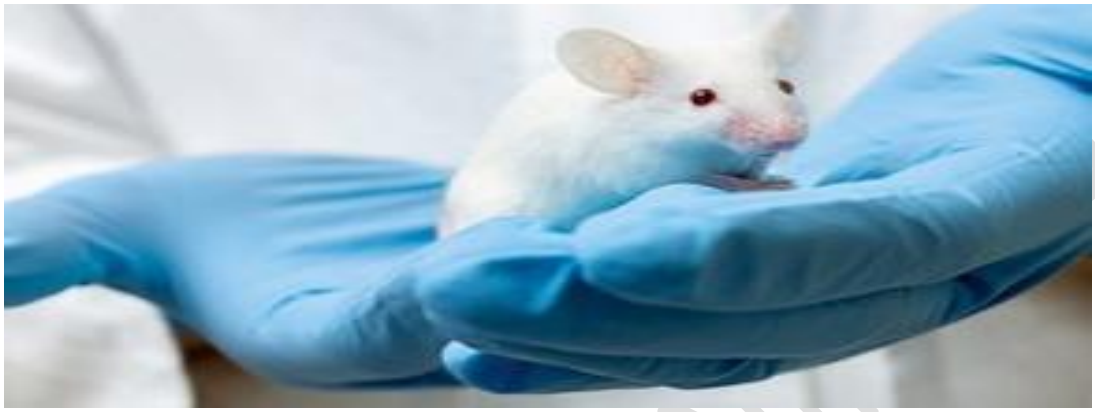
TRANSGENIC ANIMAL



INTRODUCTION

- A transgenic animal is one that carries a foreign gene that has been deliberately inserted into genome.
- Transgenic is the process by which mixing up of genes takes place.
- Foreign genes are inserted into the germ line of the animal, so it can be transmitted to the progeny.
- Transgenic technology has led to the development of fishes live stock and other animals with altered genetic profiles which are useful to mankind.
- First transgenic animal was a "supermouse" created by Ralph Brinster [U Pennsylvania] and Richard Palmiter [University of Washington] in 1982
- It was created by inserted a human growth hormone gene in mouse genome.
- The offspring was much larger than the parents.
- Mouse common transgenic expt.
- Other animals include pig, goat ,cow, shop, fishs etc.
- The foreign gene is constructed using recombinant DNA methodology

ADVANTAGES

- Increased growth rate
- Improve disease resistance
- Improve food conversion rates
- Increase muscle mass
- Improve nutritional quality
- improve call quality quality

DISADVANTAGES

- Inserted gene has multiple a functions
- Breathing problems
- Sometime lead to mutagenesis and the function
- The solemn survival rate of the transaction animal

PRODUCTION OF TRANSGENIC ANIMALS

METHADOLOGY-

STEP 1- Construction of a transgene

- Transgene made of 3-
  - Promoter
  - Gene into expressed
  - Termination sequence

STEP 2- Introduction of foreign gene into the animal

- Pronuclear microinjection method
- Embryonic stem cell method

STEP 3- Screening for the transgenic positives

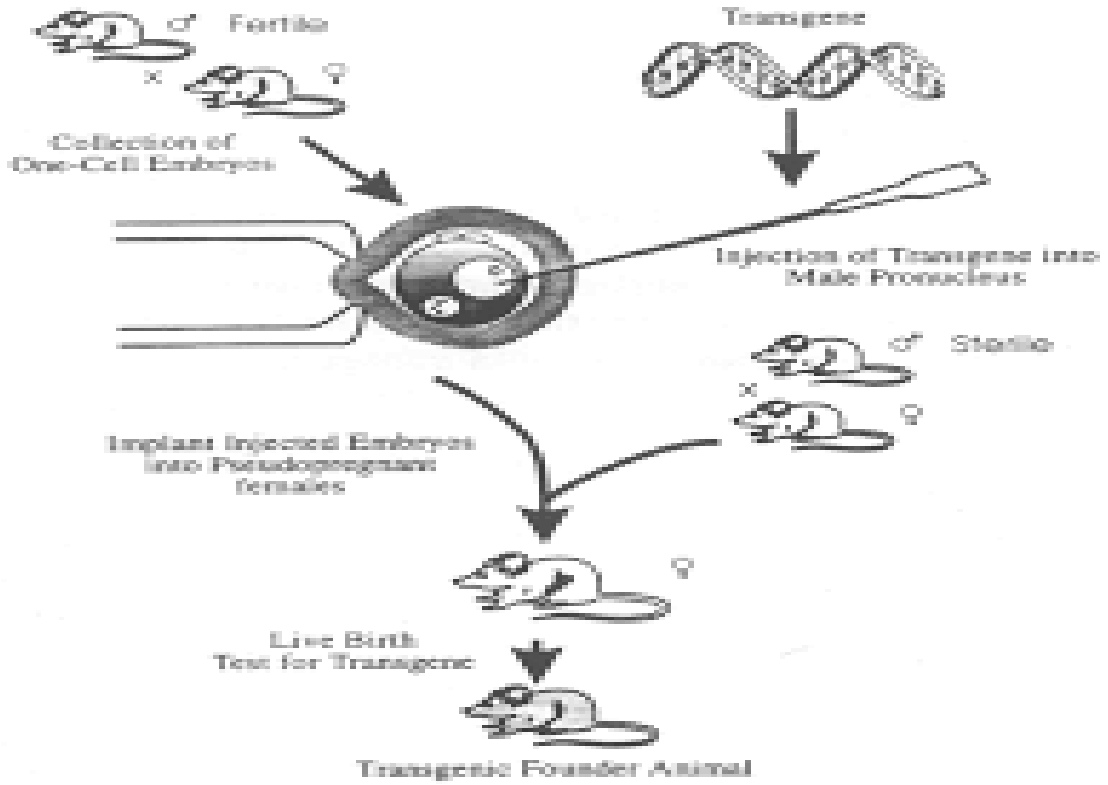
- Transgenics progenies are screened by PCR to examine the site of incorporation of the gene
- Some transgenic may not be expressed if integrated into transcriptionally inactive site

