**Lecture**

**«** **Objects of Biotechnology»**

**(Animal Biotechnology)**

**Almaty, 2021**

**Lecture 1**

**Objects of Animal biotechnology.**

**Biotechnology in Animal Agriculture: status and current issues.**

**Regulation of the release of genetic modified organisms.**

**Aim of the lesson:** Acquaintance with the objects and tasks of Animal biotechnology and their achievements.

Animal biotechnology is a broad field encompassing the polarities of fundamental and applied research, including molecular modeling, gene manipulation, development of diagnostics and vaccines and manipulation of tissue. It accounts for the use of biotechnology tools, including molecular markers, stem cells, and tissue engineering. Molecular markers are increasingly being used to identify and select the particular genes that lead to desirable traits and it is now possible to select superior germ plasma and disseminate it using artificial insemination, embryo transfer and other assisted reproductive technologies. These technologies have been used in the genetic improvement of livestock.

Transgenesis offers considerable opportunity for advances in medicine and agriculture. In livestock, the ability to insert new genes for such economically important characteristics as fecundity, resistance to or tolerance of other environmental stresses would represent a major breakthrough in the breeding of commercially superior stock. Another opportunity that transgenic technology could provide is in the production of medically important proteins such as insulin and clotting factors in the milk of domestic livestock. A comprehensive evaluation of strategies for developing, testing, breeding and disseminating transgenic livestock in the context of quantitative improvement of economic traits is being done.

Genetic improvement of livestock depends on access to genetic variation and effective methods for exploiting this variation. Genetic diversity constitutes a buffer against changes in the environment and is a key in selection and breeding for adaptability and production in a range of environments.

Animal cell culture technology in today's scenario has become indispensable in the field of life sciences, which provides a basis to study regulation, proliferation, differentiation, and to perform genetic manipulation. It requires specific technical skills to carry out successfully. Application of tissue culture includes the study and understanding of intracellular activity, intracellular flux, pharmacology, cell-cell interaction, cell products, toxicology, tissue engineering, genomics, and immunology. Knowledge acquired from these studies can be used in the biomedical applications.

Biotechnology helps to meet our basic needs:

- Food, clothing, shelter, health and safety.

- Improvements by using science. Science helps in production plants, animals and other organisms.

- Also used in maintaining a good environment that promotes our wellbeing.

- Using scientific processes to get new organisms or new products.

*Biotechnology* is technology that utilizes biological systems, living organisms or parts of this to develop or create different products. Biotechnology is the use of biological processes, organisms, or systems to manufacture products intended to improve the quality of human life. The earliest biotechnologists were farmers who developed improved species of plants and animals by cross pollenization or cross breeding. In recent years, biotechnology has expanded in sophistication, scope, and applicability.

*The interdisciplinary nature of Biotechnology.* Biotechnology involves many disciplines or branches of learning Includes all areas of Life Sciences. Depending on the tools and applications, it often overlaps with related scientific fields. Biotechnology is based on the basic biological sciences (e.g. molecular biology, biochemistry, cell biology, embryology, genetics, microbiology) and conversely provides methods to support and perform basic research in biology. Large area – Includes many approaches and methods in science and technology.

*Animal biotechnology* is an integral component of agriculture. Animal agriculture is being transformed by rapid advances in biotechnology " a term that encompasses a variety of technologies, including genetic engineering (GE), genetic modification, transgenics, recombinant DNA techniques, and cloning, among others.

Animal biotechnology is a branch of biotechnology in which molecular biology techniques are used to genetically engineer (i.e. modify the genome of) animals in order to improve their suitability for pharmaceutical, agricultural or industrial applications. Animals provide a number of products we use in everyday life. Animals provide a number of products we use in everyday life: –Milk –Leather –Meat –Wool –Egg –Enzymes –And many more e-g medicine.

Major areas of animal biotechnology: Animal breeding; Animal vaccines; Animal nutrition; Embryo transfer; Transgenic animal; Xenotransplfntation.

