METHODICAL RECOMMENDATIONS FOR LABORATORY WORK

OBJECTS OF BIOTECHNOLOGY

(ANIMAL BIOTECHNOLOGY)

Almaty, 2021

Laboratory work №1.

The structure of an animal cell. Safety rules in laboratory.

Aim of the work: Acquaintance students with structure of an animal cell and safety rules of work in biotech laboratory.

Animal Cell Structure. Animal cells are typical of the eukaryotic cell, enclosed by a plasma membrane and containing a membrane-bound nucleus and organelles. Unlike the eukaryotic cells of plants and fungi, animal cells do not have a cell wall. This feature was lost in the distant past by the single-celled organisms that gave rise to the kingdom Animalia. Most cells, both animal and plant, range in size between 1 and 100 micrometers and are thus visible only with the aid of a microscope

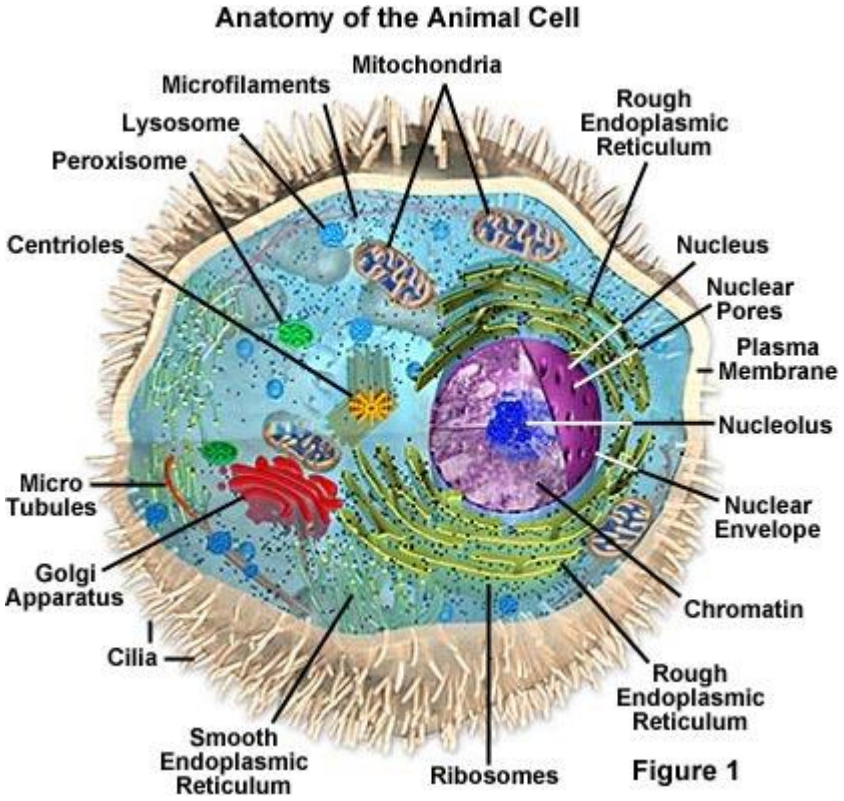

Figure 1

The lack of a rigid cell wall allowed animals to develop a greater diversity of cell types, tissues, and organs. Specialized cells that formed nerves and muscles—tissues impossible for plants to evolve—gave these organisms mobility. The ability to move about by the use of specialized muscle tissues is a hallmark of the animal world, though a few animals, primarily sponges, do not possess differentiated tissues. Notably, protozoans locomote, but it is only via nonmuscular means, in effect, using cilia, flagella, and pseudopodia.

The animal kingdom is unique among eukaryotic organisms because most animal tissues are bound together in an extracellular matrix by a triple helix of protein known as collagen. Plant and fungal cells are bound together in tissues or

aggregations by other molecules, such as pectin. The fact that no other organisms utilize collagen in this manner is one of the indications that all animals arose from a common unicellular ancestor. Bones, shells, spicules, and other hardened structures are formed when the collagen-containing extracellular matrix between animal cells becomes calcified.

Animals are a large and incredibly diverse group of organisms. Making up about three-quarters of the species on Earth, they run the gamut from corals and jellyfish to ants, whales, elephants, and, of course, humans. Being mobile has given animals, which are capable of sensing and responding to their environment, the flexibility to adopt many different modes of feeding, defense, and reproduction. Unlike plants, however, animals are unable to manufacture their own food, and therefore, are always directly or indirectly dependent on plant life.