STEP 4- Further animal breeding is done to the obtained maximal expression

- Heterozygous of spring are method to form homozyous strain

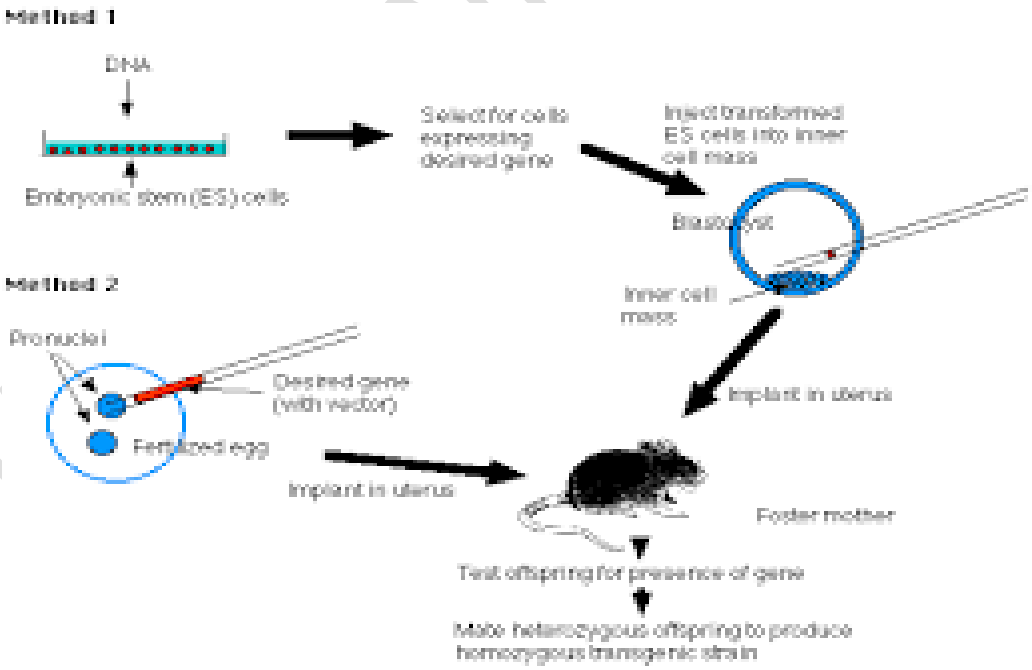
MICROINJECTION METHOD-

- Female animal is the super superovulated and eggs collected
- The eggs are fertilized in vitro
- The transgenic containing solution is injected into male pronucleus using a micropipette
- Egg with the transgenes are kept overnight in an incubator to develop to 2 cell stage
- The egg are them implanted into the uterus of the pseudo-pregnant female [female which have been mated with a vasectimized male the previous night]



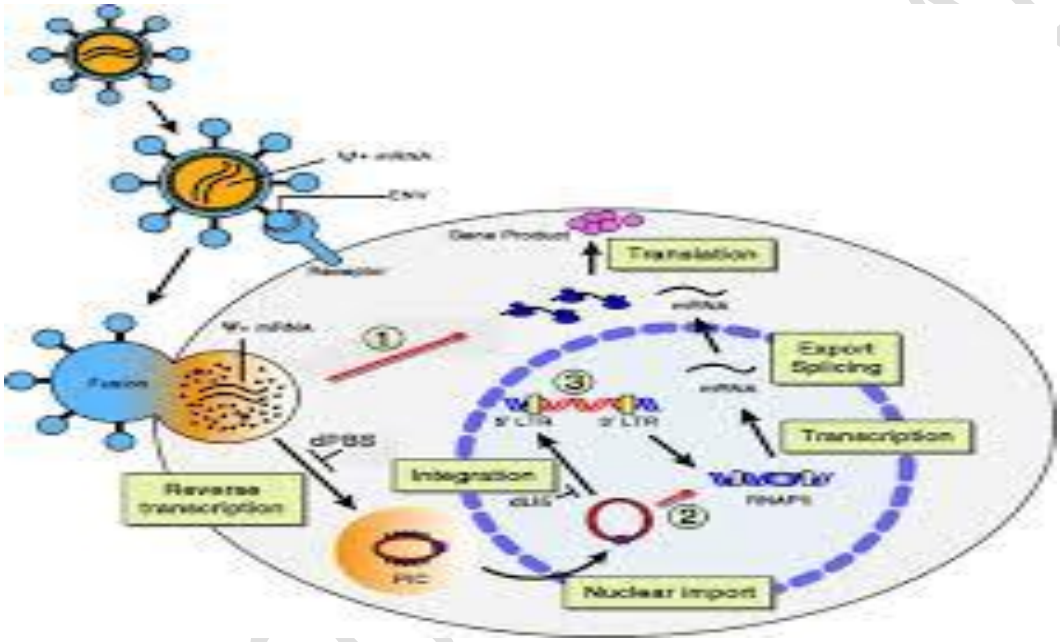
EMBRYONIC STEM CELL METHOD-

- Transgenic animal can be created by manipulating embryonic system c
- Embryonic system cell obtained from inner cell mass of a blastocyst
- Transgene is incorporated into the embryonic stem cell by-
  - Microinjection
  - By a retrovirus
  - By electroporation
- Transgenic system cell are grown in vitro
- Then they are the inserted in into a blastocyst and implanted into a host's uterus to grow normally



RETROVIRUS VECTOR METHOD-

- Retrovirus -vector the integrating the transgene into the genome of a recipient cell
- Many limitation-
    - ➢ transfer only small piece of DNA
    - ➢ risks of retroviral contamination biggest drawback
    - ➢ not in the regular use today



