Animal Biotechnology

16BTU504A Semester V

Total Hours/Week: L : 4: T: 0 : P: 0 Marks: Internal; 40 : External : 60 Total 100

Course Objectives

To understand the application of biotechnology in animals and the challenges facing the intensive and extensive livestock industries, as well as wildlife management and conservation, in the context of biotechnology approach

The contribution of biotechnology to laboratory animal models for human and animal disease will be addressed. In addition, the use of biotechnology for transgenic animal production will be focused.

A range of genetic, immunological and reproductive technologies will be introduced with some practical exposure and the integration of these technologies to improve animal production, health and welfare will be explored.

Learning outcomes:

- The limitations and challenges facing the animal industries and disciplines.
- Various biotechnological approaches for the production of transgenic animals

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Unit: I

Animal Tissue Culture: Laboratory design: Aseptic techniques- handling instruments: Microscopes, Clean Bench etc., and biosafety. Animal Cell Culture Media: Natural and Artificial Media- their constituents. Physio-chemical properties of media; Serum supplemented and serum free media. Sterilization methods.

Unit II:

Primary Cell Culture: Methods of tissue disaggregation – isolation of tissues from chick embryo, mouse and human; Continuous and established culture; Cell separation and characterization; Organ culture – types.

Unit III:

Gene transfer methods in Animals-Microinjection, Embryonic Stem cell, gene transfer, Reterovirus& Gene transfer. Introduction to transgenesis. Transgenic Animals – Mice, Cow, Pig sheep, goat, bird and insect.

Unit IV

Animal Propagation – Artificial insemination, Animal Clones. Conservation Biology – Embryo transfer techniques. Introduction to stem cell technology and its applications. Expression of bovine growth hormone; production of human proteins in milk and meat. Animal disease need help of biotechnology – Foot and mouth disease. Coccidiosis, Trypanosomiasis, Theileriosis

Unit V

Production and applications; transgenic animals: mouse, cow, sheep, fish, hen. Genetic modification in medicine- gene therapy-types and vectors in gene therapy, molecular engineering Human genetic problem and ethics

References

1. Text Book of Animal Biotechnology. 2006. R.Sasidhara. MJP Publishers (T1)
2. Animal Cell Culture; IVth edition, John & Wiley Sons, New York. Freshney R.I 2010. (T2)
3. www. biotechnology4u.com/animal biotechnology (W1)
4. Glick and Pasternack, 2003, Molecular Biotechnology, BlackWell Science.UK (R1)
5. Houdebine, 1997.Transgenic animal generation and use (R2)
6. Danniel Cressy. Transgenics: A new breed. 2013. Nature/newsfeature/1997 (J1)
7. Textbook of animal biotechnology 2nd edition by M.M.Ranga 2008 (T3)
8. Textbook of animal biotechnology- Dr.P.Ramdass & Dr.S. Meerarani 2010 (T4)

LECTURE PLAN

S.No	I	Topics to be covered	Support Materials
UNIT I			**8hr**
1.	1	Laboratory design	T1: Pg: 29
2.	1	Aseptic techniques- handling instruments: Microscopes, Clean Bench etc., and biosafety	T1: Pg: 31 - 36
3.	1	Animal Cell Culture Media	W1: Pg: 1
4.	1	Natural and Artificial Media- their constituents	W1: Pg: 2 -5.
5.	1	Physio-chemical properties of media	W1: Pg: 6
6.	1	Serum supplemented and serum free media	W1: Pg: 7 -11
7.	1	Sterilization methods	W1: Pg: 18 -37
8.	1	Unit test	
UNIT II			**8hr**
9.	1	Primary Cell Culture	T2: Pg: 148
10.	1	Methods of tissue disaggregation	T2: Pg: 149- 157
11.	1	Isolation of tissues from chick embryo	T2: Pg: 159 – 161
12.	1	Isolation of tissues from mouse and human	T2: Pg: 161 – 163
13.	1	Continuous and established culture	T2: Pg: 169 – 71
14.	1	Cell separation and characterization	T2: Pg: 172- 173
15.	1	Organ culture – types.	T2: Pg: 399 -406
16.	1	Unit test	
		Total hours = (Unit I + II) = 16 hr	

UNIT III			**8hr**
17.	1	Gene transfer methods in Animals	R1: Pg: 332 - 358
18.	1	Microinjection	T2: Pg: 456
19.	1	Embryonic Stem cell, gene transfer, Reterovirus & Gene transfer	T2: Pg: 461
20.	1	Introduction to transgenesis	J1
21.	1	Transgenic Animals – Mice, Cow	R2: Pg: 190 - 192
22.	1	Transgenic Animals – Pig, Sheep	J1
23.	1	Transgenic Animals – Goat, Bird and Insect	J1
24.	1	Unit test	
UNIT IV			**12 hr**
25.	1	Animal Propagation	T3: Pg: 201
26.	1	Artificial insemination	T2: Pg: 202
27.	1	Animal Clones	T2: Pg: 204
28.	1	Conservation Biology	T2: Pg: 205
29.	1	Embryo transfer techniques	T2: Pg: 205 – 217
30.	1	Introduction to stem cell technology	T1: Pg: 421- 427
31.	1	Application of stem cell technology	T1: Pg: 428 – 441
32.	1	Expression of bovine growth hormone	T2: Pg: 232
33.	1	Production of human proteins in milk and meat	T2: Pg: 309 - 320
34.	1	Animal disease – Foot and Mouth disease	T2: Pg: 589 -602
35.	1	Coccidiosis, Trypanosomiasis, Theileriosis	T2: Pg: 603 -610
36.	1	Unit test	
		Total hours = (Unit III + IV) = 20 hr	

UNIT V			**12 hr**
37.	1	Transgenic Animal – Production and Applications	T1: Pg: 421- 427
38.	1	Transgenic Animal - Mouse	T1: Pg: 136 - 138
39.	1	Transgenic Animal - Cow, sheep	T1: Pg: 148 - 151
40.	1	Transgenic Animal - Fish, hen	T1: Pg: 158 - 161
41.	1	Genetic Modification in medicine	T1: Pg: 275
42.	1	Gene therapy-types and vectors in gene therapy	T1: Pg: 213 - 256
43.	1	Molecular Engineering – Human Genetic Engineering	T1: Pg: 297 - 310
44.	1	Human genetic problem and ethics	T1: Pg: 311
45.	1	Unit test	
46.	1	ESE question paper revision	
47.	1	ESE question paper revision	
48.	1	ESE question paper revision	
49.	**Total Hours = (Unit I+ II + III + IV + V) = 48 hr**		

References

1. Text Book of Animal Biotechnology. 2006. R.Sasidhara. MJP Publishers (T1)
2. Animal Cell Culture; IVth edition, John & Wiley Sons, New York. Freshney R.I 2010. (T2)
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Animal Tissue Culture: Laboratory design: Aseptic techniques- handling instruments: Microscopes, Clean Bench etc., and biosafety. Animal Cell Culture Media: Natural and Artificial Media- their constituents. Physio-chemical properties of media; Serum supplemented and serum free media. Sterilization methods

Laboratory design

- The specific requirements of a cell culture laboratory depend mainly on the type of research conducted; for example, the needs of mammalian cell culture laboratory specializing in cancer research is quite different from that of an insect cell culture laboratory that focuses on protein expression.
- However, all cell culture laboratories have the common requirement of being free from pathogenic microorganisms (i.e., asepsis), and share some of the same basic equipment that is essential for culturing cells.
- This chapter lists the equipment and supplies common to most cell culture laboratories, as well as beneficial equipment that allow the work to be performed more efficiently or accurately, or permits wider range of assays and analyses.

Equipments

Equipments		
Basic Equipment	**Expanded Equipment**	**Additional Supplies**
1.Cell culture hood (i.e., laminar-flow hood or biosafety cabinet) 2. Incubator (humid CO2 incubator recommended) 3. Water bath 4.Centrifuge 5. Refrigerator and freezer (–20°C) 6. Cell counter (e.g., CountessR Automated Cell Counter or hemacytometer) 7. Inverted microscope 8. Liquid nitrogen (N2) freezer or cryostorage container 9. Sterilizer (i.e., autoclave)	1.Aspiration pump (peristaltic or vacuum) 2.pH meter 3.Confocal microscope 4.Flow cytometer	1. Cell culture vessels (e.g., flasks, Petri dishes, roller bottles, multi-well plates) 2. Pipettes and pipettors 3. Syringes and needles 4.Waste containers 5. Media, sera, and reagents 6. Cells

Cell Culture Hood

- The cell culture hood provides an aseptic work area while allowing the containment of infectious splashes or aerosols generated by many microbiological procedures.
- Three kinds of cell culture hoods, designated as Class I, II and III, have been developed to meetvarying research and clinical needs.

Classes of Cell Culture Hoods

- **Class I** cell culture hoods offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques, but they do not provide cultures protection from contamination. They are similar in design and air flow characteristics to chemical fume hoods.
- **Class II** cell culture hoods are designed for work involving BSL-1, 2, and 3 materials, and they also provide an aseptic environment necessary for cell culture experiments. A Class II biosafety cabinet should be used for handling potentially hazardous materials (e.g., primate-derived cultures, virally infected cultures, radioisotopes, carcinogenic or toxic, reagents).
- **Class III** biosafety cabinets are gas-tight, and they provide the highest attainable level of protection to personnel and the environment. A Class III biosafety cabinet is required for work involving known human pathogens and other BSL-4 materials.

Air-Flow Characteristics of Cell Culture Hoods

- Cell culture hoods protect the working environment from dust and other airborne
- contaminants by maintaining a constant, unidirectional flow of **HEPA-filtered air** over the work area.
- The flow can be **horizontal**, blowing parallel to the work surface, or it can be **vertical**, blowing from the top of the cabinet onto the work surface.
- Depending on its design, a **horizontal flow hood** provides protection to the culture (if the air flowing towards the user) or to the user (if the air is drawn in through the front of the cabinet by negative air pressure inside).
- **Vertical flow hoods**, on the other hand, provide significant protection to the user and the cell culture.

Clean Benches

- Horizontal laminar flow or vertical laminar flow "clean benches" are **not** biosafety cabinets; these pieces of equipment discharge HEPA-filtered air from the back of the cabinet across the work surface toward the user, and they may expose the user to potentially hazardous materials.
- These devices only provide product protection. Clean benches can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices, and they should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials.

Cell Culture Hood

- **Layout** A cell culture hood should be large enough to be used by one person at a time, be easily cleanable inside and outside, have adequate lighting, and be comfortable to use without requiring awkward positions. Keep the work space in the cell culture hood clean and uncluttered, and keep everything in direct line of sight.
- Disinfect each item placed in the cell culture hood by spraying them with 70% ethanol and wiping clean. The arrangement of items within the cell culture hood usually adheres to the following right-handed convention, which can be modified to include additional items used in specific applications.

A wide, clear work space in the center with your cell culture vessels

- Pipettor in the front right, where it can be reached easily
- Reagents and media in the rear right to allow easy pipetting
- Tube rack in the rear middle holding additional reagents
- Small container in the rear left to hold liquid waste

Incubator

- The purpose of the incubator is to provide the appropriate environment for cell growth. The incubator should be large enough for your laboratory needs, have forced air circulation, and should have temperature control to within 0.2°C.

- Stainless steel incubators allow easy cleaning and provide corrosion protection, especially if humid air is required for incubation. Although the requirement for aseptic conditions in a cell culture incubator is not as stringent as that in a cell culture hood, frequent cleaning of the incubator is essential to avoid contamination of cell cultures.

Types of Incubators

There are two basic types of incubators, dry incubators and humid CO2 incubators.

- **Dry incubators** are more economical, but require the cell cultures to be incubated in sealed flasks to prevent evaporation. Placing a water dish in a dry incubator can provide some humidity, but they do not allow precise control of atmospheric conditions in the incubator.
- **Humid CO_2 incubators** are more expensive, but allow superior control of culture conditions. They can be used to incubate cells cultured in Petri dishes or multi-well plates, which require a controlled atmosphere of high humidity and increased CO2 tension.

Storage

- A cell culture laboratory should have storage areas for liquids such as media and reagents, for chemicals such as drugs and antibiotics, for consumables such as disposable pipettes, culture vessels, and gloves, for glassware such as media bottles and glass pipettes, for specialized equipment, and for tissues and cells.
- Glassware, plastics, and specialized equipment can be stored at ambient temperature on shelves and in drawers; however, it is important to **store all media, reagents, and chemicals according to the instructions on the label**.
- Some media, reagents, and chemicals are sensitive to light; while their normal laboratory use under lighted conditions is tolerated, they should be stored in the dark or wrapped in aluminum foil when not in use.

Refrigerators

- For small cell culture laboratories, a domestic refrigerator (preferably one without a autodefrost freezer) is an adequate and inexpensive piece of equipment for storing reagents and media at 2–8°C.

- For larger laboratories, a cold room restricted to cell culture is more appropriate. Make sure that the refrigerator or the cold room is cleaned regularly to avoid contamination.

Freezers

- Most cell culture reagents can be stored at –5°C to –20°C; therefore an ultradeep freezer (i.e., a –80°C freezer) is optional for storing most reagents. A domestic freezer is a cheaper alternative to a laboratory freezer.
- While most reagents can withstand temperature oscillations in an autodefrost (i.e., self-thawing) freezer, some reagents such as antibiotics and enzymes should be stored in a freezer that does not autodefrost.

Cell Culture Basics

Cell Culture Laboratory

Cryogenic Storage

- Cell lines in continuous culture are likely to suffer from genetic instability as their passage number increases; therefore, it is essential to prepare working stocks of the cells and preserve them in cryogenic storage . Do not store cells in –20°C or –80°C freezers, because their viability quickly decreases when they are stored at these temperatures. There are two main types of liquid-nitrogen storage systems, vapor phase and liquid phase, which come as wide-necked or narrow-necked storage containers.
- **Vapor phase** systems minimize the risk of explosion with cryostorage tubes, and are required for storing biohazardous materials, while the **liquid phase** systems usually have longer static holding times, and are therefore more economical.
- **Narrow-necked** containers have a slower nitrogen evaporation rate and are more economical, but **wide-necked** containers allow easier access and have a larger storage capacity.

Cell Counter

- A cell counter is essential for quantitative growth kinetics, and a great advantage when more than two or three cell lines are cultured in the laboratory.

- The CountessR Automated Cell Counter is a bench-top instrument designed to measure cell count and viability (live, dead, and total cells) accurately and precisely in less than a minute per sample, using the standard Trypan Blue uptake technique.
- Using the same amount of sample that you currently use with the hemacytometer, the CountessR Automated Cell Counter takes less than a minute per sample for a typical cell count and is compatible with a wide variety of eukaryotic cells.

Aseptic Technique

- Successful cell culture depends heavily on keeping the cells free from contamination by microorganisms such as bacterial, fungi, and viruses.
- Non-sterile supplies, media, and reagents, airborne particles laden with microorganisms, unclean incubators, and dirty work surfaces are all sources of biological contamination.
- Aseptic technique, designed to provide a barrier between the microrganisms in the environment and the sterile cell culture, depends upon a set of procedures to reduce the probability of contamination from these sources.
- The elements of aseptic technique are a sterile work area, good personal hygiene, sterile reagents and media, and sterile handling.

Sterile Work Area

- The simplest and most economical way to reduce contamination from airborne particles and aerosols (e.g., dust, spores, shed skin, sneezing) is to use a cell culture hood.
- The cell culture hood should be properly set up and be located in an area that is restricted to cell culture that is free from drafts from doors, windows, and other equipment, and with no through traffic.
- The work surface should be uncluttered and contain only items required for aparticular procedure; it should not be used as a storage area.
- Before and after use, the work surface should be disinfected thoroughly, and the surrounding areas and equipment should be cleaned routinely.
- For routine cleaning, wipe the work surface with 70% ethanol before and during work, especially after any spillage.
- You may use ultraviolet light to sterilize the air and exposed work surfaces in the cell culture hood between uses.

- Using a Bunsen burner for flaming is not necessary nor recommended in a cell culture hood.
- Leave the cell culture hood running at all times, turning them off only when they will not be used for extended periods of time.

Animal Cell Culture Media:

- Cell culture is one of major techniques in the life sciences. It is the general term used for the removal of cells, tissues or organs from an animal or plant and their subsequent placement into an artificial environment conducive to their survival and/or proliferation.
- Basic environmental requirements for cells to grow optimally are: controlled temperature, substrate for cell attachment, and appropriate growth medium and incubator that maintains correct pH and osmolality.
- The most important and crucial step in cell culture is selecting appropriate growth medium for the *in vitro* cultivation. A growth medium or culture medium is a liquid or gel designed to support the growth of microorganisms, cells, or small plants.
- Cell culture media generally comprise an appropriate source of energy and compounds which regulate the cell cycle. A typical culture medium is composed of a complement of amino acids, vitamins, inorganic salts, glucose, and serum as a source of growth factors, hormones, and attachment factors. In addition to nutrients, the medium also helps maintain pH and osmolality.

Types of Cell Culture Media

Animal cells can be cultured either using a completely natural medium or an artificial/synthetic medium along with some natural products.

Natural Media

Natural media consist solely of naturally occurring biological fluids. Natural media are very useful and convenient for a wide range of animal cell culture. The major disadvantage of natural media is its poor reproducibility due to lack of knowledge of the exact composition of these natural media.

Artificial Media

Artificial or synthetic media are prepared by adding nutrients (both organic and inorganic), vitamins, salts, O2 and CO2 gas phases, serum proteins, carbohydrates, cofactors.

Different artificial media have been devised to serve one or more of the following purposes:

- immediate survival (a balanced salt solution, with specific pH and osmotic pressure)
- prolonged survival (a balanced salt solution supplemented with various formulations of organic compounds and/or serum)
- indefinite growth
- specialized functions.

Artificial media are grouped into four categories:

Serum containing media

- **Fetal bovine serum** is the most common supplement in animal cell culture media. It is used as a lowcost supplement to provide an optimal culture medium.
- Serum provides carriers or chelators for labile or water-insoluble nutrients, hormones and growth factors, protease inhibitors, and binds and neutralizes toxic moieties.

Serum-free media

- Presence of serum in the media has many drawbacks and can lead to serious misinterpretations in
- immunological studies . A number of serum-free media have been developed .
- These media are generally specifically formulated to support the culture of a single cell type, such as Knockout Serum Replacement and Knockout DMEM from Thermo Fisher Scientific for stem cells, and incorporate defined quantities of purified growth factors, lipoproteins, and other proteins, which are otherwise usually provided by the serum.
- These media are also referred to as ‘defined culture media’ since the components in these media are known.

Chemically defined media

- These media contain contamination-free ultra pure inorganic and organic ingredients, and may also contain pure protein additives, like growth factors.
- Their constituents are produced in bacteria or yeast by genetic engineering with the addition of vitamins, cholesterol, specific amino acids, and fatty acids .

Protein-free media

- Protein-free media do not contain any protein and only contain non-protein constituents. Compared to serum-supplemented media, use of protein-free media promotes superior cell growth and protein expression and facilitates downstream purification of any expressed product .
- Formulations like MEM, RPMI-1640 are protein-free and protein supplement is provided when required.

Basic Components of Culture Media

Culture media contain a mixture of amino acids, glucose, salts, vitamins, and other nutrients, and available either as a powder or as a liquid form from commercial suppliers .

The requirements for these components vary among cell lines, and these differences are partly responsible for the extensive number of medium formulations . Each component performs a specific function, as described below:

Buffering systems

Regulating pH is critical for optimum culture conditions and is generally achieved by one of the two buffering systems:

Natural buffering system

- In a natural buffering system, gaseous CO2 balances with the CO3/HCO3 content of the culture medium.
- Cultures with a natural buffering system need to be maintained in an air atmosphere with 5- 10% CO2, usually maintained by an CO2 incubator. Natural buffering system is low cost and non-toxic

HEPES

- Chemical buffering using a zwitterion, HEPES, has a superior buffering capacity in the pH range 7.2- 7.4 and does not require a controlled gaseous atmosphere.
- HEPES is relatively expensive and toxic at a higher concentration for some cell types. HEPES has also been shown to greatly increase the sensitivity of media to phototoxic effects induced by exposure to fluorescent light .

Phenol red

- Most of the commercially available culture media include phenol red as a pH indicator, which allows constant monitoring of pH. During the cell growth, the medium changes color as pH is changed due to the metabolites released by the cells.
- At low pH levels, phenol red turns the medium yellow, while at higher pH levels it turns the medium purple. Medium is bright red for pH 7.4, the optimum pH value for cell culture.

However, there are certain disadvantages of using phenol red as described below:

1) Phenol red mimics the action of some steroid hormones, particularly estrogen . Thus it is advisable to use media without phenol red for studies using estrogen-sensitive cells like mammary tissue.

2) Presence of phenol red in some serum-free formulations interferes with the sodium potassium homeostasis. This effect can be neutralized by the inclusion of serum or bovine pituitary hormone in the medium .

3) Phenol red interferes with detection in flow cytometric studies.

Inorganic salt

Inorganic salt in the media help to retain the osmotic balance and help in regulating membrane potential by providing sodium, potassium, and calcium ions .

Amino Acids

- Amino acids are the building blocks of proteins, and thus are obligatory ingredients of all known cell culture media. Essential amino acids must be included in the culture media as cells can not synthesize these by themselves.
- They ae required for the proliferation of cells and their concentration determines the maximum achievable cell density. L-glutamine, an essential amino acid, is particularly important. L-glutamine provides nitrogen for NAD, NADPH and nucleotides and serves as a secondary energy source for metabolism.
- L-glutamine is an unstable amino acid, that, with time, converts to a form that can not be used by cells, and should thus be added to media just before use.
- Caution should be used when adding more L-glutamine than is called for in the original medium formulation since its degradation results in the build-up of ammonia, and ammonia can have deleterious effect on some cell lines.
- L-glutamine concentrations for mammalian cell culture media can vary from 0.68 mM in Medium 199 to 4mM in Dulbecco's Modified Eagles's Medium. Invertebrate cell culture media can contain as much as 12.3 mM L-glutamine.
- Supplements like glutamax are more stable and can replace glutamine for long term culturing of slow cells. Nonessential amino acids may also be added to the medium to replace those that have been depleted during growth.
- Supplementation of media with non-essential amino acids stimulates growth and prolongs the viability of the cells.

Carbohydrates

Carbohydrates in the form of sugars are the major source of energy. Most of the media contain glucose and galactose, however, some contain maltose and fructose.

Proteins and Peptides

- The most commonly used proteins and peptides are albumin, transferrin, and fibronectin. They are particularly important in serum-free media.
- Serum is a rich source of proteins and includes albumin, transferrin, aprotinin, fetuin, and fibronectin.
- Albumin is the main protein in blood acting to bind water, salts, free fatty acids, hormones, and vitamins, and transport them between tissues and cells.
- The binding capacity of albumin makes it a suitable remover of toxic substances from the cell culture media.
- Aprotinin is a protective agent in cell culture systems, stable at neutral and acidic pH and resistant to high temperatures and degradation by proteolytic enzymes. It has the ability to inhibit several serine proteases such as trypsin.
- Fetuin is a glycoprotein found in fetal and newborn serum at larger concentrations than in adult serum. It is also an inhibitor of serine proteases.
- Fibronectin is a key player in cell attachment.
- Transferrin is an iron transport protein that acts to supply iron to the cell membrane.
- Fatty Acids and Lipids
- They are particularly important in serum-free media as they are generally present in serum.

Vitamins

- Many vitamins are essential for growth and proliferation of cells. Vitamins cannot be synthesized in sufficient quantities by cells and are therefore important supplements required in tissue culture.
- Again serum is the major source of vitamins in cell culture, however, media are also enriched with different vitamins making them suitable for a particular cell line. The B group vitamins are most commonly added for growth stimulation.

Trace Elements

- Trace elements are often supplemented to serum-free media to replace those normally found in serum. Trace elements like copper, zinc, selenium and tricarboxylic acid

intermediates are chemical elements that are needed in minute amounts for proper cell growth.

- These micronutrients are essential for many biological processes, e.g. the maintenance of the functionality of enzymes.

Media Supplements

- The complete growth media recommended for certain cell lines requires additional components which are not present in the basal media and serum. These components, supplements, help sustain proliferation and maintain normal cell metabolism.
- Although supplements like hormones, growth factors and signaling substances are required for normal growth of some cell lines, it is always best to take the following precautions: since the addition of supplement can change the osmolality of the complete growth media which can negatively affect the growth of cells, it is always best to recheck the osmolality after supplements are added. For most of the cell lines, optimal osmolality should be between 260 mOSM/kg and 320 mOSM/kg.
- Shelf life of the growth media changes after the addition of supplements. Complete media containing protein supplement tend to degrade faster than basal media alone.

Antibiotics

- Although not required for cell growth, antibiotics are often used to control the growth of bacterial and fungal contaminants.
- Routine use of antibiotics for cell culture is not recommended since antibiotics can mask contamination by mycoplasma and resistant bacteria.
- Moreover, antibiotics can also interfere with the metabolism of sensitive cells.

Serum in Media

- Serum is a complex mix of albumins, growth factors and growth inhibitors. Serum is one of the most important components of cell culture media and serves as a source for amino acids, proteins, vitamins (particularly fat-soluble vitamins such as A, D, E, and K), carbohydrates, lipids, hormones, growth factors, minerals, and trace elements.
- Serum from fetal and calf bovine sources are commonly used to support the growth of cells in culture. Fetal serum is a rich source of growth factors and is appropriate for cell cloning and for the growth of fastidious cells .

- Calf serum is used in contact inhibition studies because of its lower growth-promoting properties. Normal growth media often contain 2-10% of serum. Supplementation of media with serum serves the following functions .
- Serum provides the basic nutrients (both in the solution as well as bound to the proteins) for cells.
- Serum provides several growth factors and hormones involved in growth promotion and specialized cell function. It provides several binding proteins like albumin, transferrin, which can carry other molecules into the cell.
- For example: albumin carries lipids, vitamins, hormones, etc into cells.
- It also supplies proteins, like fibronectin, which promote attachment of cells to the substrate. It also provides spreading factors that help the cells to spread out before they begin to divide. It provides protease inhibitors which protect cells from preolysis.
- It also provides minerals, like Na+, K+, Zn2+, Fe2+, etc. It increases the viscosity of medium and thus, protects cells from mechanical damages during agitation of suspension cultures. It also acts a buffer. Due to the presence of both growth factors and inhibitors, the role of serum in cell culture is very complex.
- Unfortunately, in addition to serving various functions, the use of serum in tissue culture applications has several drawbacks.

Table 2 shows the advantages and disadvantages of using serum in the media.

Advantages of serum in media	Disadvantages of serum in media
Serum contains various growth factors and hormones which stimulates cell growth and functions.	Lack of uniformity in the composition of serum
Helps in attachment of the cells	Testing needs to be done to maintain the quality of each batch before using
Acts as a spreading factor	May contain some of the growth inhibiting factors
Acts as a buffering agent which helps in maintaining the pH of the culture media	Increase the risk of contamination
Functions as a binding protein Minimizes mechanical damages or damages caused by Viscosity	Presence of serum in media may interfere with the purification and isolation of cell culture products

Preparation of Media

Culture medium is available in three forms from commercial suppliers:

1. Powdered form: it needs to be prepared and sterilized by the investigator.
2. Concentrated form: to be diluted by the investigator.
3. Working solution: to be used directly without further manipulation.

- Powdered medium is the least expensive but needs to be sterilized. It is advisable to filter sterilize it prior to the addition of serum as the foaming that occurs in the presence of serum denatures the protein.
- Fetal bovine or horse sera can be added after filtration. Media should always be tested for sterility by placing it in a 37°C CO2 incubator for 72 hours prior to utilization to ensure that the lot is contamination-free.
- Medium should be stored at 4oC. Since several components of medium are light sensitive, it should be stored in dark.

Optimization of Cell Culture Media

- The complexity of composition of cell culture media provides many challenges to optimize individual components of media.
- Most of the classical culture media were devised for small-scale low-density cultures and often require serum as a key nutrient.
- However, in biotechnology industry where there is a need to sustain high cell densities and increase cellular productivity, development and optimization of culture media is very critical. Typically, media for the biotechnology industry are serum free and have much higher concentration of nutrients than classical media.

Optimization of media requires the following parameters to be considered:

Product to be made

- The type of product needed will determine the medium optimization strategy. For the rapid generation of cell numbers, cell growth rate and viability are critical. So, cell culture media should support maximal cell growth and sustain cell viability at increasing cell densities.
- For the production of virus, not just high cell densities are required but there must be abundant nutrients in the media to sustain virus replication after infection. For the production of recombinant protein, high cell density is required.
- However, nutrients required for the cell growth can compete with those required for production of proteins.
- It is, therefore, very important to carefully determine the maximum cell densities a given medium can sustain for a required level of productivity. In addition, it is very important to consider that changes to the medium during optimization must not affect product quality.

STERILIZATION TECHNIQUES

Autoclaving dry goods

- Heat-stable dry materials (including stainless steel instruments, glassware, fabrics, and plasticware) can be effectively sterilized by autoclaving, providing all surfaces of the dry material come in contact with the saturated steam at 121°C.
- This can become problematical for small items (such as forceps) that must be packaged in an outer container or wrapping that impedes the flow of steam, or for folded fabrics that tend to harbor pockets of cooler air.
- For this reason, autoclaving times for dry goods sterilization often rely on overkill, as these materials generally have much higher heat resistance

Autoclaving for decontamination of biological waste

- Biological laboratory waste is most frequently decontaminated by autoclaving unless it contains hazardous chemical materials that can volatilize in the sterilization process. In many mid- to large-sized laboratories, biological waste includes varying combinations of spent media, discarded cultures, and solid material.
- An autoclave load size or configuration can vary dramatically with each run. For this reason, autoclave cycles for decontamination most often employ the overkill approach. Validation studies prior to actual-use procedures must be performed to assure that selected operation procedures are adequate to achieve the desired conditions for successful decontamination.

