Strategy of choice of an animal model

Before starting a study it is important to well define its **aim and objectives.**

The experimental design and the animal model of the study are chosen according to the aim and the objectives of the study.

The choice of the animal model includes **the animal species** and the type of the model and it must offer **the best possible validity and reliability.**

In over all an animal model must:

-serves the aim and objectives of the study

-offers the best possible validity and reliability

Reminder on the notions of aim and objectives of a study

In research, aim and objective are two important terms that are often used interchangeably. However, there is a fundamental difference between them. Aim refers to the overall goal or purpose of a research study, while objectives are specific statements that describe the steps or actions needed to achieve the aim.

Types of scientific objectives

In experimental research there are many kinds of aims and objectives such as:

-Describing a normal or abnormal biological phenomena.
-Understanding the mechanisms involved in a biological phenomenon.
-Predicting biological response of substances.

Describing a normal or abnormal biological phenomena.

Example

-Description of the stages of prenatal and postnatal development of the nervous system.

-Description of the stages of prenatal and postnatal development of the nervous system in hyperglycemic conditions (diabetic mother).

-Description of phases of progression a disease ex, Parkinson's disease.

-Understanding the mechanisms involved in a biological phenomenon.

Example

-Studying mechanisms of synaptogenesis

-Studying mechanisms of apoptosis

-Studying mechanisms of pathogenesis of a disease

Predicting biological response of substances.

Example

-Study of the activity or toxicity of a new drug.

-Study of the toxicity of xenobitics e.g. pesticides….

Animal Species (Model organism)

-A model organism is a species that has been widely studied, usually because it is easy to maintain and breed in a laboratory setting and has particular experimental advantages, for example, they may have particularly robust embryos that are easily studied and manipulated in the lab, this is useful for scientists studying development. The most common used species are mice, rats, guinea pigs, hamsters, rabbits and Zebrafish.

Model Organisms may be classified into:

-**Invertebrates** such as flies (Drosophila), crustaceans (*Daphnia*) or worms (C. elegance) serve as versatile models to dissect fundamental cellular and molecular processes.

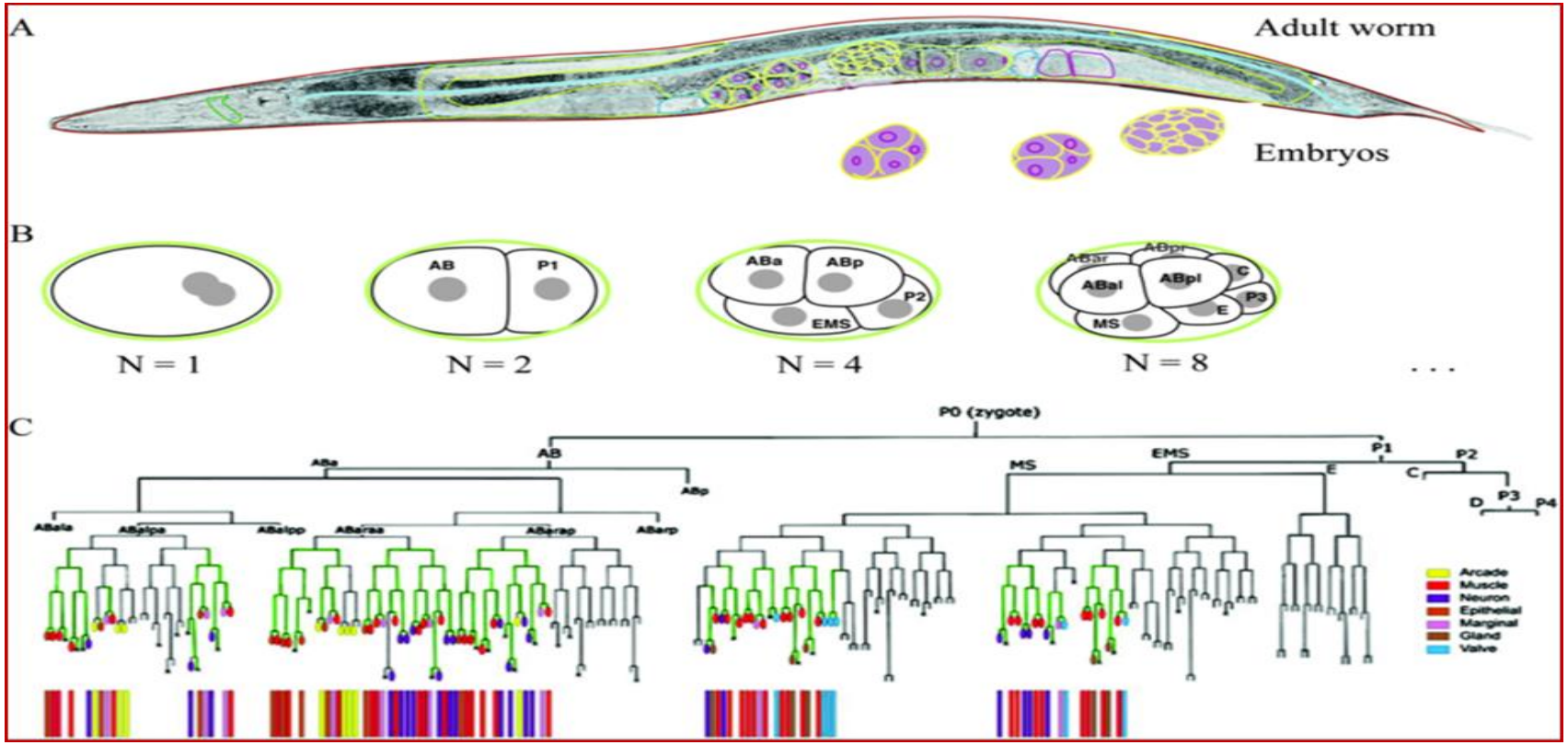
-**Vertebrates** have a vertebral column and are selected according to their similar characteristics to the human biological system. This group includes mammals , Zebrafish, chickens, and frogs.

