Indian J Anim Health (2023), 62(2)- Special Issue: 01-10
DOI: https://doi.org/10.36062/ijah.2023.spl.03223

Invited Review Article

Application and advancement of organoid technology in animal disease modeling

L. Buragohain[1], A. Bharali[1], A. Ghosh[2,3], M. Bhakat[4], S. K. Dhara[5] and A. Ghosh[2,3*]

[1]College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati- 781 022, Assam, India; [2]School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan- 2308, Australia; [3]Drug Repurposing and Medicines Research Program, Hunter Medical Research Institute, New Lambton Heights, NSW-2305, Australia; [4]Animal Physiology and Reproduction (APR) Division, ICAR- Central Institute for Research on Goats, Makdoom, Mathura- 281 122, Uttar Pradesh, India; [5]Division of Animal Biotechnology, Indian Veterinary Research Institute, Izatnagar, Bareilly- 243 122, Uttar Pradesh, India

Abstract

Organoid technology has emerged as a powerful tool in the field of disease modeling, offering a unique opportunity to recapitulate complex tissue and organ functions *in vitro*. Organoids, three-dimensional cell cultures that mimic the architecture and function of specific organs or tissues, have revolutionized our ability to study and understand various diseases in a more physiologically relevant context. This review explores the application and advancement of organoid technology in animal disease modeling. It delves into the significant contributions of organoids in modeling a wide range of animal diseases, including gastrointestinal disorders, respiratory disorders, neurological disorders, liver disorders, kidney disorders, reproductive disorders, and infectious diseases. By summarizing recent studies and breakthroughs, we highlight how organoid technology has enabled researchers to gain new insights into animal disease mechanisms, screen for potential therapeutics, and personalize treatment approaches. Furthermore, this review also explores recent advancements in the field, such as the incorporation of organoids to utilize gene-editing techniques like CRISPR-Cas9 to enhance disease modeling precision. Overall, the application and advancement of organoid technology in animal disease modeling hold immense promise for improving our understanding of disease pathogenesis and the development of novel therapies. While challenges remain, ongoing innovations in organoid culture techniques and integration with other technologies offer exciting prospects for the future of disease research and personalized medicine. This review underscores the transformative potential of organoids in advancing our knowledge of animal diseases and ultimately improving human and animal health.

Keywords: Animal diseases, Disease modeling, Gene editing techniques, Organoid, Personalized treatment

Highlights

- Provide an overview about origin of organoids, and their significance in animal disease modeling.
- Exploring potentials of organoids in drug screening and enabling personalized medicine approaches.
- Highlights involvement of genome editing techniques to modify organoids for disease modeling.
- Summarize the impact of organoid technology on advancing our understanding of animal diseases.

INTRODUCTION

In recent years, the development of organoids has revolutionized our understanding of organ biology and disease mechanisms in humans. Organoids are 3D structures comprised of cells from a particular tissue of origin. It can mimic the source tissue's exact architecture, cellular morphology, and function (Hayashi *et al.*, 2022; Jamaluddin *et al.*, 2022; Tominaga *et al.*, 2022) (Fig. 1). Through organoids, primary cells can be maintained for a long-time, compared to traditional 2D culture, where primary cells have a limited lifespan. However, the application of organoids in veterinary medicine has garnered little attention. Organoids hold immense promise as a powerful tool for studying animal physiology, modeling animal diseases, and advancing personalized veterinary medicine. This article aims to provide an overview of the potential of organoids in veterinary medicine and highlights key areas of research and applications (Fig. 1).

Generation of organoids

Organoids are generated from adult stem cells (tissue-derived stem cells), embryonic stem cells, or induced pluripotent stem cells (Kar *et al.*, 2021). Adult stem cells are derived from matured tissue, and embryonic stem cells are obtained from the embryo's inner cell mass. Organoids are developed from these stem cells as a 3D structure mimicking the source tissue in an appropriate

*Corresponding Author, E-mail: arnab.ghosh@newcastle.edu.au

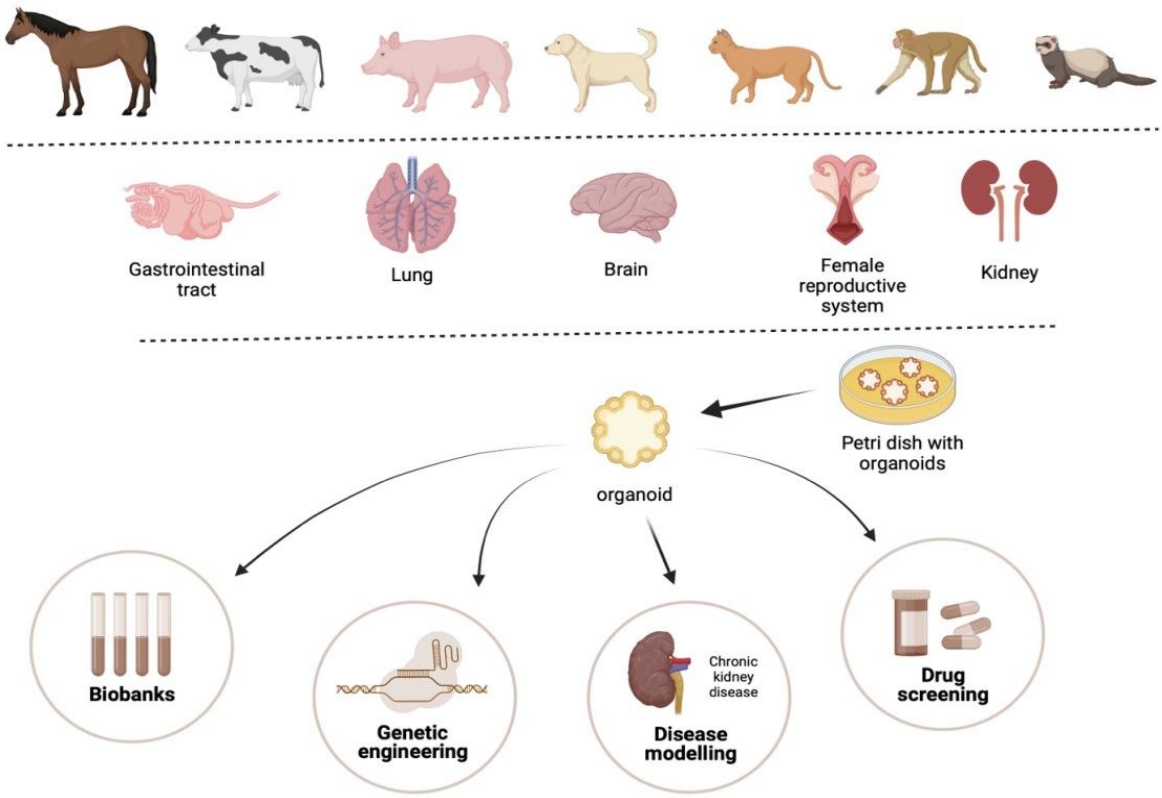

Fig. 1. Potential and current applications of organoid technology in veterinary medicine. Organoids derived from different organs of various animal species can be used for organoid biobanking, genetic engineering, disease modeling, and drug screening for personalized medicine.

environment using conditioned medium, essential and key growth factors, and extracellular matrix.

