Laboratory Animals

2

Mageshwaran Lakshmanan

Abstract

Though the current modern era has introduced equivalent alternatives for animal research like *in-silico*, *in-vitro* methods, and Computer-Assisted-Learning, animal experimentation cannot be overlooked entirely and put aside, especially in research. The 'hit molecule' should be administered to an animal before humans at some point in time for the concern of safety. Various mammals ranging from humble mice to large horses have been used in the experimental laboratory for drug research. Invertebrates, fishes like zebrafish, avians like pigeon and chicken have been used as an alternative to mammals. Every animal has its own merits and demerits. Moreover, the recent decade has seen the development of various genetic animal models that exhibit similar human pathological conditions. This chapter will review the salient feature, advantages, disadvantages, and genetic models of laboratory animals.

Keywords

Laboratory animal · Genetic model · Mouse · Rat · Guineapig

2.1 Introduction

For many decades, since modern science's evolution, animal experiments have been a fundamental part of research and medical teaching. Though the current modern era has introduced equivalent alternatives for animal research like *in-silico*, *in-vitro* methods, and Computer-Assisted-Learning, etc., animal experimentation cannot be overlooked entirely and put aside, especially in research. This is because the 'hit

M. Lakshmanan (✉)
Department of Pharmacology, Thanjavur Medical College, Thanjavur, Tamil Nadu, India

M. Lakshmanan et al. (eds.), *Introduction to Basics of Pharmacology and Toxicology*, https://doi.org/10.1007/978-981-19-5343-9_2

molecule' should be administered to an animal before humans at some point in time for safety concerns. Furthermore, the choice of an animal model with a close resemblance to humans for evaluating drugs is a vital step in drug development. Hence, the pharmacologist should be aware of the anatomical peculiarities, physiological similarities, biochemical resemblance, research suitability, and recent genetic modifications of various experimental animals.

The experimental animals are broadly divided into the following categories:

- Mammals—Rodents (Mouse, Rat, Guinea pig, Hamster, and Gerbil)
- Mammals—Non-rodents (Rabbit, Cat, Pig, Dog, Monkey, Sheep, and Horse)
- Avian (Pigeon, Chicken)
- Amphibian (Frog and toad)
- Pisces (Zebrafish)

2.2 Mouse

2.2.1 Scientific Name

- Genus*: Mus*
  - Species: *musculus (*Common*), famulus (*India*), domesticus (*Western Europe and Africa*), spretus (*Western Mediterranean*), fragalicauda (*Thailand*), macedonicus (*Eastern Mediterranean*),* and *spicilegus (*Central Europe*)*
- Genus: *Peromyscus*
  - Species*: maniculatus* (North American deer mouse), *leucopus* (American White-foot mouse)
- The genus *Mus* is commonly used, while *Peromyscus* is rarely used

2.2.2 Description

- Body Length: 6–9 cm
- Tail length: 6–10 cm
- Weight: 12–30 g
- Breeding season: Throughout the year
- Gestational age: 19–21 days
- Litter size: 3–12
- Sexual maturity: 5–7 weeks
- Average life span: 2 years

2.2.3 Salient Points of the Mouse as Experimental Models

- The mouse is one of the earliest animal model used in experimentation:
  - William Harvey in 1678 for studying circulation and reproduction
  - Joseph Priestley in the eighteenth century for studying respiration
  - Gregor Mendel in the nineteenth century for studying gene inheritance
- The mouse is the smallest experimental rodent that was used in the laboratory. Easy to keep, cheap, and requires only minimal space for housing
- Mice have the shortest gestational duration and hence, a good model for reproductive toxicity studies
- Commonly used in acute toxicity studies
- The mouse genome was sequenced in 2002, and it revealed 17,000 genes with identifiable human orthologs that can be manipulated to study more than 1000 human diseases. Mice have a similar genome to humans (>90% conservation) and hence an ideal animal of choice for genetic studies and inherited human diseases

2.2.4 Outbred Stocks of the Mouse

- *Swiss albino Mouse:*
  - It is created by the outbreeding of two albino males and seven females in Lausanne, Switzerland
  - As it is an outbred stock, it is extensively used in toxicological studies due to high variability between the animals
  - Not used as an animal model for specific genetic disease for which inbred or transgenic mice are a better model
- *J/NU Mouse:*
  - It is also called 'outbred-homozygous-athymic-nude mouse ($Foxn1^{nu}$/$Foxn1^{nu}$). It lacks thymus and hair due to a recessive mutation in the '*nu*' gene.
  - Tumor cells can be successfully transplanted in this strain and studied, as T cell development is absent due to lack of thymus.
  - It is a standard model *in vivo* model for anticancer drug testing.
- *CD-1 mouse:*
  - It is derived from the outbreeding of a group of Swiss albino mice in Lausanne, Switzerland.
  - It is commonly used for testing the reproductive toxicity of various chemicals, diphenylamine, atrazine, oxalates, carbaryl, etc., in chemical industries.
  - Despite albino status, the visual acuity of the CD-1 mouse is good.
- *ICR-mouse:*
  - The Institute of cancer research (ICR) mouse was also derived from Swiss albino outbreeding and selected as a separate mouse line for fertility.
  - It has a rapid growth rate with a high litter yield. The occurrence of spontaneous tumors is less in this outbred stock.

- Besides these, various outbred stock of mouse-like Carworth Farms-1 (CF-1), Swiss-Webster (SW), OF-1, Mouse-outbred-rock-only (MORE), and many more are available in the market but are uncommonly used.