Invertebrates: serve as versatile models to dissect **fundamental** cellular and molecular processes.

Example: Use of Caenorhabditis elegans (nematode) to study cell division and differentiation (cell lineage).

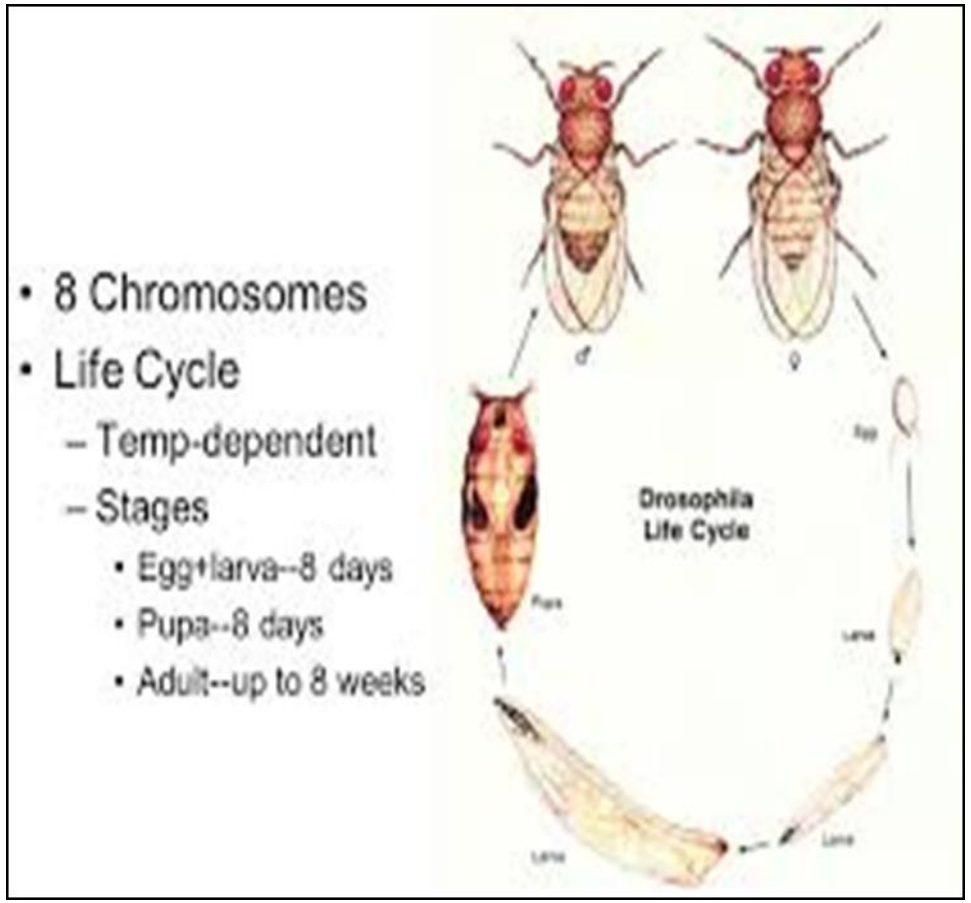
C. elegans is completely transparent. Additionally, the precise lineage of all cells in the body can be studied which make it easy to study developmental origins of diseases. These organisms have a short life cycle (3 weeks, suitable for studying the phenomenon of aging) and can be studied in large numbers.