Most animal cells are diploid, meaning that their chromosomes exist in homologous pairs. Different chromosomal ploidies are also, however, known to occasionally occur. The proliferation of animal cells occurs in a variety of ways. In instances of sexual reproduction, the cellular process of meiosis is first necessary so that haploid daughter cells, or gametes, can be produced. Two haploid cells then fuse to form a diploid zygote, which develops into a new organism as its cells divide and multiply.

The earliest fossil evidence of animals dates from the Vendian Period (650 to 544 million years ago), with coelenterate-type creatures that left traces of their soft bodies in shallow-water sediments. The first mass extinction ended that period, but during the Cambrian Period which followed, an explosion of new forms began the evolutionary radiation that produced most of the major groups, or phyla, known today. Vertebrates (animals with backbones) are not known to have occurred until the early Ordovician Period (505 to 438 million years ago).

Fluorescence Microscopy of Cells in Culture

Cells were discovered in 1665 by British scientist Robert Hooke who first observed them in his crude (by today's standards) seventeenth century optical microscope. In fact, Hooke coined the term "cell", in a biological context, when he described the microscopic structure of cork like a tiny, bare room or monk's cell. Illustrated in Figure 2 are a pair of fibroblast deer skin cells that have been labeled with fluorescent probes and photographed in the microscope to reveal their internal structure. The nuclei are stained with a red probe, while the Golgi apparatus and microfilament actin network are stained green and blue, respectively. The microscope has been a fundamental tool in the field of cell biology and is often used to observe living cells in culture. Use the links below to obtain more detailed information about the various components that are found in animal cells.

Centrioles - Centrioles are self-replicating organelles made up of nine bundles of microtubules and are found only in animal cells. They appear to help in organizing cell division, but aren't essential to the process.

Cilia and Flagella - For single-celled eukaryotes, cilia and flagella are essential for the locomotion of individual organisms. In multicellular organisms, cilia function to move fluid or materials past an immobile cell as well as moving a cell or group of cells.

Endoplasmic Reticulum - The endoplasmic reticulum is a network of sacs that manufactures, processes, and transports chemical compounds for use inside and outside of the cell. It is connected to the double-layered nuclear envelope, providing a pipeline between the nucleus and the cytoplasm.

Endosomes and Endocytosis - Endosomes are membrane-bound vesicles, formed via a complex family of processes collectively known as endocytosis, and found in the cytoplasm of virtually every animal cell. The basic mechanism of endocytosis is the reverse of what occurs during exocytosis or cellular secretion. It involves the invagination (folding inward) of a cell's plasma membrane to surround macromolecules or other matter diffusing through the extracellular fluid.

Golgi Apparatus - The Golgi apparatus is the distribution and shipping department for the cell's chemical products. It modifies proteins and fats built in the endoplasmic reticulum and prepares them for export to the outside of the cell.

Intermediate Filaments - Intermediate filaments are a very broad class of fibrous proteins that play an important role as both structural and functional elements of the cytoskeleton. Ranging in size from 8 to 12 nanometers, intermediate filaments function as tension-bearing elements to help maintain cell shape and rigidity.

Lysosomes - The main function of these microbodies is digestion. Lysosomes break down cellular waste products and debris from outside the cell into simple compounds, which are transferred to the cytoplasm as new cell-building materials.

Microfilaments - Microfilaments are solid rods made of globular proteins called actin. These filaments are primarily structural in function and are an important component of the cytoskeleton.

Microtubules - These straight, hollow cylinders are found throughout the cytoplasm of all eukaryotic cells (prokaryotes don't have them) and carry out a variety of functions, ranging from transport to structural support.

Mitochondria - Mitochondria are oblong shaped organelles that are found in the cytoplasm of every eukaryotic cell. In the animal cell, they are the main power generators, converting oxygen and nutrients into energy.

Nucleus - The nucleus is a highly specialized organelle that serves as the information processing and administrative center of the cell. This organelle has two major functions: it stores the cell's hereditary material, or DNA, and it coordinates the cell's activities, which include growth, intermediary metabolism, protein synthesis, and reproduction (cell division).

Peroxisomes - Microbodies are a diverse group of organelles that are found in the cytoplasm, roughly spherical and bound by a single membrane. There are several types of microbodies but peroxisomes are the most common.