*Animal biotechnology* is the use of science and engineering to modify living organisms. *The goal* is to make products, to improve animals and to develop microorganisms for specific agricultural uses. Examples of animal biotechnology include creating transgenic animals (animals with one or more genes introduced by human intervention), using gene knock out technology to make animals with a specific inactivated gene and producing nearly identical animals by somatic cell nuclear transfer (or cloning).

*History.* The animal biotechnology in use today is built on a long history. Some of the first biotechnology in use includes traditional breeding techniques that date back to 5000 B.C.E. Such techniques include crossing diverse strains of animals (known as hybridizing) to produce greater genetic variety. The offspring from these crosses then are bred selectively to produce the greatest number of desirable traits. For example, female horses have been bred with male donkeys to produce mules, and male horses have been bred with female donkeys to produce hinnies, for use as work animals, for the past 3,000 years. This method continues to be used today.

*Biotechnology in Animal Agriculture: Status and Current Issues.* Animal agriculture is being transformed by rapid advances in biotechnology—a term that encompasses a variety of technologies, including genetic engineering (GE), genetic modification, transgenics, recombinant DNA techniques, and cloning, among others.

Producers are interested in the application of biotechnology: to improve productivity, consistency, and quality; to introduce new food, fiber, and medical products; and to protect the environment.

Animal Biotech a) Improve animals or the products they produce. Animals may be used to produce products that promote human health. Increase milk productivity , Example Transgenic organisms are organisms that are injected with foreign DNA from another organism. Cows engineered to produce human hemoglobin.

b) Animal Cloning. Cloning is the copying animal gene into many copies,

Start with Embryo Twinning (splitting embryos in half). Advantage of cloning: preservation of endangered animals, studying the effect of drugs e.t.c. on duplicates, improve agricultural production Dolly and her surrogate mother.

c) Improvement animal Health. Animal health and wellbeing have become increasingly important issues for animal producers and consumers. Biotechnology can improve animal health by producing genetically engineered animal that resist disease. The development of genome resources and technologies allow for identification of several host resistance genes. Aim: to prepare and present about genetic bases of disease resistance in the livestock sector.

**Control questions:**

1. Objects of Animal Biotechnology.

2. Animal biotechnology.

3. Application of Animal Biotechnology.

4. The goals and tasks of Animal biotechnology.

5. Biotechnology in Animal Agriculture: Status and Current Issues.

**Lecture 2**

**Animal reproduction. The main directions of animal breeding.**

**Aim of the lesson:** Acquaintance with animal reproduction and main directions of animal breeding.

How Animals Reproduce? Some animals produce offspring through asexual reproduction while other animals produce offspring through sexual reproduction. Both methods have advantages and disadvantages. Asexual reproduction produces offspring that are genetically identical to the parent because the offspring are all clones of the original parent. A single individual can produce offspring asexually and large numbers of offspring can be produced quickly; these are two advantages that asexually reproducing organisms have over sexually reproducing organisms. In a stable or predictable environment, asexual reproduction is an effective means of reproduction because all the offspring will be adapted to that environment. In an unstable or unpredictable environment, species that reproduce asexually may be at a disadvantage because all the offspring are genetically identical and may not be adapted to different conditions.

During sexual reproduction, the genetic material of two individuals is combined to produce genetically diverse offspring that differ from their parents. The genetic diversity of sexually produced offspring is thought to give sexually reproducing individuals greater fitness because more of their offspring may survive and reproduce in an unpredictable or changing environment. Species that reproduce sexually (and have separate sexes) must maintain two different types of individuals, males and females. Only half the population (females) can produce the offspring, so fewer offspring will be produced when compared to asexual reproduction. This is a disadvantage of sexual reproduction compared to asexual reproduction.

*Asexual Reproduction.* Asexual reproduction occurs in prokaryotic microorganisms (bacteria and archaea) and in many eukaryotic, single-celled and multi-celled organisms. There are several ways that animals reproduce asexually, the details of which vary among individual species.

