11. Food safety, GMOs, animal cloning

Hello. In the lecture, we will focus on the basics of food safety, primarily in connection with GMOs, and describe the essence and significance of GMOs and animal cloning.

Food safety is currently one of the most important topics in relation to animal husbandry and food production. From this perspective, it is necessary to monitor the potential occurrence of harmful chemicals that can have mutagenic or even carcinogenic properties. Here, we most commonly encounter the presence of heavy metals and mycotoxins. In some countries, antibiotics may also be present in meat as unwanted contamination. Food must have a defined composition to prevent the occurrence of naturally occurring allergens, and in this case, consumers need to be notified of this risk. The most serious in terms of health is microbiological contamination, especially in meat and dairy products, by bacteria such as salmonella, listeria, or Campylobacter. Fortunately, the presence of these foodborne pathogens can be tested using microbiological cultivation techniques, but also DNA tests. In connection with the presence of GMOs in food, thorough testing of their health safety is carried out during their approval. With GMOs, there is a potential risk of allergenicity, so even they need to be tested and detected. Similar mechanisms for verifying health safety need to be applied to potential foods made from cloned animals.

Currently, the presence of GMOs above 0.9% must be declared in food. For this purpose, several sensitive detection methods have been developed. The diagnosis of GMOs in food can be performed by detecting the presence of GMOs through direct identification of transgenic DNA (using polymerase chain reaction - PCR) or by detecting transgenic protein - immunochemically using the ELISA method. In the case of using PCR, if a transgene is present in the sample, amplification or replication of the product occurs, which is visible as a band on an electrophoretic gel.

Quantitative methods are used to determine the proportion of GMOs in food. The most sensitive method is real-time PCR, which is extremely accurate and sensitive. The amount of GMO in the sample is determined by comparing the curve of the tested sample with the curves of known composition standards. The method is also called relative quantification.

In terms of the result, there is no difference between traditional breeding and transgenesis as the main method of creating genetically modified organisms. They have the same goal, which is to obtain individuals with desirable alleles in their genotypes. In traditional breeding, hybridization and deliberate crossing are often used, and it takes many generations to obtain desired combinations of genotypes. Transgenesis is characterized by targeted and rapid changes, requiring knowledge of the gene we want to use and transferring it to the organism using a vector, i.e., its structure, function, and sequence. Traditional breeding work is done in the population, while transgenesis can be seen as breeding an individual. The comparison of both approaches can be seen in the diagram.

First, let's define genetic modification. Genetic modifications refer to targeted interventions in genetic information. Although the result may be similar, random interventions by mutagens or ionizing radiation (such as the creation of wheat or rapeseed varieties) are not considered genetic modification. Genetic modifications include intentional changes in gene activity, changes in "site of action" (in which tissue), gene replacement with another variant, gene

blocking, and especially the introduction of foreign genes - transgenesis. A classic example of transgenesis in plants is the use of the bacterium Agrobacterium tumefaciens, which has the natural ability to introduce genes for herbicide resistance or insecticide production - this is how Bt corn, for example, was created. Alongside the development of these biotechnologies, legal regulations are emerging, such as the law on handling GMOs.

Genetic modifications are synonymous with recombinant DNA techniques. These involve direct and targeted interventions in the hereditary material of an organism (i.e., DNA). The most well-known method is transgenesis, also known as gene transfer between species and the introduction of individual genes into the genome using genetic engineering methods. A genetically modified organism (GMO) is an organism (other than a human) whose genetic material has been intentionally altered in a manner that cannot be achieved through natural recombination. GMOs can be microorganisms, fungi, plants, or animals.

The creation of GMOs presents a few problems. One of them is the low efficiency of insert incorporation. The integration of an insert and its copies often occurs randomly. The product can be formed in low or high concentrations because it is difficult to properly regulate expression of the structural gene. Incorporation of foreign DNA is often unstable and may be lost over generations. Gene manipulations are still costly, and the goal is often achieved with great uncertainty. The most advanced molecular biotechnology based on the CRISPR-Cas9 system helps solve most of these problems.