2.2.5 Inbred Strains of the Mouse

- *C57BL/6J mouse:*
  - It is also called the B6/J mouse. C57BL/6J mouse is the most commonly used inbred strain. It is the first inbred mouse strain with successful complete genome sequencing.
  - They have low bone density when compared to other strains and develop loss of hearing upon aging. They are resistant to sound-induced-seizures and anthrax toxins but are susceptible to atherosclerosis, diet-induced-obesity, and diabetes mellitus-type 2.
  - B6/J mice are commonly used in transgenic mice production.
  - Variants like C57BLKS/J, C57BL/10J, C57BL/6NJ, C57BL/6J DIO, and C57BL/10SnJ are available in the market across the world and is used for specific research fields.
- *Non-obese diabetic mouse (NOD) mouse:*
  - It is also called as NOD/ShiLtJ strain. The NOD mouse is a polygenic model commonly used for type-1 diabetes (autoimmune-mediated).
  - It has a mutation in CTLA-4 gene-exon-2 that leads to the failure of suppression of T-cell immune response.
  - NOD mouse presents with hyperglycemia due to pancreatitis with the infiltration of leukocytes in the pancreatic islets. Defects in NK cell function, antigen presentation, and complement-5 malfunction lead to inflammation of the pancreas that mimics type 1 diabetes pathophysiology.
  - Female NOD mouse develop type-1 diabetes faster than males.
  - NOD mouse often presents with SCID and hearing impairment.
- *CB-17 Severe combined immunodeficiency (CB17-SCID) mouse:*
  - CB17-SCID is an albino mouse strain derived in Fox-Chase Cancer Centre in 2005.
  - Due to spontaneous mutation in *prkdc*^{scid}, both B cell and T cells are entirely underdeveloped in this strain.
  - Around 20% of CB17-SCID mouse exhibits significant low levels of IgG even at 12 weeks of age. Antibody response to a particular antigenic material is not mounted. This makes CB17-SCID an ideal model for anticancer drug evaluation.
- *Friend Leukemia Virus B (FVB) Mouse:*
  - FVB inbred strains are derived from the National Institute of health mice in 1970 by observing that some strains were susceptible to Friend-Leukemia-Virus (FVB).

  - FVB mice are blind. They have a homozygous mutation in the *PDE6B* gene resulting in loss of rods in the retina within 9 weeks of birth. Circadian rhythm is severely affected in this strain due to early blindness.
  - The oocyte of the FVB strain is large when compared to other strain, and hence they are an ideal strain for transgenic research.
  - Squamous cell carcinoma can be induced easily in this strain.
- *Murphy Roths Large (MRL) mouse:*
  - It is also called MRL/MpJ mouse. This strain is derived in the Wistar Institute of Philadelphia, the USA, in 1999.
  - They exhibit autoimmune disorders in their later stage of life.
  - MRL mouse strain is famous for its ability to regenerate the tissue at a faster rate without any scar on healing. In their skin, the hair follicles and the sebaceous gland complex also shows a higher degree of regeneration. Cardiac tissues can also be regenerated in this strain. Female MRL strains heal faster than males.
  - MRL mouse has an inbuilt capacity to resist muscular dystrophy. MRL strains are extraordinarily docile, and even the male MRL strains fight very rarely.
- *DBA-1 and DBA-2 mouse:*
  - DBA inbred strains were derived by Jackson Laboratory in 1909. DBA is one of the oldest inbred strains of mice. Subsequently, in 1929, two sub-strains, namely DBA-1 and DBA-2, were derived.
  - DBA-1 and DBA-2 are also called D1 and D2 mouse respectively. Both DBA-1 and DBA-2 are non-albino strains.
  - DBA-1 strains are commonly used as a model of evaluation of rheumatoid arthritis. After injection with collagen-type-II, the DBA-1 strains develop polyarthritis that is almost similar to humans. Synovitis, bone, and cartilage erosion are seen in the DBA-1 strain that mimics rheumatoid arthritis in humans.
  - DBA-2 strains are commonly used as a model of atherosclerosis, glaucoma, and cochlear pathology. DBA-2 strains mutation in the $Cdh23^{ahl}$ gene that results in progressive hearing loss by the third month of age. Upon aging, DBA-2 strains also develop glaucoma that closely mimics human hereditary glaucoma with the dispersion of iris pigmentation and atrophy, synechia, and elevated IOP.
  - DBA-2 strains show significant intolerance to morphine and alcohol. They are naturally CD94 deficient with the absence of expression of CD94/NKG2A receptors.
- *Ob/Ob mouse or obese mouse or B6-ob mouse:*
  - The B6-ob mouse strain was derived by a spontaneous mutation in V/Le strain with subsequent backcrossing for 45 generations in the Jackson Laboratory, Maine, in 1949.
  - The leptin gene (Lep^{ob}) is defective in this strain leading to hyperphagia, rapid weight gain, and obesity. The mouse gains weight three to four times higher than the regular mouse.

  - B6-Ob mouse exhibits hyperglycemia, hyper-insulinemia, altered hormonal secretion from the pituitary, and impaired wound healing, making this strain an ideal model of choice for evaluating metabolic syndrome, type-2 diabetes mellitus, and infertility.
  - This strain is hypothermic, hypo-metabolic, and sub-fertile.
  - Adipogenesis is increased in the bone marrow, and the bone mass of the hindlimbs are decreased in this strain.
- *Japanese Waltzing mouse:*
  - It is a 'Fancy' inbred mouse strain derived from the *Mus musculus* subspecies *molossinus* in Japan.
  - They have inherent cochlear defects, and hence they cannot move forward in a straight line; instead, they twirl in small circles (similar to the dance- waltz). Hence, they are called Waltzing mouse.

2.2.6 Genetically Modified Mouse

- A mouse model in which its genome is altered by biotechnology is called a genetically modified mouse. The first genetically modified mouse was produced in 1974 at the Massachusetts Institute of Technology. They can be produced via the following methods:
  - *Retroviral infection method:* Retrovirus is used as a vector to insert the 'small DNA inserts' into developing embryos of the mouse. The limitations of this technique are that only a small portion of DNA can be inserted, and random unpredicted insertion by retrovirus can lead to the expression/silencing of non-target phenotypes.
  - *DNA microinjection method:* The DNA construct of the desired transgene can be directly injected into the mouse oocyte's pronucleus to create a transgenic mouse. However, this method possesses similar limitations to the retrovirus infection method. To overcome this limitation, the technique has been upgraded to microinjection of fertilized mouse egg using 'endonuclease-based-reagent' that can create a transgenic mouse with minimal off-target gene modifications.
  - *Gene-targeted transgene method:* As a first step, the embryonic stem cells of the mouse are targeted for gene manipulation by introducing primarily 'loss-of-function' at specified loci. Later, the mouse's blastocysts were injected with this modified embryonic stem and transferred to a female mouse resulting in successful chimeric offspring. These chimeric offspring are intercrossed repeatedly to obtain the mutated homozygous transgenic mouse.

