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# Effect of genetic modification on the health status of transgenic pigs produced with the human α-1,2-fucosyltransferase gene*)

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Summary

**The aim of the study was to evaluate the influence of transgenesis on the animal health status, morbidity rate and course of diseases in a herd of transgenic pigs during 6 years of observation.**

**It was evaluated 378 potentially transgenic pigs with the human α-1,2 fucosyltransferase gene, born after artificial insemination. One of the parents was transgenic and the other non-transgenic. Molecular verification of transgenesis was performed in all piglets. Piglets with positive molecular verification were the experimental group (n = 150), and the other, non-transgenic piglets were the control group (n = 228).**

**In the transgenic group, was diagnosed 43 sick animals (28.7%). Their mortality rate was high: out of the 43 sick animals, 24 (55.81%) died of disease, which accounted for 16.0% of the total population of transgenic animals. Recovery was observed in 19 out of the 43 sick animals (44.19%). Out of 228 non-transgenic animals, 55 suffered from disease (24.12%). Twenty-seven of them (49.9%) died of disease, which constituted 11.5% of the whole non-transgenic population. Twenty-eight animals (50.9%) recovered.**

**Transgenesis had no negative influence on the health, morbidity or mortality of animals. A comparison of transgenic and non-transgenic siblings in the same litters reveals that the observed differences are due to reasons other than transgenesis. The most important potential cause is extensive inbreeding.**

**Keywords: pigs, transgenic animals, GMO, morbidity**

The increasing use of modern biotechnological methods (including transgenesis) in agriculture, industry and medicine raises many important scientific, social and ethical issues. The main controversy is over the uncontrolled use of genetically modified (GM) organisms, their impact on natural ecosystems once they are released into the environment, and the ways of preventing and dealing with negative consequences (15). Studies involving GM organisms have been carried out for many years all over the world, including Poland. The main achievement of genetic engineering in Poland is xenotransplantation research that has been conducted since 2002 on the use of transgenesis in genetic modification of pigs as a source of organs for human transplantation. The first transgenic boar bred for xenotransplantation purposes was obtained in October 2003 (7). Genetic modification involved the introduction of the human α-1,2-fucosyltransferase gene (pFut-GFPBsd, FUT II) (7, 8). The mechanism of α-1,2-fucosyltransferase is based on binding a molecule of N-acetyllactosamine (N-lac), which is a substrate for α-galactosyltransferase. The competitive action of fucosyltransferase against αGT reduces the level of the αGal epitope and helps limit the hyper-acute rejection response (7, 8). Outstanding achievements are accompanied by a discussion on the practical application of genetic engineering and genetic modifications. There

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are still no reliable studies, observations or publications to determine the possible environmental impact of GM organisms. Likewise, no information is available concerning the possible effect of genetic modifications on the bodies of animals subjected to genetic manipulation. One issue of fundamental importance is to conclusively determine whether genetic modifications have any adverse effects on animal physiology and health.

The aim of this study was to determine the effect of transgenesis on animal health in a herd of transgenic pigs during a 6-year observation.

## Material and methods

**Experimental conditions.** Six-year studies involved a herd of 37 pigs. Piglets were born by natural delivery. Pregnancies were obtained by mating sows in natural estrus. One of the parents was transgenic and had the human α-1,2-fucosyltransferase gene, and the other originated from a breeding herd and was non-transgenic. All piglets born were treated as potentially transgenic until the molecular verification of transgenesis. Throughout the experiment, animals were housed under the same conditions and remained under constant veterinary care. Pig rations were balanced for energy and protein content. Animals received *ad libitum* feed mixtures Pt Smyk, Starter Euro, Grower Euro and Finisher Euro (Provimi Polska). All animals were fed diets that were appropriate for their age and body weight.

Routine prophylactic measures included periodic deworming (twice a year) and preventive vaccinations according to the farm vaccination schedule. Animals were housed in very good conditions according to current regulations on animal welfare, the use of animals in research, and the use of genetically modified (GM) animals. The farm on which the herd of transgenic pigs was kept belongs to a pig breeding station and is permanently isolated from the breeding section to keep GM animals within the confines of their designated area. Throughout the study, animals were kept under observation to determine the herd health and morbidity of transgenic pigs.