Plasma Membrane - All living cells have a plasma membrane that encloses their contents. In prokaryotes, the membrane is the inner layer of protection surrounded by a rigid cell wall. Eukaryotic animal cells have only the membrane to contain and protect their contents. These membranes also regulate the passage of molecules in and out of the cells.

Ribosomes - All living cells contain ribosomes, tiny organelles composed of approximately 60 percent RNA and 40 percent protein. In eukaryotes, ribosomes are made of four strands of RNA. In prokaryotes, they consist of three strands of RNA.

In addition the optical and electron microscope, scientists are able to use a number of other techniques to probe the mysteries of the animal cell. Cells can be disassembled by chemical methods and their individual organelles and macromolecules isolated for study. The process of cell fractionation enables the scientist to prepare specific components, the mitochondria for example, in large quantities for investigations of their composition and functions. Using this approach, cell biologists have been able to assign various functions to specific locations within the cell. However, the era of fluorescent proteins has brought microscopy to the forefront of biology by enabling scientists to target living cells with highly localized probes for studies that don't interfere with the delicate balance of life processes.

https://micro.magnet.fsu.edu/cells/animalcell.html

Safety rules in laboratory. Students successfully completing this laboratory training should be able to:

1. Locate and properly use basic emergency equipment such as eye wash stations, first aid kits, fire extinguishers, a telephone, etc.
2. Wear proper attire for the microbiology lab at all times.
3. Wash hands prior to and following laboratories and at any time contamination is suspected.
4. Never eat or drink in the laboratory. EVER.
5. Never apply cosmetics, handle contact lenses, place objects (fingers, pencils, etc.) in the mouth, or touch the face.
6. Report all injuries immediately to the instructor.

7. Report all spills or broken glassware to the instructor and receive instructions for clean- up.

8. Disinfect the lab benches prior to and after laboratory exercises.

9. Keep lab benches clear of extraneous materials.

10. Follow appropriate steps in the event of an accident.

1.1 Introduction

Laboratory safety is critically important in the microbiology lab because we will be working with a number of potentially pathogenic organisms, Biosafety (BSL) 1 and 2 organisms, and toxic chemicals. BSL 1 organisms are defined as organisms that are not consistently known to cause disease in healthy individuals. BSL2 organisms are defined as organisms that are associated with human disease via percutaneous injury, ingestion and mucous membrane exposure. While none of the organisms or chemicals utilized in this laboratory is excessively pathogenic or toxic, any of these might cause problems if handled incorrectly or inappropriately. Therefore, proper lab procedures must be followed at all times. The purpose of this guide is to familiarize you with some basic aspects of lab safety and the facilities found in the laboratory.

1.2 Safety Procedures A). Overriding Principles

Please treat every culture used in the lab as every chemical utilized as a hazardous chemical. Follow all safety procedures so that we can ensure that no student or instructor becomes infected by any microorganisms or harmed by any chemical used in this class.

B). Safety Equipment

Identify and know the location of the following safety equipment in the lab:

- The nearest safety shower and eye- wash station (figure 1)
- The nearest fire extinguisher/fire pull (figure 2)
- The nearest fire blanket
- The nearest fire alarm pull station (figure 2)
- The first aid kit (figure 3)
- The lab bench disinfectant
- The nearest emergency phone (located on the wall in each lab)
- The evacuation route

1.3 Proper Attire

Please wear proper clothing for the microbiology lab at all times. Proper clothing consists of:

- Closed toe shoes that protect the feet from falling cultures, chemical spills, or dropped glassware.
- Absolutely no sandals or other footwear that leave toes exposed.
- Clothing without loose sleeves or scarves that may droop into cultures, burners, or onto the lab benches. No exposed bellies, either!
- Please tie back all long hair to prevent it from falling into burners or cultures.

- Remove gloves BEFORE leaving the lab.
- Purchase a lab coat which will protect your clothing from stains and exposure to microorganisms. Lab coats stay housed in the microbiology lab.
- Goggles

1.4 Hand Washing

Students must wash their hands with disinfectant soap prior to beginning the laboratory and upon completion of the laboratory exercises. Always keep hands away from your mouth, eyes, nose, and face during the laboratory period. Keep all objects away from your face, mouth, or eyes during the microbiology lab. DO NOT APPLY MAKE- UP WHILE IN THE LAB!