- *Types:*
  - *Transgenic mouse:* Gene modification by the methods mentioned earlier can result in the random insertion of the desired genome anywhere in the host genome and produces a transgenic mouse. The primary use of transgenic mice in research is in understanding the pathophysiology of cancer and non-communicable disease. The following are a few examples of a transgenic mouse.
    - MEF2A mouse: Overexpression of muscle-specific MEF2A protein due to a mutation in *mef2a* gene resulting in diabetes phenotype
    - GLUT4 series mouse: Alteration in expression of *Glut4* gene resulting in diabetes phenotype
    - LKB1-DN/B mouse: Overexpression of skeletal-muscle specific LKB1 resulting in metabolic syndrome phenotype
    - AMPK series mouse: Overexpression of AMPK protein due to alteration in *ampk* gene resulting in obesity and diabetes phenotype
    - Coll-11a2-Smad6 mouse: Alteration in expression of chondrocyte-specific smad6 resulting in dwarfism and osteopenia phenotype
    - Apart from these, more than 100 types of transgenic mice are commercially available in the market, and describing all is beyond this chapter's scope
  - *Constitutive knock-out mouse:* Gene modification method results in permanent loss of function of the gene in the entire cells of the mouse produces Constitutive knock-out mouse. This type of mouse is often employed to understand the change in anatomy, physiology, biochemical process, and behavior. Also, constitutive knock-out mice are used in the identification of novel cancer genes. The following are a few examples of constitutive knock-out mice used in research
    - BKO mouse: Acid-beta-galactosidase enzyme is deficient due to *Glb1* gene loss resulting in GM1-gangliosidosis phenotype
    - ATF4 mouse: Activating transcription factor-4 is absent due to the loss of the *Atf4* gene resulting in microphthalmia phenotype
    - ADAMTS13 mouse:Loss of function of ADAMTS13 metalloproteinase due to knock-out of *Adamts13* gene resulting in Thrombotic thrombocytopenic purpura phenotype
    - PEX11a mouse: Loss of function of Pex11 protein in kidney and liver lead to the development of hepatic steatosis with renal disorder phenotypes
    - FAM16a-L53P-tg mouse: Used to study hypomyelinating leukodystrophies in the central nervous system
  - *Conditional knock-out mouse:* Gene modification method results in loss of gene function in a specified organ in a specified time produces conditional knock-out mouse. This model mimics better than the constitutive knock-out model in terms of the development of cancer in humans. The gene knock-out can be regulated spatially and temporally in this model Example:
    - TAK1flox mouse and Ubc13 flox mouse: used to study immune abnormality
    - NDRG1lfox mouse: used to study Charcot-Marie-Tooth disease type4D
    - FIR flox mouse: used to study various cancers

Sik-3 flox mouse: Used to study abnormal metabolism of lipids and carbohydrates

BIG1 and arf1 mouse: Used to study cellular vesicular trafficking mechanisms

– *Knockin mouse:* In this mouse, the genome is inserted, resulting in overexpression or production of a new protein that mimics humans' disorders. Example

nNOS-Cre mouse: used to study various neurological disorders related to nitric oxide synthase systems

MBP- Cre mouse: Used to study myeline generative diseases

CD72-Y7F mouse: Used to study systemic lupus erythematous

2.2.7 Limitations of the Mouse as Experimental Models for Human Diseases

- Owing to their tiny size, the detection and dissection of lesions developed in the mice are very difficult.
- The atherosclerotic mouse model shows an extremely low incidence of rupture of plaque leading to thrombus/myocardial infarction in mice, while in humans, plaque rupture is the primary cause of myocardial infarction.
- An anticancer drug that was shown effectiveness in mouse was later found to be ineffective in humans due to underlying different regulatory mechanisms between mice and humans. Example: Endostatin.
- Chemical moiety that was proven carcinogenic in mice had been proven non-carcinogenic in humans and later was approved for use in humans. Example: Chloramphenicol.
- Majority of cancer models of mouse show development of mesothelial sarcomas while in humans epithelial carcinoma are the most common cancers.
- Spontaneous regressions of cancers are uncommon in adult humans, while it is common in the adult mouse.
- The basal metabolic rate per gram of body tissue in the mouse is seven-time higher than that of adult humans. Moreover, mice also have a higher mitochondrial density in the cell than humans. Thus the mouse is very prone to oxidative damages due to reactive oxygen species than humans that may confound the study of anti-oxidant drugs.

2.3 Rat

2.3.1 Scientific Name

- Genus: *Rattus*
- Species:
  - *novergicus* (Norway brown rat- most commonly used)
  - *rattus* (Black/Roof rat—less commonly used)
  - *xanthurus* (Sulawesian white-tailed rat—less commonly used with the longest tail of 25–35 cm)
  - *osgoodi* (Southern Vietnam Osgood's rat—less commonly used)

2.3.2 Physical Description

- Body Length: 12–14 cm
- Tail length: 12–15 cm
- Weight: Male: 300–500 g, Female: 250–350 g
- Breeding season: Throughout the year
- Gestational age: 21–22 days
- Duration of estrous cycle: 4–5 days
- Litter size:6–12
- Sexual maturity: 9–14 weeks
- Average life span: 2–4 years

2.3.3 Salient Points of the Rat as an Experimental Animal

- Rat is the most commonly used lab animal besides mouse and guinea pig in academic and pharmaceutical research sectors, especially in acute and chronic toxicity studies, carcinogenicity, and mutagenicity studies.
- A rat can be trained easily when compared to other animals. Hence rat is the lab animal of choice for studying behavior, conditional reflexes, and various neurological disorders.
- The rat has two parts of the stomach—the upper two-fifth is translucent and non-secretory (rumen), and the lower three-fifth is glandular (antrum with pylorus). The lower portion of the stomach of a rat is identical to the human stomach. Moreover, Rat lacks gall bladder, and hence constant bile presence in the duodenum causes the continuous secretion of HCl even under fasting state. Hence antipeptic ulcer drugs are evaluated better in rat models.
- The rat has the fastest liver regeneration period (less than 7 days) and is commonly used for liver physiology, effects of drugs on the liver, and outcome of various types of liver surgeries.