**Verification of transgenesis.** Sections of the auricle were taken from all piglets within 7 to 14 days of birth to verify and confirm transgenesis. Transgenesis was verified by the molecular analysis of genomic DNA at the Department of Biochemistry and Biotechnology of the Poznań University of Life Sciences. Animals with confirmed transgenesis were assigned to the transgenic herd. Animals with unconfirmed transgenesis were introduced into breeding.

**Clinical observations.** During the study, clinical observations of the health of transgenic animals and their non-transgenic siblings were made daily. Animals were divided into two groups. The experimental group consisted of transgenic animals with the human α-1,2-fucosyltransferase gene, and the control group comprised non-transgenic animals (siblings of the transgenic animals), in which the human α-1,2-fucosyltransferase gene had not been confirmed. The incidence, course and termination of diseases, as well as deaths, were recorded in both groups.

The results were analysed statistically by Student's t-test. Disease frequency, the course and termination of diseases, and the number of deaths were compared in the experimental groups, in the control group, and between the groups.

## Results and discussion

The results are presented in tables 1 and 2. Transgenesis was confirmed in 150 out of 378 potentially transgenic piglets (39.6%). All non-transgenic animals entered breeding and were subjected to routine clinical observation.

In the herd of 150 transgenic animals, forty-three incidences of disease were found. During the 6-year observations of the transgenic population with the human α-1,2-fucosyltransferase gene, morbidity was high, at 28.7% ($p \leq 0.05$). The mortality of sick animals was also high. Out of 43 sick piglets, 24 died of disease (55.81%, $p \leq 0.05$), which constituted 16.0% ($p \leq 0.05$) of the total transgenic population. Out of the 43 sick piglets, recovery was achieved by 19 (44.19%, $p \leq 0.05$), of which 9 (47.4%, $p \leq 0.05$) recovered completely and returned to their pre-disease condition, and the other 10 (52.63%, $p \leq 0.05$) never fully recovered. Another 3 piglets died of causes other than disease: 2 were crushed by their mothers and 1 died after being bitten by a sibling. Six disease entities were diagnosed, 4 of which were infectious diseases common in pig breeding. Among the 43 cases of disease, there were 15 cases of enzootic pneumonia, 4 cases of streptococcosis, 3 cases of colibacillosis of older piglets, 10 cases of oedema disease, and 10 piglets with signs of circulatory failure. Out of the 15 animals affected with enzootic pneumonia, 9 died of disease and 6 recovered but never regained their health completely. Out of the 10 animals affected with oedema disease, 3 had the hyperacute form, which led to their death, and 7 had the acute form, which ended in their complete recovery. All 4 cases of streptococcosis ended in death. Out of the 10 piglets with signs of circulatory failure, 4 had the subacute form, which ended in the death of all affected animals, and 6 had the chronic form, which ended in the death of 1 animal and the recovery of the others

**Tab. 1. Morbidity, course of diseases, and mortality in the transgenic and non-transgenic pig populations under observation**

| Pigs | Morbidity | Recovery | Complete recovery | Partial recovery | Mortality due to diseases | Mortality due to other causes |
|---|---|---|---|---|---|---|
| Transgenic (n = 150) | $43^{ad}$ (28.67%) | $19^{abc}$ (44.19%) | $9^{b}$ (47.37%) | $10^{c}$ (52.63%) | $24^{d}$ (16.0%) | 3 (2.0%) |
| Non-transgenic (n = 228) | $55^{ad}$ (24.12%) | $28^{abc}$ (49.10%) | $17^{b}$ (57.14%) | $11^{c}$ (42.86%) | $27^{d}$ (11.84%) | 6 (2.63%) |

