SZENT ISTVÁN UNIVERSITY

Animal Husbandry Doctoral School

DETAILED REPORTER GENE EXPRESSION EXAMINATION IN RABBIT CREATED BY TRANSPOSON MEDIATED TRANSGENESIS

ANDREA KEREKES MSC

GÖDÖLLŐ

2019

The doctoral school's name: Animal Husbandry Doctoral School

Field of science: Animal Husbandry

Leader: **Professor Dr. Miklós Mézes D.Sc.,**
Member of the Hungarian Academy of Sciences
Szent István University,
Faculty of Agricultural and Enviromental Sciences,
Institue of Animal Biology,
Department of Animal Nutrition

Supervisor: **Bősze Zsuzsanna D.Sc.**
Scientific consultant, group leader
National Agricultural Research and Innovation Center,
Agricultural Biotechnology Institute,
Department of Animal Biotechnology

Co-supervisor: **László Hiripi PhD.**
Senior research fellow, group leader,
National Agricultural Research and Innovation Center,
Agricultural Biotechnology Institute,
Department of Animal Biotechnology

....

Head of the PhD school Supervisors

1 ANTECEDENTS OF THE WORK, OBJECTIVES TO BE MET

1.1. ANTECEDENTS OF THE DISSERTATION

Due to transgenic animals, it is possible to study the function of different genes, examine their relationship with a particular disease, and model these diseases, whether they are human or animal related. The perception of animals that carry the artificial genetic modification is rather varied in the general public. In 2009, the FDA authorized the use of a transgenic goat's milk derived human protein for therapeutic purposes ("ATryn (Recombinant Antithrombin) FDA Approval History - Drugs.com", n.d.), which was followed in 2014 by a therapeutic human protein purified from GMO rabbit milk (" Recombinant Ruconest (C1 esterase inhibitor) FDA Approval History - Drugs.com", n.d.), and in 2015 by recombinant enzyme produced in transgenic hen eggs ("Kanuma (sebelipase alfa) FDA Approval History - Drugs.com", n.d.). The process of authorizing genetically modified Atlantic salmon was completed in 2015, which may provide a partial solution to replace exhausted natural resources (Commissioner, n.d.).

These ideas and practical applications underline the fact that biotechnology, including transgenesis and hereby produced GMO animals, is an important task not only in basic and applied research, but also in practice, which is expanding day by day.

The therapeutic proteins produced in genetically modified animals are already used in medicine, a good example of which is the previously mentioned Ruconest®, which is produced in the milk of transgenic rabbits to treat angioedema.

A good choice for producing recombinant proteins is biofarming, where the production of pharmaceutical proteins in milk in transgenic animals results in a lower physiological load and reduced stress for the animal than recombinant protein production in blood or seminal plasma.

One of the most commonly used laboratory animal is rabbit in which recombinant proteins can be produced at high quantity in milk under a mammary

gland tissue specific promoter. Though the efficiency of transgenic rabbit production by classical microinjection is low, but the possibility of using it is wide. The transgenic rabbit is a commonly used model for the study of human diseases as well, such as metabolic syndrome, atherosclerosis, cardiovascular disease, and heart rhythm disorders (Bősze et al., 2016; Duranthon et al., 2012).

In addition to the production of therapeutic proteins that have been used in practice, genetically modified animals play important role in basic research. By creating transgenic animals expressing a reporter gene, it is possible, for example, to examine gene expression pattern or to monitor cell migration *in vivo*, and with the help of reporter genes, the intracellular movement of proteins can also be trace (Murakami and Kobayashi, 2012). One of the most commonly used non-tissue-specific synthetic promoters is CAG (chicken beta-actin promoter with CMV enhancer), which provides stable transgene expression in vertebrates (Sakai et al., 1995).

1.2. OBJECTIVES

1. Detailed expression study of our transgenic rabbit expressing Venus protein produced by a Sleeping Beauty (SB) transposon technique that was found to be more efficient than the classical pronucleus microinjection (Katter et al., 2013).
2. In the transgene construct, the above mentioned CAG promoter directed the Venus reporter gene expression. The construct did not contain either a mammary gland specific regulatory element or a secretory signal peptide. In the light of the previously published article (Mukherjee et al., 2016), the main purpose of our experiment was to examine our transgenic rabbit's milk and its fractions to reveal if Venus protein was present.
    2.1. The milk samples as well as its different fractions were examined macroscopically under UV excitation.

2.2. Western blot analysis was used to determine the concentration of Venus protein in milk complemented with densitometry.