Animal species suitable for organoid models

Organoid models have become a valuable biomedical research tool for studying organ development, disease modeling, and drug screening. While most organoids are generated from human tissues, recent advancements have expanded the application of organoids to various animal species. This review highlights the animal species suitable for organoid models, discusses their advantages, and presents the potential applications in veterinary and comparative medicine.

Mouse (*Mus musculus*): The mouse is the most extensively used animal species as an organoid model due to its well-characterized genetics and the availability of genetic engineering tools. Mouse organoids have been employed to understand organ development and disease pathogenesis for various organs, such as the intestine, colon, stomach, liver, pancreas, brain, kidney, male and female reproductive organs (Clevers *et al.*, 2016; Ghosh *et al*., 2020)

Sheep (Ovine): Organoids offer unique advantages to study the growth mechanisms of organs, particularly of livestock. In this regard, Liu *et al.* (2020) have developed pancreatic duct organoid of sheep and established that moderate concentration of copper is essential for development of pancreas whereas deficient or excessive copper concentration may damage pancreatic tissue and hampers the development phase.

Zebrafish (*Danio rerio*): Zebrafish organoids offer unique advantages, such as rapid development, optical transparency, and high regenerative capacity. These features make zebrafish organoids suitable for studying organ development and tissue regeneration (Rossi *et al*., 2018).

Pig (Porcine): Porcine organs share anatomical and physiological similarities with humans, making them highly relevant for translational research. Thus, porcine organoids have been employed to study gastrointestinal diseases, liver disorders, and respiratory infections (Penningand van den Boom, 2023). Porcine intestinal organoid models were utilized to investigate heat stress,

the impact of mycotoxin, Swine enteric virus infection, Porcine epidemic diarrhea virus infection, *Lawsonia intracellularis* infection, *S. Typhimurium* and *T. gondii* infection, as well as for mucus production (Beaumont *et al.*, 2021). Porcine enteroids are also used to evaluate nutrient uptake and feed efficiency (Kar *et al.*, 2021). Since it is a model animal for human physiology and anatomy, its organoids have wide applications in biomedical research like drug screening and many human diseases infections (Kar *et al.*, 2021).

Dog (Canine): Canine organoids have gained attention in veterinary medicine for modeling diseases that commonly affect dogs, such as cancer and kidney disorders. Canine organoids offer insights into disease mechanisms and potential therapies for veterinary patients (Elbadawy *et al.*, 2020). Several researchers have developed different organoid models of canine for disease modeling, which include enteroids, liver, cardiac, renal, neuronal, skin, oviduct, and corneal organoids (Lawson *et al.*, 2023; Penning and van den Boom, 2023). Besides these, many cancer organoids were generated from canines, particularly prostate cancer, bladder cancer, mammary cancer, and thyroid cancer organoids (Penningand van den Boom, 2023).

Horse (Equine): Equines are primarily used as sports and companion animals and are still used as working animals in many places. They commonly have musculoskeletal disorders and gastrointestinal disturbances. Thus, a few intestinal organoid models were established to study the gastrointestinal biology of equines, determine the interactions with nutrients, and elucidate host-pathogen interactions (Kar *et al.*, 2021). Other organoids that were established for equine were endometrial (Thompson *et al.*, 2020), mammary (Bartlett *et al.*, 2022), and oviductal organoids (Thompson *et al.*, 2022; Lawson *et al.*, 2023). Thompson *et al.* (2022) recently used an equine endometrial organoid model to develop a treatment regimen to prevent endometritis.

Cow (Bovine): Several bovine-origin organoids have been established for various purposes. Bovine organoids, particularly enteroids, were developed to study the interactions of several pathogens, such as *S. Typhimurium*, *T. gondii*, *E. coli*, and the Rotavirus (Beaumont *et al.*, 2021). Faber *et al.* (2022) developed bovine gastric organoids to elucidate the invasion mechanism of gastrointestinal nematode in the bovine intestine. Lawson *et al.* (2023) recently developed oviductal organoids of bovine, which could be utilized for assisted reproductive technologies (ART).

Cat (Feline): Several feline organoid models have been generated to understand disease processes like canine. Feline organoids reported include intestinal, liver, cardiac, endometrial, and oviductal organoids (Lawson *et al.*, 2023; Penning and van den Boom, 2023). Liver organoids of feline were used to identify two promising drug molecules, *viz.*, 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) and T863 (a diacylglycerol O-acyltransferase 1 [DGAT1] inhibitor) for treatment of hepatic lipidosis (Haaker *et al.*, 2020).

Non-human primates (NHPs): Non-human primates, including rhesus macaques (*Macaca mulatta*) and marmosets (*Callithrix jacchus*), are valuable models for understanding human biology due to their genetic and physiological similarities to humans. NHP organoids provide insights into primate-specific aspects of organ development and disease (Mariani *et al.*, 2012).

Chicken: Chicken (*Gallus gallus*) organoids have been used to study avian-specific diseases, such as avian influenza, and to explore the development of organs with evolutionary significance, such as the avian limb bud (Dye *et al.*, 2015). Chicken intestinal organoid is a promising model to study gut biology and immunity, and the intestinal organoid of chicken can be developed by hanging drop technique, which is simpler than the alternative protocols (Panek *et al.*, 2018). In another study, Acharya *et al.* (2020) reported the development of avian crypt-villus enteroids, which could be used to understand gut metabolism, physiology, and pathology.

Snake: Snake venom gland organoids were generated from embryonic cells or adult venom gland tissues, which could be used for *in-vitro* production of snake venom (Post *et al.*, 2020; Puschhof *et al.*, 2021).