SOME EXAMPLE OF TRANSGENIC ANIMALS

- **Transgenic fish**-
    - ➢ Superfish-
        - ❖ Increase growth and size
        - ❖ Growth hormones gene insert into the fertilized egg
        - ❖ Transgenic salmon grow about 10-11 times faster than normal fish
    - ➢ Gol fish-

- GM freshwater zebra fish[Danio rerio]
- Produced by integrating a fluorescent protein gene from jelly fish into embryo of fish

- **Transgenic mouse**-
  - Alzheimer's mouse-
    - In the brain of Alzheimer 's patients, dead nerve cells are entagled in a prptein called amyloid
    - Mouse made by introducing amyloid precursor gene fiertilized egg of mice
  - Oncomouse-
    - Mouse model to study cancer
    - Made by inserting activated oncogenes
  - Smart mouse-
    - Biological model engineered to overexpress NR2B receptor in the synaptic pathway
    - This make the mice learn faster like juveniles throughout their lives
- **Transgenic pig**-
  - Pig have the trouble fully digesting a compound known as phytate found in many cereal grain used to feed them
  - Transgenic pig are created by the introducing phytase gene of e coli
  - Phytase enzyme is thus produced in the salvary gland of pig
  - It is degrade indigestible phytate with the release of phosphate that readily digested by pigs

Pig for organ transplant-

- ✓ Pig with human genes, in order to decrease the chance of organ rejection by human body

- **Transgenic livestock**-
  - Bioreaction whose cell have been engineered to the synthesis marketable protein
  - More economical then proceeding desire protein in the cell culture

- **Transgenic cattle**-
  - Transgenic cows are made to produce protein lactoferrin and intrerferons in their milk
  - Prior free cows resistant to mad cow disease
- **Transgenic Sheep**-
  - For good quality of wool production
  - Dolly was the female domestic sheep and the first mammal cloned from an adult somatic cell ,using the process of the nuclear transfer born **15 July 1996**
  - She was the cloned **by** the said and **Sir Ian Wilmut,Keith Campbell** and the colleagues at the **Roslin institute part of the University of Edinburgh the Scotland**

- **Transgenic goat**-
  - Goat that could be express the tissue plasminogen activator and anti throbin 3, spider silk in milk
- **Transgenic rabbit**-
  - **Alba** ,the EGFP [Enhanced green flurescent protein] bunny
  - Created in 2000 as a transgenic artwork
- **Transgenic monkey**-
  - **Andi**, was the first transgenic monkey born in 2000
  - **Andi** stands for inserted DNA spelled backward
  - An engineered virus was the used to the inserted the harmless gene for the green fluorescent protein [GPF] into and Andi's rhesus genome
  - Andi prove that transgenic primate can be created and can be express a foreign gene delivered into the their genome

APPLICATION OF TRANSGENIC ANIMALS

Transgenic animal are divided into the five classes based on their the purpose-

- Disease models
- Transpharmers
- Xenoplanters
- Food sources
- Biological models

Disease models-

- Animal that have been the genetically alerted to the express some expect of the human disease
- Etiology of the complex disease and to be developed the potential therapies without the use of the human subject
- **Example**- AIDS mouse, Alzheimer mouse, oncomouse other models- HTN,CAD, DM ,etc

Transgenic models for HTN-

- Polygenic disease -RAAS plays on imp. role
- 1st model- Transgenic Mouse experessing rat renin & ongiotensin gene [Ren-2-gene]
- Rat Models - Microinjection of Mouse Ren-2-gene into oocyte of normotensive rats
  - Hetrozygous transgenic rats [TGRm Ren-2]
  - Fulminant HTN [SBP>200 mmHg
- Sensitive to ARBs[Losartan,Telmisartan] or ACEIs[Lininopril]