Caenorhabditis elegans



The *C. elegans* cell lineage (Mango 2007) DOI:10.1895/wormbook.1.129.1

Drosophila melanogaster (fruit fly) is a classic model used to study molecular genetics, detect mutagenicity and teratogenicity. Drosophila is known by its genome susceptibly to mutation which make it a perfect model to study chemical mutagenesis.



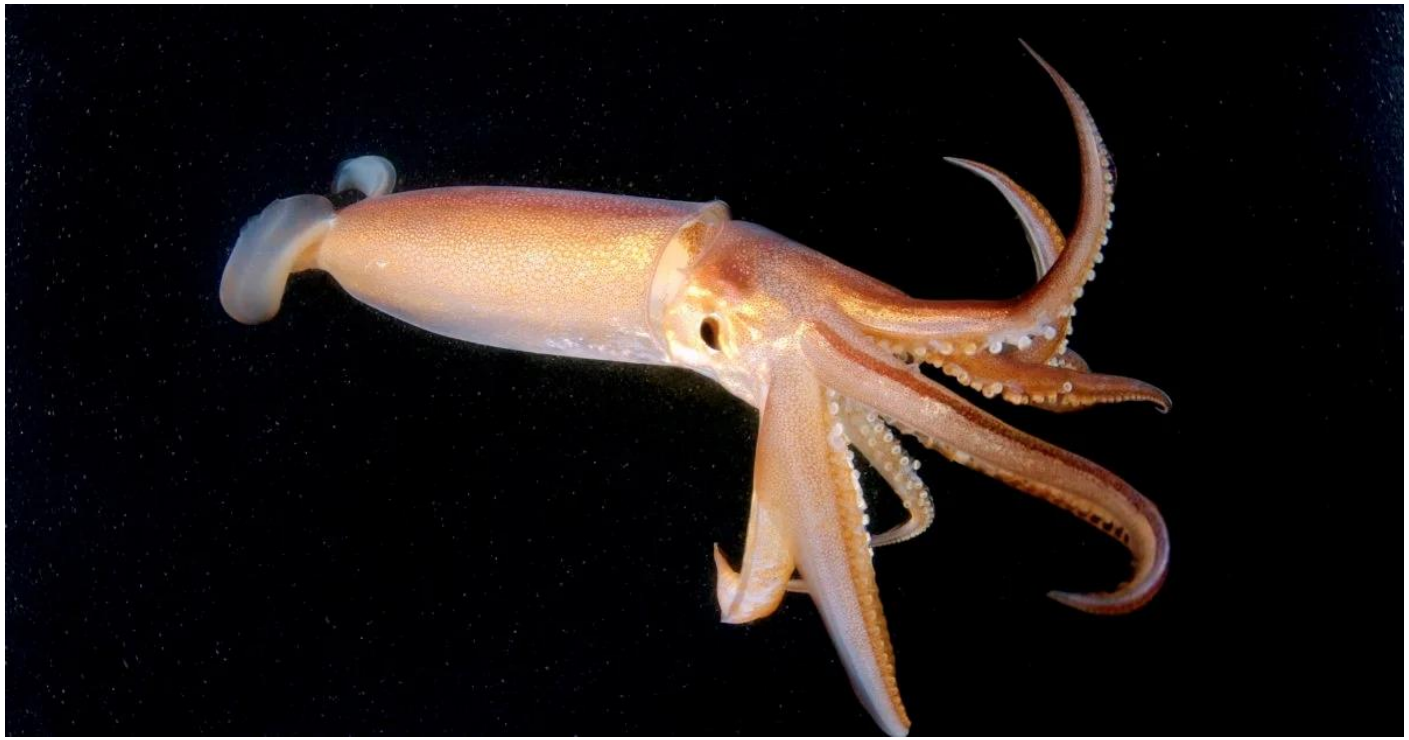
Life cycle of Drosophila melanogaster. (Flag O. Carolina Drosophila Manual)