Disease modeling with organoids

Organoid technology has transformed disease modeling in biomedical research, allowing researchers to study organ-specific diseases in a controlled and relevant environment. In the context of veterinary medicine, organoids have the potential to significantly enhance our understanding of animal diseases and contribute to improved diagnostics and treatments. This article provides an overview of the application of organoids in modeling diseases in veterinary medicine. This review discusses recent advancements in using organoids to model animal diseases such as gastrointestinal disorders, respiratory diseases, neurological conditions, liver diseases, kidney disorders, reproductive disorders, and viral pathogenesis. It highlights their potential implications for understanding

disease mechanisms, drug responses, and personalized veterinary medicine.

Organoids to model gastrointestinal (GI) disorders: Organoids have emerged as a powerful tool in veterinary medicine for studying gastrointestinal (GI) disorders. GI organoids are three-dimensional *in vitro* models that mimic the structure and function of the gastrointestinal tract, providing a relevant and controlled environment to investigate various GI diseases. Gastrointestinal organoids have been utilized to model diseases such as inflammatory bowel disease (IBD), enteric infections, and gastritis across multiple animal species (Dye *et al.*, 2015; Tekes *et al.*, 2020). These models help elucidate disease pathogenesis mechanisms and interactions between pathogens and the intestinal epithelium. GI organoids are typically derived from primary GI cells or induced pluripotent stem cells (iPSCs) from animal species like dogs, cats, pigs, and rodents (Dye *et al.*, 2015; Tekes *et al.*, 2020). These cells are cultured under specific conditions that mimic the GI microenvironment, leading to the self-organization and differentiation into complex GI-like structures, including intestinal and gastric organoids. Inflammatory bowel disease (IBD), such as canine inflammatory bowel disease (CIBD) and feline chronic enteropathy, is a common GI disorder in animals. GI organoids have been used to model IBD, allowing researchers to study the inflammatory processes, immune responses, and interactions with the gut microbiota (Sang *et al.*, 2021). Gastroenteritis, often caused by viral or bacterial infections, affects animals of various species. GI organoids have been employed to study the pathogenesis of gastroenteritis and evaluate potential anti-viral or anti-bacterial therapies (Gebert *et al.*, 2023). Gastritis and gastric ulcers are common GI disorders in small and large animals. Gastric organoids have also been used to model these conditions, providing insights into the mechanisms of gastric inflammation and ulcer formation (Idowu *et al.*, 2022). Malabsorption syndromes, such as exocrine pancreatic insufficiency (EPI) in dogs and cats, impair nutrient absorption in the GI tract. GI organoids have been utilized to study the impact of EPI on the gut epithelium and to explore potential treatments (Molnár *et al.*, 2020).

GI organoids have significant implications for drug screening and personalized veterinary medicine. Using organoids derived from individual animals can predict customized drug responses, helping veterinarians tailor treatment plans for improved efficacy and reduced side effects (Gebert *et al.*, 2023). Recent advancements in GI organoid technology have improved their functionality and physiological relevance. Incorporating various cell types, such as immune cells and microbial communities, into GI organoids has enhanced their ability to mimic the complex interactions within the GI tract (Takebe *et al.*, 2019). Moreover, gene editing techniques, such as CRISPR-Cas9, allow researchers to introduce genetic mutations associated with specific GI disorders, facilitating the study of disease mechanisms and potential therapeutic targets.

GI organoids are promising for advancing our understanding of GI disorders in veterinary medicine. By providing a more accurate and controllable model, organoids contribute to the development of targeted therapies and personalized medicine for animals with GI diseases. Continued advancements in GI organoid technology and collaboration between veterinary and human medicine will drive research forward and lead to improved diagnostics and treatments for GI disorders in animals.

Organoids to model respiratory diseases: Respiratory organoids, particularly lung organoids, have been employed to model respiratory diseases, including pneumonia, bronchitis, and viral infections, in small and large animals (Archer *et al.*, 2021; Quah *et al.*, 2023). These models enable researchers to study lung injury and repair processes and the impact of environmental factors on respiratory health. Respiratory organoids provide a relevant and controlled environment to investigate various respiratory disorders, offering insights into disease mechanisms, drug responses, and potential therapeutic interventions. Respiratory organoids are typically derived from primary respiratory cells or induced pluripotent stem cells (iPSCs) obtained from different animal species (Dye *et al.*, 2015; Sang *et al.*, 2021). These cells are cultured under specific conditions that mimic the respiratory microenvironment, leading to self-organization and differentiation into lung-like structures. Pneumonia is a common respiratory disease in animals, often caused by bacterial or viral infections. Respiratory organoids have been used to model pneumonia, enabling researchers to study infection progression, immune responses, and potential treatments (Archer *et al.*, 2021; Quah *et al.*, 2023). Bronchitis, characterized by inflammation of the bronchial tubes, affects animals of various species. Respiratory organoids have been employed to study bronchitis and the underlying molecular and cellular changes (Archer *et al.*, 2021). Viral respiratory infections, such as canine influenza (CIV) and feline calicivirus (FCV), pose significant health threats to animals. Respiratory organoids have provided a valuable platform to study the pathogenesis of these viral infections and evaluate potential anti-viral therapies (Stroulios *et al.*, 2022). Respiratory organoids have been utilized to study

lung injury and repair processes. These models allow researchers to investigate tissue regeneration and healing mechanisms following lung injury (Dye *et al.*, 2015; Kong *et al.*, 2021). This organoid has significant implications for drug screening and personalized veterinary medicine.

Recent advancements in respiratory organoid technology have improved their functionality and physiological relevance. Incorporating various cell types, such as immune cells and lung-specific cell populations, into respiratory organoids has enhanced their ability to mimic the complex cellular interactions within the respiratory system (Takebe *et al.*, 2019). Moreover, gene editing techniques, such as CRISPR-Cas9, allow researchers to introduce genetic mutations associated with specific respiratory disorders, facilitating the study of disease mechanisms and potential therapeutic targets. Respiratory organoids are promising for advancing our understanding of respiratory diseases in veterinary medicine (Archer *et al.*, 2021; Quah *et al.*, 2023). By providing a more accurate and controllable model, organoids contribute to the development of targeted therapies and personalized medicine for animals with respiratory disorders. Continued advancements in respiratory organoid technology and collaboration between veterinary and human medicine will drive research forward and lead to improved diagnostics and treatments for respiratory diseases in animals.