- Being small and easy administration of drugs (intraperitoneally), rats are commonly used to study analgesics' effects using a standardized tail-flick method.
- Various rats' organs express particular receptors in high concentration, and hence rat organs became standard tissue of choice for evaluation of agonist and antagonist of that receptor. For example:
  - Bioassay of adrenaline using rat uterus
  - Bioassay of acetylcholine using rat colon
- The MHC complex of rats has been thoroughly studied by genome sequencing, and hence rat is often used to study biological control over the functional activity of MHC complexes. Due to the same reason, the rat is also used for xenograft and allograft transplant experiments.
- The rat has a shorter gestation period, with high litter size and shorter sexual maturity duration. Because of these facts, the rat is commonly employed to test the reproductive toxicity of drugs.
- Rats have a high intrinsic healing capacity. Hence rats are most useful in assessing the various parameters of implant like residence time, rate of degradation, and safety of biomaterials that are implanted via the subcutaneous or intramuscular route.
- Seven days old rat pups are similar to human infants and are extensively studied for the hypoxic-ischemic model for stroke and evaluation of drugs for cerebral palsy.

2.3.4 Outbred Stocks of Rat

- *Wistar rat:*
  - Wistar is the first rat model that was standardized for research purposes. Wistar is an outbred stock rat developed in 1906 by Wistar Institute, USA.
  - Wistar rat is also an albino rat with a broad head, long ears, and average body length.
  - The tail length of this stock is always lesser than the body length.
  - The substrains Wistar-Unilever rat and Wistar-Hannover rats are outbred stock. On the other hand, Wistar-Furth and Wistar-Kyoto rats are inbred strains.
- *Sprague Dawley rat:*
  - It was developed in 1945 by Sprague Dawley Incorporation from the Wistar stock.
  - They are albino rats and relatively docile when compared to wild rats.
  - This stock is commonly used in all aspects of biomedical research.
  - It has a low incidence of spontaneous tumors and a high reproductive rate.
  - It has a narrow head with a longer body length when compared to other stocks. The tail is always longer than the body in Sprague Dawley rats.
- *Long Evan rat:*
  - This stock was developed in 1915 by crossing several wild grey male rats with female Wistar rats.

- This stock is also called 'hooded rat' because of the typical fur coat color. The head and its upper part of the trunk have greyish to black color fur, and other parts of the body have varying proportions of albino fur. Occasionally, a white hood with brown body parts can also be seen.
- This stock has a high resistance to respiratory problems when compared to other stocks. Thus Long Evan stock is preferred to study surgical procedures that require high inhalational anesthetic use.

2.3.5 Inbred Strains of Rat

- *Spontaneously hypertensive rat:*
  - This strain was developed in 1960 by inbreeding of Wistar-Kyoto strain with hypertension.
  - Hypertension develops spontaneously around the fifth week to the sixth week of age. The systolic blood pressure can reach up to 200 mmHg in adult rats.
  - This rat is the preferred strain for studying and understanding the pathophysiology of hypertension, stroke, and the evaluation of several anti-hypertensive drugs.
  - Further modification of this strain led to the development of 'stroke-prone SHR' in which the rat will die of stroke due to severe hypertension.
- *Zucker Fatty rat:*
  - Zucker fatty rat (ZFR) was developed by Zucker in 1961. This strain comprises another substrain called Zucker diabetic fatty rat (ZDF rat).
  - They have a mutation in the leptin receptor leading to increased food intake (hyperphagia). The rat can reach a weight of up to 1.5 kg. Obesity is apparent from the fourth week of age.
  - The ZFR is not overtly diabetic even though they show poor glucose tolerance. However, the substrain- ZDF rats also have significant hyperglycemia and dyslipidemia besides obesity and became overtly diabetic within the tenth week of their age.
  - Diabetic changes are characteristically seen in males than females in ZDF rats. Feeding females with high-calorie food convert them to overt diabetic like males.
  - This strain is extensively used in research concerned with obesity and diabetes.
- *OLETF rats:*
  - The Otsuka-Long-Evans-Tokushima fatty rats are inbred strains derived from the spontaneously diabetic Long Evans rats in 1994.
  - Late-onset hyperglycemia, mild obesity, and hyperinsulinemia are their characteristics features.
  - The diabetic changes are clinically visible by the 18th week of their age. The progressive degeneration of islets of the pancreas due to beta-cell apoptosis is the underlying pathology for the development of diabetes.

- *Goto-kakizaki rats:*
  - This inbred strain is derived from repeated inbreeding of Wistar rats that were showing glucose intolerance by Goto in 1976.
  - This strain is used as a non-obese model rat model for type II diabetes. Hyperglycemia occurs in this strain by insufficient insulin response rather than insulin resistance, as seen in obese models like ZDF rats and OLETF rats.
- *BB rat:*
  - This strain was called *biobreeding rat* and was developed from inbreeding of Wistar rats in 2005.
  - This strain shows features of diabetes in their eighth week of age. Lymphopenia is an additional feature seen in this strain.
  - This strain is used in the research area interlinking diabetes with autoimmunity.
- *Other inbred strains:*
  - Besides these strains, various inbred strains of rat-like *ZDSD rats*, *Brattleboro rats, Rowett nude rats, Fuzzy rats, Shorn rats, Royal-College of surgeon (RCS) rats*, and *Lewis rats* are also used in research.
  - Rowett nude rats lack thymus and are used in upper respiratory infection studies. Fuzzy rats are also a nude rat that develops progressive renal failure in their first year of age.
  - RCS rat has an inherent capacity to develop retinal degeneration due to mutation in the *MERTK* gene. Lewis rat has a tendency for a higher incidence of spontaneous tumor and leukemias.

