METHODICAL RECOMMENDATIONS FOR LABORATORY WORK

ANIMAL BIOTECHNOLOGY

Almaty, 2021

Laboratory work №1.
Objects used in Animal biotechnology.
Safety rules in Animal biotechnology laboratory.

Aim of the work: Acquaintance students with the objects used in Animal biotechnology and safety rules of work in biotech 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

Figure 1: Safety shower/eye wash combination located in the front of classroom.

(A)

(B)

Figure 2: (A) Fire pull and fire extinguisher. Note: If you pull the fire alarm due to an emergency you must also call 112 to make sure the fire department has been alerted. (B) First Aid Kit located at the front of every classroom.

C). Important Phone Numbers

Please know the following important phone numbers:

• Police 102, Fire 101, Ambulance: 103 (emergency number)

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.

Figure 3: Biohazard signs posted in Labs dealing with hazardous chemicals or microorganisms.

Absolutely no eating or drinking at any time.

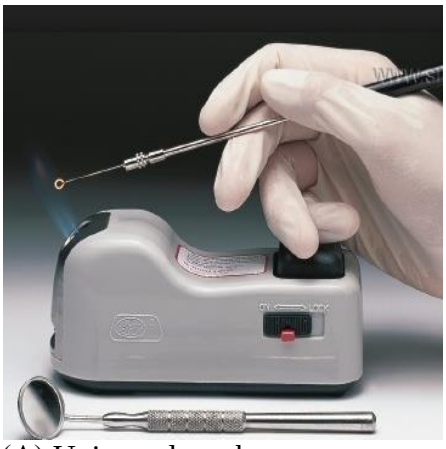
1.6 Bunsen Burners, Gas Jets and alcohol stove

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.**


(A) Universal gas burner

(B) Automatic gas burner


(C) Alcohol burner (stove)

Figure 3: Types of burners used in the microbiological laboratory

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!

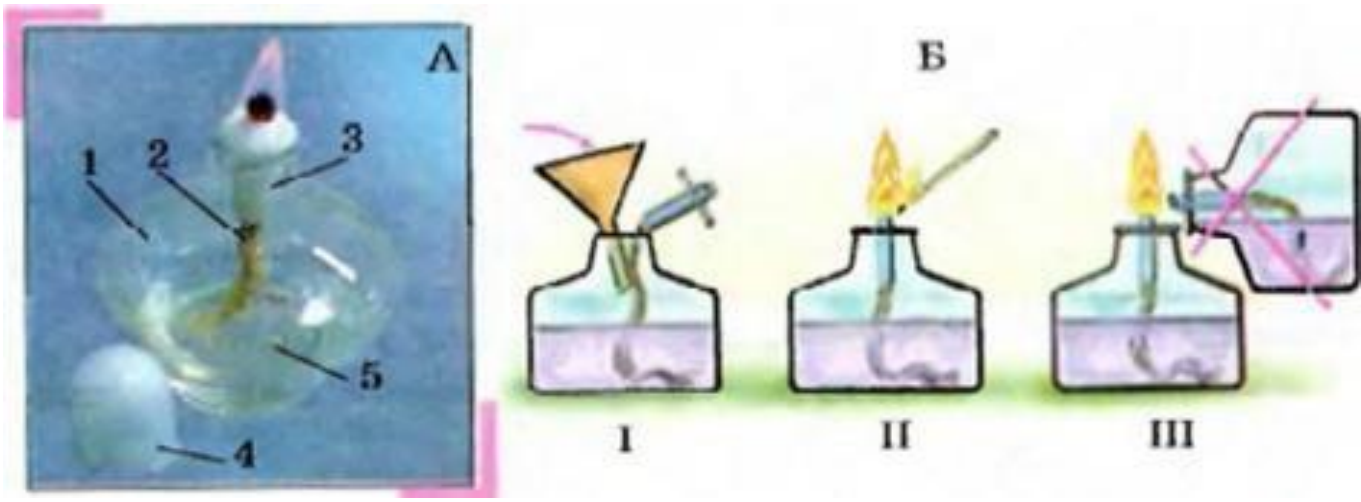


Figure 4: A. alcohol stove: 1 - tank; 2 - wick; 3 - tube; 4 - cap; 5 - alcohol. B. filling the tank circuit alcohol (I), right (II) and incorrect (III) an alcohol lamp ignition

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.

