scale. When the atmospheric pressure changes, the diaphragm moves. This makes the pointer move on the scale indicating the pressure at an instant. As it contains no liquid it is light and easily portable.

Use of Barometer:

i. Measurement of atmospheric pressure.
ii. Determination of altitude of a place.
iii. Forecasting of weather.



Fortin’s Barometer

Aneroid Barometer

Barometers

Observation:

UNIT-4

Practical No. 13 **Date:**

BASIC EQUIPMENTS TO MEASURE DIFFERENT CLIMATIC PARAMETERS

Serial No. 3: (c) MEASUREMENT OF HUMIDITY

When the air contains lots of water vapor, the weather is described as humid. The warmer the air is the more moisture it can hold. We often talk about relative humidity. This is the amount of water vapor in the air relative (compared) to the maximum it can hold at that temperature. The amount of water vapor in the air can be measured with the help of hygrometer.

A) Hair Hygrometer

It is a simple instrument to measure water content of the air that indicates wet weather by the stretching of a hair. The hair fixed inside a box stretches out when air is wet and shrinks when the air is dry. This motion of hair is converted through a system of lever to a pointer indicating relative humidity or fitted with a pen, the pointer records the relative humidity.

HAIR HYGROMETER **HYGROMETER** **WET-DRY BULB THERMOMETERs**

B) Wet and Dry Bulb Thermometers (Psychrometer):

A psychrometer is a device used to measure relative humidity of a certain area. A psychrometer has two bulbs: one wet and one dry. After some time, the water on the wet bulb evaporates and at that time, the temperature is measured of each bulb. The difference between the temperatures is noted and the temperature is noted and each is used on a chart to find the relative humidity of that temperature and area. A small difference between the temperatures of the bulbs shows a high relative humidity, because in dry air, evaporation happens faster. The other way around: high difference = low humidity.

It consists of two thermometers A and B fixed vertically on a wooden board. Below thermometer B, there is a beaker containing ice-cold with muslin and is always kept wet by dipping the end of the muslin cloth in the beaker.

Before wrapping, both the thermometers will record the same temperature. After wrapping temperature of B will gradually decrease due to the evaporation of water. The fall of temperature will depend on the rate of evaporation, which will be again dependent on the water vapour content of the atmosphere. After sometime when the temperature of the thermometer is steady, it is noted.

Table have been prepared giving the percentage humidity of various differences between the wet and dry bulb reading of the thermometers, the relative humidity is obtained from the table.

Followings are some of the psychrometer used to measure relative humidity.

i. Sling/Whirling Psychrometer:

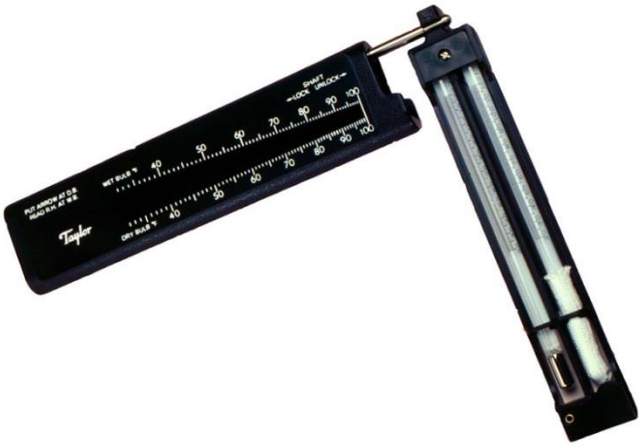
This instrument consists of two thermometers clamped in a frame, which in turn is fastened to a swivel handle. A wetted silk wick covers one thermometer and the other is bawre. The term ‘wet bulb’ and ‘dry bulb’ temperature originated from this instrument. When rapidly whirled, water evaporates from the wick, cooling the bulb. The rate of evaporation from the wick is a function of the vapour pressure gradient, determining in turn depression of the wet bulb thermometer reading below the dry bulb. From the psychrometric chart (shown overleaf) both relative humidity and vapour pressure can be read directly.

ii. Motor-Driven Psychrometer:

This instrument is also consisting of wet and dry bulb thermometers except the air motion across the thermometer bulbs is created mechanically rather than whirling by hand.