2.3.6 Transgenic and Knock-out Rats

- Similar to the mouse, the rat genome has been extensively studied. Hence the gene of rats can be knocked-out or knocked-in, resulting in the production of transgenic rats.
- Transgenic rats have similar pathophysiology to human diseases and can provide better data quality than outbred stocks and inbred rat strains.
- Long Evans and Sprague Dawley rats are commonly used to produce transgenic rats. The following methods can produce them:
  - Plasmid transgene method
  - Bacterial artificial chromosome transgene method
  - Pronuclear microinjection of Zinc-finger-nuclease-mRNA method
  - CRISPR/Cas9 gene manipulation method
- Transgenic rats like Fisher 344 rats, Crl:LE rats, CrL:Wi rats, and Crl:SD rats have been commonly used in research.

2.3.7 Limitation of the Rat as an Experimental Animal

- The rat does not have a vomiting center, and hence anti-emetic drugs cannot be evaluated using it.
- The pancreas of rats is very diffuse, and hence complete and total pancreatectomy is extremely difficult. Hence, rats are a poor model for type I diabetes mellitus studies.
- The rat has a coprophagic habit (eat their stool). Thus the pharmacokinetic parameters of drugs with GI elimination can be significantly affected when the rat is used.
- The cartilages of rats are very thin, and the joints are small. Hence rats have a limited role in chondral-defect repair studies.
- The rat has less (4 times) platelet response to thrombin and prolonged (3 times) clotting time when compared to humans. Moreover, their coagulation systems have significant interspecies differences (e.g., Severe hemorrhage occurs after Vitamin K deficient diet only in Wistar but not in Sprague-Dawley rats). Hence, rats have a limited role in the evaluation of anticoagulant drugs.
- The rat has very 'loose skin' (without any adherence strength to structures under the elastic part of its skin) that is in contrast to human skin. Moreover, the primary method of wound healing in rats is 'wound contraction' in contrast to 're-epithelization' in humans. Hence, rats have a limited role in the evaluation of drugs for soft tissue trauma.

2.4 Guinea Pig

2.4.1 Scientific Name

- Genus: *Cavia*
- Species:
  - *porcellus* (domesticated and commonly used)
  - *aperea* (Brazilian guinea pig—nondomesticated)
  - *fulgida* (Shiny guinea pig—nondomesticated)
  - *tschudii* (Montane guinea pig—nondomesticated)
  - *magna* (Largest size, nondomesticated, called as greater guinea pig)

2.4.2 Description

- Body Length: 20–40 cm
- Tail length: Not visible externally
- Weight: Male: 500–1500 g
- Breeding season: Throughout the year

- Estrous cycle: 13–20 days
- Gestational age: 59–72 days
- Litter size: 3–6
- Sexual maturity: 3–5 weeks
- Average life span: 4–5 years

2.4.3 Salient Points of the Guinea Pig as an Experimental Animal

- Vitamin C was first identified by using guinea pig in 1907. Like humans, guinea pig lacks the enzyme L-gulonolactone-oxidase and hence unable to synthesize vitamin C. Thus the diet of guinea pig must contain Vitamin C supplementation at the rate of 200 mg–1 g/l in water.
- Guinea pigs are highly susceptible to Mycobacterium tuberculosis infection. In fact, Mycobacterium tuberculosis was first discovered using guinea pig in 1882 by Robert Koch.
- Besides tuberculosis, etiological agents for infectious diseases like amoebiasis, brucellosis, diphtheria, typhus fever, and yellow fever were identified using guinea pigs.
- Guinea pigs are highly sensitive to histamine and allergens. Hence guinea pigs are commonly employed in allergen testing, skin prick testing, studies related to asthma, and anaphylactic reactions.
- Various tissues of guinea pigs act as a standardized tool for assaying chemical compounds:
  - Guinea pig bronchus—screening for bronchodilators like beta-2 agonists and muscarinic antagonists.
  - Guinea pig trachea—screening of beta-blocker activities.
  - Guinea pig terminal ileum—screening of spasmodic compound, anti-histaminic drugs, etc.
  - Guinea pig uterus—bioassay of adrenaline
  - Guinea pig aorta—alpha-adrenergic blockers
- Guinea pig serum contains an asparaginase enzyme that shows anti-leukemic activity.
- A test for deafness called Preyer reflex (whistling sound moves the outer ear) can be easily tested in guinea pigs. Moreover, the ear structure of the guinea pig resembles closely to humans. Hence guinea pigs are commonly used in hearing research. Cochlear mechanical mechanisms were discovered by using guinea pig in 1961. Nowadays, guinea pigs are commonly employed in research related to the regeneration of inner ear hair cells.
- The complement system of immunology was first discovered in the blood by using a guinea pig.
- The placental structure of guinea pigs resembles humans, and their gestation period can also be divided into three trimesters like humans. Hence guinea pigs are preferred in studies related to pre-eclampsia and gestational hypertension.

- The immune system functions of the guinea pig are closely similar to humans. Thus, guinea pigs were used extensively for the development of vaccines. Vaccines for diphtheria and TB were discovered using guinea pigs.
- Due to their relatively larger arterial size than rat/mouse, guinea pigs are preferred in studying the aortic atherosclerosis models. Moreover, guinea pig carries cholesterol in LDL lipoprotein, unlike other rodents. Hence guinea pigs act as a good model for studying interlinking lipid metabolism and atherosclerosis.

2.4.4 Strains of Guinea Pig

- Unlike rats and mice, only limited numbers of outbred stock and inbred strains are available for the guinea pig.
- *Dunkin-Hartley* stock is the most commonly used outbred stock. It is an albino stock derived from a short-haired English Guinea pig.
- *Wright strain 2 (NIH 2) and Heston strain 13 (NIH 13)* are the two most commonly used inbred strains of guinea pig in research.