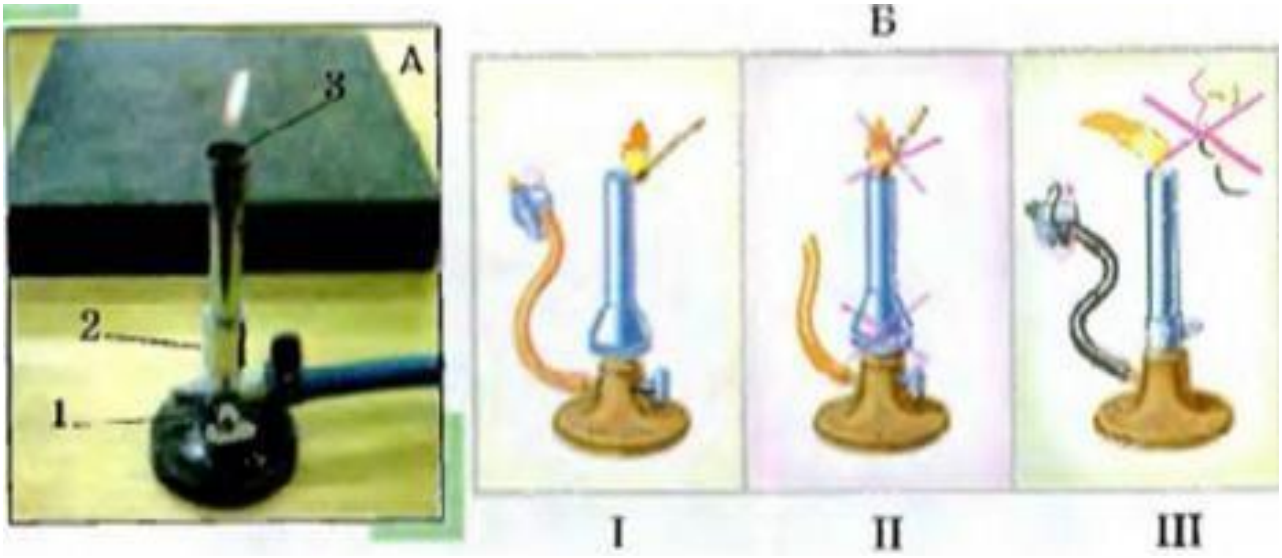


Figure 5: A. gas burner 1 - gas feed screw; 2 - regulator; 3 - burners hole. B. Scheme of correct (I) and incorrect (II) and blanking plugs (III) of the gas burner

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).

Figure 7: (A) Biohazard sharps disposal: any broken glass contaminated with blood or bacterial cultures. (B) Broken glass disposal: any broken glass NOT contaminated with blood or bacterial cultures disposed of in here. Paper products or gloves should NOT be disposed of in either container.

1.9 Culture 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.

Figure 8: (A) Dispose of culture tubes and plates in small red biohazard bag bags, secure bags then dispose of them in the larger biohazard container (B).

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.

Figure 9: Lab disinfectants.

1.12 Strict rules for students

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). <u>Not</u> 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. Animal 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

Laboratory work №2.
Cell differentiation. Artificial insemination,
In vitro fertilization, and embryo transfer in animals.

Aim of the work: Acquaintance students with the safety rules of work in biotech laboratory.

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.

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,where%20they%20develop%20to%20term.
Video –Embryo Transfer: Beef Part 2‖ https://www.youtube.com/watch?v=DkUcMnOd8g8

Laboratory work №3.
Methods for assessment of the of the somatic cells, gametes and embryos viability.

Aim of the work: Acquaintance with the methods for assessment of the of the somatic cells, gametes and embryos viability.

Cytotoxicity Assays: Determining the Number of Live and Dead Cells in a Cell Population, MultiTox-Fluor Multiplex Cytotoxicity Assay: Cell-based assays are important tools for contemporary biology and drug discovery because of their predictive potential for in vivo applications. However, the same cellular complexity that allows the study of regulatory elements, signaling cascades or test compound bio-kinetic profiles also can complicate data interpretation by inherent biological variation. Therefore, researchers often need to normalize assay responses to cell viability after experimental manipulation. Although assays for determining cell viability and cytotoxicity that are based on ATP, reduction potential and LDH release are useful and cost-effective methods, they have limits in the types of multiplexed assays that can be performed along with them. The MultiTox-Fluor Multiplex Cytotoxicity Assay (Cat. No. G9200, G9201, G9202) is a homogeneous, single-reagent-addition format that allows the measurement of the relative number of live and dead cells in a cell population. This assay gives ratiometric, inversely proportional values of viability and cytotoxicity that are useful for normalizing data to cell number. Also, this reagent is compatible with additional fluorescent and luminescent chemistries.