Explanation: a, b, c, d – significant differences in groups, $p \leq 0.05$

**Tab. 2. List of diseases diagnosed in the transgenic and non-transgenic pig populations under observation with regard to morbidity, course of diseases, and mortality**

| Name of disease | Morbidity | | Recovery | | Complete recovery | | Partial recovery | | Mortality | |
|---|---|---|---|---|---|---|---|---|---|---|
| | T | N | T | N | T | N | T | N | T | N |
| Infectious diseases | | | | | | | | | | |
| Enzootic pneumonia | 15 | 18 | 6 | 8 | | 1 | 6 | 7 | 9 | 10 |
| Streptococcosis | 4 | 7 | 1 | | | | 1 | | 4 | 6 |
| Colibacillosis of older piglets | 3 | 3 | | | | | | | 3 | 3 |
| Oedema, hyperacute | 3 | 4 | | | | | | | 3 | 4 |
| Oedema, acute | 7 | 12 | 7 | 10 | 7 | 10 | | 2 | | |
| Total | 32 | 44 | 14 | 18 | 7 | 11 | 7 | 9 | 19 | 23 |
| Non-infectious diseases | | | | | | | | | | |
| Cardiac circulatory failure, absolute | 4 | 2 | | | | | | | 4 | 2 |
| Cardiac circulatory failure, relative | 6 | 9 | 5 | 7 | 2 | 2 | 3 | 5 | 1 | 2 |
| Hypertrophic osteoarthritis | 1 | | 1 | | | | 1 | | | |
| Total | 11 | 11 | 6 | 7 | 2 | 2 | 4 | 5 | 5 | 4 |

Explanation: T – transgenic pigs, N – non-transgenic pigs

(2 of them recovered completely). One pig was affected with hypertrophic osteoarthritis.

In the group of 228 non-transgenic animals, 55 (24.12% of the population) became ill. Twenty-seven piglets died of disease (49.9%, $p \leq 0.05$), which accounted for 11.84% of the population. Twenty-eight pigs (50.9%, $p \leq 0.05$) recovered, 17 of which (57.14%, $p \leq 0.05$) recovered completely and 11 (42.86%, $p \leq 0.05$) never regained their health. Out of the six piglets that died of causes other than disease, 3 were crushed by their mothers, 2 were bitten by their siblings, and 1 was crushed by a cage. Five disease entities were diagnosed, 4 of which were infectious, as in the transgenic group, and 1 was non-infectious. As much as 70% of deaths among transgenic and non-transgenic pigs occurred within the first 2 weeks of life.

Comparison of the transgenic and non-transgenic groups revealed their close similarity in the frequency and course of diseases, and in mortality rates. The proportion of sick, recovered and dead animals in both groups was similar. The group of transgenic animals was characterized by a higher proportion of diseases and deaths, and a lower proportion of recovered animals, with non-significant differences between the groups.

The present findings are the first attempt in Poland to evaluate the health and morbidity of genetically modified animals. The only transgenic herd in Poland is the population of pigs with the human α-1,2-fucosyltransferase gene that are bred for xenotransplantation purposes.

Research on the possibility of using xenogeneic organs in human transplantology has been conducted in Poland for eight years. From the moment the first transgenic animal with the human α-1,2-fucosyltransferase gene was produced and its transgenesis was confirmed in October 2003, the animals have been constantly monitored for health, incidence and course of diseases, and deaths. The monitoring has been performed not only to evaluate the suitability of organs from GM pigs for human transplantation, but also to test the potential risk of releasing GM organisms into the environment in the form of uncontrolled genes, and to determine the effect of transgenesis on the health of transgenic animals.

The greatest controversy concerns green biotechnology and its use for agricultural purposes. No controversy exists about white biotechnology (used for industrial purposes) or red biotechnology (applied to medicine and health care), and their development is widely recognized and accepted (16). The debate that has raged for many years regarding GM organisms and the fears that their uncontrolled use may affect human health and the natural environment have sparked research in this area. It is necessary to stress that the present knowledge about potential threats from GM organisms concerns almost exclusively the plant world. Transgenic varieties of soybean, maize, rapeseed, cotton and rice are cultivated throughout the world. In the case of animals, the creation of transgenic breeds and varieties is marginal, and research has not progressed beyond the experimental stage (11). The only area with scope for using transgenic animals is human medicine, in which transgenic animals are used for the production of pharmaceuticals (11, 13, 14). One very important issue, however, is the impact of transgenesis on the health and morbidity of transgenic animals. This type of research has never been conducted in Poland, and the available foreign literature provides insufficient information concerning the effect of transgenesis on the health and morbidity of GM animals.