2.3. IMAGE-J programme was applied to follow the amount of Venus protein in the milk during the different lactation phases.

2.4. To examine the exact localization of the Venus protein histology was performed on the transgenic rabbit mammary gland sections.

3. Another objective was to reveal if the fluorescent protein could be detected in the biological fluids produced by various exocrine glands (seminal plasma, tear, saliva).

4. We were looking for Venus expression in semen as well, based on the previously described genotype independent transgenic protein in swine (Garrels, Holler, et al., 2011). Semen samples of SB-CAG-Venus heterozygous and homozygous genotyped bucks were included in the experiments.

4.1. And analysed by flow cytometry.

4.2. Genotype analysis of offspring in the SB-CAG-Venus transgenic line were performed as well

4.3. The presence of Venus specific mRNA was examined by RT-PCR.

4.4. Immunohistology was performed to detect the intercellular bridges during rabbit spermatogenesis.

5. Species specific differences in the Venus protein expression by the gametes of SB-CAG-Venus transgenic swine and mice respectively was also examined in rabbits. Exact same methods were applied to compare the sperm cells, testis and epididymal tissues from homozygous genotyped individuals from three independent SB-CAG-Venus mouse lines and that of the transgenic rabbits.

2 MATERIAL AND METHODS

2.1. EXPERIMENTAL PERMITS AND ANIMALS

Laboratory rabbits in the National Agricultural Research and Innovation Center (NARIC), the Agricultural Biotechnology Institute (ABC), the animal house of the Animal Biotechnology Department were properly placed, housed and maintained in accordance with the relevant European Union rules and the *Act XXVIII of 1998 ON THE PROTECTION AND SAVING OF ANIMALS*, *Government Decree 243/1998 (31 December) on animal testing and FVM-KÖM-GM Decree 36/1999 (02 April) on the rules of managing, keeping, transporting etc. test animals*. We have the following permissions to conduct our experiments: PEI / 001/32 and PEI / 01 / 857-3 / 2015.

We investigated the expression of Venus reporter gene in the heterozygous and homozygous individuals of SB-CAG-Venus transgenic rabbit line. The founders were created by our group applying the novel Sleeping Beauty transposon technique in 2013 (Katter et al., 2013).

Heterozygous does from another two transgenic rabbit lines both of them created by our group were used as controls. The transgenic construct in the β-MHC-G52R-KCNE1 line harbours a heart tissue specific promoter, therefore the transgene is not expressed in the milk (Major et al., 2016). In the transgenic does of the WAP-hTNAP line, a mammary gland tissue-specific promoter restricted the expression of the hTNAP transgene to milk (Bodrogi et al., 2006). It is true for both transgenic lines that the transgene is not expressed in the testis, so the transgene protein is not present in their sperm cells.

As negative control, new zealand white (NZW) rabbits produced for laboratory purposes were used.

Male specimens from homozygous transgenic mice belonging to one of the three independent SB-CAG-Venus mouse lines were used to study the species-specific expression of Venus protein (Garrels, Talluri, Ziegler, et al., 2016).

2.2. SAMPLING AND WESTERN BLOT ANALYSIS

2.2.1. MILK SAMPLES COLLECTION

Lactating SB-CAG-Venus transgenic, heterozygous and homozygous does were milked. For positive control, milk samples of the heterozygous females of the WAP-hTNAP transgenic line were used. It was published from this line that recombinant protein after butanol extraction detected in the whey fraction of their milk (Bodrogi et al., 2006).

In addition, we used milk samples from NZW lactating does. Control animals were milked in the same manner and time as the SB-CAG-Venus animals.

Total protein was isolated from the fractionated samples and the samples were loaded to SDS-PAGE gel.

2.2.2. TEAR AND SALIVA SAMPLING

Samples were collected from 3-months, 6-months, and 24-months old male SB-CAG-Venus heterozygous and NZW rabbits. For the Western blot analysis 10 μg of total protein from tear samples, while 20 μg protein from saliva samples was separated on the SDS polyacrylamide gel.

2.2.3. COLLECTING EJACULATIONS

Heterozygous and homozygous SB-CAG-Venus transgenic and NZW buck semen samples were collected. The germ cells and the seminal plasma were separated.