Dry-heat sterilization and depyrogenation

- Dry heat is used for components and materials that are resistant to the 140° to 180°C temperatures needed for effective dry sterilization; it is most often used for the sterilization of laboratory glassware and stainless steel instruments.
- It is also used for sterilization of nonaqueous, heat-stable liquids such as mineral oil. Depyrogenation of heat-tolerant materials is done with ovens capable of operating at the required processing temperatures of 220° to 350°C.

- As with autoclaving, standard dry-heat sterilization and depyrogenation times refer to the time the material is held at the prescribed temperature and not to the time the oven has been set to run.
- Dry-heat sterilization using gravity ovens generally requires a longer time than does sterilization with convection ovens, which evenly distribute the heated air throughout the chamber with blowers. In all instances, process validation of any dry-heat sterilization protocol is required.

Use of disinfectants: 70% ethanol

- Ethanol is widely used in many laboratories for benchtop or laminar-space disinfection. The antimicrobial activity of the alcoholic solution is very much dependent upon the working concentration of the solvent, proper preparation, storage, and conditions of its use.
- Ethanol is an effective disinfectant against vegetative bacterial and fungal cells, but is totally ineffective in germicidal activity against bacterial spores. Ethanol is suitable for spraying or swabbing, but is not recommended for large-volume applications.
- Ethanol is highly flammable, and spills near the flame of a Bunsen burner are always a possible safety hazard.
- Similarly, an elevated concentration of vaporized ethanol in a liberally disinfected biosafety cabinet could ignite in the presence of a flame or spark.
- Furthermore, 70% ethanol is not recommended for use in discard pans or for decontamination of biological spills in the catch basins of biological safety cabinets.

Use of disinfectants: quaternary ammonium compounds

- The discovery of the antimicrobial activity of quaternary ammonium compounds during the early 20th century was a major advancement in the development of effective germicides.
- The inherent antimicrobial activity of these compounds was soon shown to be significantly improved by the addition of long-chain alkyl groups to the nitrogen moiety of the quaternary compound.

- The various quaternary ammonium compounds commercially used as disinfectants today are chemical modifications of this original concept. The mode of action of quaternary ammonium compounds is as cationic surface-active agents, although this chemical property does not fully explain the germicidal activity of the compounds.
- All have broad-based antimicrobial activity and have proven effectiveness against algae, gram-positive bacteria, some gram-negative bacteria, fungi, and certain viruses, when used at the manufacturer's recommended concentrations (0.1% to 2.0% active ingredient, or 200 to 700 ppm).
- They are relatively nontoxic to humans when used according to manufacturer's instructions and are not chemically destructive to equipment under normal use. They can be autoclaved without formation of toxic vapors and thus are frequently used as disinfectants in discard pans.
- The limitations of quaternary ammonium compounds include lack of effectiveness at low concentrations against some commonly encountered gram-negative bacteria (e.g., *Pseudomonas* sp.). Like many other disinfectants, they are quickly inactivated by the presence of heavy organic burden.

Use of disinfectants: sodium hypochlorite

- Chlorine, in various forms, has a long history of use as a powerful disinfectant, yet the exact mode of germicidal action is unclear. Hypochlorites are the most widely used chlorine compounds for disinfection.
- Commercial liquid bleach products (e.g., Clorox) are solutions containing 5.25% (w/v) sodium hypochlorite. Sodium hypochlorite is effective against vegetative microbial cells, most spores, and many viruses.
- It has some residual effect after the treated surface dries. It can be used in sanitization procedures for laboratory floors and in laboratory coat washing. It is strongly germicidal and can be used to decontaminate small- to mid-volume spills of biological material.
- Despite their germicidal effectiveness, chlorine solutions are limited in their use as laboratory disinfectants because of their corrosiveness to metals and their human toxicity.

They should not be routinely used in discard pans or in any solutions that are autoclaved, as the chlorine fumes liberated are significant skin and respiratory irritants.

- Frequent autoclaving of chlorine solutions will corrode the chamber interior of the sterilizer. One exception to this autoclave ban is the need to sterilize any biological spill material in which bleach was used as a disinfectant during the cleanup process.
- A solution of 10% (v/v) household bleach is a strongly germicidal, containing 0.52% (w/v) sodium hypochlorite.
- Excess hypochlorite is needed in mopping up spills of biological agents, to supply additional chlorine to replace that consumed by the large amount of organic matter associated with the spill.
- Sodium hypochlorite solutions can be inactivated by organic matter (which consumes the available free chlorine that constitutes microbiocidal activity), by exposure to UV light, and by inorganic chemical reducing agents (such as ferrous or manganese cations and hydrogen sulfide). Hypochlorite solutions should be stored away from heat to avoid deterioration.

Filter Sterilization of Solutions

- All solutions that come in contact with cell cultures must be sterile in order to prevent microbial contamination. This includes non-nutritive preparations such as distilled/deionized water and reagents (e.g., dimethyl sulfoxide used as a cryoprotectant).
- Although heat-stable solutions can be sterilized by autoclaving, many solutions used in cell culture contain one or more heat-labile components (e.g., antibiotics), or are chemically formulated with ingredients that will form deleterious precipitates if subjected to steam sterilization temperatures (e.g., phosphate-buffered salines).
- Membrane filtration is the most common cold sterilization method for these types of solutions. Filter membranes with 0.2-μm pore size are used for general sterilization purposes; however, some environmentally stressed bacteria (e.g., *Pseudomonas* sp.) as well as mycoplasma can pass through filters of this porosity.
- To provide a greater degree of assurance for complete removal of these common tissue culture contaminants, cell culture media and sera should be sterilized using 0.1-μm filter membranes. Filter manufacturers offer many different types of membranes.

- With respect to cell culture applications, membranes fabricated from cellulose acetate or cellulose nitrate are used for general purpose filtration of aqueous solutions such as media and buffers, but may need prewashing with hot distilled water to remove extractable substances that may be cytotoxic.
- Nylon membranes are very low in extractable substances such as surfactants or wetting agents; polyethersulfone membranes are low in extractables and have very low protein binding The availability of presterilized, ready-to-use, disposable filter systems has eliminated much of the labor and risk of failure inherent with earlier filtration methods.
- Filter units come in a wide variety of sizes to handle small (≤10-ml) to large (≥20-liter) volumes.
- Disposable systems are available for either vacuum or positive-pressure filtration. Many manufacturers have filter systems designed specifically for cell culture applications: the sterilization membranes, housings, and receiving vessels are certified noncytotoxic and nonpyrogenic.

This section outlines selection of filters and filter-sterilization procedures for various types of liquids encountered in cell culture laboratories.

- The most common small-volume filtration technique is positive pressure using a syringe to force the liquid through the filter membrane. Volumes ranging from 50 ml to 1 liter are most efficiently processed with a vacuum.
- Larger volumes should be filter sterilized with positive pressure. The primary use of membrane filtration is in the preparation of tissue culture media; This unit focuses on problematic filtration needs that often appear in cell culture applications, such as the need to filter sterilize a hazy solution like the serum/yeast extract additives used in mycoplasma media, or the chemically aggressive reagent dimethyl sulfoxide (DMSO).
- The methods outlined in this section are equally adaptable for the preparation of tissue culture media or stock solutions of additives such as glutamine or puruvate.
- For media preparation, use noncytotoxic cellulose acetate/nitrate embranes, or similar membranes specific to the application, and food-grade silicon tubing.

Vacuum Filtration

- Solutions that are initially clean preparations, in that they are free of particulate debris and are not proteinaceous, can be directly filter sterilized with no difficulty.
- Solutions with high particle load require centrifugation and/or nonsterile prefiltration through depth filters (see Background Information) and larger-porosity membranes prior to sterile 0.2-μm filtration.

Possible questions

1. Explain aspetic techniques to be maintained in animal cell culture lab
2. Explain the laboratory design for animal cell culture
3. Explain the role of CO_2 incubators and Cenrifuge
4. Briefly explain Biosafety level
5. What is the importance of Cell counter
6. Define Biohazards
7. What is meant by sterile workplace
8. Write the importance of microscopes
9. Define Cell culture
10. What is tissue engineering?
11. Explain the different types of media
12. Write about basal and complex media
13. Briefly explain serum media
14. List out the advantages and disadvantages of serum in animal cell culture
15. Write about the physico-chemical properties of media
16. Define FBS
17. Explain the various techniques of sterilization methods
18. Write about vacuum filtration

Primary Cell Culture: Methods of tissue disaggregation – isolation of tissues from chick embryo, mouse and human; Continuous and established culture; Cell separation and characterization; Organ culture – types

What is Cell and Tissue Culture?

- **Tissue Culture** is the general term for the removal of cells, tissues, or organs from an animal or plant and their subsequent placement into an artificial environment conducive to growth. This environment usually consists of a suitable glass or plastic culture vessel containing a liquid or semisolid medium that supplies the nutrients essential for survival and growth.
- The culture of whole organs or intact organ fragments with the intent of studying their continued function or development is called **Organ Culture**. When the cells are removed from the organ fragments prior to, or during cultivation, thus disrupting their normal relationships with neighboring cells, it is called **Cell Culture**.

How Are Cell Cultures Obtained? Primary Culture

- When cells are surgically removed from an organism and placed into a suitable culture environment, they will attach, divide and grow. This is called a **Primary Culture**. There are two basic methods for doing this.
- **First**, for **Explant Cultures**, small pieces of tissue are attached to a glass or treated plastic culture vessel and bathed in culture medium. After a few days, individual cells will move from the tissue explant out onto the culture vessel surface or substrate where they will begin to divide and grow.
- The **second**, more widely used method speeds up this process by adding digesting (proteolytic) enzymes, such as **trypsin** or **collagenase**, to the tissue fragments to dissolve the cement holding the cells together.
- This creates a suspension of single cells that are then placed into culture vessels containing culture medium and allowed to grow and divide. This method is called **Enzymatic Dissociation**

Subculturing

- When the cells in the primary culture vessel have grown and filled up all of the available culture substrate, they must be **Subcultured** to give them room for continued growth. This is usually done by removing them as gently as possible from the substrate with enzymes.
- These are similar to the enzymes used in obtaining the primary culture and are used to break the protein bonds attaching the cells to the substrate. Some cell lines can be harvested by gently scraping the cells off the bottom of the culture vessel.
- Once released, the cell suspension can then be subdivided and placed into new culture vessels. Once a sample of cells is available, they can be treated with suitable cryoprotective agents, such as **dimethylsulfoxide** (DMSO) or glycerol, carefully frozen and then stored at cryogenic temperatures (below -130°C) until they are needed

Buying and Borrowing

- An alternative to establishing cultures by primary culture is to buy established cell cultures from organizations such as the American Type Culture Collection (ATCC; www.atcc.org) or the Coriell Institute for Medical Research (arginine.umdnj.edu). These two nonprofit organizations provide high quality cell lines that are carefully tested to ensure the authenticity of the cells.
- More frequently, researchers will obtain (borrow) cell lines from other laboratories. While this practice is widespread, it has one major drawback. There is a high probability that the cells obtained in this manner will not be healthy, useful cultures. This is usually due to previous mix-ups or contamination with other cell lines, or the result of contamination with microorganisms such as mycoplasmas, bacteria, fungi or yeast.

What Are Cultured Cells Like?

Once in culture, cells exhibit a wide range of behaviors, characteristics and shapes. Some of the more common ones are described below.

Cell Culture Systems

- Two basic culture systems are used for growing cells. These are based primarily upon the ability of the cells to either grow attached to a glass or treated plastic substrate

(**Monolayer Culture Systems**) or floating free in the culture medium (**Suspension Culture Systems**).

- Monolayer cultures are usually grown in tissue culture treated dishes, T-flasks, roller bottles, or multiple well plates, the choice being based on the number of cells needed, the nature of the culture environment, cost and personal preference.

Suspension cultures are usually grown either:

1. In magnetically rotated spinner flasks or shaken Erlenmeyer flasks where the cells are kept actively suspended in the medium;
2. In stationary culture vessels such as T-flasks and bottles where, although the cells are not kept agitated, they are unable to attach firmly to the substrate.

Many cell lines, especially those derived from normal tissues, are considered to be **Anchorage-Dependent,** that is, they can only grow when attached to a suitable substrate. Some cell lines that are no longer considered normal (frequently designated as **Transformed Cells)** are frequently able to grow either attached to a substrate or floating free in suspension; they are **Anchorage-Independent.** In addition, some normal cells, such as those found in the blood, do not normally attach to substrates and always grow in suspension.

Types of Cells

Cultured cells are usually described based on their morphology (shape and appearance) or their functional characteristics.

There are three basic morphologies:

1. **Epithelial-like**: cells that are attached to a substrate and appear flattened and polygonal in shape.
2. **Lymphoblast-like**: cells that do not attach normally to a substrate but remain in suspension with a spherical shape.
3. **Fibroblast-like**: cells that are attached to a substrate and appear elongated and bipolar, frequently forming swirls in heavy cultures. It is important to remember that the culture conditions play an important role in determining shape and that many cell cultures are capable of exhibiting multiple morphologies.

Using cell fusion techniques, it is also possible to obtain **hybrid cells** by fusing cells from two different parents. These may exhibit characteristics of either parent or both parents. This

technique was used in 1975 to create cells capable of producing custom tailored monoclonal antibodies.

These hybrid cells (called **Hybridomas**) are formed by fusing two different but related cells. The first is a spleen-derived lymphocyte that is capable of producing the desired antibody. The second is a rapidly dividing myeloma cell (a type of cancer cell) that has the machinery for making antibodies but is not programmed to produce any antibody.

The resulting hybridomas can produce large quantities of the desired antibody. These antibodies, called **Monoclonal Antibodies** due to their purity, have many important clinical, diagnostic, and industrial applications.

Functional Characteristics

- The characteristics of cultured cells result from both their origin (liver, heart, etc.) and how well they adapt to the culture conditions. Biochemical markers can be used to determine if cells are still carrying on specialized functions that they performed *in vivo* (e.g., liver cells secreting albumin).
- Morphological or ultra-structural markers can also be examined (e.g., beating heart cells). Frequently, these characteristics are either lost or changed as a result of being placed in an artificial environment.
- Some cell lines will eventually stop dividing and show signs of aging. These lines are called **Finite**. Other lines are, or become **immortal**; these can continue to divide **indefinitely** and are called **Continuous** cell lines.
- When a "normal" finite cell line becomes immortal, it has undergone a fundamental irreversible change or "transformation". This can occur spontaneously or be brought about intentionally using drugs, radiation or viruses.
- **Transformed Cells** are usually easier and faster growing, may often have extra or abnormal chromosomes and frequently can be grown in suspension

What is Cell Culture Used For

Cell culture has become one of the major tools used in cell and molecular biology. Some of the important areas where cell culture is currently playing a major role are:

1- **Model Systems** Cell cultures provide a good model system for studying 1) basic cell biology and biochemistry, 2) the interactions between disease-causing agents and cells, 3) the effects of drugs on cells, 4) the process and triggers for aging, and 5) nutritional studies

2- **Toxicity Testing**

Cultured cells are widely used alone or in conjunction with animal tests to study the effects of new drugs, cosmetics and chemicals on survival and growth in a wide variety of cell types. Especially important are liver- and kidney-derived cell cultures.

3- **Cancer Research** Since both normal cells and cancer cells can be grown in culture, the basic differences between them can be closely studied. In addition, it is possible, by the use of chemicals, viruses and radiation, to convert normal cultured cells to cancer causing cells. Thus, the mechanisms that cause the change can be studied. Cultured cancer cells also serve as a test system to determine suitable drugs and methods for selectively destroying types of cancer.

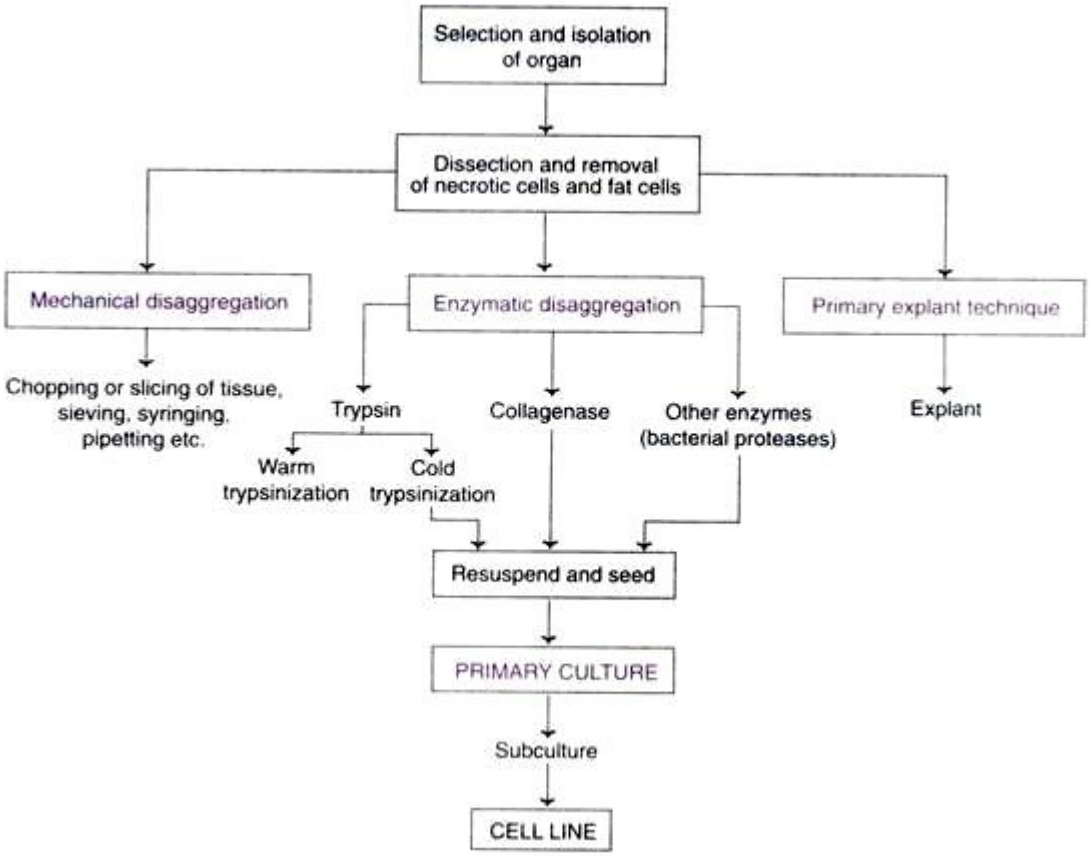
4- **Virology** One of the earliest and major uses of cell culture is the replication of viruses in cell cultures (in place of animals) for use in vaccine production. Cell cultures are also widely used in the clinical detection and isolation of viruses, as well as basic research into how they grow and infect organisms.

5- **Cell-Based Manufacturing** While cultured cells can be used to produce many important products, three areas are generating the most interest. **The first** is the large-scale production of viruses for use in vaccine production. These include vaccines for polio, rabies, chicken pox, hepatitis B and measles. **Second**, is the large-scale production of cells that have been genetically engineered to produce proteins that have medicinal or commercial value. These include monoclonal antibodies, insulin, hormones, etc. **Third**, is the use of cells as replacement tissues and organs. Artificial skin for use in treating burns and ulcers is the first commercially available product.

6- Genetic Counseling Amniocentesis, a diagnostic technique that enables doctors to remove and culture fetal cells from pregnant women, has given doctors an important tool for the early diagnosis of fetal disorders. These cells can then be examined for abnormalities in their chromosomes and genes using karyotyping, chromosome painting and other molecular techniques.

7- Genetic Engineering The ability to transfect or reprogram cultured cells with new genetic material (DNA and genes) has provided a major tool to molecular biologists wishing to study the cellular effects of the expression of these genes (new proteins). These techniques can also be used to produce these new proteins in large quantity in cultured cells for further study.

8- Gene Therapy The ability to genetically engineer cells has also led to their use for gene therapy. Cells can be removed from a patient lacking a functional gene and the missing or damaged gene can then be replaced. The cells can be grown for a while in culture and then replaced into the patient. An alternative approach is to place the missing gene into a viral vector and then "infect"" the patient with the virus in the hope that the missing gene will then be expressed in the patient"s cells.



Techniques for Primary Culture:

Among the various techniques devised for the primary culture of isolated tissues, three techniques are most commonly used: Fig. 1

1. Mechanical disaggregation.
2. Enzymatic disaggregation.
3. Primary explant technique.

Technique 1. Mechanical Disaggregation:

- For the disaggregation of soft tissues (e.g. spleen, brain, embryonic liver, soft tumors), mechanicaltechnique is usually employed.
- This technique basically involves careful chopping or slicing of tissue into pieces and collection of spill out cells.

The cells can be collected by two ways:

a. Pressing the tissue pieces through a series of sieves with a gradual reduction in the mesh size.
b. Forcing the tissue fragments through a syringe and needle.

Although mechanical disaggregation involves the risk of cell damage, the procedure is less expensive, quick and simple.

This technique is particularly useful when the availability of the tissue is in plenty, and the efficiency of the yield is not very crucial. It must however, be noted that the viability of cells obtained from mechanical techniques is much lower than the enzymatic technique

.**Technique 2. Enzymatic Disaggregation**:

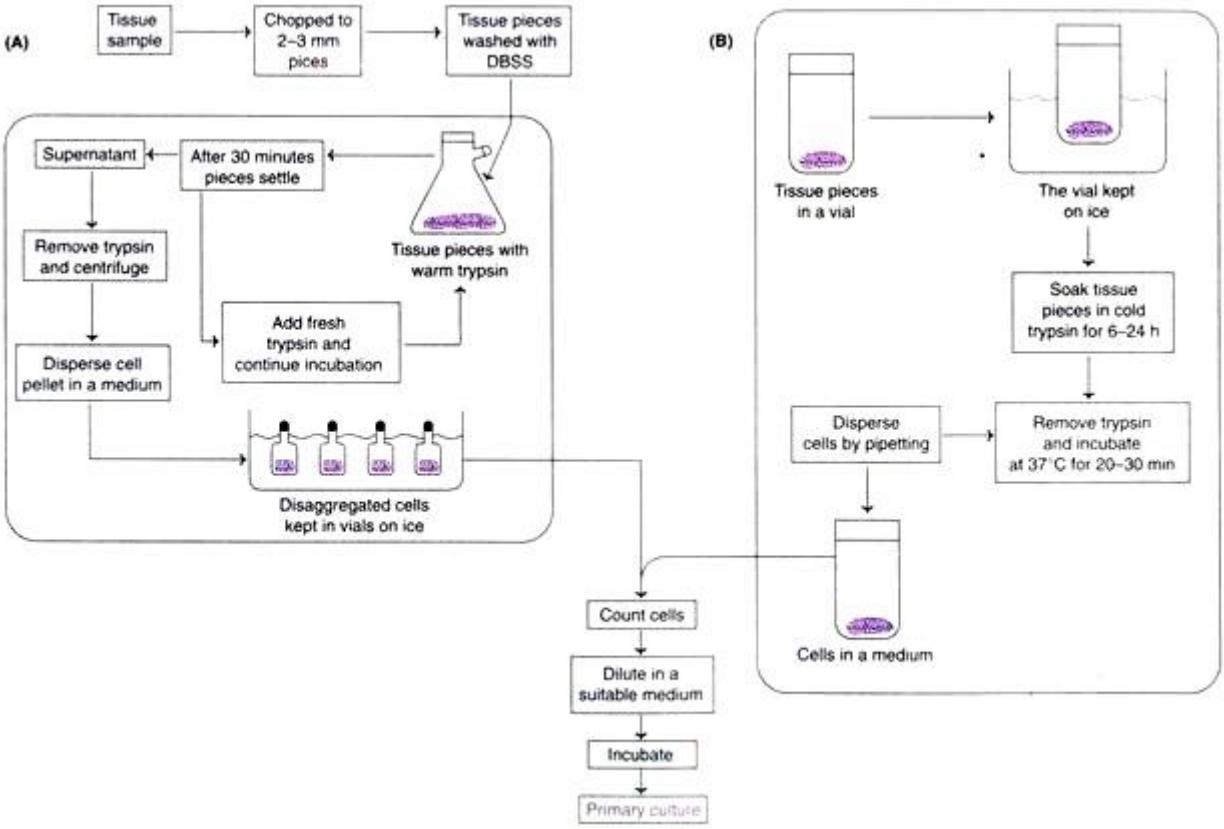
- Enzymatic disaggregation is mostly used when high recovery of cells is required from a tissue. Disaggregation of embryonic tissues is more efficient with higher yield of cells by use of enzymes.
- This is due to the presence of less fibrous connective tissue and extracellular matrix. Enzymatic disaggregation can be carried out by using trypsin, collagenase or some other enzymes.

Disaggregation by trypsin:

The term trypsinization is commonly used for disaggregation of tissues by the enzyme, trypsin. Many workers prefer to use crude trypsin rather than pure trypsin for the following reasons:

- The crude trypsin is more effective due to the presence of other proteases
- Cells can tolerate crude trypsin better.
- The residual activity of crude trypsin can be easily neutralized by the serum of the culture media (when serum-free media are used, a trypsin inhibitor can be used for neutralization).

- Disaggregation of cells can also be carried out by using pure trypsin which is less toxic and more specific in its action.
- The desired tissue is chopped to 2-3 mm pieces and then subjected to disaggregation by trypsin. There are two techniques of trypsinization-warm trypsinization and cold trypsinization (Fig. 2)



Warm trypsinization

- This method is widely used for disaggregation of cells. The chopped tissue is washed with dissection basal salt solution (DBSS), and then transferred to a flask containing warm trypsin (37° C).
- The contents are stirred, and at an interval of every thirty minutes, the supernatant containing the dissociated cells can be collected.
- After removal of trypsin, the cells are dispersed in a suitable medium and preserved (by keeping the vial on ice).
- The process of addition of fresh trypsin (to the tissue pieces), incubation and collection of dissociated cells (at 30 minutes intervals) is carried out for about 4 hours.
- The disaggregated cells are pooled, counted, appropriately diluted and then incubated.

Cold trypsinization

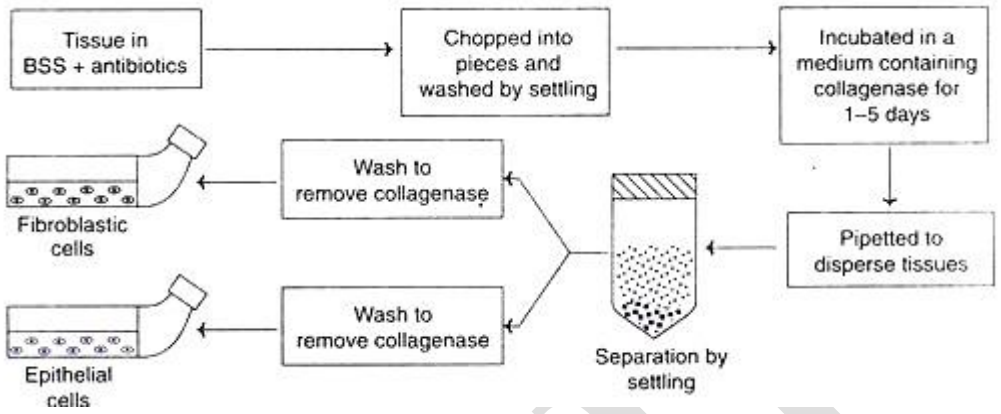
- This technique is more appropriately referred to as trypsinization with cold pre-exposure. The risk of damage to the cells by prolonged exposure to trypsin at 37°C (in warm trypsinization) can be minimized in this technique.
- After chopping and washing, the tissue pieces are kept in a vial (on ice) and soaked with cold trypsin for about 6-24 hours. The trypsin is removed and discarded. However, the tissue pieces contain residual trypsin.
- These tissue pieces in a medium are incubated at 37°C for 20-30 minutes. The cells get dispersed by repeated pi-petting's. The dissociated cells can be counted, appropriately diluted and then used.
- The cold trypsinization method usually results in a higher yield of viable cells with an improved survival of cells after 24 hours of incubation. This method does not involve stirring or centrifugation, and can be conveniently adopted in a laboratory. The major limitation of cold trypsinization is that it is not suitable for disaggregation of cells from large quantities of tissues.

Limitations of trypsin disaggregation:

- Disaggregation by trypsin may damage some cells (e.g. epithelial cells) or it may be almost ineffective for certain tissues (e.g. fibrous connective tissue). Hence other enzymes are also in use for dissociation of cells.

Disaggregation by collagenase:

- Collagen is the most abundant structural protein in higher animals. It is mainly present in the extra - cellular matrix of connective tissue and muscle. The enzyme collagenase (usually crude one contaminated with non-specific proteases) can be effectively used for the disaggregation of several tissues (normal or malignant) that may be sensitive to trypsin.
- Highly purified grades of collagenase have been tried, but they are less effective when compared to crude collagenase. The important stages in collagenase dis aggregation, depicted in are briefly described hereunder.
- The desired tissue suspended in basal salt solution, containing antibiotics is chopped into pieces. These pieces are washed by settling, and then suspended in a complete medium containing collagenase.
- After incubating for 1-5 days, the tissue pieces are dispersed by pipetting. The clusters of cells are separated by settling. The epithelial cells and fibroblastic cells can be separated. Collagenase disaggregation has been successfully used for human brain, lung and several other epithelial tissues, besides various human tumors, and other animal tissues.
- Addition of another enzyme hyaluronidase (acts on carbohydrate residues on cell surfaces) promotes disaggregation. Collagenase in combination with hyaluronidase is found to be very effective for dissociating rat or rabbit liver.
- This can be done by per-fusing the whole organ in situ. Some workers use collagenase in conjunction with trypsin, a formulation developed in chick serum, for disaggregation of certain tissues.



Use of other enzymes in disaggregation:

- Trypsin and collagenase are the most widely used enzymes for disaggregation. Certain bacterial proteases (e.g. pronase, dispase) have been used with limited success.
- Besides hyaluronidase, neuraminidase is also used in conjunction with collagenase for effective degradation of cell surface carbohydrates.