In Drosophila scientistes determined the group of genes determining pattern, called homeotic genes.

Homeotic genes specify how structures develop in different segments of the body in almost all the pluricellular organisms

Cephalopods such as Squid are useful models in neuroscience studies, due to their big neurons where the axon could be seen with naked eye, which make it easy to study these structures.

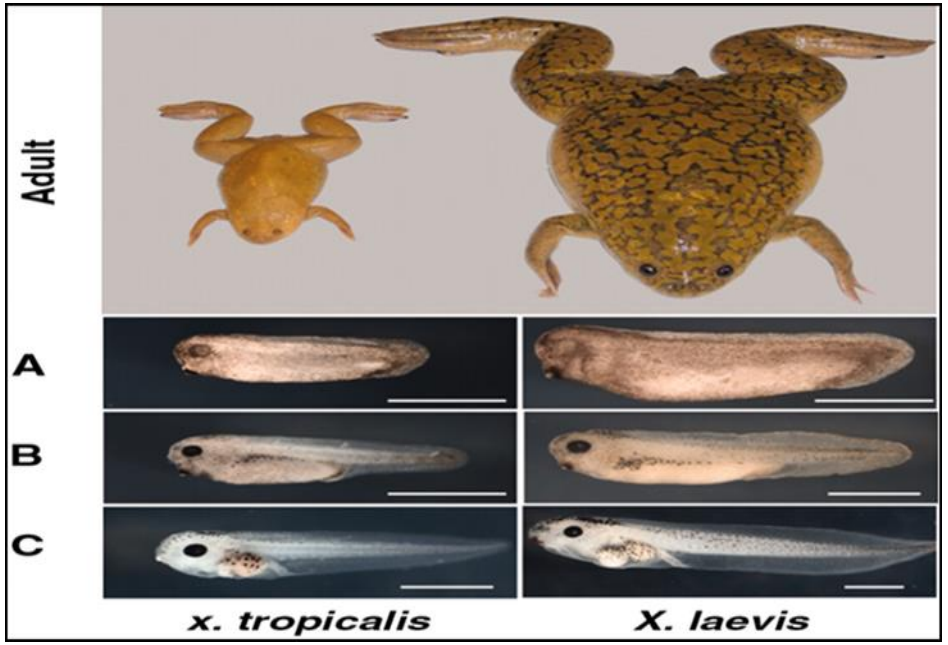
Also cephalopods have well-developed sense organs and large brains which make them a perfect model to study fundamental mechanisms of the nervous system.