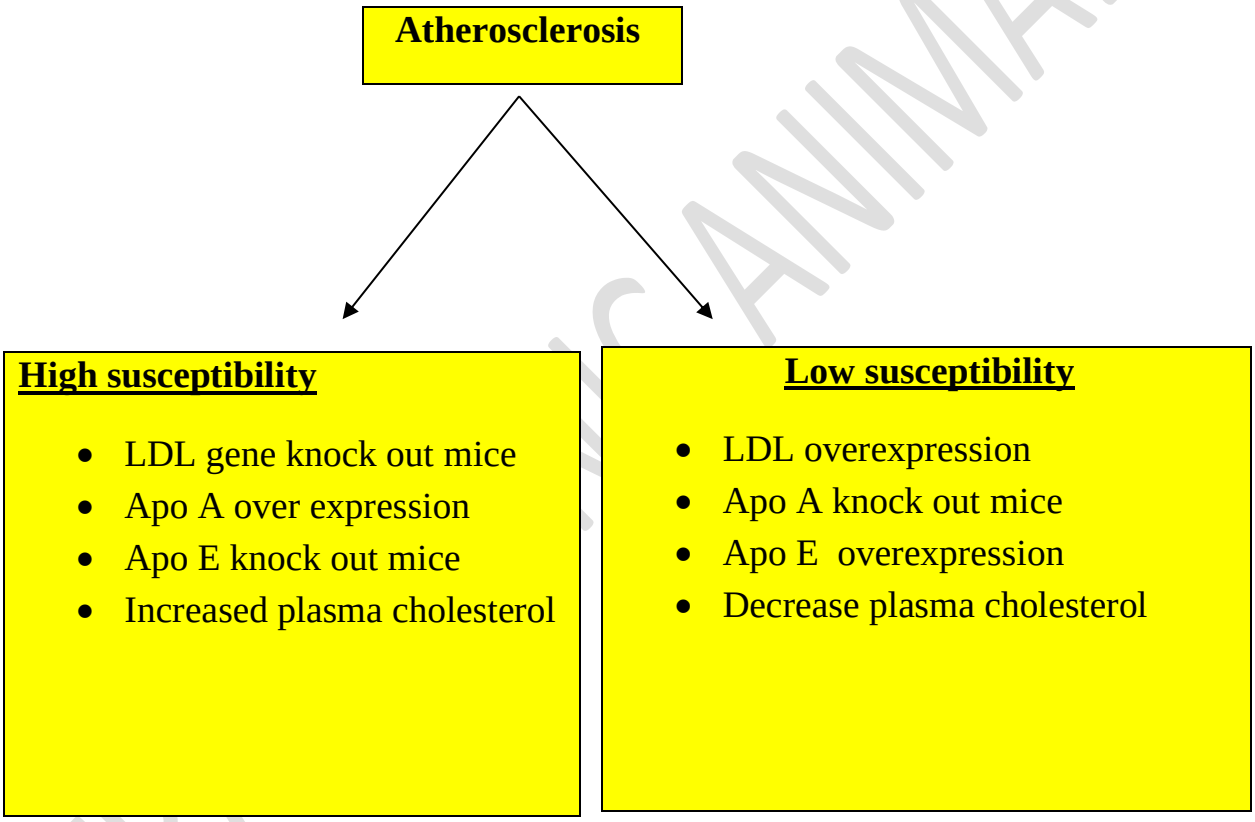
Transgenic models for diabetes –

- For studying the genes and their role in peripheral insulin action
- model of insulin secretion –
  - Glucokinase
  - Hepatic glucose production in t2 diabetes
- Transgenic mouse model- IDDM[by retrovirus factor method]

- Other-
  - Beta receptor knockout mouse
  - Uncoupling protein [UCP1]knockout mouse
  - Acute and chronic model for antidiabetic agent

Transgenic model for Atherosclerosis-

- plasma cholesterol homeostasis-
  - Receptor- Mediated endocyotsis of lipoprotein



Transpharmer -

- Transgenic animal which are the genetically engineered the procedure of **human pharmaceutical protein[recombinant protein]** in their the saliva,milk, urine or blood **–pharming**
- **1920**- Insulin extracted from the pig pancreas
- **Early 1980**- Human insulin prepared in recombinant bacteria

- Synthesis of complex protein required for post translational modification to remain stable
- Transgenic animal producing RPs
  - Milk- ex. Human antithrombin 3
  - Chicken egg white- ex. mABs, human IFNs, HAS etc.
  - Method- Microinjection and nuclear transfer

  Eg.
  - Insulin for diabetes mellitus
  - Vaccine
  - growth hormone for Rx of deficiencies
  - Alpha 1 antitrypsin for [AAT] deficiency
  - Coagulation factor
  - Lactoferrin as an infant formula food

Xenotransplanters-

- Transgenic animals that are the genetically modified to have that can be transplanted into the human
- Do not express key foreign antigens
- Promising alternative to the human donors
- Pig is the only animal used –
  - Its physiological closely matches that of human
  - Much less expensive than the monkey and other primates
- Pig have Alpha-1,3 galactose on the surface of their cell produced by enzyme Alpha- 1,2 galactosyltransferase
- Absent in human- rejection
- GGAT1- knock out pig- much lower incidence of rejection[in monkey]
- Porcine heart valves -successfully transplanted into the human heart [liver, lungs ,kidney being tested]
- Risk of transmission of porcine viruses- decrease by pre- scanning

Food sources-

- Creation of animal that grow larger without much food- more efficient ,cost effective food source
- Ethical and safety e concerns- not yet produced commercially as food source
- Only for the research purpose

Transgenic biological model-

- Create to increase our knowledge about the function of protein-
  - By over expressing the gene encoding that protein or knocking it out
- In biological and genetic studies

  Ex. Andi, smartmouse and youth mouse

ANAESTHESIA AND EUTHANASIA OF EXPERIMENTS ON ANIMALS

ANAESTHESIA

- The word anaesthesia has been drived form Greek word that means "without operation of sensibility"
- Anaesthesia is the act of providing sensation free relief from pain or pain produring procedures
- Anaesthesia must be performed by a person with knowledge of and familiarity with drug to be used in the animal species under consideration

Commonly used laboratory anaesthesia

There are numerous and anaesthe available for use in rodents. some of the more popular agent included-

- Chloralose
- Urethane
- Barbiturate
- Paraldehyde
- Magnesium sulphate
- Ketamine
- Tribromoethanol

CHLORALOSE-

- It is a compound of chloral and glucose prepared by the heating equal parts of anhydrous glucose and charcoal, when both chloralose[active form] and beta- chloraloseloss [in active form]are formed
- It is prepared as 1% solution by boiling in 0.9% NaCl or in the distilled water, and administered intravenously or intraperitoneally at the temperature of 30 -40 c before the chloralose comes out of solution

Advantages-

- It hasthe greatest advantage constancy of the depth anaesthesia
- The respiration and circulation are not depressed and the blood pressure is well maintained usually on the higher side
- Reflexes are not depressed but may be slightly exaggerated include the response to bilateral carotid occlusion

Disadvantage-

- It is suitable only for acute experiments, usually in dogs and cat, including surgical anaesthesia for 3- 4 hours or longer

Dog-

- 1% aq. Solution [hot] 80- 120mg i.v.
- 10% in polythene glycol 100mg i.v.

Cat-

- 1% aq. Solution [hot] 80mg i.v.
- 2% aq.or saline suspension 80-100mg i.p.
- 10% in propylene glycol 100mg i.p.

Rat-

- 10% in propylene glycol 80mg i.p.