Primary Explant Technique:

- The primary explant technique was, in fact the original method, developed by Harrison in 1907.
- This technique has undergone several modifications, and is still in use.
- The simplified procedure adopted for primary explant culture is depicted in Fig. and briefly described below. The tissue in basal salt solution is finely chopped, and washed by settlings. The basal salt solution is then removed.
- The tissue pieces are spread evenly over the growth surface. After addition of appropriate medium, incubation is carried out for 3-5 days. Then the medium is changed at weekly intervals until a substantial outgrowth of cells is observed.
- Now, the explants are removed and transferred to a fresh culture vessel. The primary explant technique is particularly useful for disaggregation of small quantities of tissues (e.g. skin biopsies).
- The other two techniques mechanical or enzymatic disaggregation however, are not suitable for small amounts of tissues, as there is a risk of losing the cells.

- The limitation of explant technique is the poor adhesiveness of certain tissues to the growth surface, and the selection of cells in the outgrowth. It is however, observed that the primary explants technique can be used for a majority of embryonic cells e.g. fibroblasts, myoblasts, epithelial cells, glial cells.

Separation of Viable and Non-Viable Cells:

- It is a common practice to remove the non viable cells while the primary culture is prepared from the disaggregated cells.
- This is usually done when the first change of the medium is carried out. The very few left over non-viable cells get diluted and gradually disappear as the proliferation of viable cells commences. Sometimes, the non-viable cells from the primary cultures may be removed by centrifugation.
- The cells are mixed with ficoll and sodium metrizoate, and centrifuged. The dead cells form a pellet at the bottom of the tube.

Medical Ethics and Safety Measures in Culture Techniques:

- Since the culture techniques involve the use of animal or human tissues, it is absolutely necessary to follow several safety measures and medical ethics. In fact, in some countries there are established legislation/norms for selection and use of tissues in cultures.
- For example, in United Kingdom, Animal Experiments (Scientific Procedures) Act of 1986 is followed.
- The handling of human tissues poses several problems that are not usually encountered with animal tissues. While dealing with fetal materials and human biopsies, the consent of the patient and/his or her relatives, besides the consent of local ethical committee is required.
- Further, taking any tissue (even in minute quantities) from human donors requires the full consent of the donor in a prescribed format.

The following issues need to be fully considered while dealing with human tissues:

1. The consent of the patient and/or relatives for using tissues for research purposes.
2. Ownership of the cell lines developed and their derivatives.
3. Consent for genetic modification of the cell lines.
4. Patent rights for any commercial use of cell lines.

In the general practice of culture techniques using human tissues, the donor and/or relatives are asked to sign a disclaimer statement (in a prescribed proforma) before the tissue is taken. By this approach, the legal complications are minimized.

Safety measures:

- Handling of human tissues is associated with a heavy risk of exposure for various infections. Therefore, it is absolutely necessary that the human materials are handled in a biohazard cabinet.
- The tissues should be screened for various infections such as hepatitis, tuberculosis, HIV, before their use. Further, the media and apparatus, after their use must be autoclaved or disinfected, so that the spread of infections is drastically reduced.

Organ Culture

- Organ culture: Organ culture in which whole organs or representative parts are maintained as small fragments in culture and retain their intrinsic distribution, numerical and spatial, of participating cells.
- Organ culture seeks to retain the original structural relationship of cells of the same or different types, and hence their interactive function in order to study the effect of exogenous stimuli on further development.
- Organ culture seeks to retain the original structural relationship of cells of the same or different types and hence their interactive function, in order to study the effect of exogenous stimuli on further development.

Organ Culture: Gas and Nutrient Exchange:

- A major deficiency in tissue architecture in organ culture is the absence of a vascular system, limiting the size (by diffusion) and potentially the polarity of the cells within the organ culture.
- When cells are cultured as a solid mass of tissue, gaseous diffusion and the exchange of nutrients and metabolites is from the periphery, and the rate of this diffusion limits the size of the tissue.

- The dimensions of individual cells cultured in suspension or as a monolayer are such that diffusion is not limiting, but survival of cells in aggregates beyond about 250 μm in diameter (~5000 cell diameters) starts to become limited by diffusion, and at or above 1.0 mm in diameter (~2.5 × 105 cell diameters) central necrosis is often apparent.
- To alleviate this problem, organ cultures are usually placed at the interface between the liquid and gaseous phases, to facilitate gas exchange while retaining access to nutrients. This is achieved by most system by positioning the explant in a filter well insert on a raft or gel exposed to the air, but explants anchored to a solid substrate can also be aerated by rocking the culture, exposing it alternately to a liquid medium and a gas phase or by using a roller bottle or rotating tube rack.
- Anchorage to a solid substrate can lead to the development of an outgrowth of cells from the explant and resultant alterations in geometry even though this effect can be minimized by using a hydrophobic surface.
- One of the advantages of culture at the gas–liquid interface is that the explant retains a spherical geometry if the liquid is maintained at the correct level. If the liquid is too deep, gas exchange is impaired whereas if it is too shallow, surface tension will tend to flatten the explants and promote outgrowth.
- Permeation of oxygen increases by using increasing O_2 concentrations up to pure oxygen or by using hyperbaric oxygen. As increasing the O_2 tension will not facilitate CO_2 release or nutrient metabolite exchange, the benefits of increased oxygen may be overridden by other limiting factors.

Structural Integrity:

- The maintenance of structural integrity is the main reason for adopting organ culture as an in vitro technique in preference to cell culture.
- Whereas cell culture utilizes cells dissociated by mechanical or enzymatic techniques or spontaneous migration, organ culture deliberately maintains the cellular associations found in the tissue.

Growth and Differentiation:

- There is a relationship between growth and differentiation such that differentiated cells no longer proliferate. It is also possible that cessation of growth, regardless of cell density, may contribute to the induction of differentiation, if only by providing a permissive phenotypic state that is receptive to exogenous inducers of differentiation.
- Because of density limitation of cell proliferation and the physical restrictions imposed by organ culture geometry, most organ cultures do not grow or if they do proliferation is limited to the outer cell layers.
- Hence the status of the culture is permissive to differentiation and the appropriate cellular interactions and soluble inducers are provided as an ideal environment for differentiation to occur.

Limitations of Organ Culture:

1. Organ cultures depend largely on histological techniques and they do not impart themselves readily into biochemical and molecular analyses.
2. Biochemical monitoring requires reproducibility between samples, which is less easily achieved in organ culture than in propagated cell lines, because of sampling variation in preparing an organ culture, minor differences in handling and geometry, and variations in the ratios of cell types among cultures.
3. Organ cultures are also more difficult to prepare than replicate cultures from a propagated cell line and do not have the advantage of a characterized reference stock to which they may be related.
4. Organ culture is essentially a technique for studying the behaviour of integrated tissues rather than isolated cells.
5. Organ culture has contributed a great deal to our understanding of developmental biology and tissue interactions and that it will continue to do so in the absence of adequate synthetic systems

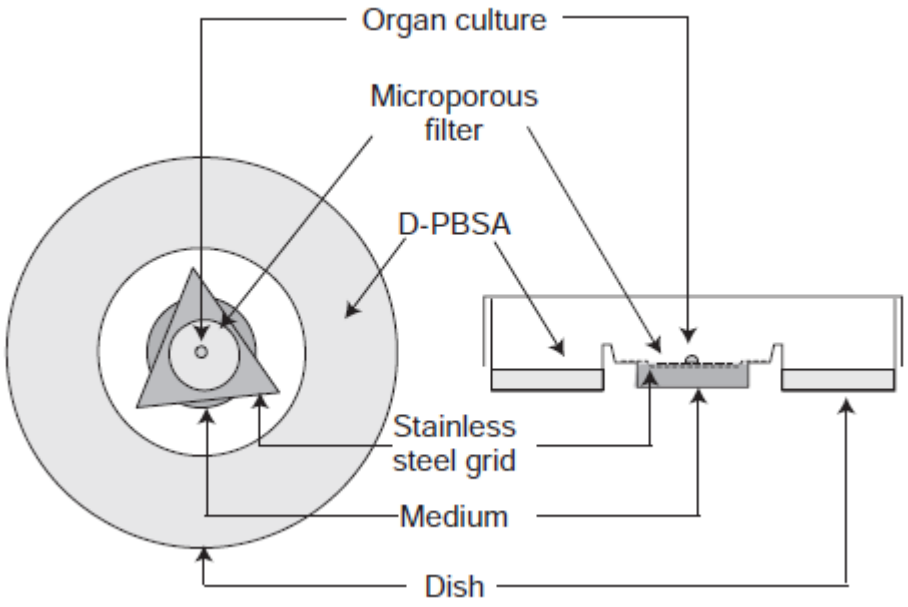


Figure 1: Organ Culture Small fragment of tissue on a filter laid on top of a stainless steel grid over the central well of an organ culture dish

Possible Questions

1. What is Cell and Tissue Culture?
2. How Are Cell Cultures Obtained?
3. Write about primary culture
4. Explain finite and non-finite cell lines
5. Explain passage cell line
6. Write about the techniques involved in disaggregating the tissue
7. Explain enzymatic disaggregation of tissue
8. Explain the applications of cell culture
9. Write about organ culture
10. Explain subculture

Gene transfer methods in Animals- Microinjection, Embryonic Stem cell, gene transfer, Reterovirus & Gene transfer. Introduction to transgenesis. Transgenic Animals – Mice, Cow, Pig sheep, goat, bird and insect

Microinjection

- DNA microinjection was first proposed by Dr. Marshall A. Barber in the early of nineteenth century.
- This method is widely used for gene transfection in mammals.
- It involves delivery of foreign DNA into a living cell (e.g. a cell, egg, oocyte, embryos of animals) through a fine glass micropipette. The introduced DNA may lead to the over or under expression of certain genes.
- It is used to identify the characteristic function of dominant genes.

Following steps are involved in production of transgenic animal by microinjection

Transgene DNA Construct:

- cDNA of a gene of interest along with the regulatory elements (promoter, terminator etc) are used to design the DNA construct.
- Promoter and 3' UTR are necessary for proper gene regulation. Some evidence shows that intron splicing plays role in gene expression in mammalian system.
- Hence introns are also included in the construct sometimes. Construct should be linearized before injection.

Embryo Collection:

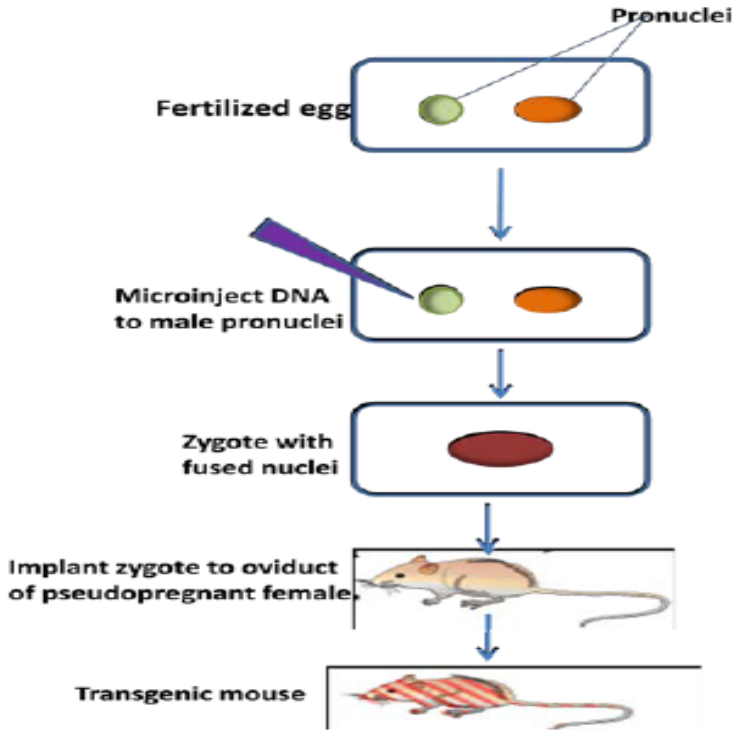
- Donor parental strain for the production of embryo are selected considering factors like response to super ovulation (ability to produce mature ova at large number), frequencies of embryo survival following microinjection, siz**e** of pronuclei and incidence of specific pathologies inherent to strain.
- Successful super ovulation depends on the strain, age and weight of the animal. Breeding should be monogamous. After post-mating, embryos are collected at single cell stage and proceed for microinjection.

DNA Microinjection:

- The purified double stranded linear DNA construct containing the transgene DNA sequence is introduced to host cell by microinjection.
- The foreign DNA must be integrated (although random) into the host genome prior to cell division.
- To facilitate this transgene DNA is introduced into zygote at the earliest possible stage (pronuclear period) immediately after fertilization. Usually male pronucleus is preferred because it is larger and easier to inject.
- The host chromosome at the site of integration generally undergoes duplication, deletion or rearrangements due to transgene incorporation.
- This may lead to insertional mutagenesis and thus producing detectable phenotypic trait.

Critical points for successful DNA microinjection technique-

- Careful collection of relatively large group of accurately single cell embryo.
- Embryo transfer to suitable recipient female (standardized in each case).
- Construction and preparation of transgene DNA fragment to be injected.



Advantage:

- No size limitation of insert DNA.
- Applicable to wide range of species.
- Less time consuming and short generation time.

Limitation:

- Low success rate
- Random integration of transgene constructs to host chromosome, resulting in undesirable effect or phenotype.
- Potential undesired insertional mutagenesis

Embryonic stem cell technology:

Embryonic stem (ES) cells are pluripotent stem cells isolated from inner cell mass (ICM) of blastocyst stage of embryo. Use of ES cell mediated transgenic animal production is quite effective specially to generate targeted gene modification at precise location.

The procedure to generate transgenic animals using modified ES cell technology is described in brief below:

a) Isolation of pluripotent ES cells from ICM of blastocyst and culturing of the cells *in vitro*.

b) Transgene introduction to ES cells using methods like DNA micro-injection, electroporation, precipitation reaction, transfection etc. DNA transfection to ES cells can be carried out using different vectors likeliposome, retroviral vectors etc.

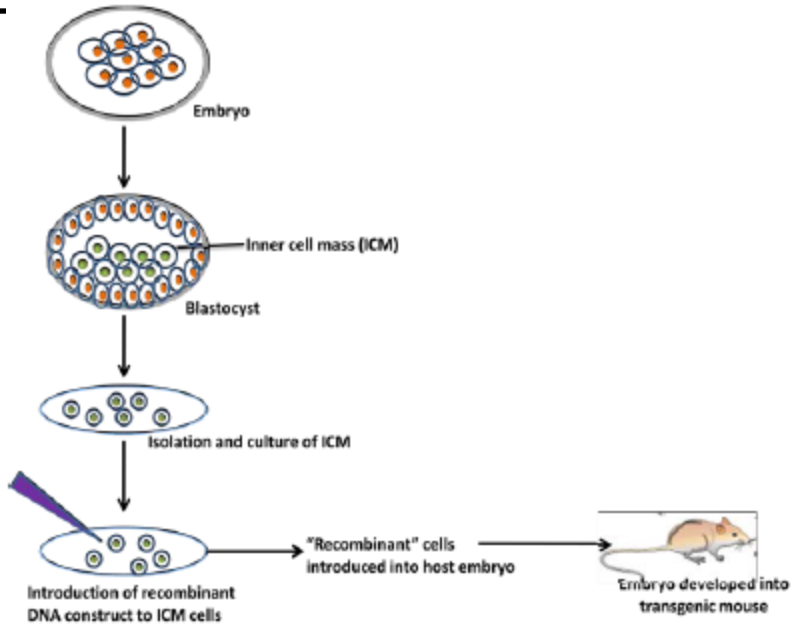
c) . Selection of transformed ES cells for either knockin or knockout constructs.

d) Transformed cells are injected into blastocyst followed by implantation in a surrogate female.

e) After birth, the offsprings are screened for chimerism.Inbreeding of the genetic chimeras is performed to obtain homozygous transgenic animal carrying both the mutated alleles for that character.

Limitation:

- Difficulty in *in vitro* culturing and maintenance of pluripotent ES cells.
- Germ line transfection with low frequency.



Retroviral mediated gene transfer:

- Retrovirus is a virus of family "Retroviridae" that has RNA as its genetic material and replicate inside the host cell using reverse transcription machinery (to convert RNA to DNA).
- Retroviruses are commonly used as vectors to transfer genetic material into the host cell because of its high efficiency to transfer RNA to the host. Recombinant retrovirus RNA genome is then copied by reverse transcriptase (encoded by retrovirus) to yield a DNA copy, which then becomes integrated into the host genome at random sites.
- The offspring results from such transformation are chimeric, an organism consisting of tissues of diverse genetic constitution. To transmit transgene to next generation, any gene transfer technique including retroviral mediated should target germline cells.
- Generally, early 4-16 celled embryos are used to get maximum of retrovirus infection resulting in chimeric animal. Although homozygosity is attained just after several generations of selfing, these chimeric animals are inbred for 10-20 generations to obtain homozygous transgenic animals.
- Although retroviral vectors have high efficiency of transgene integration into host cell, there are certain limitations of this approach.

Limitations:

- Low copy number integration.
- Additional steps needed for construction of recombinant retrovirus.
- Limited size of DNA insert (~<15kb).
- Mosaicism (same individual having two different types of genotypes) of the recovered animals.
- Possible interference of the proviral LTR sequences with the expression of transgene.

Introduction to transgenesis

- Trans-genesis refers to the phenomenon of introduction of exogeneous DNA into the genome to create and maintain a stable heritable character.
- The foreign DNA that is introduced is called transgene. And the animal whose genome is altered by adding one or more transgenes is said to be transgenic.
- The transgenes behave like other genes present in the animals' genome and are passed on to the offspring's.
- Thus, transgenic animals are genetically engineered or genetically modified organisms (GMOs) with a new heritable character.
- It was in 1980s, the genetic manipulation of animals by introducing genes into fertilized eggs became a reality.

Importance of Transgenic Animals-General

- Trans-genesis has now become a powerful tool for studying the gene expression and developmental processes in higher organisms, besides the improvement in their genetic characteristics.
- Transgenic animals serve as good models for understanding the human diseases.
- Further, several proteins produced by transgenic animals are important for medical and pharmaceutical applications.
- Thus, the transgenic farm animals are a part of the lucrative world-wide biotechnology industry, with great benefits to mankind. Trans-genesis is important for improving the quality and quantity of milk, meat, eggs and wool production, besides creating drug resistant animals.

Milk as the Medium of Protein Production:

- Milk is the secretion of mammary glands that can be collected frequently without causing any harm to the animal. Thus, milk from the transgenic animals can serve as a good and authenticated source of human proteins for a wide range of applications.
- Another advantage with milk is that it contains only a few proteins (casein, lactalbumin, immunoglobulin etc.) in the native state, therefore isolation and purification of a new protein from milk is easy.

Commonly used Animals for Trans-genesis:

- The first animals used for trans-genesis was a mouse. The 'Super Mouse', was created by inserting a rat gene for growth hormone into the mouse genome.
- The offspring was much larger than the parents. Super Mouse attracted a lot of public attention, since it was a product of genetic manipulation rather than the normal route of sexual reproduction.
- Mouse continues to be an animal of choice for most transgenic experiments. The other animals used for trans-genesis include rat, rabbit, pig, cow, goat, sheep and fish.

Position Effects:

- Position effect is the phenomenon of different levels of gene expression that is observed after insertion of a new gene at different position in the eukaryotic genome. This is commonly observed in transgenic animals as well as plants.
- These transgenic organisms show variable levels and patterns of transgene expression. In a majority of cases, position effects are dependent on the site of transgene integration.
- In general, the defective expression is due to the insertion of transgene into a region of highly packed chromatin.
- The transgene will be more active if inserted into an area of open chromatin.The positional effects are overcome by a group of DNA sequences called insulators. The sequences referred to as specialized chromatin structure (SCS) are known to perform the functions of insulators. It has been demonstrated that the expression of the gene is appropriate if the transgene is flanked by insulators.

Animal Bioreactors:

- Trans-genesis is wonderfully utilized for production proteins of pharmaceutical and medical use. In fact, any protein synthesized in the human body can be made in the transgenic animals, provided that the genes are correctly programmed.
- The advantage with transgenic animals is to produce scarce human proteins in huge quantities. Thus, the animals serving as factories for production of biologically important products are referred to as animal bioreactors or sometimes pharm animals.

Transgenic Animals in Xenotransplantation:

- Organ transplantation (kidney, liver, heart etc.) in humans has now become one of the advanced surgical practices to replace the defective, nonfunctional or severally damaged organs.
- The major limitation of transplantation is the shortage of organ donors. This often results in long waiting times and many unnecessary deaths of organ failure patients.
- Xenotransplantation refers to the replacement of failed human organs by the functional animal organs. The major limitation of xenotransplantation is the phenomenon of hyper acute organ rejection due to host immune system.

The organ rejections is mainly due to the following two causes:

a) The antibodies raised against the foreign organ.
b) Activation of host's complement system.

Pigs in Xenotransplantation?

- Some workers are actively conducting research to utilize organs of pigs in xenotransplantation. It is now identified that the major reason for rejection of pig organs by primates is due to the presence of a special group of disaccharides (Gal-α 1, 3-Gal) in pigs, and not in primates.
- The enzyme responsible for the synthesis of specific disaccharides in pigs has been identified. It is α 1, 3-galactosyltransferase, present in pigs and not in primates. Scientists are optimistic that knockout pigs lacking the gene encoding the enzyme α 1, 3-galactosyltransferase can be developed in the next few years.
- Another approach is to introduce genes in primates that can degrade or modify Gal-α 1, 3-Gal disaccharide groups (of pigs). This will reduce immunogenicity.

- Besides the above, there are other strategies to avoid hyperactive organ rejection by the hosts in xenotransplantation.
    - Expression of antibodies against the pig disaccharides.
    - Expression of complement— inactivating protein on the cell surfaces.
- By the above approaches, it may be possible to overcome immediate hyperactive rejection of organs. The next problem is the delayed rejection which involves the macrophages and natural killer cells of the host.
- Another concern of xenotransplantation is that the endogenous pig retroviruses could get activated after organ transplantation. This may lead to new genetic changes with unknown consequences.
- The use of transgenic animals in xenotransplantation is only at the laboratory experimental stages, involving animals. It is doubtful whether this will become a reality in the near future.
- There is a vigorous debate concerning the ethics of xenotransplantation and the majority of general public are against it.

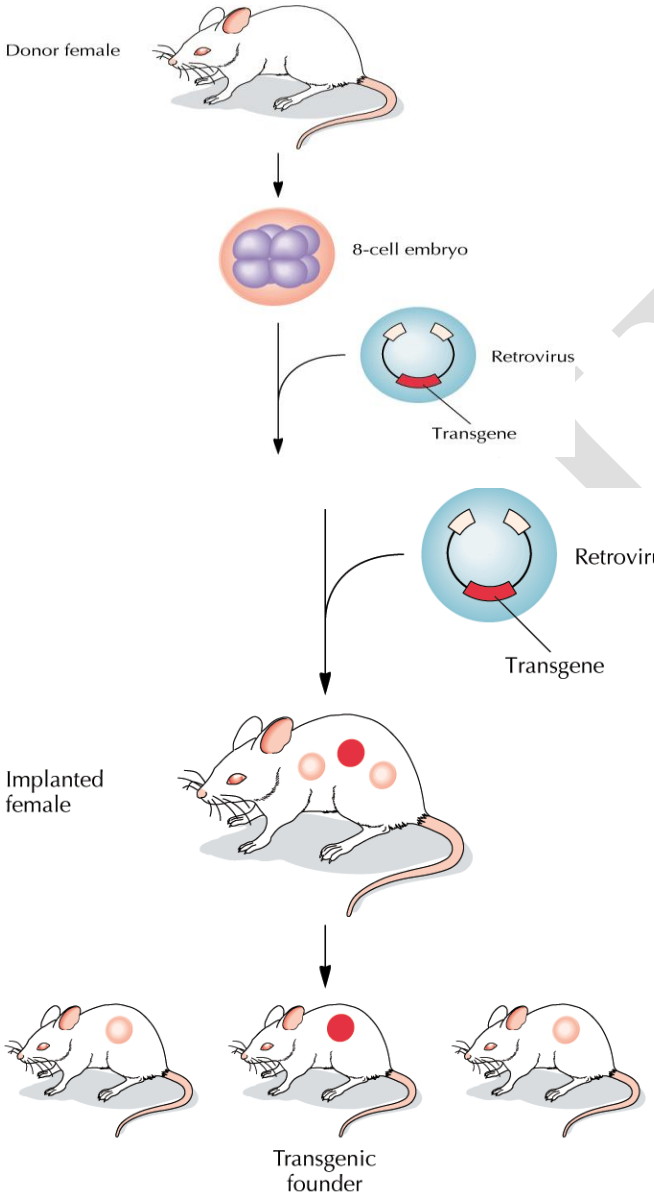
Transgenesis in Mice – Methodology

- A genetically modified mouse (Mus musculus) is a mouse that has had its genome altered through the use of genetic engineering techniques.
- Genetically modified mice are commonly used for research or as animal models of human diseases, and are also used for research on genes.
- There are two basic technical approaches to produce genetically modified mice. The first involves pronuclear injection into a single cell of the mouse embryo, where it will randomly integrate into the mouse genome.
- This method creates a transgenic mouse and is used to insert new genetic information into the mouse genome or to over-express endogenous genes.
- The second approach, pioneered by Oliver Smithies and Mario Capecchi, involves modifying embryonic stem cells with a DNA construct containing DNA sequences homologous to the target gene. Embryonic stem cells that recombine with the genomic DNA are selected for and they are then injected into the mice blastocysts.
- This method is used to manipulate a single gene, in most cases "knocking out" the target gene, although more subtle genetic manipulation can occur (e.g. only changing single nucleotides)

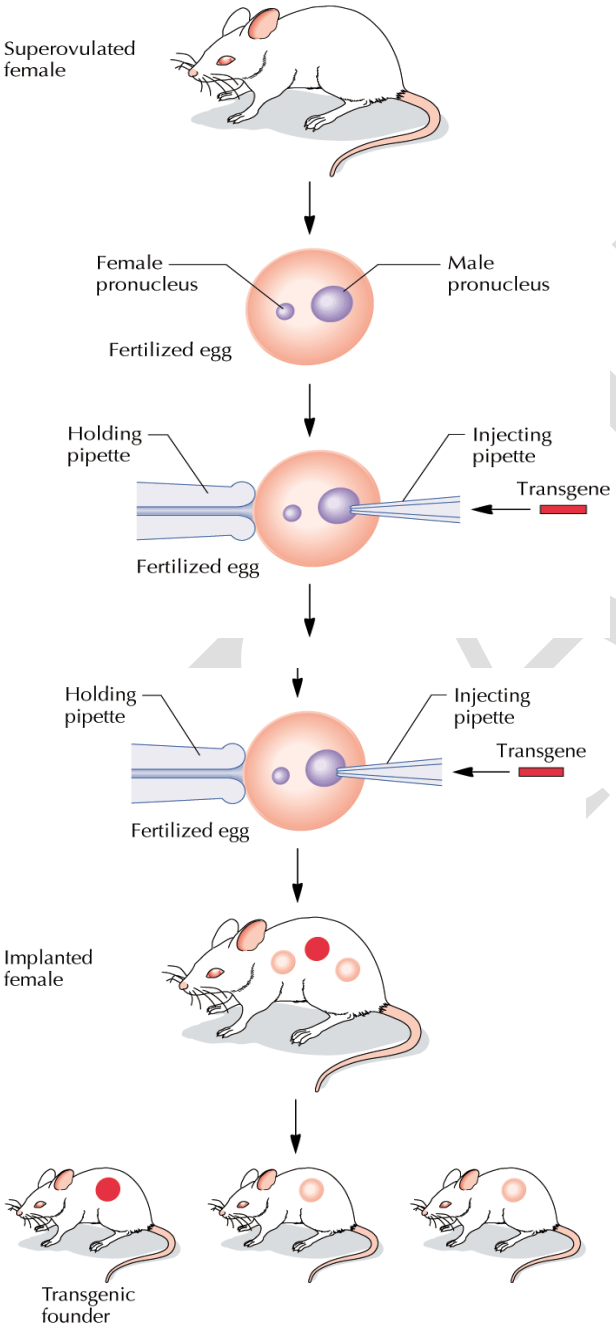
Transgenic Mouse – Uses and applications

- Genetically modified mice are used extensively in research as models of human disease. Mice are a useful model for genetic manipulation and research, as their tissues and organs are similar to that of a human and they carry virtually all the same genes that operate in humans.
- They also have advantages over other mammals, in regards to research, in that they are available in hundreds of genetically homogeneous strains. Also, due to their size, they can be kept and housed in large numbers, reducing the cost of research and experiments.
- The most common type is the knockout mouse, where the activity of a single (or in some cases multiple) genes are removed. They have been used to study and model obesity, heart disease, diabetes, arthritis, substance abuse, anxiety, aging and Parkinson disease.
- Transgenic mice generated to carry cloned oncogenes and knockout mice lacking tumor suppressing genes have provided good models for human cancer. Hundreds of these oncomice have been developed covering a wide range of cancers affecting most organs of the body and they are being refined to become more representative of human cancer. The disease symptoms and potential drugs or treatments can be tested against these mouse models.
- A mouse has been genetically engineered to have increased muscle growth and strength by overexpressing the insulin-like growth factor I (IGF-I) in differentiated muscle fibers.
- Another mouse has had a gene altered that is involved in glucose metabolism and runs faster, lives longer, is more sexually active and eats more without getting fat than the average mouse (see Metabolic supermice).