1.5 Food and Drink in the Microbiology Lab

There should be absolutely no food, drink or open food containers in the microbiology lab (figure 3). This includes chewing gum. If you need a short break, carefully wash your hands, remove the lab coat and leave the laboratory.

Absolutely no eating or drinking at any time.

1.6 Bunsen Burners, Gas Jets and alcohol stove

When using the Bunsen burners and alcohol burners it is important to remember basic safety rules:
- Keep long hair tied back out of the way.
- Secure any loose clothing.
- Turn burner off when not in use. The lab gets very hot when all the burners are going at once, so please turn off unnecessary burners.
- Turn gas jets completely off when not in use.

Before ignition alcohol stove (Fig. 4, A) check the availability of alcohol in her tank and the quality of the wick. Alcohol should fill the tank to 1/3 - 2/3 of its volume. It is necessary that the wick was the same thickness, freely touching the tank bottom and not very tightly held in a tube. The alcohol can be poured only in repayment of the alcohol stove.

To ignite the alcohol stove, remove the cap, spreading the wick and brought him a lighted match. Do not light the alcohol stove from the other alcohol stove lit! Do not blow on the flame of an alcohol stove to extinguish it!

Before ignition of the gas burner (Fig. 5, A) make sure that no device failures and damage the tubes attached to the tap of the gas pipeline.

1.7 Spills and Exposure

Proper technique for microbial spills involves:

1. Do not try to catch dropped culture tubes or dishes before they hit the ground. This puts your face right down into the spray of droplets from the contaminated materials.
2. Do not touch the spill.

3. Flood the spill area and any contaminated materials with disinfectant and cover with paper towel.

4. Let stand for at least 15 minutes.

5. Alert your instructor to ensure proper disposal.

For all injuries including ALL cuts (no matter how minor) and burns alert your instructor immediately. For simple exposure to cultures, please wash the infected area immediately and carefully with disinfectant soap.

1.8 Broken Glass or Other Sharps

Do not touch broken glass or sharps with your hands. Use the broom and dustpan to collect the sharp materials and discard them in the appropriate container (the broken glass disposal container or the red sharps container for sharps that have been contaminated with blood/cultures) (Figure 7).

1.9Culture Handling and Materials Disposal

All cultures must be disposed of in the red Biohazard labeled bags and Biohazard disposal can (figure 7). Chemical waste including stain waste must also be disposed of in the proper collection container (figure 8). You will be further instructed in all proper laboratory and disposal procedures and will be expected to know and follow those procedures.

1.10 Health Issues

If you are pregnant or immune compromised please contact your instructor so that a few additional appropriate safety measures can be taken. Also talk to you health care provider and provide them with a list of organisms that you will be working with.

1.11 Laboratory Work

Before beginning any laboratory exercise you should:

- Carefully read all instructions noting all safety procedures for the laboratory exercises assigned for the given laboratory period;
- Store all unnecessary personal items in the cubbyholes in the laboratory;
- Wash your hands with disinfectant soap
- Wipe down the lab bench with disinfectant
- Only have materials required for the exercise on the lab bench.

At the end of each laboratory period you should:

- Place all cultures in the proper incubators or racks
- Dispose of all materials as required by the instructor
- Check to see that all Bunsen burners are off and the gas valve is completely closed.
- Wipe the lab bench with disinfectant (figure 9)

- Carefully wash your hands with disinfectant soap.

Students should adhere to the following rules:

1). Comply with the dress code established by the university and explained by the instructor. 2). Wear protective clothing (goggles, gloves, labcoats, close toed shoes) at all times when working in the microbiology lab.

3). Not eat any food, chew gum, smoke, or drink any beverage in the laboratory.

4). Dispose of waste in the manner prescribed by the instructor. 5). Report any and all accidents to the instructor immediately.

6). Work in a safe manner and leave a neat and clean station for the next student. 7). Not bring visitors (any person not registered in the course) into the laboratory.

Students must understand the consequences of not following any of the above rules may result in a zero grade for the given exercise. Multiple violations will result in dismissal from the laboratory class for the semester.

References:

1. R. Renaville and A. Burny (eds.), Biotechnology in Animal Husbandry, 2001. Kluwer Academic Publishers. Printed in the Netherlands. P. 209-223.

2.Anim al Biotechnology. Technologies, Markets & Companies – Edited by Prof. K.K. Jain. Jain PharmaBiotech. A Jain Pharma Biotech Report. 2013. 215 p.