2.5 Hamster

2.5.1 Scientific Name

- Eighteen species of hamsters are currently identified and have been classified under seven genus
- Genus: *Mesocricetus*
  - Species:
    auratus (Syrian or Golden hamster or Teddy Bear Hamster—commonly used)
    brandti (Turkish hamster or Brandt's hamster)
    newtoni (Romanian Hamster)• Genus: *Cricetulus*

- Species:
  griseus (Chinese hamster—commonly used)
  barabensis (Chinese striped hamster or striped dwarf hamster)
  longicaudatus (long-tailed hamster)
- Other genus includes A*llocricetulus, Cansumys, Cricetus, Phodopus,* and *Tscherskia*. Only the Syrian golden hamster and Chinese hamster are commonly used in research

2.5.2 Description

- Body Length: 15–20 cm
- Tail: diminutive and fluffy
- Weight: 100–400 g
- Breeding season: Throughout the year
- Gestational age: 2–3 weeks
- Litter size: 5–6
- Sexual maturity: within 6 weeks
- Average life span: 1.5–3 years

2.5.3 Salient Points of Hamster as an Experimental Animal

- Hamsters have distinct cheek pouches, unlike other rodents. The cheek pouches are devoid of intact lymphatic drainage. Hence this site is used as the best experimental model for tissue transplantation research.
- Chinese hamsters have an inherent tendency to spontaneously develop diabetes mellitus due to defective beta cells of the pancreas.
- Unlike a mouse, hamsters can accept the human cytokines, IL-12, and GM-CSF to the full extent and shows the corresponding responses. Hence hamsters are a better model for immunogenicity testing than other rodents.
- Hamsters were extensively used to study the pathogenesis of various viruses like West Nile virus, Yellow fever virus, Nipah virus, Ebola, and Marburg Virus.
- Hamsters were also extensively used to study disease pathogenesis of leptospirosis, Leishmaniasis, Clostridium infections, Schistosomiasis, and amoebiasis.
- Hamster cheek pouch can also be used for evaluation of various chemicals with carcinogenic potential (e.g., DMBA can cause oral squamous cell carcinoma in hamsters).
- Hamster cheek pouch also act as excellent tissue of choice to:
  - Assay microcirculation using various prostaglandins
  - Study the reperfusion injury after ischemia
- Hamsters are also an excellent model for chronic smoke inhalation-induced carcinoma in the respiratory tract. European hamsters are more suited than Syrian hamsters for this purpose due to their large size.
- Hamsters exhibit a peculiar feature that cancer could act as a contagious agent amongst them. E.g., Reticulum cell sarcoma can be transmitted between hamsters via the *Aedes aegypti* mosquito.

2.6 Gerbil

- *Scientific name:* More than 180 species of gerbil has been identified and classified under 16 genera. The commonly used gerbil in the lab is *Meriones unguiculatus* (Mongolian gerbils).

- *Description:* It is also known as a sand rat or clawed jirds. The body length is between 10 and 15 cm, and the tail length is as same as the body length. An average adult gerbil weighs about 50–100 g. Gerbils often exhibit monogamy and attain sexual maturity by the 12th week of age. The gestational age is about 21–26 days, and the litter size ranges from 1 to 8 pups. Gerbils are expected to live for 2–5 years.
- *Salient points of gerbil as an experimental model:*
  - Gerbil exhibits similar hearing patterns and curves to humans and is extensively used in auditory research like guinea pigs.
  - Gerbil can act as a good model for studying pathological aspects of aging.
  - Gerbils spontaneously exhibit epilepsy when placed in a new lab environment or by repeated handling. Hence gerbil can act as a suitable animal model for analyzing seizures.
  - The gerbil is a well-known animal model for the study of gastric carcinogens.
  - Due to a high average life span than mouse/rat, gerbils are used to study the carcinogenic effects of various compounds with long latency periods.
  - While aging, gerbils exhibit the features of amyloidosis spontaneously. It can also occur when gerbils are experimentally infected with the filarial worm. Hence gerbils are suitable animal models for amyloidosis.
  - Gerbils also tend to form aural cholesteatoma, with a prevalence of about 50% at 24 months of age. Thus gerbils are used in the evaluation of the pathogenesis of aural cholesteatoma.

2.7 Rabbit

- *Scientific name:* 20 species of rabbits have been discovered and classified under 11 genera. The commonly used rabbit in the lab is *Oryctolagus cuniculus* (European rabbit) and their outbred stocks and inbred strains.
- *Description:* An average adult male rabbit weighs about 4–5 kg. Females are slightly heavier than males. Rabbits exhibit polygamy and attain sexual maturity by the 8th - 12th week of age. Their gestational age is about 28–35 days, and the litter size ranges from 1 to 14 pups (average 8). The average life span of the rabbit is around 1–2 years.
- *Strain and stocks:* New Zealand albino rabbit is a commonly used strain. Other strains like Dutch, Watanabe heritable hyperlipidemic (WHHL) rabbit, and Flemish Giant are also used.
- *Salient points of the rabbit as an experimental model:*
  - Rabbits are very docile and do not show aggression. Hence they are effortless to handle and make observations effectively.
  - Have shorter reproductive cycle duration, and hence rabbits are utilized in reproductive toxicity studies like rats and mice.

  - Rabbits are the excellent animal model of choice for pyrogen testing as rabbits' skin is susceptible to irritants.
  - The ears of rabbits are large with visible veins in them. Hence studies involving repeated blood collection can choose the rabbit as an animal model.
  - Due to the relatively large size of eyes compared to other small lab animals, rabbits are often used to study the action of drugs on the pupil, ophthalmic antifungal and antibiotic preparations, and study the pathogenesis of fungal, viral, and bacterial keratitis.
  - The anterior chamber of the rabbit's eye can accommodate the IOL designed for human purposes. Hence rabbit can be used for the evaluation of novel surgical techniques involving IOL.
  - Rabbits are an excellent animal model to evaluate nano-emulsion eye drops and other novel ophthalmic drug delivery systems.
  - Traditionally, rabbits were used for pregnancy identification in humans. Injection of serum from a pregnant human female induces ovulation in female rabbits.
- *Limitations:*
  - Wild rabbits with intact fur exhibit expression of atropinase enzyme leading to failure of the abolition of light reflex by atropine. New Zealand albino rabbit lacks this enzyme, and hence atropine can be tested in this strain.
  - Rabbit lacks adrenergic vasodilator nerves. Hence the phenomenon of vasomotor reversal of Dale could not be performed in rabbits.
  - Rabbit lack a vomiting center, and hence the evaluation of anti-emetic cannot be done in them.
  - The retina of the rabbit is poorly vascularized, with higher rod cell concentration when compared to humans. Thus effects of drugs on rabbit retina cannot be translated to retinal damages of glaucoma and other human retinal diseases.