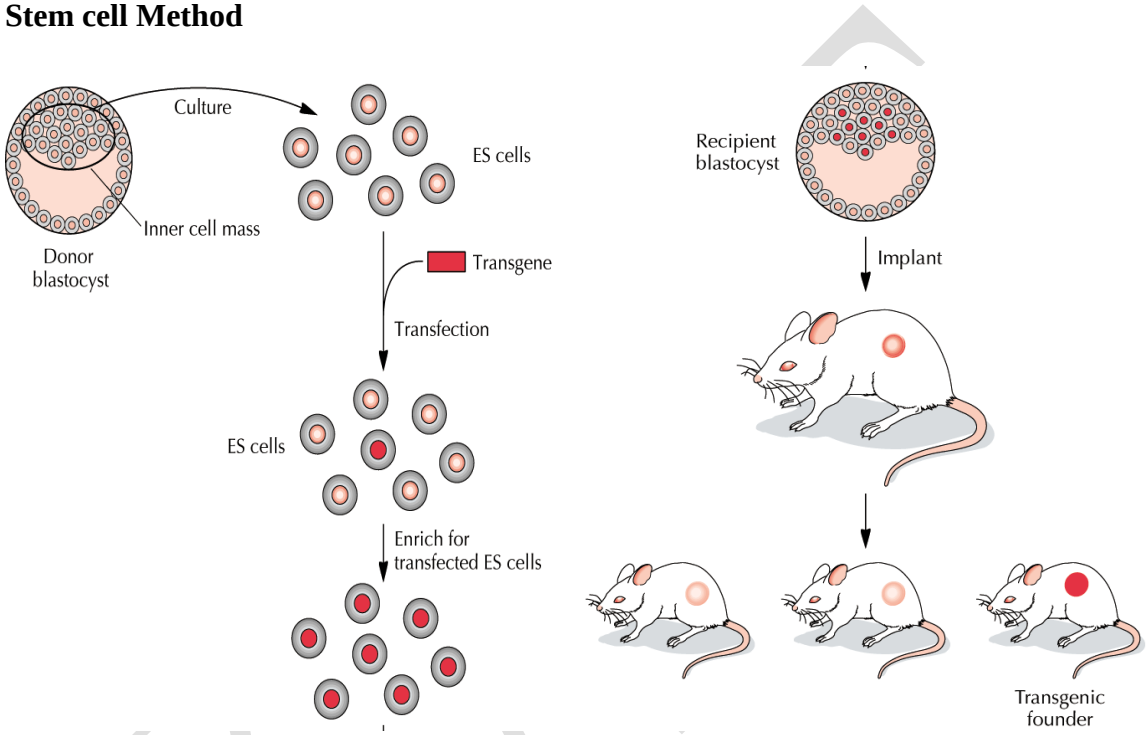
Retroviral Vector



Microinjection



Stem cell Method



Transgenic Cow

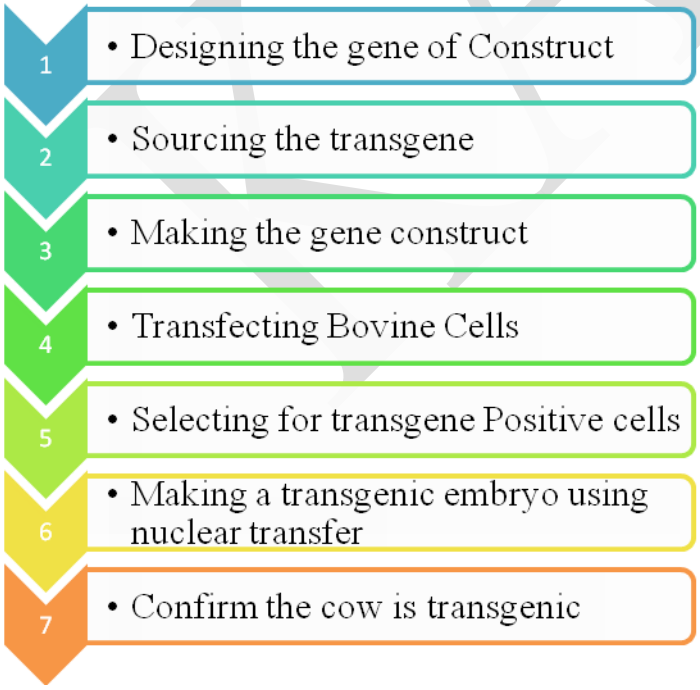
- Transgenic cows are genetically modified (GM) cows. They have an extra gene or genes inserted into their DNA. The extra gene may come from the same species or from a different species.
- The extra gene (transgene) is present in every cell in the transgenic cow. However, it's only expressed in mammary tissue. This means that the transgene's protein will only be found in the cow's milk and can only be extracted from there.

Techniques used to make transgenic cows

Step 1. Designing the gene construct

The first step is to design a gene construct. The gene construct is a unit of DNA that includes:

a) an antibiotic resistance gene – to select cells that have taken up the gene construct
b) a tissue-specific promoter sequence – to signal the start of expression of the protein in cells of the appropriate tissue, for example, in mammary cells in lactating cows
c) the desired gene – for example, bovine casein or human myelin basic protein
d) a stop sequence – to define the end of the information for making the protein.



Step 2. Sourcing the transgene

- In the past, the gene would have been extracted from the source organism's DNA. Now, however, if the desired gene sequence is known, it can simply be synthesised in a lab.
- There are companies that make genes to order within a couple of weeks.

Step 3. Making the gene construct

- A gene construct contains all the information needed for transfection into a bovine cell and expression of the desired gene in a cow. This includes an antibiotic resistance marker, a tissue-specific promoter, the transgene/gene of interest and a stop sequence.
- The gene is usually supplied in a vector. A vector is a small piece of DNA, often a plasmid, into which a foreign piece of DNA can be inserted. When the gene of interest is in a vector, it can be sent from one lab to another, it can be stored, it can be manipulated or it can be used to transform bacteria to produce more copies of the gene of interest.
- Vectors have multiple restriction enzymes sites (also called multiple cloning sites) so the gene can be inserted into the vector and then cut out from the vector using restriction enzymes. This article has more information on restriction enzymes.
- After the gene is cut from the vector, it is then pasted into the multiple cloning site of the gene construct using a method known as ligation. This article has more information on DNA ligation.

Step 4. Transfecting bovine cells

- The gene construct is incorporated into the genome of a bovine (cow) cell using a technique called transfection. During transfection, holes are made in the cell membrane that allow the DNA to enter.
- The holes can be made by applying an electrical pulse or by adding chemicals to the cells. Once inside the cell, the gene construct may enter the nucleus and incorporate into the cell's genome.

Step 5. Selecting for transgene positive cells

- After transfection, an antibiotic is added to select the bovine cells that have incorporated the gene construct. Transgenic bovine cells will survive treatment with an antibiotic, because they contain an antibiotic resistance gene making them resistant to the antibiotic.
- Cells without the gene construct will have no resistance to the antibiotic and will die. In addition to antibiotic selection, polymerase chain reaction (PCR) is used to check that the bovine cell contains the transgene.

Step 6. Making a transgenic embryo using nuclear transfer

- Nuclear transfer is used to create a whole animal from a single transgenic bovine cell.
- The transgenic bovine cell is fused with a bovine oocyte that has had its chromosomes removed (called an enucleated oocyte). An electrical pulse is applied to help fuse the cells. Once fused with the oocyte, the transgenic cell's chromosomes are reprogrammed to direct development into an embryo.
- After 7 days, the transgenic embryo will have about 150 cells and can be transferred into a recipient cow for further development to term.

Step 7. Confirming the cow is transgenic

- If the embryo develops to full term, after 9 months, the cow will give birth to a calf. To confirm that the calf is transgenic, scientists can check using:
- PCR to determine the presence or absence of the transgene
- quantitative PCR (q-PCR) to determine the number of copies of the transgene
- fluorescent in situ hybridization (FISH) to visualize where the transgene is in the chromosome and whether the transgene has integrated into more than one chromosome.
- When the calf is lactating (either after being induced to lactate or after having its own progeny), its milk is checked to determine if the transgenic protein is being expressed.

Transgenic sheep, goat

- The birth of Dolly was soon followed by that of a cloned transgenic sheep named Polly (Schnieke et al., 1997).
- The advantages of this method of adding genes are multiple. About two to five times fewer sheep are needed to generate transgenic sheep than by microinjection. The integrated gene may be examined in cells before nuclear transfer.
- Cells in which the foreign gene is rearranged or has too many copies may be discarded. The sex and, more generally, the genotype of the nuclear donors may be chosen. The founder animals are never mosaic for the transgene.
- Several animals having the same genotype, including the same transgene, can be generated simultaneously.
- Although cloning is a laborious technique, it offers some flexibility to the experimenters. The nuclear donor cells can be kept frozen and used at the most appropriate moment to generate cloned transgenic animals.

Transgenic Sheep and Goats

- Until recently, the transgenes introduced into sheep inserted randomly in the genome and often worked poorly.
- However, in July 2000, success at inserting a transgene into a specific gene locus was reported. The gene was the human gene for alpha1-antitrypsin, and two of the animals expressed large quantities of the human protein in their milk.

This is how it was done

Sheep fibroblasts (connective tissue cells) growing in tissue culture were treated with a vector that contained these segments of DNA:

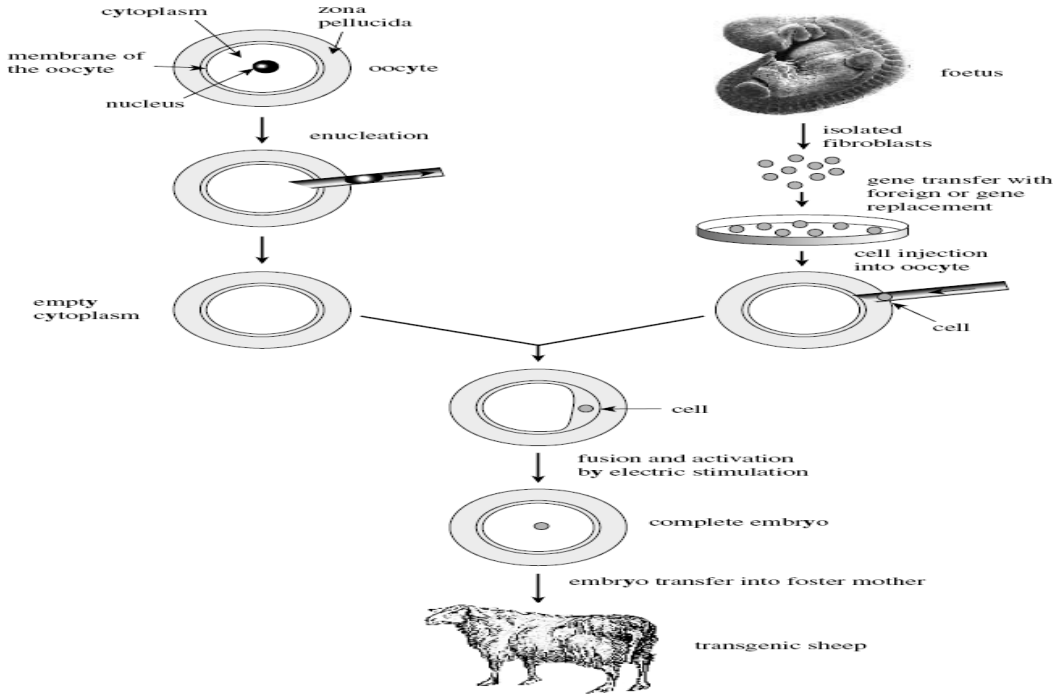
1. 2 regions homologous to the sheep *COL1A1* gene. This gene encodes Type 1 collagen. (Its absence in humans causes the inherited disease osteogenesis imperfect.)

   This locus was chosen because fibroblasts secrete large amounts of collagen and thus one would expect the gene to be easily accessible in the chromatin.

2. A neomycin-resistance gene to aid in isolating those cells that successfully incorporated the vector. The human gene encoding alpha1-antitrypsin.

   Some people inherit two non- or poorly-functioning genes for this protein. Its resulting low level or absence produces the disease **Alpha1-Antitrypsin Deficiency** (**A1AD** or **Alpha1**). The main symptoms are damage to the lungs (and sometimes to the liver).
3. Promoter sites from the **beta-lactoglobulin** gene. These promote hormone-driven gene expression in milk-producing cells.
4. Binding sites for ribosomes for efficient translation of the mRNAs.

Successfully-transformed cells were then fused with enucleated sheep eggs and implanted in the uterus of a ewe (female sheep). Several embryos survived until their birth, and two young lambs have now lived over a year. When treated with hormones, these two lambs secreted milk containing large amounts of alpha1-antitrypsin (650 µg/ml; 50 times higher than previous results using random insertion of the transgene).

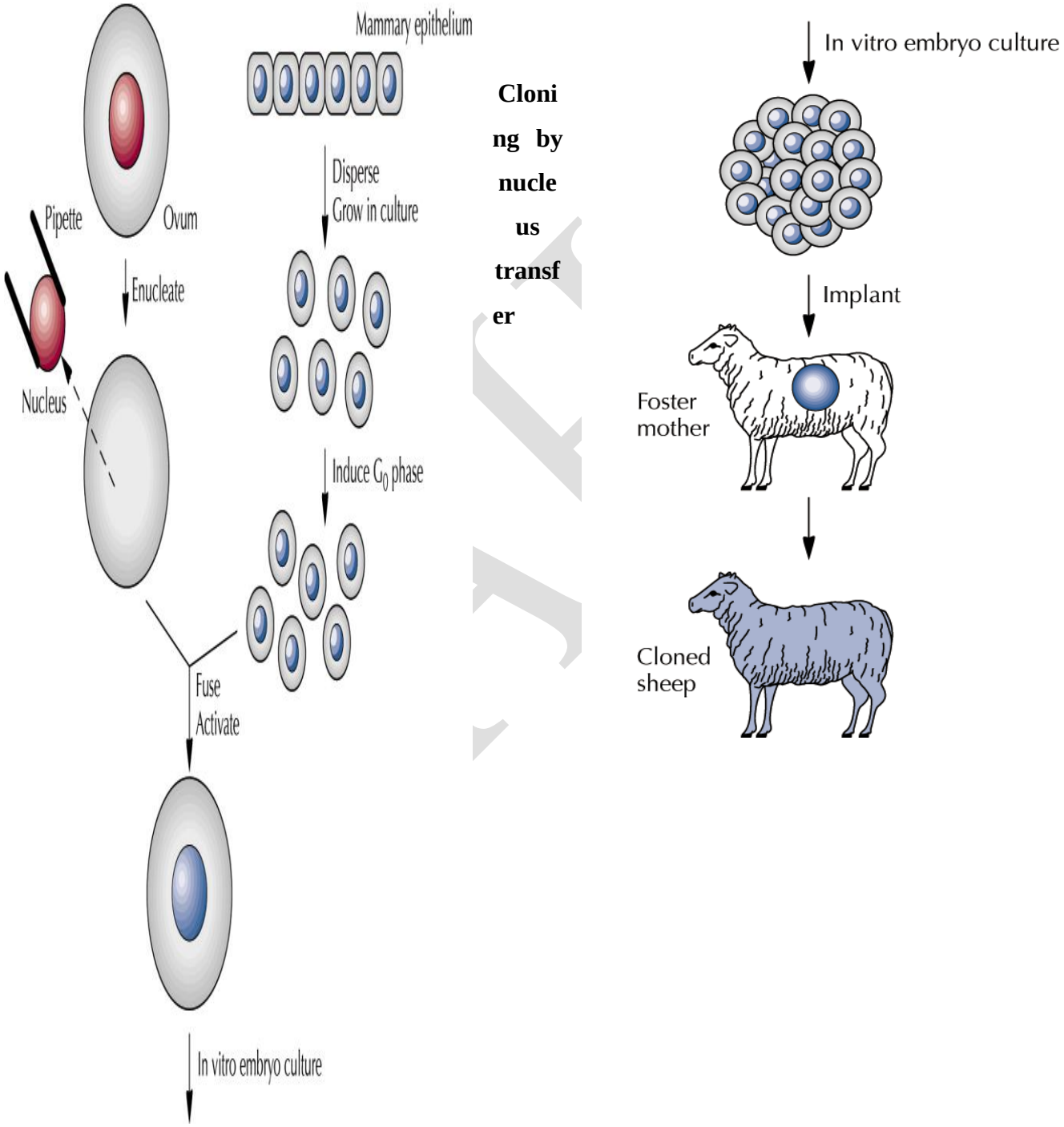


On June 18, 2003, the company doing this work abandoned it because of the great expense of building a facility for purifying the protein from sheep's milk. Purification is important because even when 99.9% pure, human patients can develop antibodies against the tiny amounts of sheep proteins that remain.

However, another company, GTC Biotherapeutics, has persevered and in June of 2006 won preliminary approval to market a human protein, antithrombin, in Europe. Their protein the first made in a transgenic animal to receive regulatory approval for human therapy was secreted in the milk of transgenic goats.

Transgenic Pigs

- Transgenic pigs have also been produced by fertilizing normal eggs with sperm cells that have incorporated foreign DNA. This procedure, called sperm-mediated gene transfer (SMGT) may someday be able to produce transgenic pigs that can serve as a source of transplanted organs for humans.
- Gene replacement was achieved in sheep, mice and pigs. This method is very laborious and still poorly controlled. A recent study showed that homologous recombination of two genes could be obtained in sheep cells but that this was followed by the death of the newborn animals obtained by cloning. This failure may be attributed to the culture of the cells, which is required to select those in which the homologous recombination has occurred.
- The culture conditions modify the physiology of the cells, which, for unknown reasons, become less capable of generating living cloned animals. A better understanding of these phenomena is necessary before gene replacement in large animals can be considered as a truly viable method.



Cloning by nucleus transfer

Transgenic fish

- Genetically modified fish (GM fish) are organisms from the taxonomic clade which includes the classes Agnatha (jawless fish), Chondrichthyes (cartilaginous fish) and Osteichthyes (bony fish) whose genetic material (DNA) has been altered using genetic engineering techniques. In most cases, the aim is to introduce a new trait to the fish which does not occur naturally in the species, i.e. transgenesis.
- The first transgenic fish were produced in China in 1985. As of 2013, approximately 50 species of fish have been subject to genetic modification. This has resulted in more than 400 fish/trait combinations. Most of the modifications have been conducted on food species, such as Atlantic salmon (*Salmo salar*), tilapia (genus) and common carp (*Cyprinus carpio*).
- Generally, genetic modification entails manipulation of DNA. The process is known as cisgenesis when a gene is transferred between organisms that could be conventionally bred, or transgenesis when a gene from one species is added to a different species. Gene transfer into the genome of the desired organism, as for fish in this case, requires a vector like a lentivirus or mechanical/physical insertion of the altered genes into the nucleus of the host by means of a micro syringe or a gene gun

Transgenic fish are used in research covering five broad areas-

a) Enhancing the traits of commercially available fish
b) Their use as bioreactors for the development of bio-medically important proteins
c) Their use as indicators of aquatic pollutants
d) Developing new non-mammalian animal models
e) Functional genomics studies

- Most GM fish are used in basic research in genetics and development. Two species of fish, zebrafish and medaka, are most commonly modified because they have optically

clear chorions (shells), develop rapidly, the 1-cell embryo is easy to see and micro-inject with transgenic DNA, and zebrafish have the capability of regenerating their organ tissues.

- They are also used in drug discovery.[10] GM zebrafish are being explored for benefits of unlocking human organ tissue diseases and failure mysteries. For instance, zebrafish are used to understand heart tissue repair and regeneration in efforts to study and discover cures for cardiovascular diseases.
- Transgenic rainbow trout (*Oncorhynchus mykiss*) have been developed to study muscle development. The introduced transgene causes green fluorescence to appear in fast twitch muscle fibres early in development which persist throughout life. It has been suggested the fish might be used as indicators of aquatic pollutants or other factors which influence development.
- In intensive fish farming, the fish are kept at high stocking densities. This means they suffer from frequent transmission of contagious diseases, a problem which is being addressed by GM research. Grass carp (*Ctenopharyngodon idella*) have been modified with a transgene coding for human lactoferrin, which doubles their survival rate relative to control fish after exposure to Aeromonas bacteria and Grass carp hemorrhage virus. Cecropin has been used in channel catfish to enhance their protection against several pathogenic bacteria by 2–4 times.

Transgenic Chickens

Chickens

- grow faster than sheep and goats and large numbers can be grown in close quarters;
- synthesize several grams of protein in the "white" of their eggs.
- Two methods have succeeded in producing chickens carrying and expressing foreign genes.
- Infecting embryos with a viral vector carrying
- the human gene for a therapeutic protein
- promoter sequences that will respond to the signals for making proteins (e.g. lysozyme) in egg white.

- Transforming rooster sperm with a human gene and the appropriate promoters and checking for any transgenic offspring.
- Preliminary results from both methods indicate that it may be possible for chickens to produce as much as 0.1 g of human protein in each egg that they lay.

Not only should this cost less than producing therapeutic proteins in culture vessels, but chickens will probably add the correct sugars to glycosylated proteins — something that E. coli cannot do.

Applications of transgenic animals:

Transgenic animals have potentially broad application for the improvement of animal production quality, the enhancement of production capacity, the studies of human disease models and the production of biomedical materials.

The benefits of these animals to human welfare can be grouped into the following areas:

Agricultural applications

The application of biotechnology to farm animals has the potential to benefit both humans and animals in significant ways.

a. Breeding: Farmers have always used selective breeding to produce animals that exhibit desired traits (e.g., increased milk production, high growth rate). Traditional breeding is a time-consuming, difficult task. When technology using molecular biology was developed, it became possible to develop traits in animals in a shorter time and with more precision. In addition, it offers the farmer an easy way to increase yields. Take ES cell technology as an example, chimeric nuclear transfer technology and production technology is improving, as ES cells are widely used in animal cloning. Proliferation of ES cells derived from donor as the nucleus, produced cloned animals. ES cells in germline chimeric, then develop into sperm or eggs to produce offspring. Animal cloning technology can produce excellent breeding, combination of genes and their high proportion in the population in short time.

b. Quality: Transgenic cows exist that produce more milk or milk with less lactose or cholesterol, pigs and cattle that have more meat on them, and sheep that grow more wool. In the past, farmers used growth hormones to spur the development of animals but this technique was problematic, especially since residue of the hormones remained in the animal product. At present the production of transgenic animals in low efficiency is one of the main problems. The results of the testing work are carried out at the individual level. Using ES cells as a carrier, directed

transformation of ES cells, the integration of inserted genes, expression level and stability of interested genes can be screened. The work is carried out at the cellular level, which is easy to obtain stable cell line with expression of satisfaction, accessing to the target gene carrying the transgene for animals. One success story is artificial insemination: the use of this technology from 1950s to 1990s in US, increased the average milk production per cow over 300%.

Medical applications

a. Xenotransplantation: Transplant organs may soon come from transgenic animals. Transgenic pigs may provide the transplant organs needed to alleviate the shortfall. Currently, xenotransplantation is hampered by a pig protein that can cause donor rejection but research is underway to remove the pig protein and replace it with a human protein. For organ and tissue transplantation, which is known as a "species of daughter cells ", for the clinical organization, organ transplantation offers great amount of material knockout cells. U.S. ACT companies put the nucleus of human skin into bovine oocytes without the genetic information, nurturing issued totipotency cell. If they could be successfully used in clinical, in future, many difficult diseases such as Parkinson's disease will be cured.

b. Nutritional supplements and pharmaceuticals: Milk-producing transgenic animals are especially useful for medicines. Products such as insulin, growth hormone, and blood anti-clotting factors may soon be or have already been obtained from the milk of transgenic cows, sheep, or goats. Research is also underway to manufacture milk through transgenesis for treatment of debilitating diseases such as phenylketonuria (PKU), hereditary emphysema, and cystic fibrosis. ES cell culture techniques are used in some special body, then the cost can be a huge improvement. For example, some special drugs (interferon, antithrombin, erythropoietin and other biological systems agents or genetically modified), in body fluids from animals (milk, blood, etc.) or tissue extract achieve the body of the animal drug production factory.

c. Human gene therapy: A transgenic cow exists that produces a substance to help human red cells grow. Human gene therapy involves in adding a normal copy of a gene (transgene) to the genome of a person carrying defective copies of the gene. The potential for treatments for the 5,000 named genetic diseases is huge and transgenic animals could play a role. The most current human serious medical diseases are cancer, genetic diseases, including birth defects, These diseases are caused by abnormal cell transformation and differentiation, such as Lesch, Nyhan.

Fully understanding the process of cell differentiation and development will be able to cure the diseases. Many scientists have established many mouse disease models, and expressed human disease gene in mice for further treatment of human disease. For example, U.S. National Institute of Molecular Neurology Laboratory used mice ESC to induce neuroepithelial cells, implanted them into the brain, and got a large number of small conflicts like cells and glial cells. It can be envisaged to treat multiple sclerosis diseases.

Possible Questions

1. What is transgene
2. Explain Transgenesis
3. Explain in detail production of transgenic mouse
4. Briefly explain DOLLY
5. With examples, discuss Gene Therapy
6. Explain the production of transgenic sheep
7. Write in detail about production of transgenic fish
8. Briefly explain the applications of transgenic animal
9. How can a foreign gene can be inserted in cow? Explain
10. Brief about transgenic Pig.

ARTIFICIAL INSEMINATION

Introduction

- Artificial insemination is the technique in which semen with living sperms is collected from the male and introduced into female reproductive tract at proper time with the help of instruments. This has been found to result in a normal offspring.
- In this process, the semen is inseminated into the female by placing a portion of it either in a collected or diluted form into the cervix or uterus by mechanical methods at the proper time and under most hygienic conditions.
- The first scientific research in artificial insemination of domestic animals was performed on dogs in 1780 by the Italian scientist, Lazanno Spalbanzani. His experiments proved that the fertilizing power reside in the spermatozoa and not in the liquid portion of semen.
- Few further studies under research station conditions helped this technique to be used commercially all over the world including India.Artificial insemination is not merely a novel method of bringing about impregnation in females.
- Instead, it is a powerful tool mostly employed for livestock improvement. In artificial insemination the germplasm of the bulls of superior quality can be effectively utilized with the least regard for their location in far away places. By adoption of artificial insemination, there would be considerable reduction in both genital and non-genital diseases in the farm stock.

SYMPTOMS OF HEAT

The various symptoms of heat are

- The animal will be excited condition. The animal will be in restlessness and nervousness.
- The animal will be bellow frequency.
- The animal will reduce the intake of feed.
- Peculiar movement of limbo sacral region will b observed.
- The animals which are in heat will lick other animals and smelling other animals.
- The animals will try to mount other animals

- The animals will standstill when other animal try to mount.. This period is known as standing heat. This extends 14-16 hours.
- Frequent maturation (urination) will be observed.
- Clear mucous discharge will be seen from the vulva, sometimes it will be string like the mucous will be seen stick to the near the pasts of valva.
- Swelling of the valva will be seen.
- 11 Congestion and hyperemia of membrane.
- The tail will be in raised position.
- Milk production will be slightly decreased.
- On Palpation uterus will be turgid and the cervix will be opened.

Artificial insemination (A.I.) is deposition of semen into the female genital tract by means of instruments.

Advantages and disadvantages of Artificial insemination:

- There are several advantages by artificial insemination over natural mating or servicing.
- There is no need of maintenance of breeding bull for a herd; hence the cost of maintenance of breeding bull is saved.
- It prevents the spread of certain diseases and sterility due to genital diseases.
- Eg: contagious abortion, vibriosis.
- By regular examination of semen after collection and frequent checking on fertility make early detection of interior males and better breeding efficiency is ensured.
- The progeny testing can be done at an early age.
- The semen of a desired size can be used even after the death of that particular sire.
- The semen collected can be taken to the urban areas or rural areas for insemination.
- It makes possible the mating of animals with great differences in size without injury to either of the animal.
- It is helpful to inseminate the animals that are refuse to stands or accept the male at the time of oestrum.
- It helps in maintaining the accurate breeding and cawing records.
- It increases the rate of conception.

- It helps in better record keeping.
- Old, heavy and injured sires can be used.

Disadvantages of A.I:

- Requires well-trained operations and special equipment.
- Requires more time than natural services.
- Necessitates the knowledge of the structure and function of reproduction on the part of operator.
- Improper cleaning of instruments and in sanitary conditions may lead to lower fertility.
- If the bull is not properly tested, the spreading of genital diseases will be increased.
- Market for bulls will be reduced, while that for superior bull is increased.

Semen collection methods and evaluation:

Various methods of collection of semen have been devised from time to time. The older unsatisfactory methods have gradually replaced by the new modern techniques.

There are three common methods.

1. Use of artificial vagina
2. By Electro-stimulation method.
3. By massaging the ampulae of the duct us differences through rectal wall.

SEMEN STORAGE

- There are two methods of freezing and storing semen: dry ice and alcohol (-100 degrees F) and liquid nitrogen (-320 degrees F).
- Liquid nitrogen is preferred because there is no evidence of fertility deterioration with age. Fertility gradually declines in semen stored in dry ice-alcohol.
- Frozen semen can be stored indefinitely if proper temperature is maintained.

INSEMINATION METHODS

There-are different methods insemination in different species of animals

1. speculum method
2. vaginal method
3. recto vaginal method.

RECTO VAGINAL METHOD

- In cattle the safe and best method of insemination is "Recto vaginal method of insemination". Cow which is in heat is well controlled placing it in a Travis.
- The inseminator will get ready by wearing a plastic apron, gumboots and gloves.
- The semen straw after thawing (keeping the semen straw in warm water for a minute to convert the freezed semen into liquid and the sperms become motile) is loaded in a sterilized A.I. gum and is covered with a plastic sheath.
- The inseminator will insert the gloved left hand into the rectum after applying the soft soap or other lubricant on the glove and back racked the animal, and the hand is further inserted and will catch hold the cervix through rectal wall.
- The A.I gum loaded with semen straw is passed.
- Through the vulva to 'vagina and cervix and observed with the hand in rectum that the A. I gum reaches the cervix, then the semen is deposited by injecting the gun, and after depositing the semen the gun is removed, the empty straw and sheath are disordered.