2.2.4. WESTERN BLOT ANALYSIS

The samples were separated on denaturing 12% SDS–polyacrylamide gel and were blotted to PVDF membrane (Hybond-P, Amersham). After the overnight blocking with 5% glycerine defatted dry milk, the membranes were incubated for 4

hours at 4°C with an anti-EGFP polyclonal antibody (1:2000, Thermo Fisher Scientific), then incubated for 2 hours at 4°C with a horseradish peroxidase conjugated anti-rabbit-IgG secondary antibody (1:10000, Sigma A5906). The blots were developed using the ECL-Advanced chemiluminescence detection system (Amersham) on Hyperfilm ECL autoradiography film (Amersham).

2.3. HISTOLOGY OF MAMMARY GLAND , TESTIS AND EPIDIDYMIS

2.3.1. MAMMARY GLAND TISSUE

Mammary gland tissue samples of a SB-CAG-Venus transgenic doe were collected during lactation and fixed in 4% (w/v) formaldehyde (PFA) at 4 °C for 24 h. After incubation in PFA, samples were replaced in 30% (w/v) sucrose-PBS solution and stored until embedding at 4°C. Mammary gland samples were embedded into cryomedium before cryosectioning (Cryomatrix, Thermo Fisher Scientific, USA) and cut into 10 µm thick sections on a cryostat (Microm, Heidelberg, Germany). Nuclei were stained with Topro-3-iodide (Thermo Fisher Scientific, USA, T3605). The images of the lactating mammary gland were obtained with a Leica TCS SP8 confocal microscope equipped with a PMT detector. The detection range of the Venus channel was 510±550 nm. Nuclear staining was recorded at 650±726 nm.

2.3.2. RABBIT TESTIS AND MOUSE EPIDIDYMIS

The rabbit testis and mouse epididymis samples were fixed with 4% buffered formaldehyde, and cryoprotected in 30% sucrose solution. Afterwards, the samples were embedded in cryostat-embedding compound (Tissue-Tek, Torrance, CA) and cut into 10-µm thick sections on a cryostat (Microm, Heidelberg, Germany). The samples were nuclear stained with TO-PRO-3 Iodide (Thermo Fisher Scientific, T3605) and covered with FluoroSave Reagent (Merck Millipore).

2.3.3. IMMUNOHISTOLOGY OF RABBIT TESTIS

Immunohistology were performed on 10-μm thick sections of rabbit testis. Briefly, after a basic heat-induced epitope retrieval protocol, the sections were blocked with 5% BSA and incubated overnight with Tex-14 polyclonal goat primary antibody (Santa Cruz Biotechnology Inc., sc169574). The primary antibody was removed by recurring washing with TBST. The fluorescent donkey anti-goat secondary antibody (Thermo Fisher Scientific, A-21082) was incubated for one hour and the remainder was washed away. The nuclei were visualised with a 30 minute staining (TO-PRO-3 Iodide, Thermo Fisher Scientific, T3605) and the slides were covered with a mounting media (FluorSave Reagent, Merck Millipore).

3 RESULTS

3.1. VENUS PROTEIN EXPRESSION IN RABBIT SPERM CELLS

3.1.1. COMPARTMENTALIZED DISTRIBUTION OF VENUS FLUOROPHORE IN THE MATURE RABBIT SPERMATOZOA

Venus fluorescence was apparent in the Venus transgenic rabbit spermatozoa under fluorescent microscope, although the fluorescence showed a pronounced accumulation to the postacrosomal sheath (PAS), just below the equatorial rim, the midpiece and the tail.

3.1.2. FLOW CYTOMETRY

The FACS analysis showed an intensity difference between the hemizygote and homozygote spermatozoa. The genotypes of the transgenic bucks were confirmed both by Q-PCR and by Mendelian segregation of the Venus reporter when mated with wild type and hemizygote transgenic does. Although the Venus fluorescence of the hemizygote sperms is less intensive compared to the homozygote's, all hemizygote sperm cells were found uniformly Venus-positive

3.1.3. VENUS TRANSGENE INHERITANCE

Transgene inheritance SB-CAG-Venus hemi- and homozygote bucks underlined, that as expected by the Mendelian lows only 50% of all sperm cells of a hemizygous male's carries the transgene.

3.2. EVIDENCE FOR THE FUNCTIONAL INTERCELLULAR BRIDGES IN RABBIT TESTES

3.2.1. ABSENCE OF VENUS SPECIFIC MRNS IN MATURE SPERMATOZOA (SPERM)

RT-PCR failed to amplify the 334 bp Venus specific mRNA fragment from transgenic buck's sperm RNA samples. The obtained RT-PCR data point to the

functional role of intercellular bridges in sharing the transgenic protein product between syncytias

3.2.2. INTERCELLULAR BRIDGE SPECIFIC IMMUNHISTOLOGY

Detection of TEX-14 the critical component of mammalian intercellular bridges in the SB-CAG-Venus rabbit testis. points to the functional role of intercellular bridges, which exist between spermatocytes and spermatids and which could explain the even distribution of the Venus fluorophore protein between transgenic and non-transgenic sperm cells.