Additional visual material for study:

1. Video ―Safety Equipment / Lab Safety‖ https://www.youtube.com/watch?v=IiHEYtnKfF0 https://youtu.be/rneeZlxyl_Y
2. Aseptic Techniques: Cell Culture Basics https://www.youtube.com/watch?v=nr1tV_LuqJk

Laboratory work №2.

The techniques of embryo transfer.

Aim of the work: Acquaintance students with The techniques of embryo transfer.

Cellular differentiation is the process in which a cell changes from one cell type to another. Usually, the cell changes to a more specialized type. Differentiation occurs numerous times during the development of a multicellular organism as it changes from a simple zygote to a complex system of tissues and cell types.

Artificial insemination (AI) is the deliberate introduction of sperm into a female's cervix or uterine cavity for the purpose of achieving a pregnancy through in vivo fertilization by means other than sexual intercourse. It is a fertility treatment

for humans, and is common practice in animal breeding, including dairy cattle (see Frozen bovine semen) and pigs.

Artificial insemination may employ assisted reproductive technology, sperm donation and animal husbandry techniques. Artificial insemination techniques available include intracervical insemination and intrauterine insemination. The beneficiaries of artificial insemination are women who desire to give birth to their own child who may be single, women who are in a lesbian relationship or women who are in a heterosexual relationship but with a male partner who is infertile or who has a physical impairment which prevents full intercourse from taking place. Intracervical insemination (ICI) is the easiest and most common insemination technique and can be used in the home for self-insemination without medical practitioner assistance.[1] Compared with natural insemination (i.e., insemination by sexual intercourse), artificial insemination can be more expensive and more invasive, and may require professional assistance.

Embryo transfer refers to a step in the process of assisted reproduction in which embryos are placed into the uterus of a female with the intent to establish a pregnancy. This technique (which is often used in connection with in vitro fertilization (IVF)), may be used in humans or in animals, in which situations the goals may vary.

Embryo transfer can be done at day two or day three, or later in the blastocyst stage, which was first performed in 1984. Factors that can affect the success of embryo transfer include the Endometrial receptivity, Embryo quality and Embryo transfer technique.

Procedure. The embryo transfer procedure starts by placing a speculum in the vagina to visualize the cervix, which is cleansed with saline solution or culture media. A soft[9] transfer catheter is loaded with the embryos and handed to the clinician after confirmation of the patient's identity. The catheter is inserted through the cervical canal and advanced into the uterine cavity.[22]

There is good and consistent evidence of benefit in ultrasound guidance,[9] that is, making an abdominal ultrasound to ensure correct placement, which is 1–2 cm from the uterine fundus. There is evidence of a significant increase in clinical pregnancy using ultrasound guidance compared with only "clinical touch".[11] Anesthesia is generally not required. Single embryo transfers in particular require accuracy and precision in placement within the uterine cavity. The optimal target for embryo placement, known as the maximal implantation potential (MIP) point, is identified using 3D/4D ultrasound.[23] However, there is limited evidence that supports deposition of embryos in the midportion of the uterus.[9]

After insertion of the catheter, the contents are expelled and the embryos are deposited. Limited evidence supports making trial transfers before performing the procedure with embryos.[9] After expulsion, the duration that the catheter remains inside the uterus has no effect on pregnancy rates.[24] Limited evidence suggests avoiding negative pressure from the catheter after expulsion.[9] After withdrawal, the catheter is handed to the embryologist, who inspects it for retained embryos.

In the process of zygote intrafallopian transfer (ZIFT), eggs are removed from the woman, fertilised, and then placed in the woman's fallopian tubes rather than the uterus.

Control questions:

1. Cell differentiation.
2. Artificial insemination.
3. In vitro fertilization.
4. Embryo transfer in animals.

References:

1. R. Renaville and A. Burny (eds.), Biotechnology in Animal Husbandry, 2001. Kluwer Academic Publishers. Printed in the Netherlands. P. 209-223.

2. Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: ed. by W. H. Freeman; 2000.

3. B.R. Glick & J.J. Pasternak. Molecular Biotechnology - Principles and Applications of Recombinant DNA. 3rd Edition). 2003

4. I.R. Gordon. Reproductive Technologies in Farm Animals. 2004. DOI 10.1079/9780851998626.0000

5. Animal Biotechnology. Technologies, Markets & Companies – Edited by Prof. K.K. Jain. Jain PharmaBiotech. A Jain Pharma Biotech Report. 2013. 215 p.