SPECTRUM METHOD

- In this method spectrum is placed in the vagina of the cow, which provides passage outside to the site of insemination, then inseminating tube is passed through the speculum and semen is deposited at the cervix insemination method.

VAGINAL METHOD

- Hand is passed through the vagina and the inseminating tube is guided by hand to the site of insemination and semen is deposited.
- Here there is a risk of contamination and injury of female genitalia.

Artificial insemination (AI) of cattle

Artificial insemination (AI) is the process of collecting sperm cells from a male animal and manually depositing them into the reproductive tract of a female.

Benefits of artificial insemination.

Increased efficiency of bull usage

- During natural breeding, a male will deposit much more semen than is theoretically needed to produce a pregnancy. In addition, natural breeding is

physically stressful. Both of these factors limit the number of natural mating a male can make.

- However, collected semen can be diluted and extended to create hundreds of doses from a single ejaculate. Also, semen can be easily transported; allowing multiple females in different geographical locations to be inseminated simultaneously, and semen can be stored for long periods of time, meaning that males can produce offspring long after their natural reproductive lives end.

Increased potential for genetic selection

- Because artificial insemination allows males to produce more offspring, fewer males are needed. Therefore, one can choose only the few best males for use as parents, increasing the selection intensity.
- Furthermore, because males can have more offspring, their offspring can be used in a progeny test program to more accurately evaluate the genetic value of the male.
- Finally, individual farmers can use artificial insemination to increase the genetic pool with which his or her animals can be mated, potentially decreasing effects of inbreeding.

Decreased costs

- Male animals often grow to be larger than females and can consume relatively larger amounts of feed.
- Also, male animals are often more strong, powerful, and potentially ill-mannered and thus require special housing and handling equipment.

Increased safety for animals and farmers

- As mentioned, male animals can become large and aggressive. These factors mean that maintaining a bull on a farm may be dangerous.
- Also, because of the relatively larger size of adult males than females, natural mating is more likely to result accidents and injury to either the cow or the bull than is artificial insemination.

Reduced disease transmission

- Natural mating allows for the transfer of venereal diseases between males and females. Some pathogens can be transmitted in semen through artificial insemination, but the collection process allows for the screening of disease agents.
- Collected semen is also routinely checked for quality, which can help avoid problems associated with male infertility.
- Artificial insemination has some potential drawbacks, however, that must be considered. First, it can be more laborious. Male animals instinctively detect the females that are in the correct status for conception.
- With artificial insemination the detection work falls on the responsibility of the farmer. Poor detection results in decreased rates of fertility.
- Also, increasing the number of offspring per male has selective advantages only if the best males can be accurately determined.
- Otherwise this process only decreases the genetic variability in a population. Increasing the number of offspring per male always reduces the gene pool.
- The benefits of more intense selection must be balanced against the negative effects of decreased variation.

Cloning

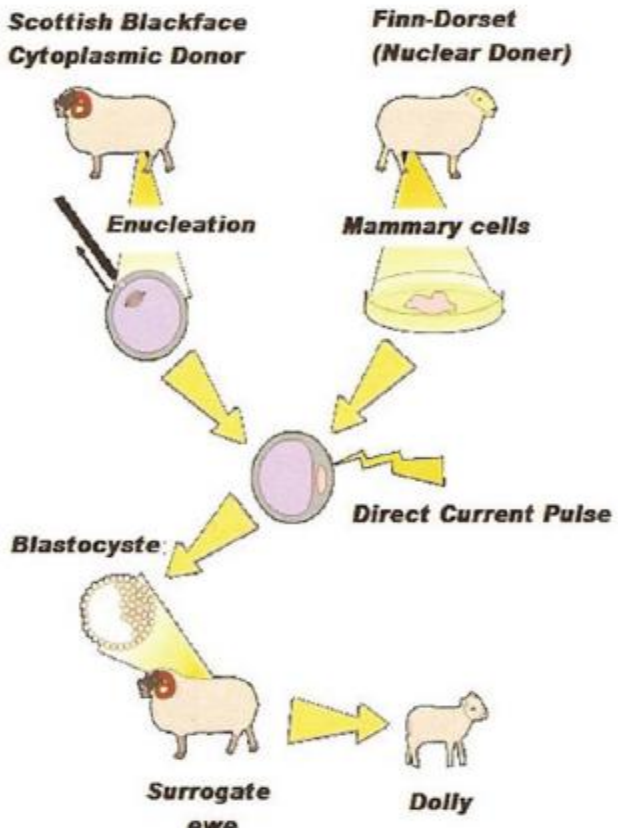
- **Cloning** is the production of an exact copy of an animal by means of asexual reproduction. Any two animals which contain exactly the same genes are called genetically identical. An animal which is genetically identical to its parents is called **clone.** The cloning of a large animal was successfully done for the first time by **Ian Wilmut** and his colleagues at the **Roslin Institute in Edinburg**.They cloned a sheep named Dolly from its parent sheep called Finn Dorest sheep. **Dolly** was born on **5th** July,**1996**.Dolly sheep was the first mammal to be cloned.
- The cloning in animals is done by the transfer of nucleus of a cell. The nucleus of a normal body cell of the animal is transferred into an empty egg cell .The newly formed egg cell is allowed to develop normally.An exact copy of the animal is produced. Ewe is a female sheep.The two sheep which are involved in the cloning of Dolly Sheep were **Finn Dorset ewe** and **Scottish Blackface ewe**.
- A normal body cell was removed from the mammary gland of a Female Dorset sheep. An unfertilised egg cell was taken from a Female Scottish Blackface sheep and its nucleus was removed, leaving the egg cell empty.
- The nucleus of normal body cell of Finn Dorset sheep was inserted into the empty egg cell of Scottish Blackface sheep.
- The new egg cell was implanted in the uterus of another female Scottish Blackface sheep making it pregnant. After 148 days, this pregnant Scottish Blackface sheep gave birth to Dolly sheep.
- Though Dolly sheep was given birth by Scottish blackface sheep, it was found to be exactly identical to the original Finn Dorset Sheep from whose cell nucleus was taken.
- All the body cells of Dolly sheep contained the same set of chromosomes having exactly the same genes as the Finn Dorset Cells.
- Dolly was a clone of Finn Dorset sheep and produced several offsprings of her own through sexual means in due course of time.Dolly had exactly the a same

genetic material as her real mother Finn Dorset sheep.Dolly dies in 14th February in 2003 due to a lung disease.

- The cloning of superior cows which gives high milk yield has become possible now. If a high yielding cow was to be mated with a bull for reproduction, then the resulting offspring cow may or may not be of high milk yielding breed.

Advantages of cloning

- This technique of cloning enable us to produce exactly identical copies of domestic animals having favourable characteristics.
- A cow which gives high milk yields can be cloned to produce exactly identical Cows which will give high milk yield.
- A sheep which gives high yield of superior quality wool can be cloned to produce exactly identical sheep which will give high yield of superior quality wool



Embryo transfer

The process by which an embryo is collected (flushed) from one female (the donor) and transferred to another female (the recipient) to complete the gestation period.

Purpose of Embryo transfer

- Increase the number of offspring, either male or female, from a genetically superior female. Allow ease of import/export.
- Genetic testing of bulls for inherited defects.
- Produce twins, although 12-15% of the offsprings will be freemartins (i.e. infertile females).
- Decrease variability in research subjects—for the study of the physiology, pathology and immunology of reproduction or other traits. Purpose
- Long-term storage by freezing.
- Disease control. Many diseases present in the dam will not be transmitted by the embryo.
- Treatment of infertility.
- Rapid genetic change within a small population

Procedure

- When a donor is selected, her estrous cycles are recorded.
- The producer decides whether a single fertilized ovum is going to be recovered or if super ovulation is desired.
- If a single fertilized ovum is desired, the donor can be flushed 6-8 days after a normal estrus and breeding.
- The recipient must be in the same stage of the estrous cycle as the donor. Therefore, it will be necessary to synchronize estrus in the donor and recipient.
- If super ovulation is the choice, the cow is treated with the gonadotropin (FSH - Follicle Stimulating Hormone), between day 9 and 14 of the estrous cycle. FSH treatment is given twice a day for 4 or 5 days resulting in the development of multiple follicles on the ovaries of the donor. 2-3 days after beginning the FSH treatment, prostaglandin injections

are given to both the donor and the selected recipients to initiate a synchronized estrous period 2 to 3 days later.

- At least 10 recipients should be in the same stage of the estrous cycle as the donor cow. Therefore, 14-18 recipients will need to be synchronized. When in standing estrus or shortly thereafter, the super ovulated donor is bred with frozen semen.
- Usually multiple inseminations at 12, 24 and 36 hours after the onset of estrus are recommended.
- Preferably, more than one vial or ampule of high-quality semen is used per insemination.
- The correct site for semen placement is in the body of the uterus. The resulting embryo(s) is (are) flushed from the donor's uterus 6 to 8 days later.
- Flushing is done by inserting a catheter with an inflatable balloon into the donor's uterus and washing a limited area with continuous or intermittent flushes of 30-200 ml. of a saline solution or other suitable culture media.
- Each uterine horn is flushed separately.
- The embryos are flushed out in the saline solution, which is collected. After 30 minutes, embryos settle and can be located using a stereoscopic microscope.
- When an embryo is found, it is washed and transferred to fluid containing bovine serum.
- The embryo is evaluated for stage of development and quality.
- Healthy embryos can be transferred to synchronized recipients (in estrus ± 1 day from the donor).

Major criteria for evaluation

- Regularity of shape of the embryo
- Compactness of the blastomeres (the dividing cells within the boundaries of the embryo)
- Variation in cell size
- Color and texture of the cytoplasm (the fluid within the cell wall)
- Overall diameter of the embryo
- Presence of extruded cells
- Regularity of the zona pellucida (the protective layer of protein and polysaccharides around the
- single celled embryo)

- Presence of vesicles (small bubble-like tructures in the cytoplasm)

Selection and preparation of recipient females

- Reproductively sound
- Exhibit calving ease
- Good milking and mothering
- Body condition score 6 for beef cows and dairy body condition score 3 to 4 for dairy breed recipients.
- Sound herd health The embryo may be surgically or non-surgically transferred into the uterus of the recipient.
- Today, non-surgical transfers are becoming more common as success rates are approaching those obtained with surgery.
- An artificial insemination Cassou gun is used to deposit the embryo into the uterine horn.
- Care must be taken to be non-traumatic and to prevent the introduction of microorganisms. After 30 minutes, embryos settle and can be located using a stereoscopic microscope.
- When an embryo is found, it is washed and transferred to fluid containing bovine serum.
- The embryo is evaluated for stage of development and quality.
- Healthy embryos can be transferred to synchronized recipients (in estrus ± 1 day from the donor).

Transfer of Embryos

- Loading of the embryo into a 1/4-ml insemination straw.
- Done under microscopic view, with the aid of a 1-ml syringe.
- Just prior to embryo transfer, the ovaries of the recipient are palpated rectally to determine which ovary has ovulated.
- The transfer gun or insemination rod is carefully passed through the cervix towards uterus. Pregnancy rates following transfer range from 30 to 70%; depending on embryo quality, synchronizations, and method of transfer.
- Embryos may be stored up to 24 hours with little trouble. This enables easy transport to
- other farms and even to other countries.
- Embryos kept at 37°C will continue to develop.

- When kept at low temperatures (O to 10°C) development will halt. If long-term storage is required, embryos must be frozen.
- Freezing, however, will damage 30 to 50% of the embryos because of ice crystal formation within the embryo during freezing and thawing.

Selection of donor cow

- Regular heat cycles commencing at a young age.
- Cycle lengths should be 18 to 24 days.
- A history of no more than two breedings per conception.
- Previous calves having been born at approximately 365-day intervals.
- No parturition difficulties or reproductive irregularities.
- No conformational or detectable genetic defects.
- Appropriate nutritional status for her body size and level of milk production.
- Both the very obese cow and the thin cow will have reduced fertility, so it is important that the donor cow be in an appropriate body condition score at the time of embryo transfer

Recent developments in Embryo transfer

Embryo splitting

- Using a device called a micromanipulator, it is possible to split or bisect the mass inside an early
- developing embryo.
- When half of the cells are removed and put into an evacuated zona (the protecting shell-like coating), both embryos will continue to develop resulting in identical twins.
- This technique is successful, but equipment costs and the need for manipulative techniques have
- limited its usefulness.

Embryo Sexing

- Embryos can be sexed by identification of the male and female chromosomes.
- This procedure is slow and detrimental to embryo survival.
- Recently, however, progress in embryo sexing has been made using a simple and fast antibody test to react with the male chromosome.

- The dairy industry eagerly awaits the perfection of this scientific advancement.
- *In Vitro* Fertilization
- The fertilization of an egg in a test tube is not commercially available for cattle, but it has been done.
- The number of potential ova in the ovaries of a heifer exceeds 75,000 when she is born.
- After maturation any number of these ova could be harvested for in vitro fertilization.
- Collectively these procedures would make the impact of ET limitless.

Advantages

- Increased number of calves out of genetically superior cows.
- Increased marketing opportunities through the sale of offspring, pregnancies, and embryos.
- Generate more offspring from rare and valuable semen.
- Larger numbers of offspring can help prove the genetic merits of a female at an earlier age in life.

Disadvantages

- Increased expenses and higher breakeven costs for calves.
- Requires a higher level of management.
- Increased potential for spread of certain diseases.
- Not all potential donors respond positively to treatment.

Stem cells

- Stem cells are defined as cells that have clonogenic and self-renewing capabilities and differentiate into multiple cell lineages. Stem cells are found in all of us, from the early stages of human development to the end of life.
- Stem cells are basic cells of all multicellular organisms having the potency to differentiate into wide range of adult cells
- Self renewal and totipotency are characteristic of stem cell. Though totipotency is shown by very early embryonic stem cells, the adult stem cells possess multipotency and differential plasticity which can be exploited for future generation of therapeutic options
- All stem cells may prove useful for medical research, but each of the different types has both promise and limitations.
- For decades, researchers have been studying the biology of stem cells to figure out how development works and to find new ways of treating health problems.
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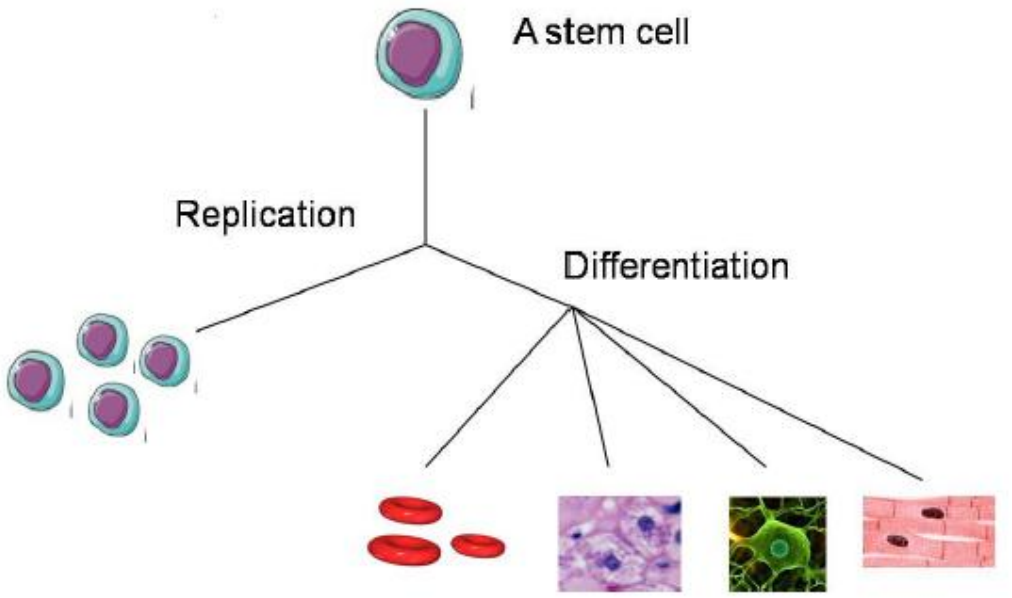
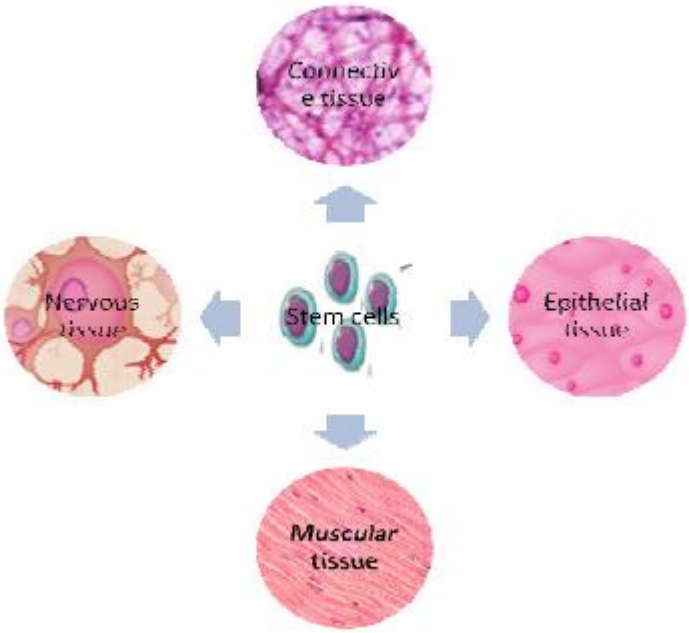


Figure 1. Characters of stem cell: replication and differentiation

- The scientific researchers and medical doctors of today hope to make the legendary concept of regeneration into reality by developing therapies to restore lost, damaged, or aging cells and tissues in the human body.
- This research has opened new horizons for stem cell research. Stem cell research holds tremendous promise for the development of novel therapies for many serious diseases and injuries.
- While stem cell-based treatments have been established as a clinical standard of care for some conditions, such as hematopoietic stem cell transplants for leukemia and epithelial stem cell-based treatments for burns and corneal disorders, the scope of potential stem cell-based therapies has expanded in recent years due to advances in stem cell research .
- Stem cells can now be grown and transformed into specialized cells with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture.
- Highly plastic adult stem cells from a variety of sources, including umbilical cord blood and bone marrow, are routinely used in medical therapies. This review focuses on types of stem cells, their sources, stem cell research and future aspects.
- A stem cell is a non-specialized, generic cell which can make exact copies of itself indefinitely and can differentiate and produce specialized cells for the various tissues of the body2 .Stem cells are cells found in most, if not all, multi-cellular organisms.
- They are characterized by selfrenewal and potency i.e. - the ability to renew themselves through mitotic cell division and differentiating into a diverse range of specialized cell types. They are vital to the development, growth, maintenance, and repair of our brains, bones, muscles, nerves, blood, skin, and other organs .
- Laboratory studies of stem cells enable scientists to learn about the cells' essential properties and what makes them different from specialized cell types. Scientists are already using stem cells in the laboratory to screen new drugs and to develop model systems to study normal growth and identify the causes of birth defects.
- Research on stem cells continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. Stem cell research is one of the most fascinating areas of contemporary biology, but, as with

many expanding fields of scientific inquiry, research on stem cells raises scientific questions as rapidly as it generates new discoveries.

- Over the past year, adult stem cells have been used either exclusively or in combination with other treatments to achieve significant "healthcare benefits" for sufferers of the every tissue of human body.



Classification of stem cells on the basis of potency Stem cells can be classified by the extent to which they can differentiate into different cell types.

These four main classifications are

1. totipotent,
2. pluripotent,
3. multipotent, or unipotent.
4. Totipotent

- **Totipotent:** The ability to differentiate into all possible cell types. Examples are the zygote formed at egg fertilization and the first few cells that result from the division of the zygote.

- **Pluripotent:** The ability to differentiate into almost all cell types. Examples include embryonic stem cells and cells that are derived from the mesoderm, endoderm, and ectoderm germ layers that are formed in the beginning stages of embryonic stem cell differentiation.
- **Multipotent:** The ability to differentiate into a closely related family of cells. Examples include hematopoietic (adult) stem cells that can become red and white blood cells or platelets.
- **Oligopotent**: The ability to differentiate into a few cells. Examples include (adult) lymphoid or myeloid stem cells.
- **Unipotent:** The ability to only produce cells of their own type, but have the property of selfrenewal required to be labeled a stem cell. Examples include (adult) muscle stem cells

Classification of stem cells on the basis of their sources

- The easiest way to categorize stem cells is by dividing them into two types: Early or embryonic and mature or adult. Early stem cells, often called embryonic stem cells, are found in the inner cell mass of a blastocyst after approximately five days of development.
- Mature stem cells are found in specific mature body tissues as well as the umbilical cord and placenta after birth.

Embryonic stem cells

- Embryonic stem cells are self replicating pluripotent cells that are potentially immortal. They are derived from embryos at a developmental stage before the time of implantation would normally occur in the uterus.
- The embryos from which human embryonic stem cells are derived are typically four or five days old and are a hollow microscopic ball of cells called the blastocyst.

Adult stem cells

- Adult stem cells are undifferentiated totipotent or multipotent cells, found throughout the body after embryonic development that multiply by cell division to replenish dying cells and regenerate damaged tissues.
- The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Unlike embryonic stem cells, which are defined by their

origin (the inner cell mass of the blastocyst), the origin of adult stem cells in some mature tissues is still under investigation.

Pluripotent stem cells

- Recently, a third type of stem cell, with properties similar to embryonic stem cells, has emerged. Scientists have engineered these induced pluripotent stem cellsi (iPS cells) by manipulating the expression of certain genes - 'reprogramming' somatic cells back to a pluripotent state.

Stem cell culture

- Growing cells in the laboratory is known as cell culture. Human embryonic stem cells (hESCs) are generated by transferring cells from a preimplantation stage embryo into a plastic laboratory culture dish that contains a nutrient broth known as culture medium.
- The cells divide and spread over the surface of the dish. However, if the plated cells survive, divide and multiply enough to crowd the dish, they are removed gently and plated into several fresh culture dishes.
- The process of replating or sub culturing the cells is repeated many times and for many months. Each cycle of subculturing the cells is referred to as a passage. Once the cell line is established, the original cells yield millions of embryonic stem cells.
- Embryonic stem cells that have proliferated in cell culture for six or more months without differentiating, are pluripotent, and appear genetically normal are referred to as an embryonic stem cell line. At any stage in the process, batches of cells can be frozen and shipped to other laboratories for further culture and experimentation.

Stem cell lines

- A stem cell line is a family of constantly dividing cells, the product of a single parent group of stem cells.
- They are obtained from human or animal tissues and can replicate for long periods of time in vitro ("within glass"; or, commonly, "in the lab", in an artificial environment). They are frequently used for research relating to embryonic stem cells or cloning entire organism.
- Once stem cells have been allowed to divide and propagate in a controlled culture, the collection of healthy, dividing, and undifferentiated cells is called a stem cell line.

Applications of stem cells

- The goal of any stem cell therapy is to repair a damaged tissue that can't heal itself. Ongoing research on stem cell therapies gives hope to patients who would normally not receive treatment to cure their disease but just to alleviate the symptoms of their chronic illness.
- Stem cell therapies involve more than simply transplanting cells into the body and directing them to grow new, healthy tissue. It may also be possible to coax stem cells already in the body to work overtime and produce new tissue.

Possible treatments by stem cells

- A number of stem cell therapeutics exist, but most are at experimental stages and/or costly, with the notable exception of bone marrow transplantation
- . Medical researchers anticipate that adult and embryonic stem cells will soon be able to treat cancer, Type 1 diabetes mellitus, Parkinson's disease, Huntington's disease, Celiac Disease, cardiac failure, muscle damage and neurological disorders, and many others.
- They have suggested that before stem cell therapeutics can be applied in the clinical setting, more research is necessary to understand stem cell behavior upon transplantation as well as the mechanisms of stem cell interaction with the diseased/injured microenvironment.

Bone marrow transplants

- (BMT) are a well known clinical application of stem cell transplantation. BMT can repopulate the marrow and restore all the different cell types of the blood after high doses of chemotherapy and/or radiotherapy, our main defense used to eliminate endogenous cancer cells. The isolation of additional stem and progenitors cells is now being developed for many other clinical applications. Several are described below.

Skin replacement

- The knowledge of stem cells has made it possible for scientists to grow skin from a patient’s plucked hair. Skin (keratinocyte) stem cells reside in the hair follicle and can be removed when a hair is plucked35. These cells can be cultured to form an epidermal equivalent of the patients own skin and provides tissue for an autologous graft, bypassing the problem of rejection.

Brain cell transplantation

- Stem cells can provide dopamine - a chemical lacking in victims of Parkinson's disease. It involves the loss of cells which produce the neurotransmitter dopamine. The first double-blind study of fetal cell transplants for Parkinson's disease reported survival and release of dopamine from the transplanted cells and a functional improvement of clinical symptoms. However, some patients developed side effects, which suggested that there was an over sensitization to or too much dopamine. Although the unwanted side effects were not anticipated, the success of the experiment at the cellular level is significant.

Treatment for diabetes

- Diabetes affects millions of people in the world and is caused by the abnormal metabolism of insulin. Normally, insulin is produced and secreted by the cellular structures called the islets of langerhans in the pancreas. Recently, insulin expressing cells from mouse stem cells have been generated.
- In addition, the cells self assemble to form structures, which closely resemble normal pancreatic islets and produce insulin. Future research will need to investigate how to optimize conditions for insulin production with the aim of providing a stem cell-based therapy to treat diabetes to replace the constant need for insulin injections.

What is Foot and Mouth Disease (FMD)?

- Foot and Mouth Disease (FMD) is a severe, highly contagious viral disease of livestock with significant economic impact. The disease affects cattle and swine as well as sheep, goats, and other cloven-hoofed ruminants.
- All species of deer and antelope as well as elephant, and giraffe are susceptible to FMD. In a susceptible population, morbidity approaches 100%. Intensively reared animals are more susceptible to the disease than traditional breeds. The disease is rarely fatal in adult animals but there is often high mortality in young animals due to myocarditis or by lack of milk when the dam is infected by the disease.
- FMD is characterized by fever and blister-like sores on the tongue and lips, in the mouth, on the teats and between the hooves. The disease causes severe production losses and while the majority of affected animals recover, the disease often leaves them weakened and debilitated.
- The organism which causes FMD is an aphthovirus of the family Picornaviridae. There are seven strains (A, O, C, SAT1, SAT2, SAT3, Asia1) each one requiring a specific vaccine strain to provide immunity to a vaccinated animal.
- FMD is a disease listed in the World Organization for Animal Health (OIE) Terrestrial Animal Health Code and must be reported to the OIE (OIE Terrestrial Animal Health Code). FMD is the first disease for which the OIE established an offi cial list of free countries and zones with or without vaccination. Member Countries can also ask the OIE to officially recognize their national programmers for FMD control.

How is the disease transmitted and spread?

- FMD is found in all excretions and secretions from an infected animal. The virus may be present in milk and semen for up to 4 days before the animal shows clinical signs of disease. Animals that have recovered from infection may serve as carriers of the virus. Infected animals notably breathe out a large amount of aerosolized virus, which can infect other animals via the respiratory or oral routes.
- The significance of FMD is related to the ease of virus spread through any or all of the following: – new animals carrying the virus (saliva, milk, semen, etc.) may introduce the disease to a herd; – contaminated pens, buildings or vehicles used to house and move susceptible animals; – contaminated materials such as hay, feed, water, milk or biologics; – people wearing contaminated clothes or footwear, or using contaminated equipment; – meat or animal products, raw or improperly cooked food infected with the virus and fed to susceptible animals, and; – aerosol spread of virus from an infected property via air currents.

What are the clinical signs of the disease?

- The severity of clinical signs will depend on the strain of virus, the age and species of animal. The signs can range from a mild infection to severe. Clinical signs are more severe in cattle and intensively reared pigs than in sheep and goats.
- The typical clinical sign is the occurrence of blisters (or vesicles) on the nose, tongue, lips, oral cavity, between the toes, above the hooves, teats and pressure points on the skin. Ruptured blisters can result in extreme lameness and reluctance to move or eat.
- Secondary bacterial infection of open blisters can also occur. Other symptoms often seen are fever, depression, hypersalivation, loss of appetite and weight, drop in milk production. Health of young calves, lambs, kids, and piglets may be compromised by lack of milk from infected dams.
- If infected with the FMD virus, death can occur in young animals before development of blisters due to damage to the heart muscle caused by the virus. Blisters usually heal within 7 days or longer, however the impact of the disease

on growth or milk production rates may persist after recovery. Animals that have recovered from infection may sometimes carry the virus and initiate new outbreaks of disease

Prevention and control measures

- The initial measures in the global strategy for dealing with FMD are early detection and warning systems and prevention measures in place according to OIE Guidelines for the Surveillance of Foot and Mouth Disease (OIE Terrestrial Animal Health Code). This contributes to monitoring the occurrence, prevalence and characterization of FMD viruses.
- Protection of FMD free countries, areas or zones is enhanced with stringent import and cross-border animal movement controls and surveillance. It is essential for livestock owners and producers to maintain sound biosecurity practices to prevent introduction/spread of the virus.
- Measures that are recommended at the farm level include: – control over access to livestock by people and equipment; – control the introduction of new animals to existing stock; – maintain sanitation of livestock pens, buildings, vehicles and equipment ; – monitor and report illness; – appropriate disposal of manure and dead carcasses
- Contingency planning for potential outbreaks will identify the elements included in a response effort to eradicate the disease, such as: – humane destruction of all infected, recovered and FMD-susceptible contact animals (OIE Terrestrial Animal Health Code); – appropriate disposal of carcasses and all animal products (OIE Terrestrial Animal Health Code); – surveillance and tracing of potentially infected or exposed livestock; – strict quarantine and controls on movement of livestock, equipment, vehicles, and; – thorough disinfection of premises and all infected material (implements, cars, clothes, etc.).