3.3. SPECIES-SPECIFIC EXPRESSION OF VENUS FLUOROPHORE IN SB TRANSGENIC MICE

3.3.1. THE LACK OF VENUS FLUORESCENCE IN SPERMATOZOA OF EPIDIDYMIS FROM THREE TRANSGENIC HOMOZYGOTE MOUSE STRAIN.

Spermatozoa obtained from the epididymis of homozygous males of three different transgenic lines were Venus-negative. Contrary to the transgenic pig's (Garrels et al., 2011b), and rabbit's (Katter et al., 2013) although those transgenic animals were created with the exact same transgene and technique. The mouse testis tissue sections revealed that the interstitial tissue, the Leydig cells and smooth muscle cells were strongly Venus positive, like in the adult transgenic rabbit. However, contrary to the rabbit the round and elongated spermatids and spermatozoa not showed a Venus fluorescence.

3.3.2. DEVELOPMENTAL STAGE-DEPENDENT EXPRESSION OF VENUS FLUOROPHORE IN RABBIT TESTIS

Taking advantage of the traceability of Venus expression, developmental stage-specific expression was examined. We compared the testes of young bucks of the Vénus transgenic line at the pre-spermatogenesis stage (42dpp), at 60 dpp, the beginning of spermatogenesis and in adult buck's testis (120 dpp). At 42 dpp the

differentiating germ cells do not express the Venus protein at detectable levels, contrary to the somatic cells, which do show transgene expression. At 60 dpp the differentiation to spermatogonia has started and accompanied by faint Venus expression. In the adult testis Venus-expressing spermatocytes, and round and elongated spermatids were identified both in hemi- and homozygote bucks. Based on the obtained information it could be concluded, that Venus expression in the SB-CAG rabbit male germ line begin in the spermatogonial cells and became uniformly strong in spermatocytes and spermatids.

3.4. Results of Venus transgene protein expression examinations in milk and mammary gland tissue samples

3.4.1. Detection if Venus fluorescence in the milk fractions

We demonstrated the expression of the Venus recombinant protein driven by the ubiquitous CAG promoter in milk of SB-CAG-Venus transgenic rabbits despite the absence of a signal peptide sequence for the secretory pathway. Milk samples of the SB-CAG-Venus transgenic does showed specific Venus fluorescence, whereas this was not observed in milk samples of WAP-hTNAP transgenic does and in a NZW doe under the same conditions. The fat and milk cell fractions from both homozygote and heterozygote SB-CAG-Venus transgenic does were Venus positive, the strongest Venus fluorescence was found in the whey fraction of the homozygote SB-CAG-Venus transgenic doe. Venus protein was detected by Western blot analysis in all milk fractions of the SB-CAG-Venus transgenic does during the whole lactation period. The dynamics of recombinant protein expression was also evaluated. We did not find notable differences between them throughout the lactation period.

This expression pattern was also verified with Western blot analysis in milk fractions.

3.4.2. Results of the histological examination of the mammary gland tissue

When examining mammary gland tissue, the Venus fluorophore protein showed a cytoplasmic position. Venus expression in the mammary epithelial cells clearly shows that the source of recombinant protein in rabbit milk could be the exfoliated epithelial cells, which reached the end of their secretory life.

3.5. Examining Venus protein expressions in the biological fluids produced by exocrine glands

To assess whether the secretion of the signal peptide-less reporter is a unique property of the mammary gland, the fluids of other exocrine glands of the transgenic animals were analyzed. Of the biological fluids of SB-CAG-Venus transgenic rabbits, tear saliva and seminal plasma were Venus-positive under specific excitation light illumination. The tear and

oral saliva samples of homozygote bucks showed stronger Venus fluorescence than those of heterozygote bucks, according to the genotype.

4 NEW SCIENTIFIC RESULTS

1. I have proved the presence of the Venus transgenic protein in the mature spermatozoa of heterozygous SB-CAG-Venus transgenic bucks, which is the first evidence of cell-to-cell information and molecular distribution through intercellular bridges in this species. I have also confirmed that the ectopically expressed transgene protein does not alter significantly the sperm quality.
2. I have proved that there are species-specific differences in the Venus fluorescent protein expression during spermatogenesis and in mature spermatozoa in rabbit and mouse created with the exact same transgene.
3. I have shown first time that for recombinant protein production in rabbit a strong tissue non-specific promoter could be a viable alternative in milk as well as in seminal plasma, tear and saliva.
4. My results show that transgenic rabbit expressing a transgene under the strong tissue non-specific CAG promoter a protein coding sequence without secretion signal produces the transgenic protein by its exocrine glands and excrete it into its biological fluids, therefore it is potentially a novel alternative of recombinant protein production.