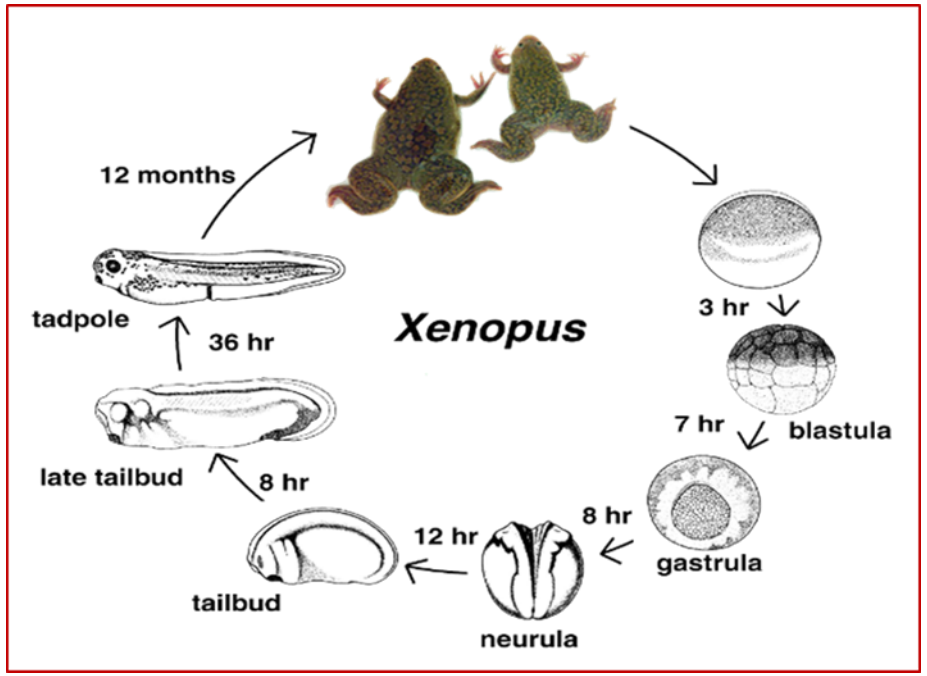


Vertebrate organisms

Are more convenient to study complex mechanisms of pathogenesis and efficacy and toxicity of molecules, due to their complexity and genome similarity to human.

Xenopus Laevis *Xenopus laevis* is an amphibian that has been used for decades as the principal vertebrate model to study embryonic development. Among the advantages of this species, is its closeness to higher vertebrates in terms of physiology, gene expression and organ development. In recent years, this model has gradually attracted the attention of ecotoxicologists, since it shows good indicator of habitat diversity, biological variety and local stressors.





Comparison of adults and tadpoles of X. tropicalis and X. laevis and Life cycle of xenopus laevis (Hellsten et al 2010) 10.1126/science.1183670

Zebra fish: Classically, the zebrafish model organism has been used to elucidate the genetic and cellular mechanisms related to development since the embryo forms and grows externally following fertilization. This provides insight into the genetic control of developmental processes in humans because their genomes are similar (70%).

Adult zebrafish have homologous brain structures to those found in mammals, as well as equivalent cellular and synaptic structure and function. Moreover, like humans, zebrafish exhibit age-related declines in cognitive functions, and a convergence of evidence has indicated that subtle changes in cellular and synaptic integrity underlie these changes. Therefore, the zebrafish is a powerful model organism for studying the neurobiological consequences of aging-related behavioral and biological changes, which offers the potential to identify possible interventions that would promote healthy aging. In addition, it is a very sensitive model for the study of neurotoxicity, and particularly developmental neurotoxicity.

Mammals

-Rodents: among the most used as experimental models of pathologies

- Cats, dogs: Used in specific studies particularly as natural models of obesity, aging and cognitive decline. These species are used as well in advanced studies of toxicology.

-Primate: used in advanced stages of preclinical studies. Used in studies which require a high analogy of the model with the human being (constructive validity), EX, studies of the mechanisms of infections.

Experimental approach: from the cell to the animal

Experimental approaches must be chosen to serve the aim and the objectives of the study, for example if the objective is to study the effect of a molecule on a specific target or a molecular pathway, it is more convenient to use cell culture than using in vivo models.

Also due to ethical considerations, it is important to chose the approach that guaranties the use the less number of animals and that requires the less surfing of animals such using anesthesia and non-invasive approaches. Although each approach has its own advantages and limits.