Coccidiosis

- Coccidiosis is widely distributed in Illinois poultry. flocks. The specific cause is a group of microscopic protozoa known as coccidia. There are at least three species of coccidia that are capable of causing serious disease in chickens.
- Symptoms of the acute form of the disease, which is usually confined to young chickens, include bloody diarrhea, weakness, and paleness, accompanied by high mortality. In the chronic malady, which generally occurs in pullets or cockerels, varying degrees of unthriftiness are observed. Fowls affected with either type of the disease perpetuate the disease on the premises and are thus a constant threat to healthy stock.
- Diagnosis of coccidiosis may require careful consideration of the history' of the outbreak, symptoms, and autopsy findings, although usually the presence of the disease is conclusively demonstrated by the finding of coccidia in intestinal contents or droppings when these are subjected to microscopic examination.
- Prevention of coccidiosis is largely a problem of sanitary management. Frequent cleaning of brooder houses, the providing of well-drained, uncontaminated grounds, and the adoption of sanitary feeding methods are valuable control methods.
- Coccidiosis in poultry is spread both directly in polluted feed or water and indirectly by more remote mechanical means.

Direct spread.

- The pollution of feed and water with the droppings of infested birds is the most common and direct means by which this disease is spread. A fact often overlooked is that some birds, although apparently recovered from an attack of cecal coccidiosis may, for as long as six months, continue to pass viable coccidia.
- There is also evidence that older birds may harbor and spread other species indefinitely even tho they are not exposed to reinfestation. That coccidia may be transmitted within the egg is only remotely possible, for it has been shown that oocysts placed on the shells of chicken eggs do not survive the incubation period of the eggs.

Indirect spread.

- Mechanical transmission of coccidiosis by various animals, rodents, birds, as well as the shoes and clothing of man, are factors in the indirect spread of the disease. Particles of dust may bear coccidia from one place to another by wind or air currents.
- Surface drainage and streams likewise may carry coccidia. Poultry crates and other contaminated equipment carried from farm to farm may also serve to spread coccidiosis. In addition to flies and other insects, other of the smaller forms of animal life may serve as mechanical spreaders by transporting coccidia in or on their bodies and leaving them where they may be ingested by susceptible fowls
- Another mode of spread is by different barnyard fowls carrying coccidia to which they are not susceptible. The turkey, for instance, may ingest coccidial oocysts from the droppings of chickens and later expel them uninjured in the droppings, where they ultimately may be picked up by susceptible chickens. Furthermore, sparrows and pigeons may be indirectly responsible for transporting and spreading coccidiosis from one poultry flock to another.
- While the importance of this indirect mode of spreading coccidiosis is not definitely established, it cannot be entirely disregarded in some localities.

Preventive measures are of primary importance

1. Avoid contaminated soil and range
2. Avoid contamination of feed and water.
3. Common disinfectants useless against coccidia.
4. Heat effective against coccidia.
5. Cull out carriers of intestinal type.
6. Guard traffic between flocks and flock units.

Trypanosomiasis

- Trypanosomiasis is a group of protozoal infections of both man and animals caused by trypanosome parasites. The species of trypanosomes consist of *T. brucei, T. vivax, T. congolense, T. simiae and T. evans*i.
- Animal trypanosomiasis in Africa represents one of the most serious veterinary problems in the world, and while most other animal diseases have been successfully controlled during this century, trypanosomiasis continues to represent a major threat to animal production in sub-Saharan Africa.
- The parasites causing the disease are largely transmitted by tsetse flies. Approximately ten million km2 of Africa are infested with tsetse flies and approximately 30 percent of the 150 million cattle in these countries are exposed to the infection.
- Most infected cattle die if they are not treated. No vaccines are available, however, the disease can be prevented by the use of prophylactic drugs. Another effective control method is to prevent tsetse flies from biting animals.

Clinical signs:

- Anaemia and general loss of condition are the first clinical signs, when trypanosomes invade and multiply in the bloodstream of the affected animals. About one or two weeks later, the sick animals usually have recurrent fevers for up to three months.
- There are about twelve days between the bouts of fever. Although after the first bout of fevers, the number of parasites in the circulation declines, the animals continue to be anaemic and lose condition. Some animals can survive many months before dying. A few animals can survive the disease.
- In the case of *T. brucei* and *T. evansi* infections, the parasites can invade brain, eyes and skin and clinical signs like nervous signs, discharges from the eyes, oedematous swellings under the skin can occur.

Treatment:

- Trypanosomiasis can be treated by trypanocidal drugs. In order for the drugs to be effective, early treatment is essential. The drugs can be classified as for therapeutic and prophylactic purposes.

- The therapeutic drugs for cattle include diminazene aceturate, homidium chloride and homidium bromide. The prophylactic drugs for cattle include homidium chloride, homidium bromide and isometamidium. Because most of these drugs have been in use for many years, many stains of trypanosomes have developed drug resistance towards these drugs.
- In order to try to overcome this difficulty, some countries have policies in restricting the use of drugs. Some drugs are reserved in the case of that drug resistance develops towards the drugs in use.

Control measures:

- Trypanosomiasis can be prevented by the use of prophylactic drugs. The prophylactic drugs currently used for cattle are homidium and isometamidium. After properly injecting into the animals, these drugs can usually provide three months protection.
- Prophylactic drugs are very effective to be used to protect animals at times when they are exposed to constant disease challenge. Unfortunately, because these drugs have been in use for many years and in many places they were not properly used, resistance has been developed in some places.
- Another effective control method is to eradicate the vectors, tsetse flies, which transmit the disease. Various strategies have been used to control tsetse flies. These include spraying insecticide on tsetse habitat, destruction of tsetse habitat and alteration of vegetation so that it becomes not suitable for tsetse flies.
- It appears that trypanosomiasis can be eliminated, at least from much of Africa, by eradicating tsetse flies. However, this method is very costly and requires a high level of management, organization and specialist expertise.

Theileriosis

- Theileriosis refers to diseases caused by Theileria, a very small parasites called protozoa.
- There are many different types of Theileria, which cause different diseases in animals (all tend to be called theileriosis). In South Africa we are mainly interested in corridor disease.
- Turning sickness is another theilerial disease that can occur. Theilerial diseases are tick-borne diseases, which mean that they are spread between animals by the bites of infected ticks.

How are cattle infected?

- The ticks that can spread this disease to cattle in South Africa are the brown ear tick and the lowveld brown ear tick.
- Cattle are infected when they are bitten by ticks that have fed on infected buffaloes. Therefore, the disease only occurs in cattle grazing pastures on which buffaloes are or have recently been present.
- This can occur when the two species share the same pasture, when cattle are moved into or through buffalo areas or when buffaloes stray from their natural habitat or game reserves into farming areas.
- The presence of a single buffalo for a relatively short period may cause a serious outbreak of disease among cattle.
- Buffaloes do not normally become sick when they are carrying the parasite or have these parasites on their bodies. Not all buffaloes are infected.
- The disease does not normally spread between cattle. Therefore, the disease does not continue spreading once cattle are removed from the buffaloes in the case of an outbreak.

Signs in sick animals

The signs seen in sick animals can include the following:

a) Fever (shown by depression, listlessness, standing apart)
b) Enlargement of glands (especially the one below the ear and the ones in front of the shoulder and the knee)
c) Decreased milk production
d) Loss of appetite

e) Watery discharge from eyes
f) Gums and mucous membranes of eyes may be pale
g) Weakness
h) Difficulty in walking
i) Suppressed cough
j) Animals have great difficulty in breathing before death occurs
k) Frothy fluid coming from the nose just before death
l) May show nervous signs such as walking in circles and paralysis
m) In at least 80% of infected animals, death follows 3 to 4 days after the first signs of disease

Can this disease be treated?

- Because the disease has a very quick progression, treatment is usually not possible.
- In South Africa the treatment of sick animals is also illegal because treated animals may become carriers of the disease and infect ticks.
- When an outbreak occurs, the cattle are moved to uninfected pasture and strict tick control is introduced.

How can it be prevented?

- By prohibiting buffalo contact with cattle, by movement control and fencing between cattle and game areas.
- Buffaloes can only be introduced into farming areas if they test free of infection (blood test)

Possible Questions

1. Explain Artificial Insemination
2. Describe the production of animal using clone technology
3. Write about embryo transfer techniques in cattle
4. Elaborate stem cell and its applications
5. Give a brief note on production of human protein in milk using transgenic approach
6. Explain Foot and mouth diseases
7. Write about Coccidiosis
8. Explain Trypanosomiasis
9. Comment on Theileriosis
10. Write about super ovulation
11.

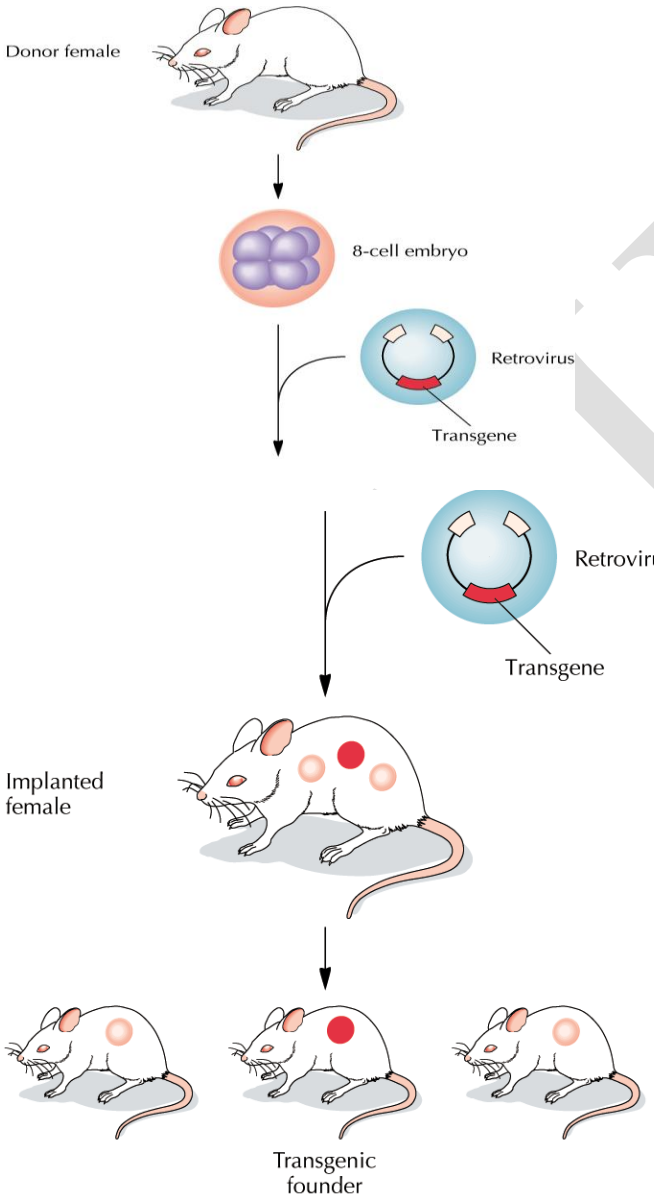
Transgenesis in Mice – Methodology

- A genetically modified mouse (Mus musculus) is a mouse that has had its genome altered through the use of genetic engineering techniques.
- Genetically modified mice are commonly used for research or as animal models of human diseases, and are also used for research on genes.
- There are two basic technical approaches to produce genetically modified mice. The first involves pronuclear injection into a single cell of the mouse embryo, where it will randomly integrate into the mouse genome.
- This method creates a transgenic mouse and is used to insert new genetic information into the mouse genome or to over-express endogenous genes.
- The second approach, pioneered by Oliver Smithies and Mario Capecchi, involves modifying embryonic stem cells with a DNA construct containing DNA sequences homologous to the target gene. Embryonic stem cells that recombine with the genomic DNA are selected for and they are then injected into the mice blastocysts.
- This method is used to manipulate a single gene, in most cases "knocking out" the target gene, although more subtle genetic manipulation can occur (e.g. only changing single nucleotides)

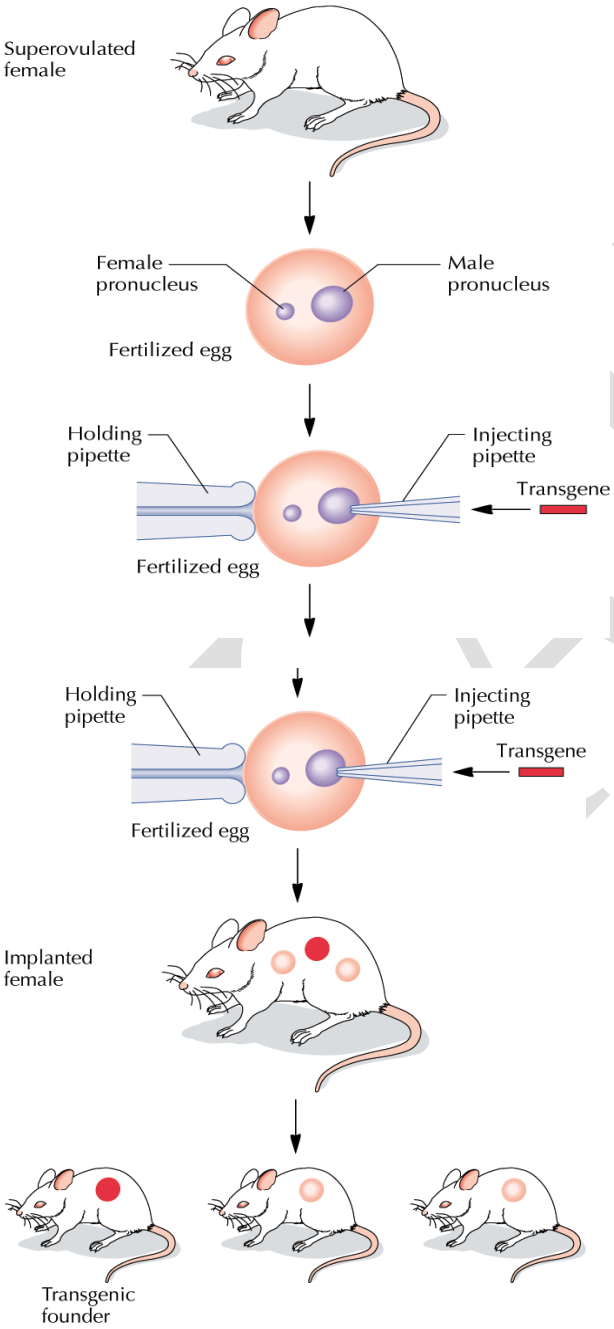
Transgenic Mouse – Uses and applications

- Genetically modified mice are used extensively in research as models of human disease. Mice are a useful model for genetic manipulation and research, as their tissues and organs are similar to that of a human and they carry virtually all the same genes that operate in humans.
- They also have advantages over other mammals, in regards to research, in that they are available in hundreds of genetically homogeneous strains. Also, due to their size, they can be kept and housed in large numbers, reducing the cost of research and experiments.
- The most common type is the knockout mouse, where the activity of a single (or in some cases multiple) genes are removed. They have been used to study and model obesity, heart disease, diabetes, arthritis, substance abuse, anxiety, aging and Parkinson disease.
- Transgenic mice generated to carry cloned oncogenes and knockout mice lacking tumor suppressing genes have provided good models for human cancer. Hundreds of these oncomice have been developed covering a wide range of cancers affecting most organs of the body and they are being refined to become more representative of human cancer. The disease symptoms and potential drugs or treatments can be tested against these mouse models.
- A mouse has been genetically engineered to have increased muscle growth and strength by overexpressing the insulin-like growth factor I (IGF-I) in differentiated muscle fibers.
- Another mouse has had a gene altered that is involved in glucose metabolism and runs faster, lives longer, is more sexually active and eats more without getting fat than the average mouse (see Metabolic supermice).

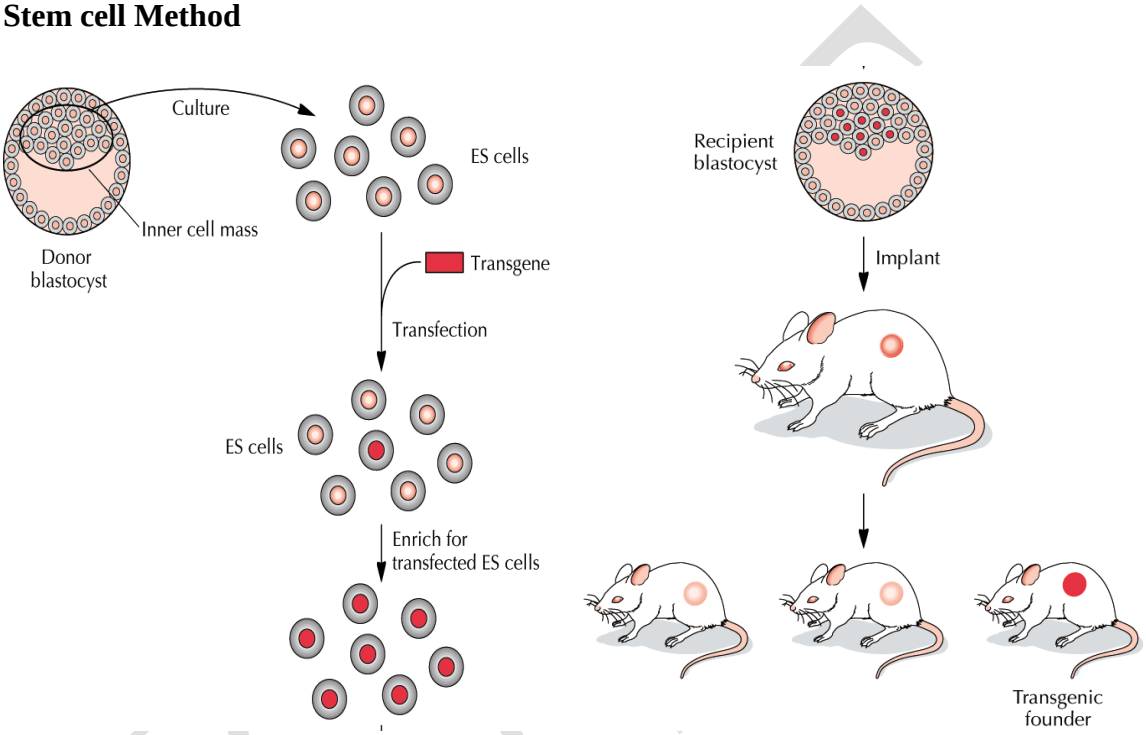
Retroviral Vector



Microinjection



Stem cell Method



Transgenic Cow

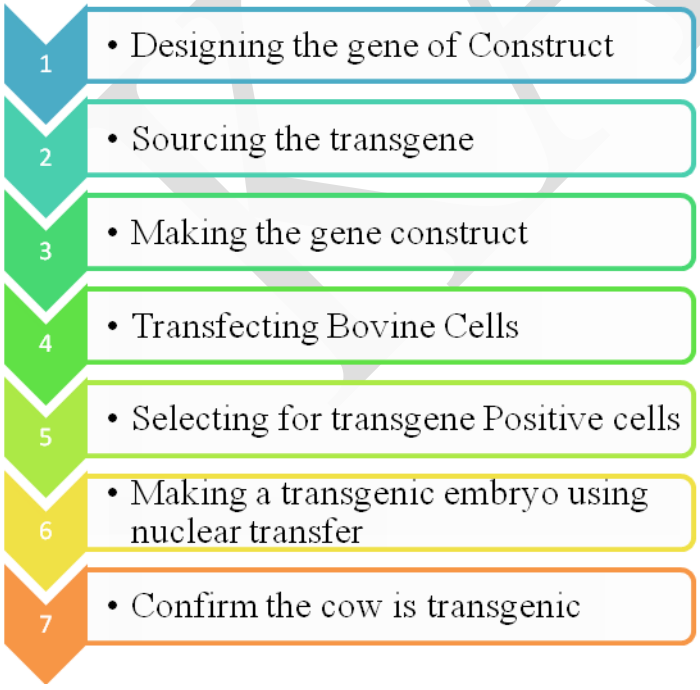
- Transgenic cows are genetically modified (GM) cows. They have an extra gene or genes inserted into their DNA. The extra gene may come from the same species or from a different species.
- The extra gene (transgene) is present in every cell in the transgenic cow. However, it's only expressed in mammary tissue. This means that the transgene's protein will only be found in the cow's milk and can only be extracted from there.

Techniques used to make transgenic cows

Step 1. Designing the gene construct

The first step is to design a gene construct. The gene construct is a unit of DNA that includes:

a) an antibiotic resistance gene – to select cells that have taken up the gene construct
b) a tissue-specific promoter sequence – to signal the start of expression of the protein in cells of the appropriate tissue, for example, in mammary cells in lactating cows
c) the desired gene – for example, bovine casein or human myelin basic protein
d) a stop sequence – to define the end of the information for making the protein.



Step 2. Sourcing the transgene

- In the past, the gene would have been extracted from the source organism's DNA. Now, however, if the desired gene sequence is known, it can simply be synthesised in a lab.
- There are companies that make genes to order within a couple of weeks.

Step 3. Making the gene construct

- A gene construct contains all the information needed for transfection into a bovine cell and expression of the desired gene in a cow. This includes an antibiotic resistance marker, a tissue-specific promoter, the transgene/gene of interest and a stop sequence.
- The gene is usually supplied in a vector. A vector is a small piece of DNA, often a plasmid, into which a foreign piece of DNA can be inserted. When the gene of interest is in a vector, it can be sent from one lab to another, it can be stored, it can be manipulated or it can be used to transform bacteria to produce more copies of the gene of interest.
- Vectors have multiple restriction enzymes sites (also called multiple cloning sites) so the gene can be inserted into the vector and then cut out from the vector using restriction enzymes. This article has more information on restriction enzymes.
- After the gene is cut from the vector, it is then pasted into the multiple cloning site of the gene construct using a method known as ligation. This article has more information on DNA ligation.

Step 4. Transfecting bovine cells

- The gene construct is incorporated into the genome of a bovine (cow) cell using a technique called transfection. During transfection, holes are made in the cell membrane that allow the DNA to enter.
- The holes can be made by applying an electrical pulse or by adding chemicals to the cells. Once inside the cell, the gene construct may enter the nucleus and incorporate into the cell's genome.

Step 5. Selecting for transgene positive cells

- After transfection, an antibiotic is added to select the bovine cells that have incorporated the gene construct. Transgenic bovine cells will survive treatment with an antibiotic, because they contain an antibiotic resistance gene making them resistant to the antibiotic.
- Cells without the gene construct will have no resistance to the antibiotic and will die. In addition to antibiotic selection, polymerase chain reaction (PCR) is used to check that the bovine cell contains the transgene.

Step 6. Making a transgenic embryo using nuclear transfer

- Nuclear transfer is used to create a whole animal from a single transgenic bovine cell.
- The transgenic bovine cell is fused with a bovine oocyte that has had its chromosomes removed (called an enucleated oocyte). An electrical pulse is applied to help fuse the cells. Once fused with the oocyte, the transgenic cell's chromosomes are reprogrammed to direct development into an embryo.
- After 7 days, the transgenic embryo will have about 150 cells and can be transferred into a recipient cow for further development to term.

Step 7. Confirming the cow is transgenic

- If the embryo develops to full term, after 9 months, the cow will give birth to a calf. To confirm that the calf is transgenic, scientists can check using:
- PCR to determine the presence or absence of the transgene
- quantitative PCR (q-PCR) to determine the number of copies of the transgene
- fluorescent in situ hybridization (FISH) to visualize where the transgene is in the chromosome and whether the transgene has integrated into more than one chromosome.
- When the calf is lactating (either after being induced to lactate or after having its own progeny), its milk is checked to determine if the transgenic protein is being expressed.

Transgenic sheep, goat

- The birth of Dolly was soon followed by that of a cloned transgenic sheep named Polly (Schnieke et al., 1997).
- The advantages of this method of adding genes are multiple. About two to five times fewer sheep are needed to generate transgenic sheep than by microinjection. The integrated gene may be examined in cells before nuclear transfer.
- Cells in which the foreign gene is rearranged or has too many copies may be discarded. The sex and, more generally, the genotype of the nuclear donors may be chosen. The founder animals are never mosaic for the transgene.
- Several animals having the same genotype, including the same transgene, can be generated simultaneously.
- Although cloning is a laborious technique, it offers some flexibility to the experimenters. The nuclear donor cells can be kept frozen and used at the most appropriate moment to generate cloned transgenic animals.

Transgenic Sheep and Goats

- Until recently, the transgenes introduced into sheep inserted randomly in the genome and often worked poorly.
- However, in July 2000, success at inserting a transgene into a specific gene locus was reported. The gene was the human gene for alpha1-antitrypsin, and two of the animals expressed large quantities of the human protein in their milk.

This is how it was done

Sheep fibroblasts (connective tissue cells) growing in tissue culture were treated with a vector that contained these segments of DNA:

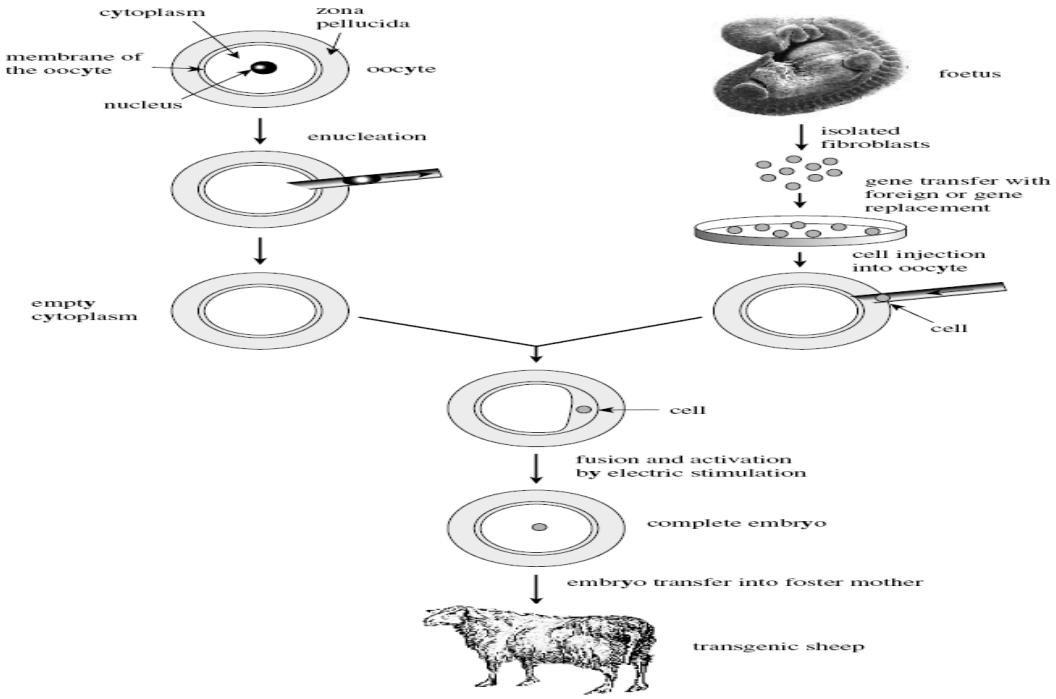
1. 2 regions homologous to the sheep *COL1A1* gene. This gene encodes Type 1 collagen. (Its absence in humans causes the inherited disease osteogenesis imperfect.)

   This locus was chosen because fibroblasts secrete large amounts of collagen and thus one would expect the gene to be easily accessible in the chromatin.
2. A neomycin-resistance gene to aid in isolating those cells that successfully incorporated the vector. The human gene encoding alpha1-antitrypsin.

Some people inherit two non- or poorly-functioning genes for this protein. Its resulting low level or absence produces the disease **Alpha1-Antitrypsin Deficiency** (**A1AD** or **Alpha1**). The main symptoms are damage to the lungs (and sometimes to the liver).

3. Promoter sites from the **beta-lactoglobulin** gene. These promote hormone-driven gene expression in milk-producing cells.
4. Binding sites for ribosomes for efficient translation of the mRNAs.

Successfully-transformed cells were then fused with enucleated sheep eggs and implanted in the uterus of a ewe (female sheep). Several embryos survived until their birth, and two young lambs have now lived over a year. When treated with hormones, these two lambs secreted milk containing large amounts of alpha1-antitrypsin (650 µg/ml; 50 times higher than previous results using random insertion of the transgene).