2.8 Cats

- *Scientific name: Felis catus.*
- Cats are a good model of choice for neurological research, especially in balance, movement, hearing, and motor neuron disorders of spinal injury.
- Feline Leukemia Virus and Feline immunodeficiency virus (FIV) have been studied using domestic cats, and the results have been correlated with human leukemia and HIV, respectively. Thus FIV infected cats act as a good model for studying the pathogenesis of AIDS.
- Cats are highly susceptible to *Helicobacter felis* infection, similar to humans for *H.pylori.* Hence the feline helicobacter model helps in understanding the pathogenesis of ulcer by Helicobacter infection.
- Cat nictitating membrane is the good tissue of choice to bioassay adrenaline and histamine.

- Unlike humans, a paradoxical response by histamine is observed in cats. Histamine produces bronchodilation and vasoconstriction in cats.
- Traditionally, cats are used for studying the hemodynamic effects of various cardiovascular drugs.
- *Limitation:* Righting reflex cannot be studied in cats as it quickly regains this reflex even after falling from a high altitude.

2.9 Pig

- *Scientific name: Sus-scrofa domestica.*
- *Breeds:* Duroc pigs, Landrace pigs, Yorkshire pig, Large white pig, Mexican hairless pig, and Clawn miniature pigs.
- Pigs share anatomical and biochemical similarities like humans. Hence pigs are a better research model when compared with other non-rodent species.
- Coronary artery circulation, size of the heart, and blood vessels of the pig are identical to humans. Similar to the right-side dominant blood supply in the heart observed in 90% of the human population, pigs also share the same right-side dominant system. Moreover, collateral coronary circulation is absent in pigs (unlike dogs and monkeys where it is present) result in complete infarct on occlusion. Hence pigs are an excellent animal model to study heart circulation and myocardial infarction.
- Pig's pancreas is similar to humans in terms of histological, functional, and anatomical effects. A surgical pancreatotomy-induced diabetes model can be produced effectively in pigs.
- Pigs act as a standard model for reconstructive surgical methods and wound healing models as pig's skin has a similar dermal and epidermal composition to humans.
- Pigs were extensively used for studying intravascular coronary stents leading to the development of recent stents like bioresorbable stents and drug-eluting stents.
- Pigs are a popular model for studying aneurysms. Surgical induction of saccular aneurysms in pigs has been standardized.
- Pigs are preferred for analyzing ventricular assist devices and other novel transplant techniques.

2.10 Dog

- *Scientific name: Canis familiaris*
- *Breeds:* Mongrel and Beagles are commonly used breeds for experimentation
- Dogs are mainly used for studying the effects of drugs on the cardiovascular system
- Dogs are a good model for the surgical induction of diabetes. Diabetes and the role of the pancreas and insulin in diabetes were discovered by using experimentation on dogs

- The Vagus nerve and cervical sympathetic nerve run together in dogs, and hence stimulation can lead to complex variations in blood pressure
- Dogs are the animal of choice for studies on digestion and gastric secretion as they can be 'conditioned' easily
- Dogs can also develop hypertension spontaneously like humans

2.11 Monkeys and Other Non-human Primates

- *Scientific name:* Out of many non-human primates like lemurs, lorises, tarsiers, gibbon, gorilla, Orang-utan, Chimpanzees, the rhesus monkey (*Macaca mulatta*) is the commonly used species in the lab.
- Monkeys are used mainly when results from rodent species are not effectively translated to humans. Thus results from monkeys have closer resemblances to humans.
- Monkeys are the best animal model of choice for psychopharmacology due to their high neurological development and similar brain structure to humans.
- Monkeys have been used to develop polio vaccines, analyze the effects of the life-support system in infants, study novel techniques of dialysis, transplant rejection, and various stroke rehabilitation therapies.
- Research on Parkinsonism involving deep brain stimulation utilizes monkeys often.
- The pharmacokinetic profile, allergic sensitivity, and safety profile of the drug are very similar to the humans when estimated using monkeys.

2.12 Horse

- *Scientific name: Equus caballus.*
- Horse genome sequencing was completed in 2009. To date, more than 90 hereditary diseases of horses have been identified with similar patterns in humans.
- The insulin resistance pattern of the horse is similar to humans. Like humans, horses also develop 'Equine metabolic syndrome' with insulin resistance and abnormal fat distribution. Hence, horses are used to study insulin resistance and its response to overfeeding.
- Domestic horses are used to study the behavioral pattern in depression.
- Horses have been utilized in the lab to generate anti-serum for snake venom, diphtheria toxin, tetanus toxin, etc., in the past decades.
- *Limitations:*.
  - Due to its larger body size, feeding and housing are very difficult. Moreover, due to the high cost/animal in the horse, data generation from larger samples cannot be done.
  - Horses cannot vomit routinely but do in an extreme situation due to the presence of a powerful esophageal sphincter. Hence evaluation of anti-emetic drugs is limited in horses.

2.13 Sheep

- *Scientific name: Ovis aries.*
- Sheep has a similar anatomical structure and physiological function of the respiratory system to humans. Sheep can be used as an animal model for studying asthma, COPD, and interstitial lung diseases.
- The sheep model sensitized with a nematode (*Ascaris suum*) is a standard model to study the basic mechanism of lower respiratory tract inflammatory reactions and evaluate drugs affecting the lower respiratory tract's inflammation.
- The sheep model for asthma by dermatophagoides is a standard ovine model developed in Australia for studying aeroallergens in asthma.
- Premature lambs were used to study the pathogenesis of surfactants in the development of infant respiratory distress syndrome, leading to surfactant development as a drug for the same.
- Due to their long life span of 10–12 years, sheep are used to study neurodegenerative disorders like Alzheimer's disease, Huntington's disease, and Parkinsonism. The transgenic sheep model (created by microinjection of mutant HTT gene) is a standardized model for Huntington's disease.