5 CONCLUSIONS AND RECOMMENDATIONS

5.1. DISCUSSING THE RESULTS OF SEMEN EXAMINATIONS

Transposon-mediated genetic modification is an efficient way of additive transgenesis and enabled to create transgenic mouse, rat, swine lines and the rabbit line by our group with the same SB-CAG-Venus transgene (Garrels, et al., 2011a; Ivics, et al., 2014b; Ivics, et al., 2014a; Ivics, et al., 2014c; Katter et al., 2013). Very similar transgene expression patterns were found in the somatic cells of SB-CAG-Venus transgenic mice, rats, rabbits and pigs with the same construct, and no variable expression or position effect was observed, but most of them exhibited a reporter gene expression pattern characteristic of the CAG promoter. (Garrels et al., 2016b).

Genotype independent expression of the fluorescent transgenic protein in mature spermatozoa was observed in SB-CAG-Venus transgenic swine (Garrels *et al.*, 2011b). On the basis of this we examined the sperm samples of transgenic male rabbits created with the same SB-CAG-Venus construct. Our results showed that all haploid spermatides of the SB-CAG-Venus heterozygous bucks Venus fluorescent.

Surprisingly, these results did not coincide with those seen in mice where Venus expression was not observed in sperm. I applied the same methods to examine the germ cells in the testicular tissue sections of transgenic rabbits produced by the SB-CAG-Venus construct and of males from three independent transgenic mice generated by this construct.

My results showed, that SB transgenesis with the CAG-Venus transgene resulted in fluorophore expressing spermatocytes, spermatids and mature spermatozoa in the laboratory rabbit. The intensity of expression correlated with the transgene copy number, lining up with the observations made in the SB-transgenic pig lines. In stark contrast, mouse spermatozoa obtained from the

epididymis of three independent transgenic lines were negative for Venus expression.

My experiments underline that the terminally differentiated spermatozoas are exceptions from the uniform Venus reporter gene expression pattern observed in somatic tissues of four different SB transgenic species.

The even Venus protein distribution between the SB-CAG-Venus heterozygous rabbit sperm cells suggests that either the transgene mRNA or the transgene protein moves via the intercellular bridges connecting the neighbouring cells. The presence of intercellular bridges in male rabbits has already been reported in 1959 (Fawcett et al., 1959), but evidence for its function in this species have not been published. In mice it has been shown that TEX 14 is an essential protein of intercellular bridges, its absence leads to inappropriate spermatogenesis (Greenbaum et al., 2006).

Genotype-independent transgenic phenotype of spermatids was first reported in a sperm specific protamine 1 promoter- human growth hormone transgenic hemizygote mouse model, where even distribution of transgene mRNA and protein were detected in testis sections (Braun et al., 1989). Recently, genotype-independent transmission of Venus protein by spermatozoa was reported by SB-transgenic hemizygote transgenic boars (Garrels, et al., 2011a), however to our knowledge beyond those two examples none were published in mammals.

We have demonstrated the presence of TEX 14 protein in the testicles of transgenic male rabbits. The fact that we were unable to detect the presence of Venus-specific mRNA by RT-PCR in mature spermatozoa proves that transgenic protein is not the result of active transcription during sperm maturation. This result and the uniform transgene protein distribution in the sperm of heterozygous rabbits supposes the active role of intercellular bridges between spermatocytes and spermatids, which is the first proof of the function of intercellular bridges in this species.

I have shown that ectopic protein expression has no effect on the fertility of sperm. 50% of the offspring of heterozygous animals carries the Venus transgene, and the gene is inherited according to Mendel's rules.

The motility of the sperm cells of heterozygous and homozygous bucks, and freezability were also assessed. These characteristics did not differentiate from the values determined in NZW rabbits. The SB-CAG-Venus bucks were used for artificial insemination for four generations, and neither decrease in fertility nor in litter size were detected.

5.1.1. RECOMMENDATIONS

A more detailed analysis of rabbit spermatogenesis, which might also help to solve human fertility problems.