The base of recombinant DNA technology (used in genetic engineering) lies in the use of restriction enzymes, which originate from bacteria and serve as a defense mechanism against foreign DNA. These enzymes can cleave DNA at specific sites known as restriction sites. By using the same enzyme to open the vector and create both ends of the gene, the likelihood of correct insertion of the gene into the plasmid can be increased. The DNA ligase enzyme is used for this joining process of originally unrelated DNA segments, and it is called DNA recombination. The vector can then be integrated into the genome of the host cell, thereby transferring the sequence of the gene itself.

The main problem with transgenesis is how to effectively introduce recombinant DNA into the cell and nucleus. Many methods have been developed, of which I will first mention the so-called biological methods. Lipofection involves the use of lipid micelles that encapsulate nucleic acids into liposomes, which can naturally penetrate the cell nucleus. Transfection using plasmid vectors is a relatively simple method but with low efficiency. Viral vectors are now more commonly used in animals because they have a natural ability to penetrate the cell and nucleus. In the case of adenoviruses, DNA enters the chromatin, not directly the DNA, while in the case of retroviruses and lentiviruses, foreign DNA directly integrates into the genome. The image on the right shows the schema of a lentivirus, which, in addition to RNA, contains enzymes for integrating nucleic acids into the host's DNA. During transgenesis, care is taken to ensure that viral vectors are safe and modified so that they cannot reproduce in the host cell.

Physical methods mainly involve microinjection, which is the introduction of DNA into a fertilized egg or embryonic stem cells. This method is simple and foreign genes are effectively expressed. The method cannot be used in later developmental stages, and the disadvantage is low success rate and random insertion of the insert. Gene transfer using embryonic stem cells, known as ESC, is a specialized method where pluripotent cells from a blastocyst with DNA inserted in vitro are inserted into a foreign embryo, which is then transferred to a surrogate

mother's uterus. The result is the birth of an offspring that is a genetic chimera, meaning it has some tissues with the transgene and others without.

Other methods include transfer of DNA through various particles and shooting them into the cell, as well as electroporation (i.e. creating pores in the cell using electrical impulses). Thermal shock or coated magnetic particles can also be used. However, all of these methods have relatively low efficiency (up to 5%) and their application depends on the specific species of animals and the experience of the respective laboratory.

In this scheme, we see an example of microinjection technique used to transfer a transgene, with the aim of expressing the transgene only in the mammary gland and producing a readily isolatable product, i.e. protein, from transgenic milk. These genetically modified animals used to produce specific transgenic proteins are called animal bioreactors.

Various uses of microinjection can be seen in the images here. Injection can be done into the pronucleus just before the fusion of nuclei and the formation of a zygote - the result is a completely transgenic individual. Another approach is the insertion of the transgene into embryonic stem cells, which are then inserted into the host blastocyst. Chimeras are born, which can be further crossed and in subsequent generations, a completely transgenic individual can be obtained again. The advantage of this method is that we can culture and select embryonic stem cells, which increases the likelihood of success in transgenesis. Another frequently used method is nuclear transfer, where a DNA construct is inserted into cultured somatic cells (usually undifferentiated, such as fibroblasts), and then the nucleus is removed and inserted into an enucleated egg. The zygote is then implanted into a surrogate mother and in all cells transgenic individual can be born again.

And what is the purpose of creating GM animals? First, to increase the better production and quality of animal food. This is also related to the production of new and better foods, for example the lactase gene in cattle will reduce the lactose content in milk. Another example is the replacement of allergenic proteins in milk with human proteins. There is great potential for the production of high-quality recombinant proteins (pharmaceuticals, etc.) or new materials in industry with the help of so-called "living bioreactors". High hopes are placed in research on resistance to disease and the adverse effects of a changing environment, for example, an experiment with the transfer of a gene for a protein that protects against freezing into the genome of salmon. The use of animal models is important for research into human diseases, and research into the use of transgenic animals for xenotransplantation is also underway.

The most significant application of genetically modified animals is in the field of pharmaceutical production. Many important drugs, often for the treatment of genetic diseases, are of protein nature. Proteins, as complex substances, cannot be produced chemically like simple drugs, but synthesis must occur in biological systems. The simplest way is the production of drugs using microorganisms, such as bacteria. Insulin has been produced this way for many years. However, insulin is a relatively simple protein, and bacteria cannot produce more complex proteins with specific post-translational modifications. Therefore, eukaryotes - animals such as mammals or birds - must be used. One of many examples of drug production is obtaining human protein lipoprotein lipase from chicken eggs. Goats or rabbits are often used for similar purposes in mammals.