URETHANE [ETHYL CARBAMATE]-

It is rapidly soluble in water given a natural solution usually 25% solution in water is used

Disadvantage –

- It is suitable only for acute experiment since it has delayed toxic effect on liver, and may also cause agranulocytosis and pulmonary adenomata
- Mice develop an exceptionally high incidence of lung tumors regardless of the route of administration

Dog -

- 25% aq. solution 1.5g i.v.

Cat-

- 25% aq. solution 1.0 to 1.5g i.v.

Rabbit-

- 25% aq. solution 0.5 to 1.75g i.v.
- 50% aq. solution 1.5- 2g i.p.

Guinea Pig-

- 25% or 50% aq. solution 1.5g i.p.

Rat-

- 25% aq. solution 1.25 - 1.75g i.m. or s.c.
- 20% aq. solution 1.5g i.p

BARBITURATES-

- Barbiturate interfere with the nerve impulse transmission both in the central nervous system and in the ganglia producing depression of cardiovascular and spinal cord reflexes
- In rabbit pedal reflex(leg retraction) is the lost first,then papillary and finally palpebral reflex
- Pentobarbital is a barbiturate and, historically the most commonly used anaesthesia in rodent

Advantage-

- At recombinant doses it cause minimal cardiovascular depression
- It is also related long-acting can provide approximately 45 minutes of surgical anaesthesia

Disadvantage-

- Pentobarbital is the potent inducer of the hepatic microsomal enzyme system causes the respiratory depression as well as hypothermia particularly when the repeated doses are given

PENTOBARBITONE SODIUM-

Phenobarbitone sodium and barbitone sodium are used for prolonged experiments.

- Dog and cat - 10% aq. Solution 180-200mg i.p.
- Monkey - 6% solution 25mg i.p. or i.v.
- Dog- and cat 6% solution 30-50mg i.p.
- Rabbbit- 6% solution 50 -60mg i.v.
- Guinea pig - 1% solution 30-50mg i.p.
- Rat and mouse- 0.6% solution 30-60mg I.p.
- Frog- 0.6% solution 50mg intraabdominally.

TIOPENTONE SODIUM-

Thiopentone sodium (pentothal) is used for surgical operations of short duration. It produces rapid induction with minimum excitation.

- Dog -2.5% fresh solution.
  - 12-16 mg i.v. (for brief duration)
  - 20-26mg i.v. (for longer duration)

PARALDEHYDE-

Advantages-

- It has a wide margin of safety because it depresses only the cerebrum and not the medullary centres.
  - Intravenous injection is likely to produce cardiac dilatation and pulmonary congestion and oedema

Disadvantages-

- Under its influence the basal blood pressure as well as the response to vasopressor and depressor drugs are low.

- Bilateral carotid occlusion produces poor pressor response or even a depressor response.
- Dog- 6% solution 1.2ml i.p.
- Cat - 6% solution 2.1ml i.m.

MAGNESIUM SULPHATE-

- A 20% magnesium sulphate solution 5ml/kg intravenously produces anaesthesia for about an hour.
- Calcium gluconate intravenously will counteract its depressanteffect immediately.
- Its principal use is in producing euthanasia

TRIBROMOETHANOL-

Advantages-

- In most rodents, tribromoethanol produces good surgical anesthesia, with good skeletal muscle relaxation and only a moderate degree of respiratory depression.
- It is relatively inexpensive and not a controlled agent.

Disadvantages-

- It is a potential for causing peritonitis. When exposed to either light or temperatures >40°C, tribromoethanol degrades into two byproducts: hydrobromic acid and dibromoacetaldehyde. Both of these compounds are highly irritating when administered IP and result in peritonitis and visceral adhesions which may be fatal

KETAMINE HYDROCHLORIDE-

Ketamine hydrochloride, a dissociative anesthetic, disrupts pain transmission and suppresses spinal cord activity with some action at opioid receptors. Visceral pain is not abolished with dissociative anesthetics and there is poor muscle relaxation and analgesia.

Disadvantages-

- Ketamine is a poor anesthetic when used alone, but is more often combined with other agents. When combined with other drugs, it is usually administered IP. Ketamine is acidic, can be irritating, and cause muscle necrosis when administrated IM.
- Ketamine-induced nerve damage can cause selfmutilation in rodents.
- Ketamine is a controlled substance. Store in a locked cabinet and maintaina log of its use

EUTHANASIA

- The term euthanasia is derived from the Greek terms **"eu"** meaning "**good**" and "**thanatos** " meaning "**death**". A "good death" would be one that occurs with minimal pain and distress.

  In the context-
- Euthanasia is the act of inducing humane death in an animal.
- Sacrificing the experimental animal after use by gentle procedure causing minimum of physical and mental suffering is called euthanasia (Painless killing).

Methods of Euthanasia-

Methods of euthanasia fall into two broad categories.

- Chemical methods
- Physical methods

Chemical methods-

• Inhalant agents-

Ex- Ether, halothane, methoxyflurane, isoflurane, enflurane, chloroform, nitrogen, nitrous oxide, carbon di oxide, carbon monoxide, argon, hydrogen cyanide.

- Injectable agents-

  Ex- Barbiturates, chloral hydrate, ethanol, ketamine, magnesium sulphate , neuromuscular blocking agents, potassiumchloride

Physical methods-

- Penetrating captive bolt
- Euthanasia by a blow to the head
- Gunshot
- Cervical dislocation
- Decapitation
- Electrocution
- Microwave irradiation
- Thoracic (Cardiopulmonary, Cardiac) Compression
- Kill traps
- Maceration
- Adjunction Methods
    - Exsanguination
    - Stunning
    - Pithing

Penetrating captive bolt-

A penetrating captive bolt is used for euthanasia of ruminants, horses, swine, laboratory rabbits, and dogs. Its mode of action is concussion and trauma to the cerebral hemisphere and brainstem.

Advantages -

- The penetrating captive bolt is an effective method of euthanasia for use in slaughterhouses, in research facilities, and on the farm when use of drugs is inappropriate.