To reveal Venus transgenic protein distribution in the ovarium of the SB-CAG-Venus transgenic does would help to underline our hypotheses on the function of intercellular bridges.

Spermatogonial stem cell transplantation from SB-CAG-Venus homozygous bucks could allow to observe the formation of bridges, cell-to-cell connections, and the functioning intercellular bridges in the recipient NZW bucks.

5.2. DISCUSSING THE RESULTS OF TEAR AND SALIVA SAMPLING

The presence of the transgenic protein was detected in all three fractions of the SB-CAG-Venus transgenic rabbit milk (whey, fat, casein). Our results are supported by the findings in the milk samples of the transgenic swine generated with the same transgenic construct (Garrels, et al., 2011b), Venus protein was observed both in whey and milk cell fractions, albeit with reduced amounts in the pig fat fractions. In addition, mCherry recombinant protein controlled by the same CAG promoter was obviously enriched in an another transgenic swine milk cells (Mukherjee et al., 2016).

Based on our results along with Mukherjee et al. (2016), the expression pattern in transgenic animals produced by CAG promoter-regulated constructs- without secretion signal peptide- shows species-specific differences in rabbit and pig milk , which may be due to different somatic cell count in milk and the cell type distribution (Boutinaud and Jammes, 2002). In rabbits, the total somatic cell count is 0.5-1x10^6 cells/ml, similar to that of pigs (1x10^6 cells / ml), with the proportion of epithelial cells being 60-90% (Le Jan, 1996).

The recombinant Venus protein in the biological fluids produced by the secreted apocrine and merocrine glands of the SB-CAG-Venus transgenic animals foresees a new possibility for therapeutic protein production in transgenic animals. The expression of the Venus reporter protein is restricted to the cytoplasm and therefore the transgenic protein does not undergo post-translational modifications, and the protein does not cross the normal secretory pathway as the construct did not contain secretory signal peptide. This can be an advantage for some of the recombinant proteins. The recombinant protein produced under the control of the tissue-specific promoter receives translational modifications , but there are cases when these modifications are not performed perfectly by the mammary gland. For example, the efficiencies of the essential γ -carboxylation of the recombinant human protein C (rhPC) and the recombinant human Factor IX driven by the whey acid protein gene promoter were different at similar expression levels in transgenic pigs (Van Cott et al., 1999).

Heterogeneity of the glycosylation has also been observed in transgenic goats producing human antithrombin with the β-Casein promoter driven construct (Zhou et al., 2005). Incomplete post-translational modifications can reduce the activity of the recombinant protein and alter its immunogenicity.

This type of recombinant protein production system, which I have studied can be further polished in combination with a tissue specific recombinase system. The Cre recombinase integrated into the mammary gland-specific promoter-driven construct combined with the loxP cassette incorporated into the other CAG

promoter-driven construct enable the mammary gland specific gene expression, while the CAG promoter is responsible for high expression levels. The new system can be combined with in the cassette or can be used in a binary approach ((Mukherjee et al., 2016).

Western blot analysis confirmed the presence of fluorescent reporter protein in the tears and saliva of SB-CAG-Venus heterozygous and homozygous animals of different ages. Our findings show that the heterozygous and homozygous animals differ in transgenic protein concentrations but the protein production is independent of the age of animals and the time of sampling.

The presence of a recombinant protein expressed in tear and saliva samples under the control of a CAG promoter is an important finding, because it is difficult to find tear or saliva specific promoters. Based on our results, this can be a very good system if, for example, someone intends to produce a protein in tears.

5.2.1. RECOMMENDATIONS

We would like to continue characterizing the expression pattern of SB-CAG-Venus transgenic animals by examining blood and urine.

It would be useful to examine the presence of the recombinant protein in various biological fluids on a transgenic rabbit line created with another non tissue specific promoter, and to implement the system, for example in the case of therapeutic proteins for which post-translational modifications are undesirable.

6 REFERENCE

"ATryn (Antithrombin (Recombinant)) FDA Approval History - Drugs.com". (n.d.). , available at: https://www.drugs.com/history/atryn.html (accessed 6 August 2018).

Bodrogi, L., Brands, R., Raaben, W., Seinen, W., Baranyi, M., Fiechter, D. and Bősze, Z. (2006), "High Level Expression of Tissue-Nonspecific Alkaline Phosphatase in the Milk of Transgenic Rabbits", *Transgenic Research*, Vol. 15 No. 5, pp. 627–636.