Proteins have very diverse properties that can be used as materials for various advanced technologies. For example, spider silk protein is a material that is about 7 times stronger than high-quality steel in terms of volume and weight, and it is called Biosteel. The source of this protein is genetically modified goats that produce this protein in their milk.

There are not many examples of genetically modified food of animal origin approved for consumption. A well-known example is GM salmon, which grows 11 times faster than regular farmed salmon. The approval process in the United States was very complicated and took 20 years. To prevent the escape of GM salmon into the wild, they are bred in isolated tanks.

Genetic modification is also associated with ethical problems. Is the new product acceptable to customers? European consumers tend to reject GMO foods. In the past, concerns arose regarding the risk of tumor formation or neurodegenerative diseases in transgenic animals due to the integration or expression of transgenes, following the initial failed experiments. It is logical that side effects due to gene modification cannot always be excluded. An ethical issue for many people may be that humans may benefit from genetically modified animals even if the transgenic animals themselves do not. In any case, it is necessary to ensure that GM does not cause any harm to animals. There are often concerns that foreign genes affect hosts and whether there may be a threat to ecological balance and species diversity. However, GM animal husbandry is usually closed, although there is no 100% guarantee. Currently, rapidly developing genome editing as a method causing specific and only minor changes in the genome eliminates most potential risks. Nevertheless, all GMOs are subject to detailed verification of potential risks, so safety should be ensured.

And now a few words about animal cloning. Cloning, in this sense, is a reproductive technique for creating genetically identical offspring. However, it is often also used for techniques related to genetic modification. Cloning techniques in mammals include microsurgical embryo dissection, isolation and proliferation, or aggregation of individual blastomeres, and especially nucleus transfer, which may or may not be modified. Cloning in mammals can be divided into reproductive, where a new genetically identical individual is created, and therapeutic for the purpose of treatment. The first type applies exclusively to animals, while the second has great potential in human medicine.

The first successful cloning of a mammal from an adult cell was carried out by Professor Wilmut of the Roslin Institute in Scotland, when the sheep Dolly was born in 1996. The nuclear transfer technique was used by cell fusion. Dolly was genetically identical to the sheep from which the nucleus was taken from a somatic cell of the mammary gland epithelium. Interestingly, Dolly had three mothers: the first donated the genetic information, the second provided an empty egg, and the third carried the offspring.

A number of problems have arisen in connection with animal cloning. Low pregnancy rates, developmental defects such as early miscarriage, stillbirths, early deaths after birth, short lifespan, obesity, malformations of various organs, and poor immunity have been observed in many animals. Cloned animals are generally not accepted by breeders - for example, horses are not included in studbooks. Legislative and ethical problems arise. Regarding food products from cloned animals (also known as "cloned meat"), the U.S. Food and Drug Administration (FDA) states that consuming meat from cloned animals is without risk. However, economically, this breeding is currently highly inefficient due to the high costs of cloning. The European Food

Safety Authority (EFSA) also declared products of animal origin from clones to be safe, but there are concerns about the welfare of surrogate mothers and the cloned animals themselves.

A large number of different animal species have been cloned, from model organisms such as mice and rats to most livestock. The picture shows 6 clones of one mare, which the owner rode during horse polo. Interestingly, although the mares are highly similar, they are not genetically identical in terms of coloring. The distribution and size of color markings in this case are not directly genetically determined. It can be said that cloning is often done for money or for entertainment or publicity purposes.

I see the main significance of cloning techniques primarily in the potential for so-called therapeutic cloning, which could enable the treatment of previously incurable diseases. The mechanism of this treatment involves replacing damaged cells with genetically identical cells from the patient's own body, or cells with corrected genetic information (in the case of treatment of genetic diseases). The use of embryonic stem cells and, especially, induced pluripotent stem cells (iPSCs) created directly from the somatic cells of a specific patient, appears promising.

Unlike GMO foods, food from cloned animals is more acceptable as it does not contain anything foreign. However, both biotechnological approaches require improvement in procedures, especially with regard to the welfare of the lives of these created animals. Neither the consumer nor a perfect laboratory analyzer can discern the differences between these pieces.

The issue of GMOs and animal cloning is controversial, and everyone must form their own opinion on these techniques. The aim of this short presentation was to provide objective information to help form that opinion. Thank you all for your attention.