Disadvantages-

- It is aesthetically displeasing.
- Death may not occur if equipment is not maintained and usedproperly.

Euthanasia by a blow to the head-

Must be evaluated in terms of the anatomic features of the species on which it is to be performed. The anatomic features of neonatal calves. however, make a blow to the head in this species unacceptable.

• Personnel performing euthanasia by use of a blow to the head must be properly trained and monitored for proficiency with this method of euthanasia, and they must be aware of its aesthetic implications.

Gunshot-

A properly placed gunshot can cause immediate insensibility and humane death. In some circumstances, a gunshot may be the only practical method of euthanasia.

Advantages-

- Loss of consciousness is instantaneous if the projectile destroys most of the brain.
- Given the need to minimize stress induced by handling and human contact, gunshot may at times be the most practical and logical method of euthanasia of wild or free-ranging species.

Disadvantages-

- Gunshot may be dangerous to personnel.
- It is aesthetically unpleasant.
- Under field conditions, it may be difficult to hit the vital target area

Cervical dislocation-

Cervical dislocation is a technique that has been used for many years and, when performed by well trained individuals, appears to be humane. However, there are few scientific studies to confirm this observation

Advantages-

- Cervical dislocation is a technique that may induce rapid loss of consciousness.
- It does not chemically contaminate tissue.

- It is rapidly accomplished.

Disadvantages-

- cervical dislocation may be aesthetically displeasing to personnel
- Cervical dislocation requires mastering technical skills to ensure loss of consciousness is rapidly induced.
- Its use is limited to poultry, other small birds, mice, and immature rats and rabbits.

Decapitation-

Decapitation can be used to euthanatize rodents and small rabbits in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated.

Advantages-

- Decapitation is a technique that appears to induce rapidloss of consciousness.
- It does not chemically contaminate tissues.
- It is rapidly accomplished

Disadvantages-

- Handling and restraint required to perform this technique may be distressful to animals.
- The interpretation of the presence of electrical activity in the brain following decapitation has created controversy and its importance may still be open to debate.

Electrocution-

Electrocution, **using** alternating current, has been used as a method of euthanasia for species such as dogs, cattle, sheep, swine, foxes, and mink. Electrocution induces death by cardiac fibrillation, which causes cerebral hypoxia. However, animals do not lose consciousness for 10 to 30 seconds or more after onset of cardiac fibrillation.

Advantages-

- Electrocution is humane if the animal is first rendered unconscious.
- It does not chemically contaminate tissues.
- It is economical.

Disadvantages-

- Electrocution may be hazardous to personnel.
- When conventional single-animal probes are used, it may not be a usefulmethod for mass euthanasia because so much time is required per animal.

Microwave irradiation-

Heating by microwave irradiation is used primarily by neurobiologists to fix brain metabolites in vivo while maintaining the anatomic integrity of the brain. Microwave instruments have been specifically designed for use in euthanasia of laboratory mice and rats:

Advantages-

- Loss of consciousness is achieved in less than 100ms, and death in less than I second.
- This is the most effective method to fix brain tissue in vivo for subsequent assay of enzymatically labile chemicals

Disadvantages-

- Instruments are expensive.
- Only animals the size of mice and rats can be euthanatized with commercial instruments that are currently available.

Thoracic (cardiopulmonary ,cardiac) compression-

- It is used to euthanatize small- to medium-sized free ranging birds when alternate techniques described in these guidelines are not practical

Advantages –

- This technique is rapid.
- It is apparently painless.
- It maximizes carcass use for analytical/contaminant studies.

Disadvantages –

- It may be considered aesthetically unpleasant by onlookers.
- The degree of distress is unknown.

Traps-

- Mechanical kill traps are used for the collection and killing of small. free-ranging mammals for commercial purposes (fur, skin, or meat). scientific purposes, to stop property damage, and to protect human safety.

Advantage-

- Free-ranging small mammals may be killed with minimal distress associated with handling and human contact.

Disadvantages-

- Traps may not afford death within acceptable time periods.
- Selectivity and efficiency is dependent on the skill and proficiency of the operator.

Maceration-

- Maceration, via use of a specially designed mechanical apparatus having rotating blades or projections, causes immediate fragmentation and death of day-old poultry and embryonated eggs.

Advantages-

- Death is almost instantaneous.
- The method is safe forworkers
- Large numbers of animals can be killed quickly

Disadvantages-

- Special equipment is required.
- Macerated tissues may present biosecurity risks.

Adjunctive methods-

- Stunning and pithing, when properly done, induce loss of consciousness but do not ensure death. Therefore, these methods must be used only in conjunction with other procedures, such as pharmacologic agents. exsanguination, or decapitation to euthanatize the animal.

➢ **Exsanguination**-

Exsanguination can be used to ensure death subsequent to stunning, or in otherwise unconscious animals. Because anxiety is associated with extreme hypovolemia, exsanguination must not be used as a sole means of euthanasia. Animals may be exsanguinated to obtain blood products. but only when they are sedated, stunned, or anesthetized.

➢ **Stunning**-

Animals may be stunned by a blow to the head, by use of a non penetrating captive bolt, or by use of electric current. Stunning must be followed immediately by a method that ensures death.

➢ **Blow to the head**-

Stunning by a blow to the head is used primarily in small laboratory animals with thin craniums. A single sharp blow must be delivered to the central skull bones with sufficient force to produce immediate depression of the central nervous system. When properly done, consciousness is lost rapidly.

- **Non-penetrating captive bolt**-

A non-penetrating captive bolt may be used to induce loss of consciousness in ruminants, horses, and swine. Signs of effective stunning by captive bolt are immediate collapse and a several second period of tetanic spasm, followed by slow hind limb movements of increasing frequency. Other aspects regarding use of the

non-penetrating captive bolt are similar to the use of a penetrating captive bolt. as previously described.