Bősze, Z., Major, P., Baczkó, I., Odening, K.E., Bodrogi, L., Hiripi, L. and Varró, A. (2016), "The potential impact of new generation transgenic methods on creating rabbit models of cardiac diseases.", *Progress in Biophysics and Molecular Biology*, Vol. 121 No. 2, pp. 123–30.

Boutinaud, M. and Jammes, H. (2002), "Potential uses of milk epithelial cells: a review.", *Reproduction, Nutrition, Development*, Vol. 42 No. 2, pp. 133–47.

Braun, R.E., Behringer, R.R., Peschon, J.J., Brinster, R.L. and Palmiter, R.D. (1989), "Genetically haploid spermatids are phenotypically diploid", *Nature*, Vol. 337 No. 6205, pp. 373–376.

Commissioner, O. of the. (n.d.). "For Consumers - FDA Has Determined That the AquAdvantage Salmon is as Safe to Eat as Non-GE Salmon", https://www.fda.gov/ForConsumers/ucm472487.htm, available at: https://www.fda.gov/ForConsumers/ucm472487.htm.

Van Cott, K.E., Butler, S.P., Russell, C.G., Subramanian, A., Lubon, H., Gwazdauskas, F.C., Knight, J., et al. (1999), "Transgenic pigs as bioreactors: a comparison of gamma-carboxylation of glutamic acid in recombinant human protein C and factor IX by the mammary gland.", *Genetic Analysis : Biomolecular Engineering*, Vol. 15 No. 3–5, pp. 155–60.

Duranthon, V., Beaujean, N., Brunner, M., Odening, K.E., Santos, A.N., Kacskovics, I., Hiripi, L., et al. (2012), “On the emerging role of rabbit as human disease model and the instrumental role of novel transgenic tools”, *Transgenic Research*, Vol. 21 No. 4, pp. 699–713.

Fawcett, D.W., Ito, S. and Slautterback, D. (1959), “The occurrence of intercellular bridges in groups of cells exhibiting synchronous differentiation.”, *The Journal of Biophysical and Biochemical Cytology*, Vol. 5 No. 3, pp. 453–60.

Garrels, W., Holler, S., Taylor, U., Herrmann, D., Struckmann, C., Klein, S., Barg-Kues, B., et al. (2011), “Genotype-independent transmission of transgenic fluorophore protein by boar spermatozoa.”, edited by Mueller, F.*PloS One*, Vol. 6 No. 11, p. e27563.

Garrels, W., Mátés, L., Holler, S., Dalda, A., Taylor, U., Petersen, B., Niemann, H., et al. (2011), “Germline transgenic pigs by Sleeping Beauty transposition in porcine zygotes and targeted integration in the pig genome.”, edited by Wutz, A.*PloS One*, Vol. 6 No. 8, p. e23573.

Garrels, W., Talluri, T.R., Apfelbaum, R., Carratalá, Y.P., Bosch, P., Pötzsch, K., Grueso, E., et al. (2016), “One-step multiplex transgenesis via sleeping beauty transposition in cattle”, *Scientific Reports*, Nature Publishing Group, Vol. 6 No. February, pp. 1–6.

Garrels, W., Talluri, T.R., Ziegler, M., Most, I., Forcato, D.O., Schmeer, M., Schleef, M., et al. (2016), “Cytoplasmic injection of murine zygotes with Sleeping Beauty transposon plasmids and minicircles results in the efficient generation of germline transgenic mice”, *Biotechnology Journal*, Vol. 11 No. 1, pp. 178–184.

Greenbaum, M.P., Yan, W., Wu, M.-H., Lin, Y.-N., Agno, J.E., Sharma, M., Braun, R.E., et al. (2006), “TEX14 is essential for intercellular bridges and fertility in male mice”, *Proceedings of the National Academy of Sciences*,

Vol. 103 No. 13, pp. 4982–4987.

Ivics, Z., Garrels, W., Mátés, L., Yau, T.Y., Bashir, S., Zidek, V., Landa, V., et al. (2014), “Germline transgenesis in pigs by cytoplasmic microinjection of Sleeping Beauty transposons.”, *Nature Protocols*, Vol. 9 No. 4, pp. 810–27.

Ivics, Z., Hiripi, L., Hoffmann, O.I., Mátés, L., Yau, T.Y., Bashir, S., Zidek, V., et al. (2014), “Germline transgenesis in rabbits by pronuclear microinjection of Sleeping Beauty transposons”, *Nature Protocols*, Vol. 9 No. 4, pp. 794–809.