Organoids to model neurological conditions: In veterinary medicine, the study and treatment of neurological conditions in animals have long posed significant challenges (Garosi *et al.*, 2006; Pain *et al.*, 2021). These disorders can range from epilepsy and neurodegenerative diseases to traumatic brain injuries, affecting our beloved pets' quality of life and overall health. Traditional research methods often need to accurately model these conditions, hindering diagnosis and therapy development progress. However, a promising breakthrough has emerged in recent years – the use of organoids (Shou *et al.*, 2020). These miniature, lab-grown structures have paved the way for novel insights and therapeutic approaches in veterinary neurology. Here, we will explore the innovative applications of organoids in modeling neurological conditions in veterinary medicine.

One of the most common neurological disorders in animals is epilepsy. Studying this condition in traditional animal models can be challenging due to differences in brain structure and function between species (Paternain *et al.*, 2012). Organoids, however, offer a unique advantage by allowing researchers to grow miniature brains that closely mimic those of the animal in question. Researchers can then induce epileptic-like activity within these organoids, providing insights into the mechanisms underlying seizures and potential treatment strategies (Waldbaum *et al.*, 2010; Blümcke *et al.*, 2017). Organoids have been instrumental in modeling neurodegenerative diseases like canine degenerative myelopathy and feline infectious peritonitis (Coates *et al.*, 2010; Laksono *et al.*, 2021). These conditions often involve the progressive degeneration of specific cell types in the nervous system. By growing organoids from affected animals, researchers can study the disease's pathology, test potential drugs, and develop personalized treatment approaches (Pain *et al.*, 2021). Traumatic brain injuries (TBIs) are a significant concern in veterinary medicine, especially for animals involved in accidents or sports. Organoids allow researchers to recreate the complex neural networks present in the brain and study how they respond to injury. This can revolutionize our understanding of animal TBIs, improving diagnostics and rehabilitation techniques (Noraberg *et al.*, 2005; Harvey *et al.*, 2015). Organoids have also been used to model neurological complications from infectious diseases like rabies and West Nile virus (Depla *et al.*, 2022). By infecting brain organoids with pathogens, researchers can observe how these diseases affect neural tissue and develop strategies for early diagnosis and treatment (Jain *et al.*, 2018).

Organoids have emerged as a powerful tool in veterinary medicine, particularly in studying and treating neurological conditions (Garosi *et al.*, 2006). These miniature organ models offer unprecedented insights into the complex workings of animal brains and pave the way for more targeted and effective therapies. As researchers continue to refine and expand the use of organoids in veterinary neurology, the future holds great promise for improving the lives of animals affected by neurological disorders. The collaboration between veterinarians, scientists, and the wider medical community will be crucial in harnessing the full potential of this innovative technology.

Organoids to model liver diseases: Liver diseases are a significant concern in veterinary medicine, encompassing many conditions such as hepatotoxicity, infections, and congenital hepatic disorders. Accurate modeling of these diseases is crucial for understanding their pathogenesis and developing effective treatments. Traditional animal models may not always provide relevant insights into veterinary patients, highlighting the need for innovative approaches. Organoids, three-dimensional miniature organ-like structures derived from stem cells or induced pluripotent stem cells (iPSCs), offer a promising avenue for modeling liver diseases in a

species-specific manner (Sampaziotis *et al*., 2017). Here, we explore the innovative applications of organoids in modeling liver diseases in veterinary medicine, emphasizing their potential to enhance diagnosis and treatment. Hepatitis, characterized by liver inflammation, affects various animal species. Porcine liver organoids have been utilized to investigate the pathogenesis of swine hepatitis E virus infection, providing insights into its impact on the liver (Sampaziotis *et al*., 2017). Veterinary drugs and toxins can induce hepatotoxicity, leading to liver damage. Organoids can serve as a valuable platform for assessing the potentially harmful effects of these substances on the liver. Conditions such as congenital biliary atresia and cholangitis can be challenging to model in animals. Bovine and canine liver organoids have been generated to mimic biliary diseases, offering insights into their pathophysiology (Nantasanti *et al.*, 2015; Nagao *et al.*, 2023;). Organoids can be instrumental in studying metabolic disorders like hepatic lipidosis in cats or copper storage diseases in dogs. Organoids derived from affected animals facilitate the investigation of molecular mechanisms underlying these conditions (Nantasanti *et al.*, 2015).

While organoids hold tremendous promise for modeling liver diseases in veterinary medicine, several challenges persist. Optimization of organoid culture techniques for different animal species, ensuring long-term viability, and replicating complex liver functions are ongoing areas of research (Shinozawa *et al.*, 2021). Addressing ethical considerations, standardizing protocols, and scaling up organoid production for broader veterinary applications are also essential steps for the future (Huch *et al*., 2015). As researchers continue to refine and expand the use of organoids, the potential to enhance diagnostics, treatment, and personalized medicine for liver diseases in veterinary patients is promising. Collaboration between veterinarians, scientists, and the wider medical community will be essential in harnessing the full potential of this cutting-edge technology.

Organoids to model kidney disorders: Kidney organoids offer a relevant and controlled environment to model various renal diseases, providing insights into disease mechanisms, drug responses, and potential therapeutic interventions. Kidney organoids are typically derived from primary kidney cells or induced pluripotent stem cells (iPSCs) from animal species like dogs, cats, pigs, and rodents (Kar *et al.*, 2021; Kawasaki *et al.*, 2022;). These cells are cultured under specific conditions that mimic the kidney's microenvironment, leading to self-organization and differentiation into complex kidney-like structures. Chronic kidney disease (CKD) is a prevalent condition in animals, characterized by the gradual loss of kidney function. Kidney organoids have been used to model CKD, enabling researchers to study disease progression, fibrosis, and the impact of potential therapeutic interventions (Khoshdel-Rad *et al.*, 2022). A nephrotic syndrome is a group of symptoms caused by increased permeability of the glomerular filtration barrier. Kidney organoids have been employed to study nephrotic syndrome and the underlying molecular and cellular changes (Khoshdel-Rad *et al.*, 2022). Polycystic kidney disease (PKD) is a hereditary disorder characterized by the formation of fluid-filled cysts in the kidneys. Kidney organoids have provided a valuable platform to study PKD's development and progression and evaluate potential therapeutic approaches (Cruz *et al*., 2017). Kidney organoids model renal toxicity caused by drugs, environmental toxins, and other substances. These models allow for the assessment of nephrotoxic effects and provide insights into mechanisms of kidney injury (Przepiorski *et al.*, 2022; Dilz *et al.*, 2023). Kidney organoids have a significant impact on drug screening and personalized veterinary medicine. These models can predict individualized drug responses based on an animal's specific genetic background, helping veterinarians tailor treatment plans for improved efficacy and reduced side effects (Luni *et al.*, 2016).