- **Electrical stunning-**

Alternating electrical current has been used for stunning species such as dogs, cattle, sheep, goats. hogs, fish and chickens. Experiments with dogs have identified a need to direct the electrical current through the brain to induce rapid loss of consciousness. In dogs, when electricity passes only between fore- and hind limbs or neck and feet, it causes the heart to fibrillate but does not induce sudden loss of consciousness. For electrical stunning of any animal, an apparatus that applies electrodes to opposite sides of the head. or in another way directs electrical current immediately through the brain, is necessary to induce rapid loss of consciousness.

- **Pithing-**

- In general, pithing is used as an adjunctive procedure to ensure death in an animal that has been rendered unconscious by other means. For some species, such as frogs, with anatomic features that facilitate easy access to the central nervous system, pithing may be used as a sole means of euthanasia, but an anesthetic overdose is a more suitable method.

BREEDING AND MANTAINING LABORATORY ANIMAL

BREEDING LABORATORY ANIMAL

- Bedding should be absorbent, free of toxic chemicals or other substances that could injure animals or personnel, and of a type not readily eaten by animals.
- Bedding should be used in amounts sufficient to keep animals dry
- Bedding should be removed and replaced with fresh materials as often as necessary to keep the animals clean and dry.
- The frequency is a matter of professional judgment of the animal care personnel in consultation with the investigation depending on the number of animals and size of cages.
- However it is ideal to change the bedding twice a week Nesting materials for newly delivered pups wherever can be provided
- e.g. paper, tissue paper and cotton.

Method of breeding animals

1. **Hand mating-** In this method male and female are brought together for a brief period and then separated once the mating is over.
   EX- Rabbits and Hamsters

2. **Pair mating-** In this method male and females are mated together rest of their breeding life. In this method in case of mice one male mated with one female, and left together for the rest of their breeding life. In the case of rats, pregnant females should be isolated prior to delivery.
   EX- Rats and Mice

3. **Harem mating-** In this method males and females are run together, but separated prior to parturition.In this method four females are regularly mated

with one male.The female is separated soon after the pregnancy is established, and replaced by fresh females.
EX-guinea pig

BREEDING DATA -

Items	Rat	Mouse
Breeding age – male and female	1oo day for both	50 days 50-60 days
Estrous cycle	5 days	4-5 days
Gestation	20-22 days	17-21 days
Weaning age	16-21 days	21 days
Litter size	12 days	8-12 days
Breeding life: male female	 1 year 1 year	 18 months 7-9 months
Mating group	1.3-5	1.3-4

Breeding data for some common laboratory species

Character	Mouse	Hamster	Rat	Guinea-pig	Rabbit
Typical adult weight (g)	25-30	150	250-400	500-800	1000-7000
Average longevity (years)	1-2	2-3	2-3	4-8	5-6
Age at puberty (days)	35	45-60	45-75	45-75	150-210
Usual breeding age (days)	50	56	80	80	150-210
Mating system	M,C	D,M	M,H	C	D
Length of oestrus cycle (d)	4-5	4	4-5	14-16	n.a.
Ovulation	S	S	S	S	I
Gestation period (days)	20	16	21-23	65-72	32-32
Average litter size	6-10	4-8	6-12	3-4	6-8
Weight at birth (g)	1-2	1-2	5-6	85-90	-100
Weight at weaning (g)	10-12		35-40	-250	-1000
Age at weaning (days)	19-21	21	21	14-21	50
Productivity (young per Breeding unit per year*)	25-75	25-50	25-100	12-18	15-20

M – Monogamous pair C – Colony D- Hand mating H- Harem mating S- Spontaneous I- Induced

P.G. TEACHING

Procedure of breeding

1. The exact procedure for rodent breeding for investigators is performed according to instructions in the individual animal use protocols.
    - The most important item on a rodent breeding bin is the cage ID/breeding record card.
    - This card records the breeding of 1-2 female rodents per cage. Breeding cage cards should be filled out completely,
2. Records of rodent breeding are also kept in a Breeding Book that is kept in each room used for breeding.
    - One male and 1-2 females are placed in the breeder cage. The pair should be provided with a Shepard shack or hiding box and fed a breeder diet. Females should be housed together in order tosynchronize their estrus cycle. The male is then introduced into the females' cage.
    - When sufficient offspring have been produced, the male is removed and placed back into a holding cage. Otherwise, the male can remain in the cage with a single female. Alternatively, the male may be removed before the litter is born to minimize the possibility of cannibalism.
    - Females may be separated from males near parturition or they may be permanently paired with a male. Juveniles are usually removed at 21 days, the average weaning age for rodents, or longer depending on size.
    - It is also important to identify individual breeding animals as closely as possible and record their ages and breeding data. This can be accomplished by grouping adult breeders and identifying the cage with a regular cage I.D. card. At the investigator's request, andaccording to instructions written in their animal use protocol, other methods of identification can be used (ear punching, ear tags, etc.).
    - Animals that reach or exceed their breeding life should be culled fromthe colony and replaced with offspring reserved for this purpose

(It maybe necessary to periodically bring in new blood, i.e., new animals from other Dolines in order to maintain the viability of the colony).

MAINTANCE OF LABORATORY ANIMALS

Laboratory animals in most countries are protected by the Law "Cruelty to Animals Act". One is to obtain licence from the Home Department for using them on experimental purposes.
The health and well-being of the animals depend on the care, human attitude of the animal-keepers (staff) of the animal house. To keep animals healthy, the staff has to look after cleanliness of the animal rooms and cages, provide proper food and water, to move to a cooler or warmer place and to provide air

The general principles are –

1. Fluid- They are to be provided with plentiful supply of fresh clean drinking water from a bottle (250 ml capacity) attached to the outside of the cage. The water is led in a 6-9 mm glass tubing through a rubber bung to an accessible position inside the cage; the outlet tubing is about 3 mm.