Internet resources:

https://en.wikibooks.org/wiki/Anatomy_and_Physiology_of_Animals/Reproductive_System

http://people.ucalgary.ca/~browder/transgenic.html https://www.ncbi.nlm.nih.gov/books/NBK207576/

Additional visual material for study:

https://www.uaex.edu/publications/pdf/fsa-3119.pdf http://www.fao.org/3/X6500E03.htm#:~:text=Embryo%20transfer%20is%20an%20artificial,wh ere%20they%20develop%20to%20term.

1. Video ―Embryo Transfer: Beef Part 2‖ https://www.youtube.com/watch?v=DkUcMnOd8g8

Laboratory work №3.

Cell culture media and components.

Aim of the work: Acquaintance with cell culture media and components.

Culture Media: The culture medium is the most important component of the culture environment, because it provides the necessary nutrients, growth factors, and hormones for cell growth, as well as regulating the pH and the osmotic pressure of the culture. Although initial cell culture experiments were performed using natural media obtained from tissue extracts and body fluids, the need for standardization, media quality, and increased demand led to the development of defined media. The

three basic classes of media are basal media, reduced-serum media, and serum-free media, which differ in their requirement for supplementation with serum.

Media Components. Balanced Salt Solutions: A balanced salt solution (BSS) is composed of inorganic salts and may include sodium carbonate and, in some cases, glucose. Commercial complete media will list which BSS formulation was used.

Serum: Serum is vitally important as a source of growth and adhesion factors, hormones, lipids and minerals for the culture of cells in basal media. In addition, serum also regulates cell membrane permeability and serves as a carrier for lipids, enzymes, micronutrients, and trace elements into the cell. However, using serum in media has a number of disadvantages including high cost, problems with standardization, specificity, variability, and unwanted effects such as stimulation or inhibition of growth and/or cellular function on certain cell cultures. If the serum is not obtained from reputable source, contamination can also pose a serious threat to successful cell culture experiments. Always check new batches of serum before use. The quality and the composition can vary greatly from batch to batch. Serum is inactivated by incubating it for 30 min at +56oC. Originally, heating was used to inactivate complements for immunoassays, but it may also have other, undocumented effects.

Other Supplements: In addition to serum, tissue extracts and digests have traditionally been used to supplement tissue culture media. The most common ones are amino acid hydrolysates (from beef heart) and embryo extract (chick embryo).

Basal Media: The majority of cell lines grow well in basal media, which contain amino acids, vitamins, inorganic salts, and a carbon source such as glucose, but these basal media formulations must be further supplemented with serum.

Reduced-Serum Media: Another strategy to reduce the undesired effects of serum in cell culture experiments is to use reduced-serum media. Reduced-serum media are basal media formulations enriched with nutrients and animal-derived factors, which reduce the amount of serum that is needed.

Control questions:

1. Culture Media. Media Components.
2. Serum.
3. Other Supplements.
4. Basal Media.
5. Reduced-Serum Media.

References:

1. R. Renaville and A. Burny (eds.), Biotechnology in Animal Husbandry, 2001. Kluwer Academic Publishers. Printed in the Netherlands. P. 209-223.

2. Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: ed. By W. H. Freeman; 2000.

3. B.R. Glick & J.J. Pasternak. Molecular Biotechnology - Principles and Applications of Recombinant DNA. 3rd Edition). 2003

4. I.R. Gordon. Reproductive Technologies in Farm Animals. 2004. DOI 10.1079/9780851998626.0000

5. Animal Biotechnology. Technologies, Markets & Companies – Edited by Prof. K.K. Jain. Jain PharmaBiotech. A Jain Pharma Biotech Report. 2013. 215 p.

Internet resources:

https://en.wikibooks.org/wiki/Anatomy_and_Physiology_of_Animals/Reproductive_System

Additional visual material for study:

1. Video “How to Make Dulbecco's Modified Eagles Media” https://www.youtube.com/watch?v=ORSisZgcRHg

Laboratory work №4.

Genetic engineering of mammals.

Aim of the work: Acquaintance with principles of genetic engineering in Animal biotechnology.

Over the last 35 years the term genetic engineering has been commonly used not only in science but also in others parts of society. Nowadays this name is often associated by the media forensic techniques to solve crimes, paternity, medical diagnosis and, gene mapping and sequencing. The popularization of genetic engineering is consequence of its wide use in laboratories around the world and, developing of modern and efficient techniques.

