TRAINING MATERIALS FOR LABORATORY WORKS.

BASICS OF BIOTECHNOLOGY

Laboratory work №1.
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.

Safety Laboratory Work

A) 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.

B) 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 successfully completing this laboratory training should be able to:
Locate and properly use basic emergency equipment such as eye wash stations, first aid kits, fire extinguishers, a telephone, etc.

Necessary Equipment, Material :

Safety Equipment

Identify and know the location of the following safety equipment in the lab:
The nearest safety shower and eye- wash station .
The nearest fire extinguisher/fire pull .
The nearest fire blanket
The nearest fire alarm pull station.
The first aid kit .
The lab bench disinfectant
The nearest emergency phone (located on the wall in each lab).
The evacuation route.

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:
Video “Safety Equipment / Lab Safety” https://www.youtube.com/watch?v=IiHEYtnKfF0
https://youtu.be/rneeZlxyl_Y

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

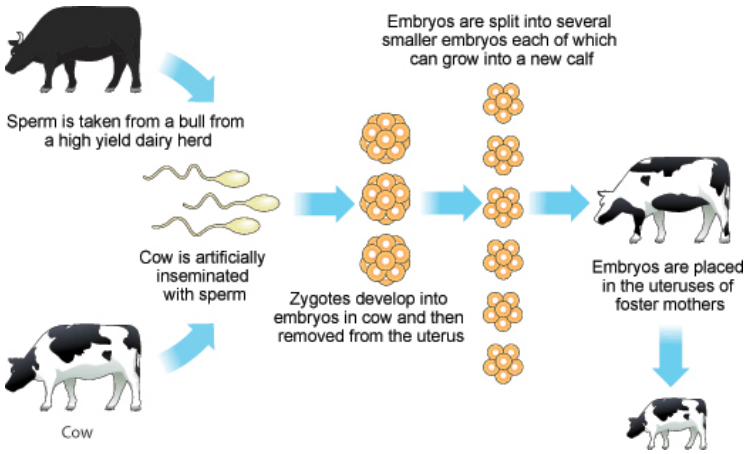
Aim of the work: Acquaintance students with the methods of artificial insemination, In vitro fertilization, and embryo transfer in animals.

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 acomplex system of tissues and cell types.

In vitro fertilisation (IVF) is a process of fertilisation where an egg is combined with sperm outside the body, in vitro ("in glass"). The process involves monitoring and stimulating a woman's ovulatory process, removing an ovum or ova (egg or eggs) from the woman's ovaries and letting sperm fertilise them in a liquid in a laboratory. After the fertilised egg (zygote) undergoes embryo culture for 2–6 days, it is implanted in the same or another woman's uterus, with the intention of establishing a successful pregnancy.

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.

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.



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.

Protocol for Cryopreserving Cultured Cells.

Aim of the work: Acquaintance with the protocol for cryopreserving cultured cells.
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

Necessary Equipment, Material and Reagents:

1. Fluorescent Microscope,
2. TUNEL assay kits
3. Slides
4. Cover slides.

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.

Aim of the work: Acquaintance with the methods of embryonic cloning.

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 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.

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.

Methods of introducing the foreign DNAs into animal cells.

Aim of the work: Acquaintance with the methods of introducing the foreign DNAs into animal cells.

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.

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.

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