*Fission.* Fission, also called binary fission, occurs in some invertebrate, multi-celled organisms. It is in some ways analogous to the process of binary fission of single-celled prokaryotic organisms. The term fission is applied to instances in which an organism appears to split itself into two parts and, if necessary, regenerate the missing parts of each new organism. For example, species of turbellarian flatworms commonly called the planarians, such as Dugesia dorotocephala, are able to separate their bodies into head and tail regions and then regenerate the missing half in each of the two new organisms. Sea anemones (Cnidaria), such as species of the genus Anthopleura (Figure 13.2), will divide along the oral-aboral axis, and sea cucumbers (Echinodermata) of the genus Holothuria, will divide into two halves across the oral-aboral axis and regenerate the other half in each of the resulting individuals. *Budding.* Budding is a form of asexual reproduction that results from the outgrowth of a part of the body leading to a separation of the “bud” from the original organism and the formation of two individuals, one smaller than the other. Budding occurs commonly in some invertebrate animals such as hydras and corals. In hydras, a bud forms that develops into an adult and breaks away from the main body.

*Fragmentation*. Fragmentation is the breaking of an individual into parts followed by regeneration. If the animal is capable of fragmentation, and the parts are big enough, a separate individual will regrow from each part. Fragmentation may occur through accidental damage, damage from predators, or as a natural form of reproduction. Reproduction through fragmentation is observed in sponges, some cnidarians, turbellarians, echinoderms, and annelids. In some sea stars, a new individual can be regenerated from a broken arm and a piece of the central disc. This sea star is in the process of growing a complete sea star from an arm that has been cut off. Fisheries workers have been known to try to kill the sea stars eating their clam or oyster beds by cutting them in half and throwing them back into the ocean. Unfortunately for the workers, the two parts can each regenerate a new half, resulting in twice as many sea stars to prey upon the oysters and clams.

*Sexual Reproduction*. Sexual reproduction is the combination of reproductive cells from two individuals to form genetically unique offspring. The nature of the individuals that produce the two kinds of gametes can vary, having for example separate sexes or both sexes in each individual. Sex determination, the mechanism that determines which sex an individual develops into, also can vary. *Hermaphroditism.* Hermaphroditism occurs in animals in which one individual has both male and female reproductive systems. Invertebrates such as earthworms, slugs, tapeworms, and snails (Figure 13.5) are often hermaphroditic. Hermaphrodites may self-fertilize, but typically they will mate with another of their species, fertilizing each other and both producing offspring. Self-fertilization is more common in animals that have limited mobility or are not motile, such as barnacles and clams. Many species have specific mechanisms in place to prevent self-fertilization, because it is an extreme form of inbreeding and usually produces less fit offspring.

 Animal breeding, nowadays, is a field that is influenced by a whole range of biotechnological applications and developments (Bazer and Spencer, 2005). Various biotechnology methods are used in improving the breeding stock of animals. Animal breeding, nowadays, is a field that is influenced by a whole range of biotechnological applications and developments (Bazer and Spencer, 2005). Various biotechnology methods are used in improving the breeding stock of animals.

*Artificial Insemination (AI).* One of the earliest perfected technology is artificial insemination (AI) where new breeds of animals are produced through the introduction of the male sperm from one superior male to the female reproductive tract without mating. Various technologies have evolved that led to the efficient use of AI in developing desired livestock, including the methods of freezing semen or cryopreservation and sperm sexing.

*Embryo Transfer (ET).* Embryo transfer from one mother to a surrogate mother makes it possible to produce several livestock progenies from a superior female. Week-old embryos are flushed out of the donor’s uterus, isolated, examined microscopically for number and quality, and inserted into the lining of the uterus of surrogate mothers.

*In-vitro Fertilization*. In case other artificial reproductive techniques fail due to difficulties such as blocked reproductive systems, non-responsive ovaries in the females, marginal semen quality and quantity in the male, and presence of disease, in vitro fertilization (IVF) is used. The fertilization of the sperm and the egg is conducted in vitro (outside the animal’s body) at specific environmental and biochemical conditions.

**Control questions:**

Describe advantages and disadvantages of asexual and sexual reproduction

Discuss asexual reproduction methods

Discuss sexual reproduction methods

Discuss internal and external methods of fertilization

**Lecture 3**

**Animal cell technologies. Aseptic Techniques.**

**Aim of the lesson:** Acquaintance with animal cell technologies and aseptic Techniques.

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.