The genetic engineering, often used with trivia, involves sophisticated techniques of gene manipulation, cloning and modification. Many authors consider this term as synonymous as genetic modification, where a synthetic gene or foreign DNA is inserted into an organism of interest. Organism that receives this recombinant DNA is considered as genetically modified (GMO). Its production are summarized in simplified form in five steps: 1) Isolation of interested gene, 2) Construction, gene of interested is joined with promoters (location and control the level of expression), terminator (indicates end of the gene) and expression marker (identify the gene expression), 3) transformation (when the recombinant DNA is inserted into the host organism), 4) Selection (selection of those organisms that express the markers), 5) Insertion verification of recombinant DNA and its expression. https://www.intechopen.com/books/genetic- engineering/genetic-engineering-and-cloning-focus-on-animal-biotechnology.

Genetic engineering technology has numerous applications involving companion, wild, and farm animals, and animal models used in scientific research. The majority of genetically engineered animals are still in the research phase, rather than actually in use for their intended applications, or commercially available.

Companion animals. By inserting genes from sea anemone and jellyfish, zebrafish have been genetically engineered to express fluorescent proteins — hence the commonly termed ―GloFish.‖ GloFish began to be marketed in the United States in 2003 as ornamental pet fish; however, their sale sparked controversial ethical debates in California — the only US state to prohibit the sale of GloFish as pets (5). In addition to the insertion of foreign genes, gene knock-out techniques are

also being used to create designer companion animals. For example, in the creation of hypoallergenic cats some companies use genetic engineering techniques to remove the gene that codes for the major cat allergen Fel d1: (http://www.felixpets.com/technology.html).

Companion species have also been derived by cloning. The first cloned cat, ―CC,‖ was created in 2002 (6). At the time, the ability to clone mammals was a coveted prize, and after just a few years scientists created the first cloned dog, ―Snuppy‖ (7).

With the exception of a couple of isolated cases, the genetically engineered pet industry is yet to move forward. However, it remains feasible that genetically engineered pets could become part of day-to-day life for practicing veterinarians, and there is evidence that clients have started to enquire about genetic engineering services, in particular the cloning of deceased pets (5).

Wild animals. The primary application of genetic engineering to wild species involves cloning. This technology could be applied to either extinct or endangered species; for example, there have been plans to clone the extinct thylacine and the woolly mammoth (5). Holt et al (8) point out that, ―As many conservationists are still suspicious of reproductive technologies, it is unlikely that cloning techniques would be easily accepted. Individuals involved in field conservation often harbour suspicions that hi-tech approaches, backed by high profile publicity would divert funding away from their own efforts.‖ However, cloning may prove to be an important tool to be used alongside other forms of assisted reproduction to help retain genetic diversity in small populations of endangered species.

Farm animals. As reviewed by Laible (9), there is ―an assorted range of agricultural livestock applications [for genetic engineering] aimed at improving animal productivity; food quality and disease resistance; and environmental sustainability.‖ Productivity of farm animal species can be increased using genetic engineering. Examples include transgenic pigs and sheep that have been genetically altered to express higher levels of growth hormone (9).

Genetically engineered farm animals can be created to enhance food quality (9). For example, pigs have been genetically engineered to express the Δ12 fatty acid desaturase gene (from spinach) for higher levels of omega-3, and goats have been genetically engineered to express human lysozyme in their milk. Such advances may add to the nutritional value of animal-based products. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3078015/

Control questions:

1. Genetic engineering of mammals.
2. Method of Genetic engineering of mammals..
3. GMO.
4. Application of Mammals genetic engineering..
5. Ethical Concerns.

References:

1. R. enaville and A. Burny (eds.), Biotechnology in Animal Husbandry, 2001. Kluwer Academic Publishers. Printed in the Netherlands. P. 209-223.

2. Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: ed. By W. H. Freeman; 2000.

9. B.R. Glick & J.J. Pasternak. Molecular Biotechnology - Principles and Applications of Recombinant DNA. 3rd Edition). 2003

10. I.R.Gordon. Reproductive Technologies in Farm Animals. 2004. DOI 10.1079/9780851998626.0000

11. Animal Biotechnology. Technologies, Markets & Companies – Edited by Prof. K.K. Jain. Jain PharmaBiotech. A Jain Pharma Biotech Report. 2013. 215 p.