Assays to Detect Apoptosis: A variety of methods are available for detecting apoptosis to determine the mechanism of cell death. The Caspase-Glo® Assays are highly sensitive, luminescent assays with a simple ―add, mix, measure‖ protocol that can be used to detect caspase-8, caspase-9 and caspase-3/7 activities. If you prefer a fluorescent assay, the Apo-ONE® Homogeneous Caspase-3/7 Assay is useful and, like the Caspase-Glo® Assays, can be multiplexed with other assays. A later marker of apoptosis is TUNEL analysis to identify the presence of oligonucleosomal DNA fragments in cells. The DeadEnd™ Fluorometric and the DeadEnd™ Colorimetric TUNEL Assays allow users to end-label the DNA fragments to detect apoptosis

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 Countess; 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 countess. 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.

Multiplexing Cell Viability Assays: The latest generation of cell-based assays includes luminescent and fluorescent chemistries to measure markers of cell viability, cytotoxicity and apoptosis, as well as to perform reporter analysis. Using these tools researchers can investigate how cells respond to growth factors, cytokines, hormones, mitogens, radiation, effectors, compound libraries and other signaling molecules. However, researchers often need more than one type of data from a sample, so the ability to multiplex, or analyze more than one parameter from a single sample, is desirable.

Control questions:

1. Methods for assessment of the of the somatic cells, gametes and embryos viability.
2. Cytotoxicity Assays: Determining the Number of Live and Dead Cells in a Cell Population.
3. Multiplex Cytotoxicity Assay.
4. Assays to Detect Apoptosis.
5. Cell Counter. Multiplexing Cell Viability Assays.

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:
Video ‒Click-iT® Plus TUNEL apoptosis assays‖
https://www.youtube.com/watch?v=wJAk762VtjI

Laboratory work №4.
Method of embryonic cloning.
Cloning method using the somatic cell nuclear transplantation.

Aim of the work: Acquaintance with the methods of embryonic cloning. Cloning method using the somatic cell nuclear transplantation.

Embryo cloning is a scientific advancement, that can -- when used responsibly -- provide innumerable benefits. As suggested by the name, it is the process of cloning, or creating a copy, of an embryo. Somatic cell nuclear transfer is one type of cloning technique that relies on the transfer of genetic material from one organism to another.

Embryo Cloning Basics. An animal cloning process produces a biologically identical copy of a living creature. The biologic copy -- which is sometimes called a clone -- has the same genetic makeup as the original. An embryo is an organism that is early in its developmental cycle; fertilized eggs that have begun cell division and are up to eight weeks old are sometimes referred to as embryos. Embryo cloning, then, is the process of making a biologic copy of a fertilized egg that has begun the process of cell division -- in theory, creating a biologic "twin."

Embryo Cloning Techniques. While there are a number of techniques that can be used in embryonic cloning, somatic cell nuclear transfer, or SCNT, is one of the most common. In SCNT, scientists start by removing the DNA-containing nucleus -- which houses all of the organism's genetic material -- from a somatic, non-reproductive cell. This nucleus is then transferred to an egg cell, whose nucleus and DNA have also been extracted. After a series of laboratory "tweaks," the egg cell with the new DNA is allowed to grow into an embryo which, through a process of embryo transplant, is transferred to a surrogate mother, and is carried to term.

Embryo Cloning Benefits. Embryonic cloning is often touted for its potential in the field of medical research -- in fact, some US scientists suggest that embryonic cloning can lead to breakthroughs in the field of stem cell research, including the production of a variety of cell and tissue types. In theory, these materials could be for organ repair and transplantation, potentially saving millions of lives. When used in agriculture, embryonic cloning has the potential of increasing food supply by increasing the production of plants and animals with desirable traits. Similarly, embryonic cloning may prove useful in preventing the extinction or rare and endangered animals.

Ethical Concerns. Despite its numerous benefits, embryonic cloning is not without flaw. In fact, the health problems faced by many cloned creatures have caused some to question the safety of their use. Researchers in Tokoyo have found that cloned mice generally die sooner than their "natural" counterparts -- and even those who do survive often suffer from a host of birth defects, according to the National Human Genome Research Research Institute. Similarly, female animals implanted with cloned fetuses may experience an increased risk of death as a result of cloning-related complications.

Control questions:

1. Embryo Cloning Basics.
2. Method of embryonic cloning.
3. Embryo Cloning Benefits.
4. Cloning method using the somatic cell nuclear transplantation.
5. Ethical Concerns.

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:

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:

Video ‒Cloning process‖ https://www.youtube.com/watch?v=kyr2OVyjia4

Laboratory work №5.
The principles of genetic engineering in Animal biotechnology. Methods of introducing the foreign DNAs into animal cells.

Aim of the work: Acquaintance with the 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. What is genetic engineering?
2. The principles of genetic engineering in Animal biotechnology.
3. Construction of genes for expressing in mammalian cells.
4. Selectable markers.
5. Methods of introducing the foreign DNAs into animal cells.

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.
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