*Aseptic Techniques.* To minimize the risk of contamination, follow these 5 rules:

1. Always check the cells carefully before handling (by eye and on a microscope). Become familiar with the indicators of abnormal cell growth.

2. Whenever possible, maintain cultures without antibiotics for at least part of the time, to reveal cryptic contamination.

3. Check sterility of all reagents before use.

4. Use dedicated media and reagents; do not share with other cell lines.

5. Maintain a high standard of sterility at all steps.

Mycoplasma contamination, which may slow cell growth, cannot be checked under a regular microscope. To confirm or rule out such contamination, use a mycoplasma test (e.g. Roche Applied Science Mycoplasma PCR ELISA Kit).

*Environment:* There should be a laminar flow hood in the room dedicated to cell culture, and this hood should be used for all culture manipulations and storage of all equipment. The hood must be placed away from traffic or equipment that might generate air currents (e.g., centrifuges, refrigerators and freezers). Always carefully clean the hood before and after your procedure.

Remove all unneeded items. It is crucial to always keep the work surface clean and tidy. To achieve this, follow these rules:

- Use 80% ethanol to clean the surface before starting.

- Place and keep on this surface only the items required for your procedure. This will reduce the possibility of contact between sterile and non-sterile items and facilitate culture manipulations.

A. Clear space in the center of the bench, not just the front edge.

B. Avoid spills, if they happen immediately clean the area.

C. Remove everything when you are done, and again clean the work surface.

Reagents and media obtained from commercial suppliers will already have undergone strict quality testing. Most of the bottles are wrapped in polyethylene. The wrapping should be removed outside the hood. Unwrapped bottles should be cleaned with 80% ethanol whenever they are removed from the refrigerator or from a water bath. Regularly clean the refrigerator, the incubator and the water bath to avoid growth of mold or fungi. Imported cell lines should always be quarantined before being incorporated into your main stock. Do not perpetually use antibiotics; they will suppress some contaminants, but will not eliminate them.

*Handling:* Special care should be taken with caps. Use deep screw caps in preference to stoppers. When working on an open bench, flame glass pipettes and necks of the bottles before and after each use. Always use the pipettes which are best adapted your procedure; regularly clean them and check their calibration. Use a multi-channel pipette instead of a single pipette if you are working with multiwell plates. This will reduce both the time required to perform the procedure and the probability of contamination. Prepare as many reagents and equipment as possible in advance, to reduce the time the cultures are kept out of the incubator.

**Control questions:**

1. Animal cell culture technology.

2. What is Cell Culture?

3. Primary Culture.

4. Subculturing. Cell line. Maintenance.

**Lecture 4**

**Biotechnology and genetic engineering of mammals.**

**Aim of the lesson:** Acquaintance with the method of genetic transformation mammals.

A somatic cell (from Ancient Greek σῶμα sôma, meaning "body"), or vegetal cell, is any biological cell forming the body of an organism; that is, in a multicellular organism, any cell other than a gamete, germ cell, gametocyte or undifferentiated stem cell.

In contrast, gametes are cells that fuse during sexual reproduction, germ cells are cells that give rise to gametes, and stem cells are cells that can divide through mitosis and differentiate into diverse specialized cell types. For example, in mammals, somatic cells make up all the internal organs, skin, bones, blood and connective tissue, while mammalian germ cells give rise to spermatozoa and ova which fuse during fertilization to produce a cell called a zygote, which divides and differentiates into the cells of an embryo. There are approximately 220 types of somatic cell in the human body.

Theoretically, these cells are not germ cells (the source of gametes); they transmit their mutations, to their cellular descendants (if they have any), but not to the organism's descendants. However, in sponges, non-differentiated somatic cells form the germ line and, in Cnidaria, differentiated somatic cells are the source of the germline. Mitotic cell division is only seen in diploid somatic cells.

Development of biotechnology has allowed for the genetic manipulation of somatic cells, whether for the modelling of chronic disease or for the prevention of malaise conditions.

Genetic engineering of somatic cells has resulted in some controversies, although the International Summit on Human Gene Editing has released a statement in support of genetic modification of somatic cells, as the modifications thereof are not passed on to offspring.