The few existing studies indicate that transgenesis has many pathological consequences that are serious and irreversible. These include lethargy, lameness, exophthalmus, pachyderma, uncontrollable movements, severe synovitis, pericarditis and endocarditis, nephritis, osteitis, and inflammation of the cartilaginous tissue (5, 12). In addition, Pursel et al. (12) noted a very high mortality (> 89%) of transgenic animals during the first 12 months of life. It is pertinent to note that these experiments were conducted on transgenic pigs generated with the human growth hormone gene. Owing to the hypersecretion of the growth hormone, this modification caused severe pathological changes in the entire body, and the experiments were abandoned.

The results obtained do not confirm the incidence of pathological changes in transgenic animals. The genetic modification of the observed animals was not systemic, and by design it did not lead to systemic and pathological changes. During the six-year observations, no pathological changes that could result from the genetic modification were found. The diseases that occurred in the herd under observation are common in pig breeding.

Most cases concerned inflammation of the respiratory tract in the form of enzootic pneumonia. The proportion of diagnosed cases was 34% in the transgenic population and 32% in the non-transgenic one. Enzootic pneumonia of pigs is an acute and often fatal infectious and contagious disease with signs of bronchitis and pneumonia. Epizootic data show that morbidity in pig herds ranges from 8 to 100%, with the average mortality of 25% (6). In the pigs under observation, the disease took the acute and chronic forms, with mortality of 60% and 55%, respectively. Among the recovered animals only one pig recovered completely, and the others were left with after-effects of the disease in the form of low weight gains and low body weight.

The second most frequent disease was colibacillosis, diagnosed as two disease entities: colibacillosis of older piglets and oedema. Colibacillosis of older piglets occurs between the second week of life and weaning, with signs of diarrhoea and emaciation. Up to 50% of piglets become ill but mortality is low (6). Among the animals under observation, 3 transgenic and 3 non-transgenic piglets became ill and died, which accounted for 2% and 1.3% of all animals. Oedema is a common disease of young pigs aged between 4 and 12 weeks with signs of severe enterotoxemia. Morbidity within litters averages 30-40% with 50-90% mortality (6). During the observations, oedema occurred in two forms. The hyperacute form affected 2% of transgenic pigs and 1.7% of non-transgenic ones, all of which died. The acute form affected 4.6% of transgenic animals and 5.2% of non-transgenic ones, all of which survived. Most animals affected by the acute form recovered completely, and only 2 piglets recovered partially, with low weight gains and low body weight, as in the case of enzootic pneumonia. The morbidity and course of oedema in transgenic and non-transgenic animals were similar, with no statistically significant differences between the groups. Comparison of both groups showed that transgenesis had no effect on the morbidity or course of oedema in transgenic pigs. In the context of transgenesis and the introduction of the human FUT2 gene into the pig genome, it is particularly important to analyse the incidence of oedema because the activation of the FUT1 gene has a considerable effect on the natural immunity of pigs to *E. coli* infections and the incidence of oedema.

One of the main aetiological agents of oedema, which is common in pigs, is the F18 enterotoxigenic *Escherichia coli* (ETEC) strain (*E. coli* F18) (2, 10). Research concerning the natural resistance of pigs to oedema has been carried out in several centres in the world (1, 2, 9). It has been proven that some pigs are genetically resistant to colonization by *E. coli* F18, and the F18 gene, which is responsible for natural resistance to intestinal colonization, is located on chromosome 6 at the FUT1 locus (2, 4). The F18 receptor (F18R) plays a crucial role in the colonization of intestinal epithelium. Pigs without the F18 receptor (F18R-) are naturally resistant to intestinal colonization by *E. coli* F18 and to the enterotoxins and verotoxins produced by these bacteria (2, 3). The presence or absence of the receptor is genetically determined, and the possibility of infection with *E. coli* F18 depends on the activity of the FUT1 gene, which codes for α(1,2)-fucosyltransferase (FUT1) (2, 3). This enzyme is responsible, among other things, for the synthesis of blood group antigenic oligosaccharides, red blood cells, and tissues of ectodermal and endothelial origin. The α(1,2)-fucosyltransferase (FUT) enzymes are key enzymes in the formation of A and O antigens in pigs, which correspond to the ABO group in humans (2). This enzyme is commonly found in all mammals. Analysis of the phylogenetic tree of FUT1 and FUT2 in mammals shows great similarities between all species. The 0~-2-fucosyltransferase gene family (including FUT1 and FUT2) developed and spread 80 million years ago, long before most of the present species of mammals came into existence. That is why the common occurrence, analogy and phylogenetic proximity of FUT1 and FUT2 in mammals seem natural (4). It has been established that the natural resistance of pigs to *E. coli* F18 adhesion is controlled by mutation at 307 (M307) and 857 bp (M858) of the FUT1 gene, and the M307 mutation is a very good marker for selection of pigs with natural resistance to oedema (2, 9).