Recent advancements in kidney organoid technology have enhanced their functionality and physiological relevance. Incorporating multiple cell types, such as endothelial cells and immune cells, into kidney organoids has improved their ability to mimic the kidney's complex cellular interactions (Low *et al*., 2019). Moreover, gene editing techniques, such as CRISPR-Cas9, have enabled researchers to introduce genetic mutations associated with specific kidney disorders, facilitating the study of disease mechanisms and potential therapeutic targets. Kidney organoids are promising for advancing our understanding of kidney disorders in veterinary medicine. Further advancements in kidney organoid technology and collaboration between veterinary and human medicine will drive research forward and lead to improved diagnostics and treatments for animal kidney diseases.

Organoids to model reproductive disorders: Organoids derived from reproductive tissues, such as the ovary, oviduct, uterus, vagina, prostate, and testis, have been utilized to model reproductive disorders in animals (Karthaus *et al.*, 2014; Ghosh *et al.*, 2020; Lawson *et al.*, 2023). These models aid in studying hormone regulation, gametogenesis, and infertility, providing valuable information for reproductive health

research. Reproductive organoids offer a relevant and controlled environment to investigate the mechanisms underlying reproductive diseases, including infertility and hormone dysregulation. Reproductive organoids can be derived from primary reproductive cells or induced pluripotent stem cells (iPSCs) from animal species like dogs, cats, pigs, cattle, and rodents (Dye *et al.*, 2015; Bourdon *et al.*, 2021). These cells are cultured under specific conditions that mimic the reproductive microenvironment, leading to self-organization and differentiation into tissue-specific structures such as ovarian and testicular organoids. Infertility is a common animal reproductive disorder resulting from various causes, including hormonal imbalances, genetic mutations, and reproductive tract abnormalities. Reproductive organoids have been used to model infertility, allowing researchers to study the impact of these factors on gametogenesis and reproductive function (Lawson *et al.*, 2023; Smela *et al.*, 2023). Ovarian disorders, such as Polycystic ovary syndrome (PCOS), can cause irregular ovulation and hormonal imbalances in animals. Ovarian organoids have been employed to study PCOS and other ovarian pathologies, providing insights into follicular development and hormone regulation (Smela *et al.*, 2023). Testicular disorders, including testicular degeneration and testicular tumors, are significant reproductive health concerns in male animals. Testicular organoids have been utilized to model these disorders, offering a platform to study spermatogenesis, testosterone production, and the impact of toxicants on testicular function (Bourdon *et al.*, 2021). Reproductive organoids have significant implications for drug screening and personalized veterinary medicine. These models can be used to evaluate the effects of drugs and potential therapeutic interventions on reproductive tissues, helping veterinarians tailor treatment plans for individual animals (Bourdon *et al.*, 2021).

Recent advancements in reproductive organoid technology have improved their functionality and physiological relevance. Incorporating multiple cell types, such as granulosa, theca, and immune cells, into reproductive organoids has enhanced their ability to mimic the complex interactions within the reproductive tissues. Moreover, gene editing techniques, such as CRISPR-Cas9, allow researchers to introduce genetic mutations associated with specific reproductive disorders, facilitating the study of disease mechanisms and potential therapeutic targets. Reproductive organoids hold great promise for advancing our understanding of reproductive disorders in veterinary medicine. By providing a more accurate and controllable model, organoids contribute to the development of targeted therapies and personalized medicine for animals with reproductive diseases.

Organoids to model viral pathogenesis: Viral infections can significantly impact animal health and welfare, and organoids provide a relevant and controlled environment to investigate viral interactions with host tissues. Organoids have been instrumental in studying viral pathogenesis in veterinary medicine (Sang *et al.*, 2021). Researchers have used organoids to investigate various viruses' entry, replication, and host responses, including Canine distemper virus (CDV), Feline coronavirus (FCoV), and Avian influenza virus. Organoids can be derived from primary cells or Induced pluripotent stem cells (iPSCs) from various animal species, including dogs, cats, pigs, cattle, and birds (Dye *et al.*, 2015; Sang *et al.*, 2021). These cells are cultured under specific conditions that promote self-organization and differentiation, leading to the development of organoid structures that mimic the host tissue of interest. Respiratory viruses, such as Canine influenza virus (CIV), Feline calicivirus (FCV), Porcine reproductive and respiratory syndrome virus (PRRSV), and Avian influenza viruses, pose significant health threats to animals. Respiratory organoids have been used to model these viral infections, providing insights into viral entry, replication, and the host immune response within the respiratory tract (Archer *et al.*, 2021; Quah *et al.*, 2023). In pigs, gastrointestinal viruses, like Feline coronavirus (FCoV) and Transmissible gastroenteritis virus (TGEV), can cause severe enteric diseases. Gastrointestinal organoids have been employed to study viral pathogenesis in the intestine, enabling researchers to investigate viral replication, epithelial barrier function, and immune responses (Gebert *et al.*, 2023). Certain viruses, such as Canine distemper virus (CDV) and Avian borna viruses, can cause animal neurological disorders. Brain organoids have been used to model these viral infections, providing a platform to study viral neurotropism, neuronal damage, and the potential development of anti-viral therapies. Organoids have provided valuable insights into the interactions between viral pathogens and host tissues. They enable researchers to investigate tissue tropism, viral replication kinetics, and the host's immune response to viral infections (Sang *et al.*, 2021). Organoids derived from different animal species allow for comparative viral pathogenesis and host susceptibility studies. Organoids have significant implications for anti-viral drug testing and the development of therapeutic strategies. Using organoids as a screening platform, researchers can evaluate the efficacy of anti-viral drugs and potential treatment modalities, thus accelerating the development of anti-viral therapies for animal patients (Stadler *et al.*, 2015).