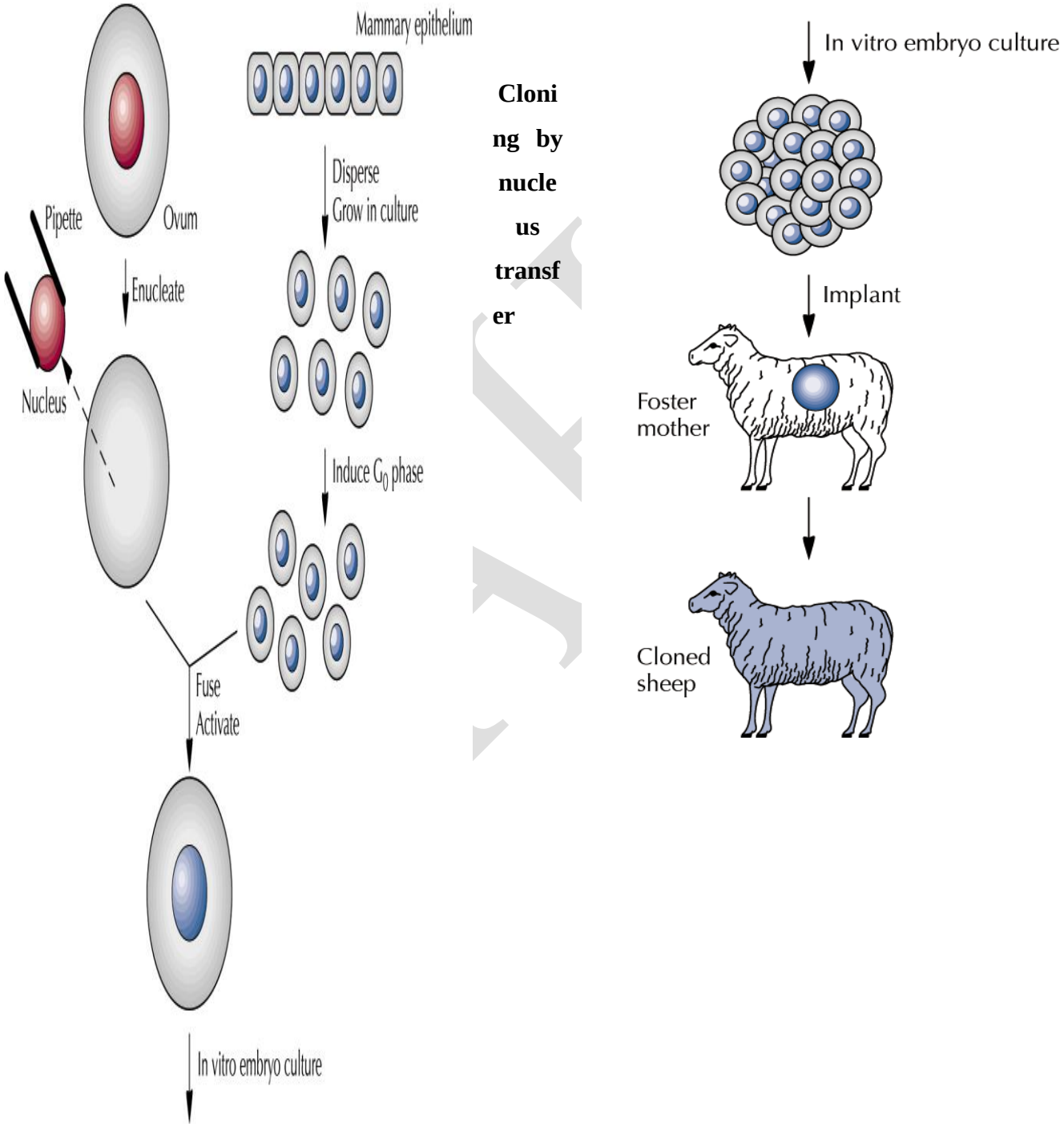


On June 18, 2003, the company doing this work abandoned it because of the great expense of building a facility for purifying the protein from sheep's milk. Purification is important because even when 99.9% pure, human patients can develop antibodies against the tiny amounts of sheep proteins that remain.

However, another company, GTC Biotherapeutics, has persevered and in June of 2006 won preliminary approval to market a human protein, antithrombin, in Europe. Their protein the first made in a transgenic animal to receive regulatory approval for human therapy was secreted in the milk of transgenic goats.

Transgenic Pigs

- Transgenic pigs have also been produced by fertilizing normal eggs with sperm cells that have incorporated foreign DNA. This procedure, called sperm-mediated gene transfer (SMGT) may someday be able to produce transgenic pigs that can serve as a source of transplanted organs for humans.
- Gene replacement was achieved in sheep, mice and pigs. This method is very laborious and still poorly controlled. A recent study showed that homologous recombination of two genes could be obtained in sheep cells but that this was followed by the death of the newborn animals obtained by cloning. This failure may be attributed to the culture of the cells, which is required to select those in which the homologous recombination has occurred.
- The culture conditions modify the physiology of the cells, which, for unknown reasons, become less capable of generating living cloned animals. A better understanding of these phenomena is necessary before gene replacement in large animals can be considered as a truly viable method.



Cloning by nucleus transfer

Transgenic fish

- Genetically modified fish (GM fish) are organisms from the taxonomic clade which includes the classes Agnatha (jawless fish), Chondrichthyes (cartilaginous fish) and Osteichthyes (bony fish) whose genetic material (DNA) has been altered using genetic engineering techniques. In most cases, the aim is to introduce a new trait to the fish which does not occur naturally in the species, i.e. transgenesis.
- The first transgenic fish were produced in China in 1985. As of 2013, approximately 50 species of fish have been subject to genetic modification. This has resulted in more than 400 fish/trait combinations. Most of the modifications have been conducted on food species, such as Atlantic salmon (*Salmo salar*), tilapia (genus) and common carp (*Cyprinus carpio*).
- Generally, genetic modification entails manipulation of DNA. The process is known as cisgenesis when a gene is transferred between organisms that could be conventionally bred, or transgenesis when a gene from one species is added to a different species. Gene transfer into the genome of the desired organism, as for fish in this case, requires a vector like a lentivirus or mechanical/physical insertion of the altered genes into the nucleus of the host by means of a micro syringe or a gene gun

Transgenic fish are used in research covering five broad areas-

a) Enhancing the traits of commercially available fish
b) Their use as bioreactors for the development of bio-medically important proteins
c) Their use as indicators of aquatic pollutants
d) Developing new non-mammalian animal models
e) Functional genomics studies

- Most GM fish are used in basic research in genetics and development. Two species of fish, zebrafish and medaka, are most commonly modified because they have optically

clear chorions (shells), develop rapidly, the 1-cell embryo is easy to see and micro-inject with transgenic DNA, and zebrafish have the capability of regenerating their organ tissues.

- They are also used in drug discovery.[10] GM zebrafish are being explored for benefits of unlocking human organ tissue diseases and failure mysteries. For instance, zebrafish are used to understand heart tissue repair and regeneration in efforts to study and discover cures for cardiovascular diseases.
- Transgenic rainbow trout (*Oncorhynchus mykiss*) have been developed to study muscle development. The introduced transgene causes green fluorescence to appear in fast twitch muscle fibres early in development which persist throughout life. It has been suggested the fish might be used as indicators of aquatic pollutants or other factors which influence development.
- In intensive fish farming, the fish are kept at high stocking densities. This means they suffer from frequent transmission of contagious diseases, a problem which is being addressed by GM research. Grass carp (*Ctenopharyngodon idella*) have been modified with a transgene coding for human lactoferrin, which doubles their survival rate relative to control fish after exposure to Aeromonas bacteria and Grass carp hemorrhage virus. Cecropin has been used in channel catfish to enhance their protection against several pathogenic bacteria by 2–4 times.

Transgenic Chickens/hen

Chickens

- grow faster than sheep and goats and large numbers can be grown in close quarters;
- synthesize several grams of protein in the "white" of their eggs.
- Two methods have succeeded in producing chickens carrying and expressing foreign genes.
- Infecting embryos with a viral vector carrying
- the human gene for a therapeutic protein
- promoter sequences that will respond to the signals for making proteins (e.g. lysozyme) in egg white.

- Transforming rooster sperm with a human gene and the appropriate promoters and checking for any transgenic offspring.
- Preliminary results from both methods indicate that it may be possible for chickens to produce as much as 0.1 g of human protein in each egg that they lay.

Not only should this cost less than producing therapeutic proteins in culture vessels, but chickens will probably add the correct sugars to glycosylated proteins — something that *E. coli* cannot do.

Gene therapy

- Gene therapy is a novel treatment method which utilizes genes or short oligonucleotide sequences as therapeutic molecules, instead of conventional drug compounds. This technique is widely used to treat those defective genes which contribute to disease development.
- Gene therapy involves the introduction of one or more foreign genes into an organism to treat hereditary or acquired genetic defects. In gene therapy, DNA encoding a therapeutic protein is packaged within a "vector", which transports the DNA inside cells within the body.
- The disease is treated with minimal toxicity, by the expression of the inserted DNA by the cell machinery. In 1990 FDA for the first time approved a gene therapy experiment on ADA-SCID in the United States after the treatment of Ashanti DeSilva.
- After that, approximately 1700 clinical trials on patients have been performed with various techniques and genes for numerous diseases. Many diseases such as ADA-SCID, X-linked SCID, Leber's congenital amaurosis (a retinal disease), Parkinson's disease, multiple myeloma, chronic and acute lymphocytic leukemia, adrenoleukodystrophy have reported of successful clinical trials.
- But these are still not approved by FDA. Some other diseases on which gene therapy based research is going on are Haemophilia, Tyrosinemia, Hyperbilirubinemia (Criglar-Nijjar Syndrom), Cystic Fibrosis and many other cancers.
- After 30 years of research and clinical trials, only one product called Glybera got approval in November 2012 which may be available in market in late 2013. It has the ability to cure lipoprotein lipase deficiency (LPLD) a very rare disease.

Types of gene therapy

- There are several approaches for correcting faulty genes; the most common being the insertion of a normal gene into a specific location within the genome to replace a non functional gene. Gene therapy is classified into the following two types:
    1. Somatic gene therapy
    2. Germ line gene therapy

Somatic Gene Therapy

- In somatic gene therapy, the somatic cells of a patient are targeted for foreign gene transfer. In this case the effects caused by the foreign gene is restricted to the individual patient only, and not inherited by the patient's offspring or later generations.

Germ Line Gene Therapy

- Here, the functional genes, which are to be integrated into the genomes, are inserted in the germ cells, i.e., sperm or eggs. Targeting of germ cells makes the therapy heritable.

Gene Therapy Approaches

Classical Gene Therapy

- It involves therapeutic gene delivery and their optimum expression once inside the target cell. The foreign genes carry out following functions.
- Produce a product (protein) that the patient lacks;
- Produces toxin so that diseased cell is killed.
- Activate cells of the immune system so as to help in killing of diseased cells.

Non-classical gene therapy

- It involves the inhibition of expression of genes associated with the pathogenesis, or to correct a genetic defect and restore the normal gene expression.

Methods of gene therapy

There are mainly two approaches for the transfer of genes in gene therapy:

1. Transfer of genes into patient cells outside the body (*ex vivo gene therapy*)
2. Transfer of genes directly to cells inside the body (*in vivo*).

Ex vivo gene therapy

- In this mode of gene therapy genes are transferred to the cells grown in culture, transformed cells are selected, multiplied and then introduced into the patient.
- The use of autologous cells avoids immune system rejection of the introduced cells.
- The cells are sourced initially from the patient to be treated and grown in culture before being reintroduced into the same individual.
- This approach can be applied to the tissues like hematopoietic cells and skin cells which can be removed from the body, genetically corrected outside the body and reintroduced into the patient body where they become engrafted and survive for a long period of time.

In Vivo Gene Therapy

- *In vivo* method of gene transfer involves the transfer of cloned genes directly into the tissues of the patient.
- This is done in case of tissues whose individual cells cannot be cultured *in vitro* in sufficient numbers (like brain cells) and/or where re-implantation of the cultured cells in the patient is not efficient.
- Liposomes and certain viral vectors are employed for this purpose because of lack of any other mode of selection.
- In case of viral vectors such type of cultured cells were often used which have been infected with the recombinant retrovirus *in vitro* to produce modified viral vectors regularly. These cultured cells will be called as vector-producing cells (VPCs)). The VPCs transfer the gene to surrounding disease cells.

- The efficiency of gene transfer and expression determines the success of this approach, because of the lack of any way for selection and amplification of cells which take up and express the foreign gene.

Vectors for gene therapy

Vectors for gene therapy can be classified into two types:

1. Viral vectors
2. Non-viral

Viral vectors

Retroviruses, adenoviruses and adeno-associated viruses (AAV) some commonly used viral vectors whereas some less commonly used viral vectors are derived from the Herpes simplex virus (HSV-1), the baculovirus etc.

Adenoviral vectors

- Adenoviruses are large linear double-stranded DNA viruses that are commonly used for preparing gene transfer vectors.
- Adenovirus vectors are known to be the second most popular gene delivery vector for gene therapy of various diseases like cystic fibrosis and certain types of cancer.
- A primary cellular receptor binds to viral fiber then the virus interacts with secondary receptors which are responsible for its internalization.
- Coxsackie and Adenovirus Receptor (CAR), Heparan sulphate glycosaminoglycans, sialic acid, CD46, CD80, CD86, alpha domain of MHC I are the primary receptors which are specific for specific strains of adenovirus. Integrins are the secondary receptors which helps in the internalization of viral particles.
- Some adenovirus directly interacts with integrins like in the case of fiber deficient Ad2 virions.

Adeno- Associated Virus (AAV)

- Adeno-associated viruses (AAVs) are a group of small, single-stranded DNA viruses which cannot usually undergo productive infection without co-infection by a helper virus, such as an adenovirus.
    1. The insert size for AAV is 4.5 kb, with the advantage of long-term gene expression as they integrate into chromosomal DNA.
    2. AAVs are highly safe as the recombinant adeno associated vectors contains onlygene of interest and 96% viral genes are deleted.

Retroviral Vectors

- Retroviruses are RNA viruses which possess a reverse transcriptase activity, enabling them to synthesize a complementary DNA. Following infection (transduction), retroviruses deliver a nucleoprotein complex (pre-integration complex) into the cytoplasm of infected cells.
- The viral RNA genome is reverse transcribed first and then integrates into a single site of the chromosome.

- **Tumor retroviruses**, example Moloney's murine leukemia virus (MoMuLV), is widely used for the generation of recombinant vectors. these are produced at low titers as all the viral genes are deleted.
- **Oncoretroviruses:** The cells that divide shortly after infection can only be transduced by oncoretrovirus. The preinitiation complex is excluded and their entry is restricted in to the nucleus as they can only enter when nuclear membrane dissolves during cell division the target cells for this viral vector is limited
- **Recombinant lentiviruses** are being developed that are non- pathogenic to humans and have the ability to transduce stationary cells.

Other Viral Vectors:

These include herpes simplex virus vectors and baculovirus.

- **Herpes simplex virus vectors**: Herpes simplex virus-1 (HSV-1) is a 150 kb double-stranded DNA virus with a broad host range that can infect both dividing and non-dividing cells. the insert size is comparatively larger (>20kb) but have a disadvantage of short-term expression due to its inability to integrate into the host chromosome

Baculovirus:

- They can take up very large genes and express them highly efficiently. They help in recombinant protein expression in insect cell. They can infect hepatocytes as an only mammalian cell type and the gene expresses under the control of either mammalian or viral promoter.

Simian Virus 40 Vectors (SV40):

- SV40 are icosahedral papovavirus with a circular double stranded DNA of 5.2kb size as genetic material. The genome encodes for early proteins viz; large T antigen (Tag) and small t antigen (tag) and late protein viz; a regulatory protein agnoprotein and three structural proteins (VP1, VP2, VP3).
- The Tag gene is removed as it is responsible for inducing immunogenicity in the recombinant SV40 vector. All the structural proteins except the major capsid protein VP1 is removed resulting in a genome of 0.5kb size which includes origin of replication (ori) and encapsidation sequence. Recombinant SV40 vectors allows expression of transduced gene

Molecular engineering Human genetic problem and ethics

- Several thousand genetic disorders of humans would appear to result from a mutation in a single gene, while many others have more complex genetic explanations and even possible interactions with environmental factors.
- Results from the Human Genome Project, discussed earlier, are now considered to offer an increased understanding of these fundamental genetic malfunctions and to give, in some cases, hope for alleviation and perhaps cure of the defect.
- However, paralleling the scientific breakthroughs and deeper understandings of gene mechanisms have come many areas of public concern

Areas of public concern on human genome research
1. Confidentiality of testing and screening results
2. Scope of genetic testing and screening
3. Discrimination and stigmatisation
4. Commercial exploitation of human genome data
5. Eugenic pressures
6. Effects of germ line gene therapies on later generations

- The major nations now committed to genome projects are also supporting research into the many ethical, legal and social issues that these studies are uncovering.
- Numerous committees now foster public debate and understanding of these highly complex issues.
- On the one hand the scientific discoveries could possibly bring much relief to millions of sufferers of genetic diseases, but on the other they give rise to questions of mind-bending implications to the way forward for the human race.

Genetic testing and screening

- Where genetic disorders have previously been observed in families it is now possible in some cases to carry out prenatal testing to discover if the foetus carries the defect. The parents may then be able to sanction an abortion or be better prepared for the needs of the full-term baby.
- There are obvious concerns that this could result in a wide range of other conditions being selected for termination, e.g. gender and diseases of a minor nature. Soon it may be possible to have a much fuller awareness of an individual's 'genetic portfolio' and to possibly diagnose future medical problems, e.g. heart disease, cancer, etc., and advise treatment well in advance of the onset of the disease.
- However, would an individual wish to know that they would develop Huntington disease (presently an untreatable, debilitating and often fatal disease) in 30–40 years time? It has been suggested that genetic testing and screening should only occur with disorders where treatment is available.
- The collection and long-term storage of biological material (blood samples, DNA, etc.) raises special ethical and policy issues about access and possible uses of the samples, which reflect around informed consent.
- Consensus opinion is that individuals should be made aware of policies for sample ownership, possible IP rights and the protection of privacy and confidentiality of genetic information.
- Perhaps the most worrying aspect of such genetic testing is the use such information could be put to by insurance and mortgage institutions. While undoubtedly such financial systems would reduce the risk aspect of their investments, the effects on the individual would be devastating.
- It is increasingly viewed by ethics committees that insurance companies should not require or be allowed access to an individual's genetic information as a prerequisite for insurance. This may well prove to be an impossible task to monitor and control. It is becoming increasingly apparent that the course of an individual's future is not 'in the stars' but in reality in their own genes.

Human gene therapy

- Gene therapy can be considered from either a somatic or germ-line approach. From an ethical viewpoint somatic gene therapy involving the insertion of single genes into a patient is really no different from the long accepted practice of transplants, e.g. hearts, lungs, etc., from other individuals.
- It is considered that such treatments should be used only to alleviate serious medical disorders and not for non-therapeutic applications. The application of gene therapy could be important to the pharmaceutical industry, but it is not yet clear whether it could be sold as an injectable 'product' or be dispensed as a service. Part of the confusion comes from the vast diversity of potential disease applications, ranging from immunotherapeutics to genetic diseases.
- In either case it will not be a cheap therapy. Somatic gene therapy must remain under close supervision to satisfy medical safety, legal implications and public concerns.
- Germ-line gene therapy is presently not being pursued because it is technically extremely difficult and is ethically and socially unacceptable.
- Interfering with germ cells raises huge problems of eugenics, and there must be extensive public debate if it is ever to be used as a meaningful medical technique. The mood in society would appear to be supportive towards gene therapy at the present limited andmostly experimental level, with the proviso that safeguards are kept and are effective. Unscrupulous 'pioneer' surgeons should be carefully monitored.
- Stem cell technology, while offering huge potential for therapeutic remedies for some of the world's worst debilitating diseases, has also generated
- major religious and moral concerns. While adult stem cells derived from various human tissues are relatively free from controversy this is not the case for hES cells derived from human blastocysts. At one side of the argument it can be considered that the moral status of the hES cells is no different from that of other cells from the human body, and it could be deemed unethical to hold back research with such cells that could offer potential relief to millions of sufferers.

- In stark contrast, the religious view is that from the moment of conception the embryo cell should receive full moral standing as a person and, thus, it would be unethical to sacrifice the life of an individual for the benefit of others!
- Is it possible that science can avoid the moral dilemmas raised by this controversial line of research?
- It is misleading to label hES cell study as immoral and to avoid the everincreasing tangled ethical division and loss of valuable research progress; scientists and religious philosophers must enlighten the public on the true nature of this technology.
- The debate revolves around the embryo or quasi-embryo. When does human life begin? Human development categorically requires implantation of the embryonic cell into the wall of the uterus, otherwise the embryo cannot develop into a new individual. Laboratory development of the embryo to a body is *not* possible.
- Reference should be made to the papers listed in the 'Further reading' for a critical examination of this most contentious area of modern biotechnology. Some general conclusions are now appearing from the increased level of discussion of these issues.
- The public does not accept or reject gene technology as a whole. Parts of it will be welcomed and utilised while others will have less or no support at all. The biotechnology community must aim to inform, not indoctrinate, the public.
- The consumers and patients of biotechnology products must be given clear and unequivocable information: a recent EEC Public Perception of Biotechnology meeting ended with the following message to biotechnology companies: 'Provide the information and listen to the public'.
- When taking into consideration the scientific complexity of most new biotechnology products and processes, companies must use public relations effectively to provide consumers with adequate information about the advantages and benefits of their products. In this way the public will be able to make informed decisions about them. Similarly, scientists must learn to communicate with the public, be willing to do so and consider it a duty to do so!
- The most significant obstacles to the full creative resolution of new biotechnology are not expected to be scientific, economic or indeed environmental, but rather cultural!

Possible Questions

1. What is transgene
2. Explain Transgenesis
3. Explain in detail production of transgenic mouse
4. Briefly explain DOLLY
5. With examples, discuss Gene Therapy
6. Explain the production of transgenic sheep
7. Write in detail about production of transgenic fish
8. Briefly explain the applications of transgenic animal
9. How can a foreign gene can be inserted in cow? Explain
10. Brief about transgenic Pig.
11. Explain Gene therapy
12. Write about the types of gene therapy
13. Explain the vectors used in gene therapy
14. Brief a note on Human genetic problem and ethics

Unit 1	Opt 1	Opt 2	Opt 3	Opt 4	Answer
Animal cells have pH…….	7-7.4	5-5.5	6-6.5	8-8.5	7-7.4
……..is used as pH indicator in ATC	Phosphate	Salt sodium	Saline	Phenol red	Phenol red
…….temperature maintained for cold blooded animals in the cell culture	15-25 °C	30-35°C	Both a and b	None of the above	15-25 °C
Glutamate is a key molecule in cellular	Respiration	Metabolism	Digestion	All cellular	Metabolism
Cells require attachment for growth is called	Anchorage dependent	Matrix dependent	Non adhesive dependent	Adhesive	Anchorage dependent
Animal tissues chopped up to ---------- pieces for explant preparation	1mm	0.1mm	0.01mm	2mm2	1mm
Which of the following is not a serum constituent	Binding	Cholesterol	Fe and Zn	Mg and Cu	Mg and Cu
In animal cell culture phenotypic stabilization requires - ---------- days	5 days	6 days	7 days	8 days	7 days
pH of HEPES is ----------	7 to 7.2	7.2 to 7.4	7.2 to 7.6	7.4 to 7.6	7.2 to 7.6
The albumin present in serum carries--------	Lipids	Minerals	Globulins	all the	all the above
_____is present only in fetal serum and it enhances cell attachment.	Fetuin	Transperin	Fibronectin	Antitrypsin	Fetuin
Fetuin is present in…..	fetal serum	Human	Both a and b	None of the	Human serum
Which of the following helps to detoxify free radicals as a co factor for GSH--------synthetase	Zinc	Selenium	Copper	Serum proteins	Selenium
Eagle's MEM supplemented with calf serum helps to maintain ----------	Human diploid	Skeletal muscles	Neurons	Melanocytes	Human diploid fibroblasts
Which of the following is the selection medium for	MEM with	M199 with	DMEM with	MEM with	MEM with CS
Serum is heat inactivated by incubating it for ----------	15min at	15min at	30min at	30min at	15min at 560C
_____used to promote cell migration from	Plasma clot	Suspention	Rolling	Calf serum	Plasma clot
Which of the following substance mitogenic to	TGF	PDGF	MDCK	all the	PDGF
Which enhances plating efficiency of different cell	Somatotroph	carticosteron	Fibroin	Fibronectin	Somatotrophin
Hepatocyte growth factor is morphogenic to-------	Hepatocytes	Kidney	keratinocytes	haematopoi	Kidney tubules
In the absence of serum, the plastic substrate needs to be coated with	Polylysine	oncostatin	transferrin	phorbol	Polylysine
Usual volume of medium to surface is….	0.1 -	0.2 -	0.3 -	0.4 -	0.2 -0.3ml/cm2
Gaseous diffusion become limited if medium is	Greater than 1 mm	Greater than 3 mm	Greater than 5 mm	c Greater than 7 mm	Greater than 5 mm

Media with reduced serum concentration is called as	Holding	Serum free	Complex	Minimal	media
Exhaustion of the media is in which phase of the	Lag	exponential	plateau	none of the	exponential
In animal tissue culture gaseous equilibrium maintained in a	Co2 incubator	Liquid nitrogen	Aerated carrels flask	None of the above	Co2 incubator
_____act as a major source of animal tissue contamination	CO2 incubator	Humidified incubator	O2 incubator	Aerated incubator	Humidified incubator
Which is the boiling point of liquid nitrogen	-196°C	-96°C	-80°C	-70°C	-96°C
Which are the cryoprotectants to freeze "spare" expanded human blastocysts	Glycerol and sucrose	Glycerol and glutamine	Progesterone and sucrose	Chloramphenicol	Glycerol and sucrose
After trypsinization, which should be added to neutralize enzyme activity?	Medium with serum	BSS with serum	Cold PBS	EDTA with serum	Medium with serum
The inner surface of the culture dish s typically coated with mouse embryonic skin cells that have been treated so that they will not divide is called ----------	feeder layers	monolayers	Nutrient matrix	Epithelial cells	feeder layers
Which of the following is not coming under enzymatic tissue disaggregation in animal cell culture?	Pronase	Collagenase	Vernase	Protease	Protease
_____are mainly used for production of cell suspensions from established cultures of	EDTA	Trypsin	Collagenase	Pronase	EDTA
Trypsin disaggregation will damage----------	epithelial	fibrous	Bone cells	None	epithelial cells
Trypsin disaggregation will ineffective in ----------	Epithelial	fibrous	Bone cells	None	fibrous tissues
The technique used for the preservation of germplasm at temperature below sub zero degree is known	Cryopreservation	Freezing	Sublimation	Dehydration	Cryopreservation
Few newly acquired cell stored in ampule is called	Token freeze	Seed stock	User stock	All the	Token freeze
Ampoules are made of ---------	Polyethylene	Silicon	Nylon	Glass	Silicon
Which of the following is used for cryopreservation?	liquid	Glycerin	DMSO	All the	All the above
3T3 cells are used in the cultivation of ----------	blood	stem cells	keratinocytes	none of the	keratinocytes
Following is not a physical tissue disaggregation…..	forcing through	forcing through silk	forcing through glass	forcing through	forcing through proteins
Minimum liquid ambient require for animal tissue	40 - 700	20 - 200	40 - 200	200 - - 1960	40 - 200
Who discovered tumor cell lines?	George and Margaret	Enders	Avery Hill	Cn Leach	George and Margaret Gey
Which of the following is a source of epithelial cells?	Liver	serum free	Plasma clots	blood	Liver

High carbonate and 5% CO2 is the concentration of ---------- BSS.	earle's	hank's	eagle's	hames's	earle's
Calcium is reduced in suspension culture in order to	density	maximize cell attachment &	maximize cell proliferation	viscosity	maximize cell attachment & aggregation
Accumulation of lactic acid in animal tissue culture medium implies--------	improper citric acid	improper glycolysis	both a and b	none of the above	improper citric acid cycle
Transformed cells derived from a single parental cell	Hybrid line	Cell line	Infinite cells	Finite cells	Cell line
_____Providing a large surface area to cells in small volumes of media.	antibody	Microcarriers	plasma clots	Serum proteins	Microcarriers
Commercially available Microcarreirs made by	PVG	Cellulose	PEG	PLGA	Cellulose
Microcarriers, macrocarriers or encapsulated beads could be used in	tissue mediium	regenration	The culture fluid is circulated in a	emulsion	The culture fluid is circulated in a
Hollow fiber reactor consist of ---------- it helps "fibers", cells to grow	Semi-permeable	Definite cut-off cells	Nutrients into the lumen	Metabolic by-products	permeable membranes
Osmolarity of animal cell is around…..	290-300 mosm/kg	260-320 mosm/kg	320-350 mosm/kg	350-390 mosm/kg	260-320 mosm/kg
Most commonly used growth factor in serum free	Insulin	Hydrocortiso	PDGF	Both a) and	Both a) and b)
Name the chemical used to reduce viscosity in animal tissue culture…..	Polyvinyl chloride	Carboxy methyl	Both a and b	None of the above	Both a and b
……solution is used to maintain embryo primary mammalian cell culture	Ringer solution	Isotonic solution	Tyrode salt solution	Balanced salt solution	Tyrode salt solution
Function of inhibitor……	Cell	Cell	Cell	Cell	proliferation
First tissue culture was done by …….	Carrel	Baker	White	Harrison	Harrison
First tissue culture was done in …..	Chick	Human cells	Frog lymph	Mice	Frog lymph
Vitamins are usually sterilized by …..	Autoclave	Filter	Dry heat	Steam	Filter
Unit 2	**Opt 1**	**Opt 2**	**Opt 3**	**Opt 4**	**Answer**
Freezing point of liquid nitrogen……	-196°C	-96°C	-296°C	-80°C	-196°C
Programmed cell death is called as	Apoptosis	Necrosis	Phagocytosis	Haemolysis	Apoptosis
Animal tissues were chopped into ……size for	1mm	0.11mm	0.01mm	2mm	1mm
…….is the chelating agent used in tissue	EDTA	Mg++	Ca++	None of the	EDTA
In cold trypsinization, the tissue soaked at….	35 °C	50 °C	40 °C	60 °C	40 °C