C. Dew point Hygrometer:

This instrument indicates the dew point temperature by providing a means for cooling a polished surface. The temperature at which moisture just begins to condense on the surface (detected by cloudy appearance) is the dew point. If the air is cooled without changing its moisture content at certain point moisture condensed out. The surface of the dew point hygrometer does just that: Cools the air to the saturation point.



SLING/ WHIRLING PSYCHROMETER

DEW-POINT HYGROMETER

Observation:

UNIT-4

Practical No. 13 **Date:**

BASIC EQUIPMENTS TO MEASURE DIFFERENT CLIMATIC PARAMETERS

Serial No. 4: (d) MEASUREMENT OF EVAPORATION

The amount of water vapour in the air (humidity) controlled the rate of evaporation of water from skin surface and mucus membranes, eg. lungs, respiratory passages, conjunctiva of eye etc. To understand the process of evaporation from these tissues into the ambient air, certain properties of liquid and vapour interfaces may be discussed. Water like other liquids, tends to saturate the surrounding space with vapour. In an open vessel, where ambient air current passes over the water continuous evaporation takes place. Three factors such as air temperature, the rate of air velocity and the degree of saturation of the ambient air governs rate of evaporation. The evaporation meter comprises of an open water container with calibration in the sidewalls, which gives difference of water level between specified time periods.

Evaporation meter

Observations:

UNIT-4

Practical No. 13 **Date:**

BASIC EQUIPMENTS TO MEASURE DIFFERENT CLIMATIC PARAMETERS

Serial No. 5: (e) MEASUREMENT OF RAINFALL

Rain-Gauge:

Rain-gauge is an instrument used for measuring the amount of rainfall.

It consists of:

1) One outer metallic cylinder

2) Another metallic cylinder, which is smaller than the previous one.

The smaller cylinder should be placed inside the bigger one during the collection of rainwater.

3) The third part is a funnel attached with a long stem. It acts as a cap for the outer cylinder. The funnel diameter is 5". While taking the reading the smaller cylinder should be placed inside the outer jacket. The stem of the funnel should be inside the smaller cylinder.

RAIN-GAUGE

In rainy days, the rain-gauge should be kept in open atmosphere before raining. The rain- water collected in funnel will be directed into small cylinder through the stem. After rainfall is over, the collected rain water can be measured with a measuring cylinder

graduated in cm or inch which is usually provided with a rain-gauge. After measuring the amount of rain water collected we can able to know the amount of rainfall occur in that particular day.

Significance:

The rainfall is one of the most important climatic factors, which control the density of animal's distribution, their body size and conformation. Rainfall is also having indirect effect on the vegetation growth, which further influences the animal's body size, feeding habit & distribution.

Rainfall is controlling the relative humidity of the atmosphere, which is having influence over evaporation of heat from animal's body and pigmentation of skin. Therefore, measurement of rainfall is a vital part to know the physiological function & geographical distribution of domestic animals.

Observation:

UNIT-4

Practical No. 13 **Date:**

BASIC EQUIPMENTS TO MEASURE DIFFERENT CLIMATIC PARAMETERS

Serial No. 6: (f) MEASUREMENT OF AIR VELOCITY

The wind has a large effect on our lives. Heat transfer by convection and evaporation between the animals and the environment is a function of movement of the ambient air. The unit associated with air motion is distance per unit time suggest different type of winds.

1.	Light air	-	Wind speed 3 Km/h
2.	Light breeze	-	Wind speed 9 Km/h
3.	Gentle breeze	-	Wind speed 15 Km/h
4.	Moderate wind	-	Wind speed 25 Km/h
5.	Fresh wind	-	Wind speed 35 Km/h
6.	Strong wind	-	Wind speed 45 Km/h
7.	Near gale	-	Wind speed 56 Km/h
8.	Gale	-	Wind speed 68 Km/h
9.	Severe gale	-	Wind speed 81 Km/h
10.	Storm	-	Wind speed 94 Km/h
11.	Severe storm	-	Wind speed 110 Km/h
12.	Hurricane	-	Wind speed 118 Km/h

Anemometers are the instruments, basically use to measure wind speed, which are as follows:

A) Propeller and Cup Anemometer:

These instruments convert the kinetic energy of the moving air mass into rotatory motion of an impeller. The simple cup anemometer has three or more cups mounted on

the end of arm that spin round a vertical pole. As the cups catch the wind, the arm spins round and the speed is recorded. Air speed is determined on the number of revolutions of the propeller in an interval timed with a stopwatch. For remote reading or recording, the rotating propeller or the cup is coupled to a D.C generator. The current generated moves a recording pen or indicator meter calibrated to read directly in meter per second.