-In Vivo *VS* Ex Vivo and in vitro

-Anesthesia *VS*. Vigilant

-Invasive *VS* non-invasive

In Vivo Vs Ex Vivo and in vitro studies

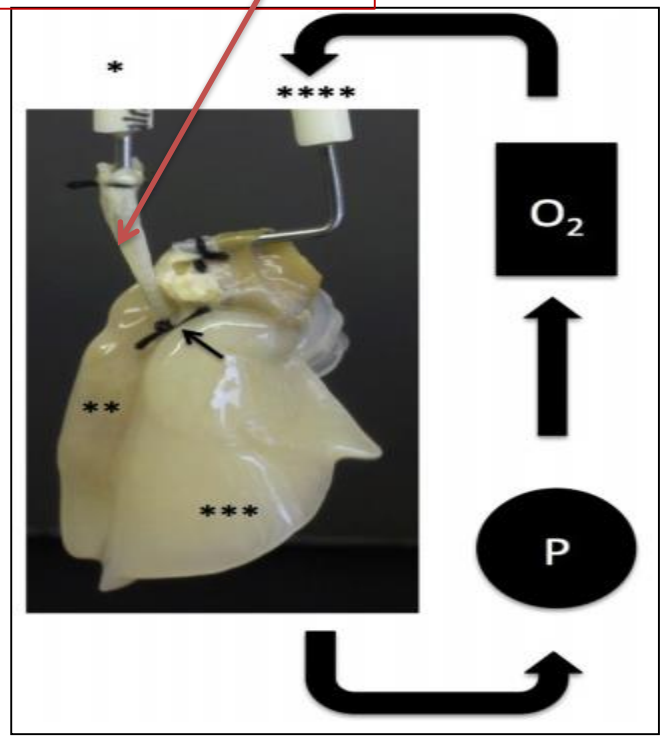
-In vitro study: the term is used when studies are carried on cells (or part of a cell; Ex mitochondria) in order to simplify studies.

-Ex vivo study: the term is used when studies are carried out on an organ or tissue isolated from an intact organism (it is still functional).

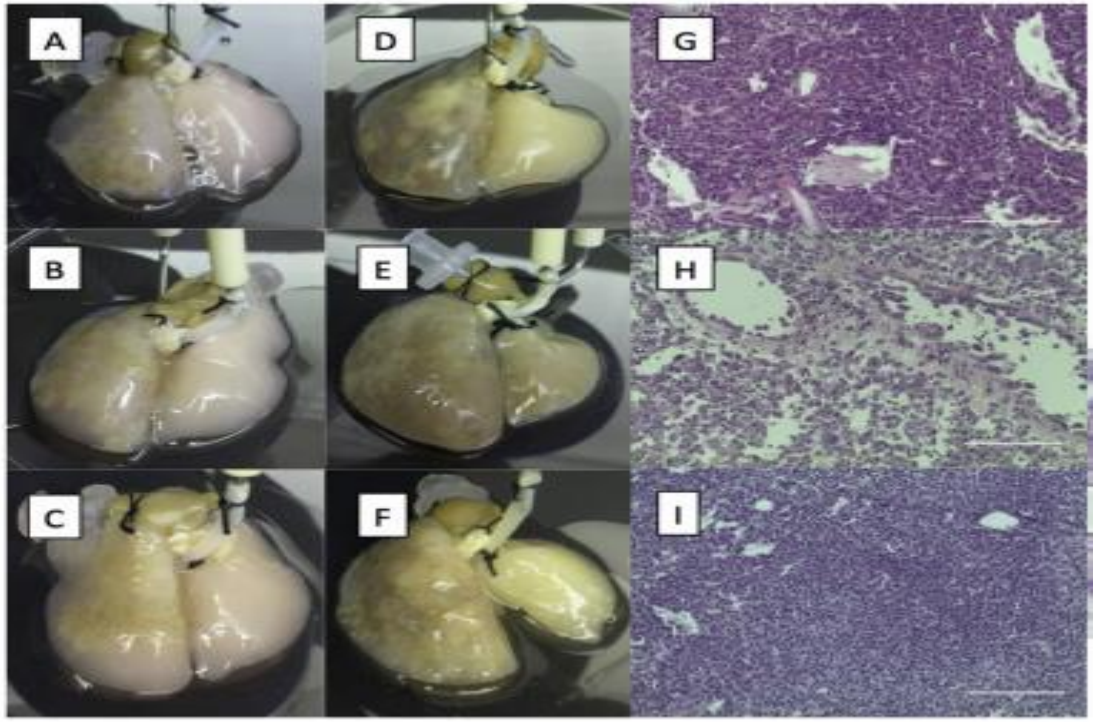
-In vivo study: the term is used when the study is carried out within a living organism.