*Gene transformation in animals.* "Transformation" may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because "transformation" has a special meaning in relation to animal cells, indicating progression to a cancerous state, the process is usually called "transfection".

In molecular biology and genetics, transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane(s). For transformation to take place, the recipient bacterium must be in a state of competence, which might occur in nature as a time-limited response to environmental conditions such as starvation and cell density, and may also be induced in a laboratory.

Transformation is one of three processes for horizontal gene transfer, in which exogenous genetic material passes from one bacterium to another, the other two being conjugation (transfer of genetic material between two bacterial cells in direct contact) and transduction (injection of foreign DNA by a bacteriophage virus into the host bacterium). In transformation, the genetic material passes through the intervening medium, and uptake is completely dependent on the recipient bacterium.

As of 2014 about 80 species of bacteria were known to be capable of transformation, about evenly divided between Gram-positive and Gram-negative bacteria; the number might be an overestimate since several of the reports are supported by single papers.

"Transformation" may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because "transformation" has a special meaning in relation to animal cells, indicating progression to a cancerous state, the process is usually called "transfection".

Introduction of DNA into animal cells is usually called transfection. In animal cells, transfection is the preferred term as transformation is also used to refer to progression to a cancerous state (carcinogenesis) in these cells. Transduction is often used to describe virus-mediated gene transfer into eukaryotic cells.

**Control questions:**

1. Animal somatic cells.
2. Genetic manipulation of somatic cells.
3. Genetic engineering of somatic cells.
4. Gene transformation in animals.
5. What are transfection and transduction?

**Lecture 5**

**Applications of animal cell culture technology.**

**Aim of the lesson:** Acquaintance with applications of animal cell culture technology.

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.

*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.

Cell potency is a cell's ability to differentiate into other cell types. The more cell types a cell can differentiate into, the greater its potency. *Potency* is also described as the gene activation potential within a cell, which like a continuum, begins with *totipotency* to designate a cell with the most differentiation potential, *pluripotency, multipotency, oligopotency*, and finally *unipotency*.

***Totipotency*** (Lat. totipotentia, "ability for all [things]") is the ability of a single cell to divide and produce all of the differentiated cells in an organism. *Spores and zygotes are examples of totipotent cells.* In the spectrum of cell potency, totipotency represents the cell with the greatest differentiation potential, being able to differentiate into any embryonic cell, as well as extraembryonic cells. In contrast, pluripotent cells can only differentiate into embryonic cells.

*The human development model* is one which can be used to describe how totipotent cells arise. Human development begins when a sperm fertilizes an egg and the resulting fertilized egg creates a single ***totipotent cell****, a zygote*. In the first hours after fertilization, this zygote divides into identical totipotent cells, which can later develop into any of the three germ layers of a human (endoderm, mesoderm, or ectoderm), or into cells of the placenta (cytotrophoblast or syncytiotrophoblast). After reaching a 16-cell stage, the totipotent cells of the morula differentiate into cells that will eventually become either the blastocyst's Inner cell mass or the outer trophoblasts. Approximately four days after fertilization and after several cycles of cell division, *these totipotent cells begin to specialize*. The inner cell mass, the source of embryonic stem cells, becomes pluripotent.

In cell biology, ***pluripotency*** (Lat. pluripotentia, "ability for many [things]") refers to a ***stem cell*** that *has the potential to differentiate into any of the three germ layers: endoderm* (interior stomach lining, gastrointestinal tract, the lungs), *mesoderm* (muscle, bone, blood, urogenital), *or ectoderm* (epidermal tissues and nervous system), but not into extra-embryonic tissues like the placenta.[19] However, cell pluripotency is a continuum, ranging from the completely pluripotent cell that can form every cell of the embryo proper, e.g., embryonic stem cells and iPSCs (see below), to the incompletely or partially pluripotent cell that can form cells of all three germ layers but that may not exhibit all the characteristics of completely pluripotent cells.

**Control questions:**

1. Animal cell culture technology.

2. What is Cell Culture?

3. Primary Culture.

4. Subculturing. Cell line. Maintenance.