The next most frequent disease was streptococcosis. It is a multiform disease that occurs as septicemia or as local lesions of the skin, lymph nodes, nervous system, joints, lungs and endocardium. Morbidity may

reach 50%, with about 60% mortality (6). During the observations, the disease affected 2.6% of the transgenic population and 3% of the non-transgenic population, with 100% mortality. During the course of the disease, we observed purulent lesions of lymph nodes with a tendency to progress to the septicemic form. In total, the infectious diseases affected 21.3% of transgenic pigs and 19.2% of non-transgenic pigs, with the mean mortality of 12.6% and 10% of the respective populations.

During the six-year observations, two non-infectious diseases: cardiac circulatory failure (absolute or relative) and hypertrophic osteoarthritis were noted. Animals affected with relative cardiac insufficiency exhibit disease symptoms only after physical exertion. In the absolute cardiac insufficiency, the symptoms also occur at rest. The disease manifests itself in dyspnea of varying intensity, bluish skin, and easy fatigability of the animal. The absolute cardiac insufficiency usually takes an acute form and is characterized by higher mortality, whereas the relative cardiac insufficiency is chronic with low mortality (6). In the transgenic and non-transgenic herds under observation, the incidence of circulatory failure was 6.6% and 4.8%, with the mortality of 50% and 36.4%, respectively. The absolute insufficiency affected 2.6% of the transgenic population and 0.8% of the non-transgenic one, with 100% mortality. The respective values for relative insufficiency were 4% and 3.9%, with 16.6% and 22% mortality. One animal was affected with hypertrophic osteoarthritis. The affected animal was the only homozygote obtained during the first 4 years of breeding and improving the line of transgenic animals. First symptoms appeared at the age of about 8-9 months as mild lameness, and the disease progressed over the following 5-6 months with marked lameness and knock-kneed front legs, more pronounced on the right side. Carpal joints showed pathological lesions. They were deformed with hypertrophy of the internal side, not painful to the touch, with the temperature of the surrounding tissues, and without swelling. The animal was euthanized because of the progressive nature of the disease and the developing circulatory failure.

In general, the incidence of diseases and deaths in both groups is high. Despite the higher incidence of diseases and deaths in the transgenic animals, there are no significant differences between the two groups. The results obtained and the similarities between the groups suggest that the high incidence of diseases and deaths could be attributed to causes other than the genetic modification. It should be taken into account that the whole population of transgenic animals and their non-transgenic siblings is descended directly from one animal, boar no. 1154, which is the only pig in Poland to have been produced by genetic engineering. One animal of the F0 generation sired the successive generations (F1, F2, F3 and F4) of transgenic pigs. F1 and F2 transgenic pigs were also sired by the boar no. 1154. Inbreeding fixes desirable characteristics but reduces genetic variation within the population as a result of increased homozygosity, thus leading to a lower viability of the progeny, higher morbidity and more deaths.

In conclusion, the performed transgenesis did not adversely affect the morbidity or mortality of animals. The comparison of transgenic and non-transgenic siblings in the same litters reveals that the observed high morbidity and mortality are due to causes other than genetic engineering. The most important potential reason is extensive inbreeding.

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