Example of Ex vivo model

Injection of tumoral cells



A representation of the ex vivo lung cancer metastasis model



In this model we can characterize tumor cells in the 3 phases of tumor progression. This model mimics the biology of lung cancer metastasis and can be used to determine its mechanism and potential therapy in the future. (**Mishra et al. 2015**) **doi10.1016/j.athoracsur.2014.08.085**

EX vivo/in vitro approach

Advantages

analysis of specific interactions at the cellular and molecular level

Ease of mathematical modeling of experimental results (low number of biological parameters to take into account)

Very good reproducibility of results (reduction in variability)

Possible reduction in the number of animals used (multiplication of the number of organ fragments taken from the same animal)

Possibility of evaluating several experimental protocols on the same organ or organ fragment (series of doses, different experimental conditions consecutively)

Disadvantages

Reduced validity (in vitro and ex vivo models)

Always invasive and leading to the eventual death of the animal (in the case of ex- vivo models).

Need for great technical skills and sophisticated equipment as in to ensure organ survival and maintain reproducible reactivity over time.

In vivo approach

Advantages

Taking into account the animal in its entirety while respecting physiological regulatory processes. (high validity comparing to ex vivo models)

Does not automatically lead to the death of the animal (non-invasive methods).

Disadvantages

-Need to know all the characters; physiological, biochemical…..of the species used

-Need to control deleterious effects in animals used in experimental pathology models.

-High variability in experimental results

-Difficult reduction in the number of animals used (only one experimental result is obtained per animal).

Anesthesia vs. Vigilant

The use of anesthesia during is important during

1/ Creation of an experimental model by an invasive method Ex, surgical route.

2/ Reduction of post-operative or manipulation pain.

3/ Evaluation of invasive experimental parameters in situ

4/ reduction in the mobility of animals during the evaluation of certain non-invasive parameters ex imaging

5/ Sacrifice of the animal

Advantages of Anesthesia

Limiting the motricity of the animal

Absence of conscious reaction from the animal;

Many parameters can be recorded in invasive means

Minimize the animal’s pain; more ethical practice

Disadvantages

-Need for a specific choice of anesthetic agent and well-determined dose choice according to the animal species and the experimental method

-Possible interference between anesthesia and the measured experimental parameters.; EX,-Alteration of biochemical parameters such as enzymatic activity

-Interaction of aesthesia with the effect of the substances studied

Vigilant animal

Advantage

-Absence of interference between the experimental procedure and the experimental results.

Disadvantages -Very limited recording of parameters in an invasive manner

- Conscious reaction like the voluntary motricity of the animal

Invasive vs non-invasive

-Invasive procedure: a procedure that breaks/breaks the skin in some way.

Ex, Surgical animal models

-Non-invasive procedure: procedure that does not break the skin.

Ex, Genetically modified animal models.

Among the parameters; Example, x-rays, ultrasound, MRI and CT scans and ECGS are examples of non-invasive procedures.

Invasive

Advantages: Large number of Registered parameters

Disadvantages: There is suffring of the animal and most often, there is a sacrifice of the animal

Non-invasive

Advantages: More ethical practice

-Less modification of the physiology animal

-Possibility of dynamic study and monitoring over time

-No necessity of euthanasia of the animal

Disadvantages: -Tests that may require anesthesia

-Limited number of the studied parameters

-Heavy and expensive techniques and requirement of high technical skills (imaging)

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