2. Diet- A balanced diet containing carbohydrate, fat, protein, vitamins and trace elements is to be given regularly. It is commercially in the form of cubes or pellets. Small quantities of green stuff are also to be supplied.

3. Cleanliness- Cleanliness of room and cages is essential unless they are kept in considerable risk of epidemic diseases. Animals, when breeding, should not have their cages changed too Clean cages should be used. Cages may be boiled in soapy water; alterna-tively, to be kept immersed in a solution of disinfe-ctant such as 3% Lysol. Lysol, however, should not be used in cleaning the cages of rabbit because its smell distresses the animals.

4. Litter- A layer of absorbent material (e.g. soft wood sawdust's, sugarcane piths) should be spread to a depth of 1/2 to 1 inch (1.25-2.5 cm) on the bottom of the cages.

5. Cages- Each species of animal require its own type of cage. It should be large enough for movement and some exercise of the animal.

6. Labelling- Every case should be provided with a holder or socket for a small card of 6-9 cm for record of the experiment (date, identifying marks of animal, nature of experiment and specimen).

7. Ventilation- Animal room should be air-conditioned; or at least ten changes of air in each hour are needed.

8. Humidity- Humidity of animal house ranges between 45% for rabbits to 65% for mice.

9. Marking animals- White or light coloured animals are marked by staining the fur with a strong dye (**e.g. carbol fuchsin , eosin**). Rabbits may be marked in ears with a needle dipped in India ink. Rats and mice are punctured in ear and fowls are marked by numbered metal tags on legs clipped through the loose skin of the wing.

10. Detection of disease in animals- A routine tour of inspection of the animals should be made at least once a day with attention to the general condition of animals, amounts of food and water consumed and the nature of faeces. To see nose movements of the animals and to see any animal remaining quiet and still. Such animals may be separated and investigated for the cause of disease.

11. Recording animals' temperature- Clinical thermometer is liberally smeared with sterile petroleum jelly and the blunt-ended rectal thermometer is introduced into the rectum or vagina to a depth of about 3 to 3.5 cm.

12. Prevention of disease-

- Newly arrived animals to be kept in a special quarantine room and kept under observation for 10-14 days. Animals falling sick during the period should be kept in quarantine and necropsis must be done for finding out the cause of the illness.

- Animal infected experimentally with bacteria or viruses should be kept in separate isolation rooms to prevent spread of infection to other animals.

13. Insect pest- Bed bugs, fleas, lice, mites, ticks, flies, mosquitoes and cockroaches may all infest the animal house. These can be controlled by 0.5% insecticidal sprays or 10% DDT

14. Handling- Animals should be handled with care. The rabbit is picked up from cage with the ears by one hand in a firm grip and another hand is placed under the hind-quarters to support the weight and then lifted gently. After removing from cage, the animal is placed in a non-slippery place as it otherwise feels insecure and becomes frightened.

15. Materials Inoculated-

- **Urine, CSF, blood and serous fluids**- These specimens are inoculated with a medium- bore needle but in case of tenacious material, like pus and sputum, is injected through a wide-bore needle.
- **Culture materials**- Liquid cultures are inoculated through a medium- bore needle. Growths on solid media are first scraped off and suspended in broth or saline, alternatively, the diluting fluid may be poured on the culture which is then emulsified with a wire loop.

3. **Tissues**- Small fragments of tissues such as brain, spleen, liver and kidney are first homogenized by crushing these materials with a suitable diluent in a tissue grinder. When tissue is well-ground, more saline is added and allowed to stand for a short time.

Table 18.1 : Shows important datas of Laboratory Animals

Features	Rabbit	Guinea-pigs	Mice	Rats	Hamsters	Fowl
Rectal temperature (No temperature below 40°C is considered as pathological)	38.7° – 39°C	37.6° – 38.9°C	37.4°C	37.5°C	36.7° – 38°C	41° – 60°C
Oestrous cycle	—	—	4-5 days	4-5 days	4-5 days	—
Normal respiration rate	55	80	—	210	—	12
Pulse rate per minute	135	150	120	—	—	140
Gestation period	28-31 days	59-72 days	19-21 days	21-23 days	16-17 days	—
Weaning age	6-8 weeks	14-21 days	19-21 days	23-28 days	3-4 weeks	—
Mating age	6-9 months	12-20 weeks	6-8 weeks	70-84 days	7-9 weeks	—
Litters	4 yearly average litters – 4	3 yearly, average litters – 3	8-12 yearly, average litters 7-8	7-9 yearly average litters – 7	3-4 yearly average litters – 5	—
Room temperature	15.5°–18.5°C	18.5°–21°C	20°–21°C	18.5°–21°C	20°–22°C	—
Humidity	40 –45%	45%	50 –60%	45–55%	40–50%	—
Weight *(adult)*	0.9–6.7 kg	120 g	25–28 g	—	—	—
CAGES : Galvanised iron	2' × 2' × 1 1/2' (for one)	4' × 6' × 1' 8" (high) (for 25)	6" × 12" × 6" (deep) (for 6)*	Same as guinea pigs	17" × 7" × 9" (for one)	24" tall and 20" × 20"
Diet *(daily)*	Pellets or daily 30 g mixture of 1 part oats plus 3 parts of bran	Pellet diet	Pellet diet	Dry pellet	Pellet diet	Pellet diet
Diet supplemented by	Green vegetables plus hay Plenty	Cabbage 60g plus hay Plenty	— Plenty	— Plenty	Fresh green foods. Milk added to bran/oats Plenty	Green food Plenty

* Cages made of thick polypropyline (plastic) are now commercially available for mice which are commonly used

Key : foot = ' inch = "