Internet resources:

https://en.wikibooks.org/wiki/Anatomy_and_Physiology_of_Animals/Reproductive_System

http://people.ucalgary.ca/~browder/transgenic.html

https://www.ncbi.nlm.nih.gov/books/NBK207576/

Additional visual material for study:

1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3078015/
2. Video ―Vitrification of embryos and oocytes‖ https://www.youtube.com/watch?v=sJ8TBqr_-Xw

Laboratory work №5.

Mammalian cell and tissues culture.

Aim of the work: Acquaintance with Mammalian cell and tissues culture.

Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), the effects of drugs and toxic compounds on the cells, and mutagenesis and carcinogenesis. It is also used in drug screening and development, and large scale manufacturing of biological compounds (e.g., vaccines, therapeutic proteins). The major advantage of using cell culture for any of these applications is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells. 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.

Tissue culture is the general term for the removal of cells from an animal or plant and their subsequent growth in a favorable artificial environment. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or theymay be derived from a cell line or cell strain that has already been already established. The culture of whole organs or intact organ fragments with the intent of studying their continued function or development is called organ culture.

Primary Culture: Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until

they occupy all of the available substrate (i.e., reach confluence). There are two basic methods for doing this.

i. 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 on the culture vessel surface or substrate where they will begin to divide and grow.

ii. Enzymatic Dissociation more widely used method speeds up this process by adding digesting 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.

Subculturing: When the cells in the primary culture vessel have grown and filled up all of the available culture substrate, they must be subcultured (i.e., passaged) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth.

Buying and Borrowing. An alternative to establishing cultures by primary culture is to buy established cell cultures from organization such as the American Type Culture Collection (ATCC) or the Coriell Institute for

Medical Research.

Cell Line: After the first subculture, the primary culture becomes known as a cell line or subclone. Cell lines derived from primary cultures have a limited life span (i.e., they are finite), and as they are passaged, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population.

Cell Strain: If a subpopulation of a cell line is positively selected from the culture by cloning or some other method, this cell line becomes a cell strain. A cell strain often acquires additional genetic changes subsequent to the initiation of the parent line.

Finite vs. Continuous Cell Lines: Normal cells usually divide only a limited number of times before losing their ability to proliferate, which is a genetically determined event known as senescence; these cell lines are known as Finite. However, some cell lines become immortal through a process called transformation, which can occur spontaneously or can be chemically or virally induced. When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a Continuous cell line.

There are two basic systems for growing cells in culture, as monolayers on an artificial substrate (i.e., adherent culture) or free-floating in the culture medium (suspension culture). The majority of the cells derived from vertebrates, with the exception of hematopoietic cell lines and a few others are anchorage-dependent and have to be cultured on a suitable substrate that is specifically treated to allow cell adhesion and spreading (i.e., tissue-culture treated). However, many cell lines can also be adapted for suspension culture. Similarly, most of the commercially available insect cell lines grow well in monolayer or suspension culture. Cells that are cultured in suspension can be maintained in culture flasks that are not tissue-culture treated, but as the culture volume to surface area is increased beyond which adequate gas

exchange is hindered (usually 0.2–0.5 mL/cm2), the medium requires agitation. This agitation is usually achieved with a magnetic stirrer or rotating spinner flasks

Control questions:

1. Mammalian cell and tissues culture.
2. Cell culture.
3. Primary Culture.
4. Subculturing.
5. Cell Strain.

References:

1. R. Renaville and A. Burny (eds.), Biotechnology in Animal Husbandry, 2001. Kluwer Academic Publishers. Printed in the Netherlands. P. 209-223.

2. Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: ed. By W. H. Freeman; 2000.

6. B.R. Glick & J.J. Pasternak. Molecular Biotechnology - Principles and Applications of Recombinant DNA. 3rd Edition). 2003

7. I.R. Gordon. Reproductive Technologies in Farm Animals. 2004. DOI 10.1079/9780851998626.0000

8. Animal Biotechnology. Technologies, Markets & Companies – Edited by Prof. K.K. Jain. Jain PharmaBiotech. A Jain Pharma Biotech Report. 2013. 215 p.

Internet resources:

http://people.ucalgary.ca/~browder/transgenic.html/
https://www.ncbi.nlm.nih.gov/books/NBK207576/

Additional visual material for study:

Video — Passaging Cells: Cell Culture Basics.
https://www.youtube.com/watch?v=CMRKKl9XSDU