Enzymes used in disaggregation	insulin	P53	Amylase	Cytokines	Amylase
Ampules are made up of ……	Selenium	Aluminium	Silicons	Silver	Silicons
Cryocanes are…..	Cryofreezers	Ampules	Freezer	Cans	Ampules
……% of glucose is used in the steps of	12	15-Oct	5	20	15-Oct
Cooling rate of cryopreservation is …..	2 °C/min	1.5 °C/min	3 °C/min	1 °C/min	1 °C/min
In cryopreservation the homebox wall thickness is ….	20mm	15mm	10mm	5mm	15mm
The principle of apoptosis was first described	Karl vogt	Walther	John foxton	Andrew	Karl vogt
The componenet helps in regulating apoptosis…..	ATP	Poly ADP ribose	Poly ATP ribose	All the above	ribose polymerase
In MTT assay number of surviving cells can be determined by……..	Dye reduction	Dye uptake	Metabolite produced	Gene expression	Dye reduction
Which of these helps to detoxify free radicals as a cofactor for GSH…..	Zinc	Selenium	Copper	Albumin	Selenium
……is used to measure cell death in LPH assay	Glucate	Lactate	Lactate dehydrogenas	None of the above	Lactate dehydrogenase
In cell characterization, cells obtained from individuals are known as ……..cells	Zenogenic cells	Allogenic cells	Isogenic cells	Autologous cells	Autologous cells
…. Cells are extensively used in cardiovascular	Isogenic	Zenogenic	Allogenic	Autologous	Zenogenic cells
Cells from the body of a donor of a same species are	Allogenic	Isogenic cells	Zenogenic	None of the	Allogenic cells
In three dimensional culture…..days old mouse embryos used for enamol formation.	18	15	10	17	17
Culturing a part of a organ whole organ invitro is called as …. Culture.	Tissue culture	Organ culture	Cell culture	None of the above	Organ culture
Media used in organ culture……..	Liquid	Solid	Liquid and	None of the	solid
……mm slide is used in single coverslip culture	10	20	5	15	20
Organ culture in liquid medium is known as……	Raft method	Grid method	Raft and grid method	Agar gel method	Raft and grid method
In agar gel method ….. parts of 1%agar prepared in	8	9	7	6	7
……metal is used in grid method of cell culture	Stainless	Silver	Aluminium	Copper	Stainless steel
In adult organ culture ……percentage of oxygen is	85	90	95	85	95
……hours old chick embryos used in embryonic	30	20	40	10	40
Doubling time of cells in finite cell culture……	72 hours	24-48 hours	24-36 hours	36-72 hours	24-48 hours
……..colour indicates cell decline during 4th day	Yellow or	Red or pink	Purple	Pink	orange

The buffer used to remove medium from cells during subculturing of cells.	Saline buffer	Phosphate buffer	Phosphate saline buffer	None of the buffer	Phosphate saline buffer
During subculture, the cells are exposed in ……ml	0.2	0.1	0.5	1	0.1
Single coverslip with plasma clot culture was	Carrel	Harrison	Jonh foxtan	Adrew	Harrison
Which type of cultures vessels provides large surface	Carrel flasks	Slides	Roller bottles	Screw cap	Roller bottles
In carrel flasks, thick clots prepared by ….ml of dilute	1.1	1	1.5	1.2	1.2
In 1956, spratt discovered	Organ culture	Flask culture	Slide cultures	Culture of chick	Culture of chick embryo
Cell culture techniques were developed in…..	1955	1956	1954	1952	1954
….% of warm trypsin is used in warm trypsinization.	2.5	0.15	0.25	2	2.5
In trypsinization ……..is used for cell counting	Hemocytom	Rift and grid	Both the	None of the	Hemocytometer
Hematopoietic cells are derived from …..	pancreas	lungs	Bone marrow	skin	Bone marrow
Which cells can survive and proliferate without being attached to a substratum	Hematopoiet ic cells	Suspension cells	Adherent cells	catherins	Suspension cells
Animal cells are stored in…..	Liquid CO_2 container	Liquid nitrogen	Liquid O_2 container	Nitrogen gas	Liquid nitrogen container
Tissue engineering is mostly used for…	Transplantat	AIDS	Chicken Pox	Fever	Transplantation
……..is used to promote cell migration from explants.	Calf serum	Suspension	Plasma clots	Rolling	Plasma clots
The first human cell type to be grown in culture, was originally obtained in 1952 from	Malignant tumor	Carcinoma of the uterine	The He La cell	All the above	The He La cell
Dye reduction is used for determination of	Cell death	Cell survival	Both a and b	None of the	Cell survival
PLA stands for….	Polyglucolic	Poly lycolic	Poly lactic	None of the	Poly lactic acid
Carbon nanotubes are example of	Tissue engineering	Cell synchronizati	Cell cell interaction	Schopers	Schopers
Invasive assay is	Organoid confirmation	Heterocytic culture	Cell transformatio	Genetic instability	Organoid confirmation
Which of the following is not a physiological cell	Glucagon	Thyroxin	Paracrine	Somatostati	Somatostatin
Hypotonic treatment for animal cell characterization is	0.050mKCl	0.075mKLl	0.095mKCl	0.10mKCl	0.075mKLl
LDH stands for …	Lactate dehydrogena	Lactate dihydrogenas	Lactate dehydroxylas	None of the above	Lactate dehydrogenase
Which of the following is used in magnetic sorting…?	Ferritin	Ferroin beads	Percoll beads	Fluorescent	Ferritin beads
Primary cell cultures contains …… types of cells	Homogenou	Heterogenou	Tumor cells	All the	cells

Cells in the same phase at cell cycle is known as…..	Cell death	Cell synchronizati	Cell preservation	None of the above	Cell synchronization
…..is used for the inhibition of DNA synthesis in cell	Dyes	Fluroscent	Cell blockates	All the	Cell blockates
…….% of DMSO is used in cryopreservation.	15	10	20	30	10
Culturing of cells or tissues invitro condition is called	Cell culture	Embryo	Organ culture	None of the	Cell culture
……Cells are matrix dependent cells.	Adherent	Non -	Both a and b	Suspension	Adherent
A medium that retains cell survival without proliferation is…	Growth medium	Maintenance medium	Minimal medium	Liquid nitrogen	Maintenance medium
Unit 3	**Opt 1**	**Opt 2**	**Opt 3**	**Opt 4**	**Answer**
GFP is……..	Green fluorescence peptide	Green fluorescent polyprotein	Green functioning protein	Green fluorescenc e protein	Green fluorescence protein
GFP contains …aminoacids	238	234	245	338	238
GFP is a	Unstable	Stable	Toxic protein	None of the	Stable protein
…….amino acid is present in the 80th position of GFP	Arginine	Glutamine	Alkaline	None of the	Glutamine
GFP emits blue light in combination with….	Sulphur	Chlorophyll	Calcium	Potassium	Calcium
Original GFP is isolated isolated from the organism	Aquarica vectoria	Acquarica bellucida	Acquarica Virginia	Acquarica Telli	Aquarica vectoria
Size of Aquarica vectoria ranges from ….	1-5 cm/diameter	3-5 cm/diameter	5-10 cm/diameter	10-12 cm/diamete	5-10 cm/diameter
Advantages of GFP	side effect	affect the function of	Non toxic to cells	Liver necrosis	All the above
Crystal structure of GFP was solved in…	1996	1991	2002	2012	1996
GFP gets activated with ……..calcium ions.	Three	Five	Six	Two	Three
Gene transfer involves transfer of genes	Recipient into a	Donor into a donor	Recipient into a donor	Donor into a recipient	Donor into a recipient
DNA ligase is used to	Join DNA	mutate DNA	replicate	translate	Join DNA
Uncontrolled growth of cells is called	Cancer	Dengue	Sickle cell	Thalassemi	Cancer
Optimum temperature used for extension process in polymerase temperature	98°C	72°C	55°C	155°C	72°C
Xgal	5-bromo-4-chloro-3-indolyl-?-D-	5-chloro-4-bromo-3-indolyl-?-D-	5-indolyl-4-chloro-3-bromo-?-D-	5-bromo-4-indolyl-3-chloro-?-D-	chloro-3-indolyl-?-D-galactoside

During 1970's … were used in gene transfer	Cat	Mice	Sheep	Pig	Mice
The DNA ligase enzyme isolated from bacteriophage…	T3 DNA	T1 DNA	T2 DNA	T4 DNA	T4 DNA Ligase
Regions present in a recombinant plasmid	Promoter	Antibiotic	Quantity	Tertiary	Promoter
Adaptors are usually	mRNA	DNA	Cysteine	Methionine	DNA Sequence
Alteration of gene for improvement is called	Gene	DNA	Transgenic	Gene	Gene Cloning
GAATTC is the restriction site	ECoRI	HaeIII	HindIII	SmaI	ECoRI
ATP Abbreviation	Alanine tri phosphate	Arginine tri phosphate	Adenosine tri phosphate	Autologous tri	Adenosine tri phosphate
Linkers are short ……sequence	DNA	Aminoacid	Protein	None of the	DNA
Alkaline phosphatase is used to remove	Sulphate	Phosphate	Carbonate	All the	Phosphate
Gene therapy can be carried out using … method	Classical and non	modern	classical and modern	ultramodern	Classical and non classical
Gene therapy can be done	In-vivo and Ex-vivo	lab	invitro	pilot	In-vivo and Ex-vivo
In 1990, gene therapy technique was used to treat……disease	SCID	Diabetes	X-linked inheritance	Alzheimer's disease	SCID
LPLD…..	Lipoprotein lipase disease	Lipoprotein lipase deficiency	Lipid protein lipase deficiciency	Lipid protein lipase	Lipoprotein lipase deficiency
Adenoviral vector contains……..as a genetic materials	dsDNA	ssDNA	dsRNA	ssRNA	dsDNA
Adeno associated virus is….?	Non Toxic	symbiotic	Entomovirus	Toxic virus	Non-toxic virus
SCID …..?	Severe combined immune	Severe combined immune	Simple combined immune	Simple combined immune	combined immune deficiency
Somatic cells are..	Body cells	Sex cells	Ovum cells	Sperm cells	Body cells
Vector used to treat gene therapy….	PBR322	Retiroviral	YAC	BAC	Retiroviral
Retroviral vector contains	ssRNA	dsRNA	ssDNA	dsDNA	ssRNA
ITR ?	Inverted terminal	Inverted tempered	Inverted terminal	Inverted tempered	Inverted terminal repeat
Direct gene transfer	Super	Microinjectio	Embryo	Lipofection	Microinjection
Which of the following is not true regarding invivo gene therapy when compared to exvivo gene theraphy ?	Less invasive	Technically simple	Vectors introduced directly	Safety check possible	Safety check possible

The drug for LPLD was approved in	Nov-12	Oct-12	Nov-13	Oct-13	Nov-12
Abbreviate GOI	Gene of information	Gene of interest	Gene of interruption	Gene of interaction	Gene of interest
The tip of the pipette used for microinjection is about …	0.5 to 5 µm in diameter	0.5 to 1 µm in diameter	0.5 to 1.5 µm in diameter	0.5 to 5.5 µm in	0.5 to 5 µm in diameter
The particles used for transformation during particle	Gold	Silver	Iron	Chlorine	Gold
Liposomes are made up of	Protein bilayers	Carbohydrade bilayers	Lipid bilayers	protein bilayer	Lipid bilayers
Genetic engineering manipulates gene products at the	Protein	Amino acid	DNA	RNA	DNA
Palindromic sequences in a DNA	Form blunt ends when cut by restriction	Reflect the same sequences on two sides	Are not useful in recombinant DNA	Very effective site	Reflect the same sequences on two sides
A cDNA version of a gene includes	Codons for a mature mRNA	Sequences corresponding to	Sequences corresponding to introns	mature DNA	Codons for a mature mRNA
Gene targeting is done on a	Sperm	Egg cell	Fertilized ovum	Early embryonic	Early embryonic cell
…. Consists of recombinant cells containing different fragments of a foreign genome	DNA probes	Homologous recombinants	Genomic libraries	Knockout organisms	Genomic libraries
…… are used to select gene of interest from a genomic library	Restriction enzymes	Cloning vectors	DNA probes	Gene targets	DNA probes
For the same gene it is possible to patent ……	mRNA	Lipids	DNA	protein	mRNA
Which gene transfer technique involves the use of a fatty bubble to carry a gene into a somatic cells	Electroporation	Liposome transfer	Microinjection	Particle bombardme	Liposome transfer
Naked DNA	Is free of nucleic acid	Is free of cell	Is free of protein	Contains just the	Is free of protein
Who invented lipofection	Gardener	Karl	Bongham	abraham	Bongham
How much amount of DNA is injected in	2 picoliter	4 picoliter	6 picoliter	10 picoleter	2 picoliter
In microinjection, the DNA of interest is injected into	Fertilized	Female	Male	zygote	Male pronuclei
cDNA is obtained from reverse transcription of	tRNA	rRNA	mRNA	protein	mRNA
Which of the following is used as a vector for gene	plasmid	protein	protozoa	enzyme	plasmid
Chemical transformants ?	Calcium	PLGA	PEG	PVC	Calcium

Gutless adeno viruses are also called as….	Third generation adenoviral	Second generation adenoviral	First generation adenoviral	All the above.	generation adenoviral vectors
Abbreviate GAT..	Gene Augmentation Therapy	Gene Arrangement therapy	Gene alignment therapy	Genome arrangement therapy	Gene Augmentation Therapy
Targeted gene mutation correction can be done at	DNA probes	hybridization	therapeutic RNA editing	Microarray	therapeutic RNA editing
Unit 4	**Opt 1**	**Opt 2**	**Opt 3**	**Opt 4**	**Answer**
……replacement triggers stem cell differentiation	Serum	BSS	Feeder layer	Amino acid	Serum
What is fertilization?	The fusion of male and female gametes	The division of the zygote into a larger and larger	The development of pattern, shape.	All the above.	The fusion of male and female gametes
Which of the following is a germ layer formed during	Ectoderm	sperm	Vacuole	Blastoderm	Ectoderm
Hollow sphere presenting cell is called ….	Morula	Blastula	Blastocoel	Blastomere	Blastula
The …..grow human embryonic stem cells in the absence of mouse derived "feeder" cell	Nature research	Renke research	All research institute	WiCell research	WiCell research institute
The term for eggs that have only a small amount of yolk that are evenly distributed	Holoblastic	Telolecithal	Isolecithal	Morula	Isolecithal
In which stage of development does a zygote go through the structural and functional specialization of	Growth	Differentiation	Morphogenesis	Fertilization	Differentiation
During gastrulation in vertebrates, the pore created by invagination will become the….	Brain	Heart	Mouth	Ear	Mouth
The fertilized egg undergoes cell division without further growth in the process called…	Cleavage	Differentiation	Morphogenesis	None	Cleavage

Generally only one sperm fertilizes an egg because…	Sperms are so few that are unlikely to arrive at the same time.	Sperm compete against one another before entering and only the fit one accepted	One small entry hole called the blastopore allows only one sperm to fit through and then seals.	When the first sperm membrane fuses with the egg membrane, it separates the fertilization	sperm membrane fuses with the egg membrane, it separates the fertilization membrane and forms a barrier to any other
Which structure in the bird egg corresponds to the egg white used in cooking ?	Albumin	Shell	Yolk	Nucleus	Albumin
Which structure in the bird egg functions to remove the	Allantois	Albumins	Chorion	kideney	Allantois
Which of the following are characteristics of reptile eggs ?	incubation	Large amount of	Sedimentation	excretion	Large amount of yolk
Unlike the sea star gastrula, the frog does not invaginate, but produces a….	Blastopore	Graycresent	Blastocoel	Primitive streak	Blastopore
Because the fertilized egg of frogs has most of the yolk at the …..pole, it is said to be	Animal, telolecithal	Animal, isolecithal	Vegetal, telolecithal	Vegetal isolecithal	Vegetal, telolecithal
Which of these best represent development?	cell death	Cells become specialized in structure and functions	Cell become flattened	Cell separated	Cells become specialized in structure and functions
The series of cellular division by which a one cell layered structure becomes a three-layer embryo is	Gastrulation	Cleavage	Blastulation	Meiosis	Gastrulation
Genes that determine how segments develop during morphogenesis are termed ….. genes.	Induction	Cleavage	Differentiation	Homeotic	Homeotic
A homeotic gene codes for a …. Protein, a sequence of 60 amino acids, sets of which determine pattern	Ion	Morphogenetic	Growth	Regulatory	Regulatory
Proteins that influence morphogenesis are….	Homebox	Mutagens	Morphogenes	Trophoblast	Homebox genes
Which stage would show the development of the ectoderm and endoderm germ layers ?]	Gastrulation	Neurulation	Blastulation	Cleavage	Gastrulation

Maternal development is the theory that	The sperm determines which genes are turned on and off.	Mother cell cytoplasm is distributed to various cells of the morula and deternines which genes	Only one sperm in four will locate the true egg rather than the polar bodies.	Boy and girl fetuses are identified at an early stage.	cytoplasm is distributed to various cells of the morula and deternines which genes are turned on and off.
Which statement is not true about homeotic genes ?	They have been found in almost all eukaryotic organisms.	They are the master genes that control all the other genes in the organism.	Each homeotic genes has a variable region and a sequence called homebox.	They are sequentially arranged in the chromosom e in the same sequences they are activated	sequentially arranged in the chromosome in the same sequences they are activated during development of the embrro in drosophila.
Spermatogenesis is not directly affected by which	GnRH	Inhibin	FSH	LH	LH
The most powerful form of the inivitro process is…..	Intracytoplas mic sperm	Intercytoplas mic sperm	Cytoplasmic sperm	invivo	c sperm injection
The technique used to conception of a human embryo outside the mother's body	Invitro fertilization	Invivo fertilization	In vitro fetus formation	invitro	Invitro fertilization
The best embryo chosen for invitro fertilization is	Initial fresh embryos	Fresh embryonic	Whole embryo	ovum	Initial fresh embryos
…..weeks to prepare a female fully and get her ready for egg retrieval.	4 to 6	5 to 6	2 to 3	4 to 8	4 to 6
The first test tube baby was born in the year….	1968	1978	1988	1975	1978
Embryonic stem cells are determined to be…	Omnipotent	Plastic	Totipotent	Pluripotent	Pluripotent
Stem cells can come from..	Adult	Umbilicl	Human	All the	All the above
To reach a stem cell, an embryo must be	Disassemble	Destroyed	Killed	All the	All the above

The issue of embryonic stem cell research has generated public debate…	Embryonic stem cell research could	Embryonic stem cell research could	Embryonic stem cell research could destroy	Both a and b.	Both a and b.
Stem cell research involves the research of … of a human being.	Primordial cells	Primary cells	Pre embryonic	Germinal cells	Primordial cells
The use of differentiated stem cells in clinical trials might	Shorten phase I	Reduce the number of animal tests	Shorten phase II	Decrease the overall timeline.	Reduce the number of animal tests
Stem cell research could yield remedies for ….	Parkinson's and Alzheimer's	AIDS	SCID	Cancer	Parkinson's and Alzheimer's disease
Embryonic stem (ES) cells are an attractive source of material for therapeutic cloning because…	They can be induced to assume any cell fate	ES cells are not targets for the host immune response, so tissue rejection is	There are no other sources of stem cells to use for therapeutic cloning , so ES cells are	Molecular cloning	They can be induced to assume any cell fate
Embryonic stem cells are……	Pluripotent	Self	Less in	All the	Pluripotent
IVF abbreviation	In-vivo	In-vitro	In-vivo	In-vitro	fertilization
Gametogenesis involves…	SpermatogenesisOogenesis	ovum	Blastulation	Gastrulation	SpermatogenesisOogenesis
Gametogenesis occur in…	Intestine	Gonads	Egg	All the	Gonads
Gametes are of …. Types	Three	Four	Two	Five	Two
The testis is formed of thousands of minute tubules called as ….	Interstitial tubules	Seminiferous tubules	Secondary tubules	T cells	Interstitial tubules
Repeated mitotic cell division takes place in…….	Multiplication phase	Growth phase	Maturation phase	B cells	Multiplication phase
Transformation of the spermatid into spermatozoon is	Spermiogen	Oogenesis	Parthenogene	Granulogen	Spermiogenesis
Acrosomes of the sperm develops from….	Mitochondri	Microtubules	Golgibodies	Graules	Golgibodies
…….is a process by which the ovum develops	Ovulation	Oogenesis	Spermiogenes	Oogonia	Oogenesis

Multiplication phase in oogenesis is also called as …….	Growth phase	Maturation phase	Multiplication phase	Developing phase	Multiplication phase
In mammals, the follicle cells and developing ovum together constitute a …..	Liquor follicle	Graffian follicle	Membrane granules	Corona radiate	Graffian follicle
The large nucleus of the oocyte is called…….	Cortical granules	Germinal vesicle	Membrane vesicles	Desosomes	Germinal vesicle
The movement of the yolk to the oocyte is facilitate by a structure called…	Microvilli	Macrovilli	Nanovilli	villi	Microvilli
During oogenesis, the oocyte is surrounded by a transparent membrane called…..	Desmosomes	Graffian follicle	Theca externa	Zona pellucid	Zona pellucid
Union of spermatozoon and egg resulting in the formation of zygote is called…..	Gametogenesis	Fertilization	Polysomeogenesis	None of the above	Fertilization
Fertilization maintains the …. no of chromosomes in	Haploid	Diploid	Both the	None of the	Diploid
The enzymes helpful for the entry of spermatozoa during fertilization…..	Heterogenase	Saccoglossus	Hyaluronidase	Dehudrogenase	Hyaluronidase
Physiological changes during fertilization……	mitochondria	Cytoplasmic movement	Nuclear transfer	cytoplasm	Cytoplasmic movement
During fertilization NAD + ATP is converted to NADP + ADP in the presence of	Kinase	Mutase	Dehydrogenase	Phosphatase	Kinase
The fertilization egg freely rotates inside the …….fluid around fertilization membrane.	Berivitelline fluid	Copulation fluid	Grey crescent	Lactic acid	Berivitelline fluid
Cleavage is initiated by the appearance of a groove or constriction called …..	Cleavage funnel	Cleavage roll	Cleavage bottom	Cleavage furrow	Cleavage furrow
In invitro fertilization, Embryo transfer involves collection ……from donor.	Fertilized ovum	Unfertilized ovum	Both	None of the above	Fertilized ovum
Unit 5	**Opt 1**	**Opt 2**	**Opt 3**	**Opt 4**	**Answer**
……method is to insert genes into eggs of animals	Micro-	Electroporati	Micromanipul	All the	Micro-injection
Who devised the scientific breeding of life-stock such as sheep ?	Jethro tull	Viscount turnip	Robert backwell	Richard longman	Jethro tull
Free living organisms that have a foreign gene inserted into them are…..	Recombinant	Forensic	Transgenic	Polymorphs	Transgenic

……is the use of transgenic farm animals to produce pharmaceuticals, the product is obtainable from the milk of pharmaceuticals.	Gene pharming	Gene remediation	Gene formulation	gene	Gene pharming
Human therapeutic cloning may be principally used to….	Manipulate embryos	Repair faulty tissues and organs	Create complete organism	Provide organs for xenotranspl	Repair faulty tissues and organs
Somatic cell nuclear transfer potentially has the following advantage over microinjection for producing	It may speed xeno-	It is easier	Requires fewer animals	Potential to make	It is easier
University of Idaho cloned Idaho gem using nuclear DNA from ……	Horse sperm	Horse egg	Taz	A mule fetus	A mule fetus
Dolly was produced by somatic cell nuclear transfer.	Stem cell harvesting	Mule production	Xeno transplantatio	Embryo splitting	Embryo splitting
Which of the following can also be described as a form	Plasmid transfer	Agrobacteriu m	Embryo splitting	Microinject	Microinjection
Which of the following is most likely to be used to create a transgenic animal	Microinjecti on	Gene therapy	Macro injection	Lipofection	Gene therapy
……..procedures to give patient healthy genes to make up for a faculty gene.	1n	2n	1n + 1n	3n	1n + 1n
Cloning of mammals involves injecting a……nucleus adult cells into a enucleated egg.	Sexually reproduced	Genetically altered	Both a and b	Cloned	Genetically altered
Sexual reproduction dilutes the genes of a…… animal.	Disease is incurable, life	Affected cell types have been	The cost is low	The normal gene has been	The cost is low
Current important criteria for selecting disease candidates for human gene therapy include the following except…….	51 in two polypeptide chains	51 in four polypeptide chains	70 in two polypeptide chains	21 in two polypeptide chains	51 in two polypeptide chains
The total number of aminoacid units in the cattle insulin is…..	Human organs	Human tissues	Human proteins	Human cell for gene theraphy	Human proteins
Scientists have tried to clone animals genetically altered to contain human genes. To this point such alterations to animals have been attempt to get the	Somatic cell transfer	Embryo splitting	Nuclear transfer	All the above	Somatic cell transfer
……is the method used to produce transgenic animal today					

Which of the following is a key difference between the production of transgenic animals and transgenic plants ?	Transgenic animals have only few of their cells transformed, while transgenic	Chlorophyll	mitochondia	chloroplast	Transgenic animals have only few of their cells transformed, while transgenic
The immediate application of cloning techniques will be in the creation of	Genetically enhanced	Human clones	For transplant into humans	All the above	enhanced livestock
Which of the following is not a type of animal patent?	Gain of function	Loss of function	Bioreactors	Landrace	Landrace
Due to lack of ….. animal cells appear to be circular	Cell wall	Nucleus	Cellulose	All the	Cell wall
Insertion of genes into eggs of animals is carried out by	Micro-	Electroporati	Micromanipul	All the	Micro-injection
Preproinsulin is synthesized in the ---------- cells of pancreas	α cells	β cells	γ cells	None of the above	α cells
Swine insulin is transformed in to human insulin by	alanine	Cysteine	Guanine	Valine	alanine residue
Sendai viral capsid stimulate human chromosome to produce----------	Interferon	Interleukin	Immunoglobu lin M	Immunoglo bulin G	Interferon
Interferon γ is produced by sensitized lymphocytes in response to the----------	Sensitizing antigen	antigens	Specific antigens	antibody	Sensitizing antigen
Human peptide hormone synthesized in bacterial cell	Estrogen	secretin	scmatoliberin	somatostati	somatostatin
Productioln of active somatostain requires ---------- --- to cleave β galactosidase from inactive somatostatin	Cyauaogcn bromide	Growth hormone	Restriction endonuclease	zymase	Cyauaogcn bromide
β- propiolactone inactivated virus in embryonated duck eggs used to develop--------	Rubella vaccine	Rabies vaccine	Plague vaccine	Tetanus vaccine	Rabies vaccine
Non-infectious influenza virus grown in chick embryos for vaccine development is by	formalin treatment	ultraviolet radiation	mutant strain formation	all the above	all the above
-------is absent in humans causes the inherited disease ovteogenesis imperfecta	COLLIAI gene	AIADAI	Alpha AI gene	COLA BI gene	COLLIAI gene
Transgenic ---------- express large quantities of the human alp hal-antitrypsin protein in their milk	Cow sheep	rabbit	embryo splitting	monkey	Cow sheep
which Serve as a source of transplanted organs for humans	Transgenic	Transgenic	Transgenic	Transgenic	Transgenic pigs

Transgenic pig organs or "bioreactors" containing pig cells to provide temporary support for	Failing spleen	Failing liver	Failing kidneys	Failing heart	Failing liver
Porcine endogenous retrovirus is ----------	Pig retrovirus	Bovine	Goat retrovirus	Human	Pig retrovirus
Transgenic technology has the potential of medical therapy, but it raises questions about these issues	Creation of new life forms and crossing	Long-term effects on human health and the	Unintended personal, social, and cultural	change	Long-term effects on human health and the environment
Retroviral method of DNA transfer successfully used in 1974 when a simian virus was inserted into ----------	Mice embryos, resulting in mice carrying	Rabbit embryos, resulting in mice carrying	Pig embryos, resulting in mice carrying this DNA	Rodent embryos, resulting in mice	Mice embryos, resulting in mice carrying this DNA
1n 1997, the first transgenic cow, Rosie, produced human protein-enriched milk at ----	1.4 grams per liter	2.4 grams per liter	3.4 grams per liter	4.4 grams per liter	2.4 grams per liter
The first death of participant in gene therapy experiment is ----------	Jesse Gelsinger	ames Wilson	Mark Batshaw	None of the above	Jesse Gelsinger
When you cross species boundaries, you combine the genetic or cellular material of two species	Is it ethical to experiment with part-human	Do the potential medical benefits	Should guidelines be constructed for such research	agaisnt ethics	Do the potential medical benefits outweigh the ethical concerns
Chimerus comprise a mixture of cells from two or more	Genetically distinct organisms of	Organisms of different species	They are mosaics at the sexual level	chimera	Genetically distinct organisms of
---------- Carrying a gene that promotes the development of various human cancers	Harvard mouse	Onco mouse	mouse	mice	Onco mouse
Small pox vaccines was first discovered by	Loulis	Paul ehlrich	Edward	Robert	Loulis Pasteur
Fusion of rat b cells with mouse myeloma cells are called as	Interspecific clone	interspecies hybridoma	hybrid cell	a;l the above	Interspecies hybridoma
Transplantation of heart valve from pig to human is	Allograft	Xenograft	Isograft	zoograft	Xenograft
Tetanus vaccine is an	Inactivated vaccine	Toxoid vaccine	Recombinant vaccine	cellular vaccine	Toxoid vaccine
Live attenuated vaiccine was first introduced by	Louis	Paul Ehlrich	Edward	Robert	Louis pasteur.
The day of detection of a mouse vaginal plug is called	Dating the	embryo	estrus	gastrulation	embryo
Optimal age for whole embryo preparation is	10 days	13 days	16 days	20 days	13 days

BCG is a ----------	Attenuated vaccine	Toxoid vaccine	Recombinant vaccine	Cellular Vaccine	Attenuated vaccine
An example for whole organism as vaccine	Tetanus	Tuberculosis	Diphtheria	Hepatitis	Tuberculosis
In DNA vaccine technology ---------- injected directly to the muscle	Plasmid DNA	DNA fragments	Genomic DNA	None of the above	Plasmid DNA
Which among the following statement is false?	D NA vaccines induces response to a	Attenuated vaccine induce cell mediated		DNA vaccines do not generate immunologi	DNA vaccines do not generate immunologic memory
Subculture interval is ---------- days	5	7	9	14	7
In Spheroid culture necrotic cells are present in	Outer layer	Middle layer	Inner layer	In the	Inner layer
In *in vivo* condition tumor necrosis starts from the	Outer layer	Middle layer	Inner layer	In the	Outer layer
Experimental liver modeling can be obtained by	Spheroids co culture	heterotypic 3D culture	Both a and b	Embryo culture	Both a and b
Oxygen regulated gene expression during embryonic development studied	Three dimentional	Organ culture	Tissue culture	Stem cell Culture	dimentional culture
Three dimensions of the animal cells are normally ……..in shape	Round	Polygonal	Rectangular	Spherical	Spherical
Due to the lack of ---------- animal cells appear to be	Cell wall	nucleus	cellulose	globin	Cell wall
Which of the following is the cytoskeleton of animal	Actin	Mititic	micro tubules	mitosis	Actin filaments