Recent advancements in organoid technology have improved their physiological relevance and complexity. Co-culture systems with immune cells, such as macrophages and lymphocytes, have been incorporated into organoids to mimic the *in vivo* environment and study host-virus interactions (Brodersen *et al*., 2010). Additionally, gene editing techniques, such as CRISPR-Cas9, have allowed researchers to introduce specific genetic modifications to study viral pathogenesis and host susceptibility. Organoids have revolutionized our approach to studying viral pathogenesis in veterinary medicine. By providing a more accurate and controllable model, organoids contribute to a better understanding of viral infections and host responses. Continued advancements in organoid technology and its integration with other advanced techniques will undoubtedly drive research forward and lead to improved diagnostics and anti-viral strategies for animal viral diseases.

Advantages of animal organoid models

Disease modeling: Organoids derived from animal tissues provide a platform to study diseases specific to that species, enabling researchers to investigate disease mechanisms and develop species-specific therapies.

Comparative medicine: Animal organoids offer the opportunity to compare and contrast disease mechanisms and responses to treatments across different species, aiding in the understanding of common and unique aspects of diseases.

Drug screening and development: Veterinary organoids are valuable platforms for drug screening and development. Organoid-based drug screening allows researchers to assess the efficacy and safety of potential therapeutics for specific animal diseases, facilitating the development of targeted veterinary medications.

Regenerative medicine: Organoids hold promise in regenerative medicine for animals. These models are utilized to study tissue regeneration and repair processes, offering insights into potential therapeutic approaches for tissue-specific diseases.

Conservation and wildlife research: Organoids from endangered or difficult-to-study animal species provide a non-invasive method for investigating disease processes and contributing to conservation efforts.

Challenges and limitations

The process of organoid generation is complex, and the production of uniform organoids is very challenging. The reproducibility of 3D organoid systems in terms of morphology and function is one of the significant bottlenecks (Zhao *et al*., 2022). Thus, the whole process should be standardized and automated with high-throughput platforms, from the medium to the yield of organoids, so that reproducibility could be increased (Bose *et al*., 2021). For the wide application of organoids in veterinary science, the cost of production should be minimized so that there can be large-scale production. To reduce the cost of organoid culture, 'hanging drop culture' can be used, reducing the 70% expenditure in Matrigel (Panek *et al*., 2018).

Conclusion

Organoids have opened new avenues in disease modeling for veterinary medicine, offering valuable insights into the pathophysiology of various animal diseases. Continued organoid technology advancements, including using different animal species and tissues, will further enhance our understanding of animal diseases and contribute to the development of personalized veterinary medicine.

Conflict of interest: Authors declare no conflict of interest.

Author's contributions: LB, AB, AG, MB, AG: Accumulated all the published data and wrote the manuscript; SKD, MB, AG: Did the proofreading, correction, and revision of the manuscript.

ACKNOWLEDGEMENTS

Cancer Institute NSW provides fellowship support to Arnab Ghosh. Figure Created with BioRender.com.

REFERENCES

Acharya M, Arsi K, Donoghue AM, Liyanage R, Rath NC *et al.*, 2020. Production and characterization of avian crypt-villus enteroids and the effect of chemicals. BMC Vet Res, 16(1): 179, doi: 10.1186/s12917-020-02397-1

Archer F, Bobet-Erny A and Gomes M, 2021. State of the art on lung organoids in mammals. Vet Res, 52(1): 77, doi: 10.1186/s13567-021-00946-6

Bartlett AP, Harman RM, Weiss JR and Van de Walle GR, 2022. Establishment and characterization of equine mammary organoids using a method translatable to other non-traditional model species. Development, 149(7): 200412, doi: 10.1242/dev.200412

Beaumont M, Blanc F, Cherbuy C, Egidy G, Giuffra E *et al.*, 2021. Intestinal organoids in farm animals. Vet Res, 52(1): 33, doi: 10.1186/s13567-021-00909-x

Blümcke I, Spreafico R, Haaker G, Coras R, Kobow K *et al.*, 2017. Histopathological findings in brain tissue obtained during epilepsy surgery. N Engl Med Rev J, 377(17): 1648-1656, doi: 10.1056/NEJMoa1703784

Bose S, Clevers H and Shen X, 2021. Promises and challenges of

organoid-guided precision. Medicine, 2(9): 1011-1026, doi: 10.1016/j.medj.2021.08.005

Bourdon G, Cadoret V, Charpigny G, Couturier-Tarrade A, Dalbies-Tran R *et al.*, 2021. Progress and challenges in developing organoids in farm animal species for the study of reproduction and their applications to reproductive biotechnologies. Vet Res, 52(1): 42, doi: 10.1186/s13567-020-00891-w

Brodersen BW, 2010. Bovine respiratory syncytial virus. Vet Clin North Am Food Anim Pract, 26(2): 323-333, doi: 10.1016/j.cvfa.2010.04.010

Clevers H, 2016. Modeling development and disease with organoids. Cell, 165(7): 1586-1597, doi: 10.1016/j.cell.2016.05.082

Coates JR and Wininger FA, 2010. Canine degenerative myelopathy. Vet Clin North Am Small Anim Pract, 40(5): 929-50, doi: 10.1016/j.cvsm.2010.05.001

Cruz NM, Song X, Czerniecki SM, Gulieva RE, Churchill AJ *et al.*, 2017. Organoid cystogenesis reveals a critical role of microenvironment in human polycystic kidney disease. Nat Mater, 16(11): 1112-1119, doi: 10.1038/nmat4994

Depla JA, Mulder LA, de Sá RV, Wartel M, Sridhar A *et al.*, 2022. Human brain organoids as models for central nervous system viral infection. Viruses, 14(3): 634, doi: 10.3390/v14030634

Dilz J, Auge I, Groeneveld K, Reuter S and Mrowka R, 2023. A proof-of-concept assay for quantitative and optical assessment of drug-induced toxicity in renal organoids. Sci Rep, 13: 616, doi: 10.1038/s41598-023-33110-5

Dye BR, Hill DR, Ferguson MA, Tsai YH, Nagy MS *et al.*, 2015. *In vitro* generation of human pluripotent stem cell derived lung organoids. Elife, 4: e05098, doi: 10.7554/eLife.05098

Elbadawy M, Abugomaa A, Yamawaki H, Usui T and Sasaki K, 2020. Development of prostate cancer organoid culture models in basic medicine and translational research. Cancers, 12(4): 777, doi: 10.3390/cancers12040777