2.14 Chicken

- *Scientific name*: *Gallus domesticus* and *Gallus gallus.*
- Chicken tissues are widely used in various in-vitro studies owing to their comfortable and surplus access.
- The fact that the virus can induce tumors was identified using chicken in 1900 by Peyton Rous.
- Chick embryos and it's amniotic/chorionic membrane have played a significant role in developing various vaccines like yellow fever vaccine, chickenpox vaccine, polio vaccine, rabies vaccine, and smallpox vaccine.
- Standard models of chicken are:
  - Chick comb method (an assay of androgenic substances)
  - Chick chorioallantoic membrane assay (to test angiogenesis)
  - Aversive discrimination in chicken (to test learning and memory)
  - UCD-200 chicken strain for obesity
  - Catalepsy antagonism using White Leghorn chicken
  - Scleroderma model in a chicken
  - Chicken embryo trachea model to measure the ciliary beat frequency
- Besides these, chicken is being utilized in broad research areas like growth, performance, embryology, fertility, toxicology, endocrinology, genetics, and neurobiology.

2.15 Pigeon

- *Scientific name: Columbia livia.*
- Pigeon belongs to the family Columbidae, which comprises 300 species.
- Pigeons are extensively used in the screening of anti-emetic activity. The pecking of the beak by a pigeon is considered as the vomiting response. Cisplatin, emetine, ipecac syrup, digitalis, and ditolyganidine are the agents used to induce emesis in pigeons.
- The following are a few standardized models developed using pigeon:
  - Intravenous injection of digitalis induced emesis in pigeon
  - Punished behavior model in pigeons for evaluation of chlorpromazine and amobarbital
  - Apomorphine-induced pecking behavior in pigeon to study the effects of neuroleptics
  - Auto-shaping procedure model in pigeons for learning and memory
  - Spontaneous arteriosclerosis model in pigeons
  - Pigeon crop method to assay prolactin hormone

2.16 Frog

- *Scientific name:* The amphibian species frogs are classified under order Anura that includes more than 7100 species. The commonly used lab frogs are *Xenopus laevis* (native African frog), Rana *tigrina* (Indian bullfrog) and *Rana hexadactyla* (Indian green frog), and *Bufo bufo* (Common toad).
- Frogs have longer legs than toads and have thinner skin than toads. Frogs lay eggs in the cluster while toads lay eggs in strands. Frogs are usually moist, and toads are dry. Toads can be found in dry areas, while frogs are usually seen in aquatic conditions.
- Frogs breathe primarily through their skin which is highly permeable to oxygen. Certain species of frog skin secretes a wide variety of substances like alkaloid (epibatidine), toxin (tetrodotoxin, zetekitoxin, chiriquitoxin), irritants, peptides (bombesin), antimicrobial-peptides (brevinin, ranlexin, nigrocins, dermaseptin), hallucinogens, and convulsants.
- The concept of bioelectricity (involvement of electrical transmitters in the generation of muscle activity) was discovered by Luigi Galvani in the eighteenth century using frog sciatic nerve preparation.
- Otto Loewi discovered Vagusstoff (later as acetylcholine) and awarded Nobel Prize for the same in 1936 using frog isolated heart experiments.
- Frogs have been extensively used in research to the extreme extent that nowadays, frogs for academic and research purposes are totally banned in several countries.

- A frog's heart contains three chambers in contrast to other lab animals (4 chambers in the heart). The frog's heart model is a standard method to study the chronotropic, inotropic, dromotropic, and bathmotropic effects of various drugs in the heart.
- In frogs, adrenaline is the neurotransmitter for the sympathetic system in contrast to humans, where non-adrenaline is the neurotransmitter for the same.
- Traditionally, frogs were used for the diagnosis of pregnancy.
- Showing light on the frog's eye bleaches rhodopsin in retinal layers and takes nearly 1 h for resynthesizing rhodopsin. Thus frogs were used for evaluating the retinal toxicity of compounds.
- The frog was used for the biological standardization of cardiac glycosides like digoxin. Digoxin produces ventricular systolic arrest and a widely dilated atrium.
- Ciliary motility of the frog esophagus was a standard method to assess the activity of spasmodic and antispasmodic drugs.

2.17 Zebra Fish

- *Scientific name: Danio rerio.*
- Zebra fishes are freshwater fish with blue horizontal stripes on each side of their body. Almost 70% of the human genome is present in the zebrafish genome, making the zebrafish a potential animal experiment model.
- Zebrafish's breeding time is 10 days, which is shorter than all the rodents and hence used in reproductive toxicity studies.
- The entire period of development of zebrafish from fertilized to young fish can be observed under a microscope.
- Transgenic zebrafish are relatively easier to create than rodents. This is because fertilized eggs of zebrafish develop externally while rodents it happens inside the body.
- The following pathological conditions have been successfully modeled using zebrafish:
  - Duchenne muscular dystrophy using *dystrophin* gene knock-out zebrafish model.
  - Human melanoma model using the *BRAF* gene knock-in zebrafish model.

Bibliography

Burggren WW, Warburton S. Amphibians as animal models for laboratory research in physiology. Inst lab animal Res J. 2007;48:260–9.

Guénet JL, Bonhomme F. Wild mice: An ever-increasing contribution to a popular mammalian model. Trends Genet. 2003;19:24–31.

Hedrich HJ, Bullock G. The laboratory mouse. London: Elsevier Academic Press; 2004.

Hoffman RA. The golden hamster. Ames, Iowa: Iowa State University Press; 1968.

Jackson F, Scott PP. Lab Anim. 1970;4:135–7.

McGrath P, Li CQ. Zebrafish: a predictive model for assessing drug-induced toxicity. Drug Discov Today. 2008;13(9–10):394–401.
Schwentker V. The gerbil. A new laboratory animal. Ill Vet. 1963;6:5–9.
Simon GA. The pig as an experimental animal in biomedical research. Israel J Vet Med. 1993;48: 161–7.
Thomas J, Ill G, Smith GJ, Robbery W. The rat as an experimental animal. Science. 1989;245 (4915):269–76.