In this instrument, the velocity pressure of the moving air stem is converted to static pressure against a counter weighted vane in the Velometer. While portable, this instrument is highly sensitive to direction.

Cup-type anemometer

windmill style of anemometer

3D ultrasonic anemometer -vertical axis and a sensor

B) Windsocks:

Windsocks are used in the small airports to show pilots the strength and direction of the wind. When strong winds blow, the socks is filled with moving air and billows in the direction the wind is blowing. A wind is described by the direction from which it is coming. For example- a west wind comes from the west and a north wind comes from the north.

Stevenson screen:

Most weather stations or meteorological centers and many institutions have a Stevenson screen. A Stevenson screen well ventilated wooden box, which may

contains a wet and dry bulb thermometer to record humidity. The wet and dry thermometer show different temperature according to the humidity. The humidity is worked out from a scale or psychrometer chart. The Stevenson screen also holds maximum and minimum thermometer inside it. Another, instrument that is also conventionally kept in the Stevenson screen is metallic thermocouple thermograph. All Stevenson screen are kept in open spaces and stand at least 4 ft. high above earth surface. The screen shades instruments from the direct heat of the sun and lowered sides allow air to circulate through the box.

UNIT-4

Practical No. 13 **Date:**

BASIC EQUIPMENTS TO MEASURE DIFFERENT CLIMATIC PARAMETERS

Serial No. 7: (g) MEASUREMENT OF SUNSHINE AND RADIATION

Various instruments are used to measure the sunshine and radiation in the environment.

They are sunshine recorder, Angstrom Pyrheliometer, Pyranograph, bimetallic and Net Pyrradiometer.

The instrument adopted by the World Meteorological Organisation is the Campbell-stokes apparatus. A water-filled globe concentrates the solar rays and burns traces on a special card. Length of ‘burn’ is an index of length of sunshine. Great care must be exercised in placement of the instrument and selection of cards to permit proper interpretation. In another system, photo electric cells are adjusted to turn on a recorder above a threshold of brightness equivalent to ‘bright’ sunshine.

Campbell–Stokes recorder

(Used at polar region)

Sunshine card

Campbell–Stokes recorder

(Used at tropical region)

Observation:

UNIT-4

Practical No. 13 **Date:**

BASIC EQUIPMENTS TO MEASURE DIFFERENT CLIMATIC PARAMETERS

Serial No. 8: (h) MEASUREMENT OF ATMOSPHERIC SOUND

Noise is an environmental stress of increasing impact in our technological society. Its importance in animal husbandry is indicated, for instance, by Lawsuits charging loss of milk production on dairy farms near jet airport.

Noise is a form of energy, transmitted from the source to the sensory organs in the form of waves of compression and refraction of the air. Ear sensitivity covers a tremendous ranges from threshold for pain, in the human ear, this is a ration of something like 1012 to 1.

The sensitivity of the ear varies at different frequencies, in the human being sensitivity is greatest in the 2000 to 4000 Hz range. Variations in sensitivity among species is also a factor. The familiar ‘supersonic’ doh whistle is supersonic to the human ear. It is obviously ‘sonic’ to the canine ear.

Sound level is a measure of the apparent loudness (psycho acoustical response), ceramic-type microphones are usually standard with the sound-level meter, but where high ambient temperature prevail, the condenser type should be used. To assure accuracy in performance acoustical instrument should be recalibrated periodically.

Data logging Sound Level Meter PC Based Sound Level Measurement

UNIT-4

Practical No. 14 **Date:**

STUDY OF BEHAVIOUR OF ANIMALS (demonstration)

Serial No. 1: Mating behavior

Serial No. 2: Feeding behavior