Faber MN, Smith D, Price DRG, Steele P, Hildersley KA *et al.*, 2022. Development of bovine gastric organoids as a novel *in vitro* model to study host-parasite interactions in gastrointestinal nematode infections. Front Cell Infect Microbiol, 12: 904606, doi: 10.3389/fcimb.2022.904606

Garosi L, Mc Connell JF, Platt SR, Barone G, Baron JC *et al.*, 2006. Clinical and topographic magnetic resonance characteristics of suspected brain infarction in 40 dogs. J Vet Intern Med, 20(2): 311-21, doi: 10.1111/j.1939-1676.2006.tb02862.x

Gebert JT, Scribano F, Engevik KA, Perry JL and Hyser JM, 2023. Gastrointestinal organoids in the study of viral infections. Am J Physiol Gastrointest Liver Physiol, 324(1): G51-G59, doi: 10.1152/ajpgi.00152.2022

Ghosh A, Syed SM, Kumar M, Carpenter TJ, Teixeira JM *et al.*, 2020. *In Vivo* cell fate tracing provides no evidence for mesenchymal to epithelial transition in adult fallopian tube and uterus. Cell Rep, 31(6): 107631, doi: 10.1016/j.celrep.2020.107631

Haaker MW, Kruitwagen HS, Vaandrager AB, Houweling M, Penning LC *et al.*, 2020. Identification of potential drugs for treatment of hepatic lipidosis in cats using an *in vitro* feline liver organoid system. J Vet Intern Med, 34(1): 132-138, doi: 10.1111/jvim.15670

Harvey AR, Lovett SJ, Majda BT, Yoon JH, Wheeler LP *et al.*, 2015. Neurotrophic factors for spinal cord repair: which, where, how and when to apply, and for what period of time? Brain Res, 1619: 36-71, doi: 10.1016/j.brainres.2014.10.049

Hayashi R, Okubo T, Kudo Y, Ishikawa Y, Imaizumi T *et al.*, 2022. Generation of 3D lacrimal gland organoids from human pluripotent stem cells. Nature, 605(7908): 126-131, doi: 10.1038/s41586-022-04613-4

Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F *et al.*, 2015. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell, 160(1-2): 299-312, doi: 10.1016/j.cell.2014.11.050

Idowu S, Bertrand PP and Walduck AK, 2022. Gastric organoids: Advancing the study of *H. pylori* pathogenesis and inflammation. Helicobacter, 27(3): e12891, doi: 10.1111/hel.12891

Jain A, Barrile R, van der Meer AD, Mammoto A, Mammoto T *et al.*, 2018. Primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics. Clin Pharmacol Ther, 103(2): 332-340, doi: 10.1002/cpt.742

Jamaluddin MFB, Ko YA, Ghosh A, Syed SM, Ius Y *et al.*, 2022. Proteomic and functional characterization of intra-tumor heterogeneity in human endometrial cancer. Cell Rep Med, 3(9): 100738, doi: 10.1016/j.xcrm.2022.100738

Kar SK, Wells JM, Ellen ED, Te Pas MFW, Madsen O *et al.*, 2021. Organoids: A promising new *in vitro* platform in livestock and veterinary research. Vet Res, 52(1): 43, doi: 10.1186/s13567-021-00904-2

Karthaus WR, Iaquinta PJ, Drost J, Gracanin A, van Boxtel R *et al.*, 2014. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. Cell, 159(1): 163-175, doi: 10.1016/j.cell.2014.08.017

Kawasaki M, Goyama T, Tachibana Y, Nagao I and Ambrosini YM, 2022. Farm and companion animal organoid models in translational research: A powerful tool to bridge the gap between mice and humans. Front Med Technol, 4: 895379, doi: 10.3389/fmedt.2022.895379

Khoshdel-Rad N, Ahmadi A and Moghadasali R, 2022. Kidney organoids: current knowledge and future directions. Cell Tissue Res, 387(2): 207-224, doi: 10.1007/s00441-021-03565-x

Kong J, Wen S, Cao W, Yue P, Xu X *et al.*, 2021. Lung organoids are useful tools for investigating epithelial repair after lung injury. Stem Cell Res Ther, 12(1): 95, doi: 10.1186/s13287-021-02172-5

Laksono BM, Tran DN, Kondova I, van Engelen HGH, Michels S *et al.*, 2021. Comparable infection level and tropism of measles virus and canine distemper virus in organotypic brain slice cultures obtained from natural host species. Viruses, 13(8): 1582, doi: 10.3390/v13081582

Lawson EF, Ghosh A, Blanch V, Grupen CG, Aitken RJ *et al.*, 2023. Establishment and characterization of oviductal organoids from farm and companion animals. Biol Reprod, 108(6): 854-865, doi: 10.1093/biolre/ioad030

Liu M, Yu W, Jin J, Ma M, An T *et al.*, 2020. Copper promotes sheep pancreatic duct organoid growth by activation of an antioxidant protein 1-dependent MEK-ERK pathway. Am J Physiol Cell Physiol, 318(4): C806-C816, doi: 10.1152/ajpcell.00509.2019

Low JH, Li P, Chew EGY, Zhou B, Suzuki K *et al.*, 2019. Generation of human PSC-derived kidney organoids with patterned nephron segments and a *de novo* vascular network.

Cell Stem Cell, 25(3): 373-387, doi: 10.1016/j.stem.2019.06.009

Luni C, Giulitti S, Serena E, Ferrari L, Zambon A *et al*., 2016. High-efficiency cellular reprogramming with microfluidics. Nat Methods, 13(5): 446-52, doi: 10.1038/nmeth.3832

Mariani J, Simonini MV, Palejev D, Tomasini L, Coppola G *et al*., 2012. Modeling human cortical development *in vitro* using induced pluripotent stem cells. Proc Natl Acad Sci USA, 109(31): 12770-5, doi: 10.1073/pnas.1202944109

Molnár R, Madácsy T, Varga A, Németh M, Katona X *et al*., 2020. Mouse pancreatic ductal organoid culture as a relevant model to study exocrine pancreatic ion secretion. Lab Invest, 100(1): 84-97, doi: 10.1038/s41374-019-0300-3

Nagao I and Ambrosini YM, 2023. Ion channel function in translational bovine gallbladder cholangiocyte organoids: establishment and characterization of a novel model system. Front Vet Sci, 10: 1179836, doi: 10.3389/fvets.2023.1179836

Nantasanti S, Spee B, Kruitwagen HS, Chen C, Geijsen N *et al*., 2015. Disease modeling and gene therapy of copper storage disease in canine hepatic organoids. Stem Cell Reports, 5(5): 895-907, doi: 10.1016/j.stemcr.2015.09.002

Noraberg J, Poulsen FR, Blaabjerg M, Kristensen BW, Bonde C *et al*., 2005. Organotypic hippocampal slice cultures for studies of brain damage, neuroprotection and neurorepair. Curr Drug Targets CNS Neurol Disord, 4(4): 435-52, doi: 10.2174/1568007054546108

Pain B, Baquerre C and Coulpier M, 2021. Cerebral organoids and their potential for studies of brain diseases in domestic animals. Vet Res, 52(1): 65, doi: 10.1186/s13567-021-00931-z

Panek M, Grabacka M and Pierzchalska M, 2018. The formation of intestinal organoids in a hanging drop culture. Cytotechnology, 70(3): 1085-1095, doi: 10.1007/s10616-018-0194-8

Paternain L, Batlle MA, De la Garza AL, Milagro FI, Martínez JA *et al*., 2012. Transcriptomic and epigenetic changes in the hypothalamus are involved in an increased susceptibility to a high-fat-sucrose diet in prenatally stressed female rats. Neuroendocrinology, 96(3): 249-60, doi: 10.1159/000341684

Penning LC and van den Boom R, 2023. Companion animal organoid technology to advance veterinary regenerative medicine. Front Vet Sci, 10: 1032835, doi: 10.3389/fvets.2023.1032835

Post Y, Puschhof J, Beumer J, Kerkkamp HM, de Bakker MAG *et al*., 2020. Snake venom gland organoids. Cell, 180(2): 233-247, doi: 10.1016/j.cell.2019.11.038

Przepiorski A, Vanichapol T, Espiritu EB, Crunk AE, Parasky E *et al*., 2022. Modeling oxidative injury response in human kidney organoids. Stem Cell Res Ther, 13(1): 76, doi: 10.1186/s13287-022-02752-z

Puschhof J, Post Y, Beumer J, Kerkkamp HM, Bittenbinder M *et al*., 2021. Derivation of snake venom gland organoids for in vitro venom production. Nat Protoc, 16(3): 1494-1510, doi: 10.1038/s41596-020-00463-4

Quah PS, Tran BM, Corbin VDA, Chang JJY, Wong CY *et al*., 2023. Development of matrix-embedded bovine tracheal organoids to study the innate immune response against bovine respiratory disease. Organoids, 2(2): 82-101, doi: 10.3390/organoids2020007

Rossi G, Manfrin A and Lutolf MP, 2018. Progress and potential in organoid research. Nat Rev Genet, 19(11): 671-687, doi: 10.1038/s41576-018-0051-9

Sampaziotis F, Justin AW, Tysoe OC, Sawiak S, Godfrey EM *et al*., 2017. Reconstruction of the mouse extrahepatic biliary tree using primary human extrahepatic cholangiocyte organoids. Nat Med, 23(8): 954-963, doi: 10.1038/nm.4360

Sang Y, Miller LC, Nelli RK and Giménez-Lirola LG, 2021. Harness organoid models for virological studies in animals: A cross-species perspective. Front Microbiol, 12: 725074, doi: 10.3389/fmicb.2021.725074

Shinozawa T, Kimura M, Cai Y, Saiki N, Yoneyama Y *et al*., 2021. High-fidelity drug-induced liver injury screen using human pluripotent stem cell-derived organoids. Gastroenterology, 160(3): 831-846, doi: 10.1053/j.gastro.2020.10.002

Shou Y, Liang F, Xu S and Li X, 2020. The application of brain organoids: from neuronal development to neurological diseases. Front Cell Dev Biol, 8: 579659, doi: 10.3389/fcell.2020.579659

Smela MDP, Kramme CC, Fortuna PRJ, Adams JL, Su R *et al*., 2023. Directed differentiation of human iPSCs to functional ovarian granulosa-like cells via transcription factor overexpression. eLife, 12: e83291, doi: 10.7554/eLife.83291

Stadler J, Zoels S, Fux R, Hanke D, Pohlmann A *et al*., 2015. Emergence of porcine epidemic diarrhea virus in southern Germany. BMC Vet Res, 11: 142, doi: 10.1186/s12917-015-0454-1

Stroulios G, Brown T, Moreni G, Kondro D, Dei A *et al*., 2022. Apical-out airway organoids as a platform for studying viral infections and screening for anti-viral drugs. Sci Rep, 12(1): 7673, doi: 10.1038/s41598-022-11700-z

Takebe T and Wells JM, 2019. Organoids by design. Science, 364(6444): 956-959, doi: 10.1126/science.aaw7567

Tekes G, Ehmann R, Boulant S and Stanifer ML, 2020. Development of feline ileum- and colon-derived organoids and their potential use to support feline coronavirus infection. Cells, 9(9): 2085, doi: 10.3390/cells9092085

Thompson RE, Johnson AK, Dini P, Turco MY, Prado TM *et al*., 2020. Hormone-responsive organoids from domestic mare and endangered Przewalski's horse endometrium. Reproduction, 160(6): 819-831, doi: 10.1530/REP-20-0266

Thompson RE, Meyers MA, Veeramachaneni DNR, Pukazhenthi BS and Hollinshead FK, 2022. Equine oviductal organoid generation and cryopreservation. Methods Protoc, 5(3): 51, doi: 10.3390/mps5030051

Tominaga K, Kechele D, Sanchez G, McCauley H, Enriquez J *et al*., 2022. Generation of human intestinal organoids containing tissue-resident immune cells. Inflam Bowel Dis, 28: S57, doi: 10.1093/ibd/izac015.090

Waldbaum S and Patel M, 2010. Mitochondrial dysfunction and oxidative stress: A contributing link to acquired epilepsy? J Bioenerg Biomembr, 42(6): 449-55, doi: 10.1007/s10863-010-9320-9

Zhao Z, Chen X, Dowbaj AM, Sljukic A, Bratlie K *et al*., 2022. Organoids. Nat Rev Methods Primers, 2: 94, doi: 10.1038/s43586-022-00174-y

Received- 29.09.2023, Accepted- 11.11.2023, Published- 25.11.2023 (Online), 01.012.2023 (Print)
Section Editor: Dr. I. Samanta, Associate Editor