Ivics, Z., Mátés, L., Yau, T.Y., Landa, V., Zidek, V., Bashir, S., Hoffmann, O.I., et al. (2014), “Germline transgenesis in rodents by pronuclear microinjection of Sleeping Beauty transposons”, *Nature Protocols*, Vol. 9 No. 4, pp. 773–793.

Le Jan, C. (1996), “Cellular components of mammary secretions and neonatal immunity: a review.”, *Veterinary Research*, Vol. 27 No. 4–5, pp. 403–17.

“Kanuma (sebelipase alfa) FDA Approval History - Drugs.com”. (n.d.). , available at: https://www.drugs.com/history/kanuma.html (accessed 6 August 2018).

Katter, K., Geurts, A.M., Hoffmann, O., Mátés, L., Landa, V., Hiripi, L., Moreno, C., et al. (2013), “Transposon-mediated transgenesis, transgenic rescue, and tissue-specific gene expression in rodents and rabbits.”, *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, The Federation of American Societies for Experimental Biology, Vol. 27 No. 3, pp. 930–41.

Major, P., Baczkó, I., Hiripi, L., Odening, K.E., Juhász, V., Kohajda, Z., Horváth, A., et al. (2016), “A novel transgenic rabbit model with reduced repolarization reserve: long QT syndrome caused by a dominant-negative mutation of the KCNE1 gene”, *British Journal of Pharmacology*, Vol. 173 No. 12, pp. 2046–2061.

Mukherjee, A., Garrels, W., Talluri, T.R., Tiedemann, D., Bosze, Z., Ivics, Z. and

Kues, W.A. (2016), “Expression of Active Fluorophore Proteins in the Milk of Transgenic Pigs Bypassing the Secretory Pathway”, *Scientific Reports*, Vol. 6 No. 1, p. 24464.

Murakami, T. and Kobayashi, E. (2012), “GFP-Transgenic Animals for In Vivo Imaging: Rats, Rabbits, and Pigs”, *Methods in Molecular Biology (Clifton, N.J.)*, Vol. 872, pp. 177–189.

“Ruconest (C1 esterase inhibitor (recombinant)) FDA Approval History - Drugs.com”. (n.d.). , available at: https://www.drugs.com/history/ruconest.html (accessed 6 August 2018).

Sakai, K., Mitani, K. and Miyazaki, J. (1995), “Efficient Regulation of Gene Expression by Adenovirus Vector-Mediated Delivery of the CRE Recombinase”, *Biochemical and Biophysical Research Communications*, Vol. 217 No. 2, pp. 393–401.

Zhou, Q., Kyazike, J., Echelard, Y., Meade, H.M., Higgins, E., Cole, E.S. and Edmunds, T. (2005), “Effect of genetic background on glycosylation heterogeneity in human antithrombin produced in the mammary gland of transgenic goats”, *Journal of Biotechnology*, Elsevier, Vol. 117 No. 1, pp. 57–72.

7 PUBLICATIONS

7.1. INTERNATIONAL, SCIENTIFIC ARTICLES WITH IMPACT FACTOR

Liptak N, Hoffmann OI, Skoda G, Gocza E, **Kerekes A**, Bosze Z, Hiripi L (2018) „Glomerulosclerosis in transgenic rabbits with ubiquitous Venus protein expression.” *Acta veterinaria hungarica* 66:(2) pp. 281-293. **IF.:** ***1,042***

Kerekes A, Hoffmann OI, Iski G, Liptak N, Gocza E, Kues WA, Bosze Z, Hiripi L (2017) „Secretion of a recombinant protein without a signal peptide by the exocrine glands of transgenic rabbits.” *PLoS One* 12:(10) p. e0187214. **IF.:** ***2,766***

Liptak N, Hoffmann OI, **Kerekes A**, Iski G, Ernszt D, Kvell K, Hiripi L, Bosze Z (2017) „Monitoring of Venus transgenic cell migration during pregnancy in non-transgenic rabbits." *Transgenic research* 26:(2) pp. 291-299. **IF.:** ***2,197***

Skoda G, Hoffmann OI, Gocza E, Bodrogi L, **Kerekes A**, Bosze Z, Hiripi L (2017) „Placenta-specific gene manipulation in rabbits." *Journal of biotechnology* 259: pp. 86-90. **IF.:** ***2,533***

Hoffmann OI, **Kerekes A**, Liptak N, Hiripi L, Bodo S, Szaloki G, Klein S, Ivics Z, Kues WA, Bosze Z (2016) „Transposon-Based Reporter Marking Provides Functional Evidence for Intercellular Bridges in the Male Germline of Rabbits." *PLoS One* 11:(5) p. e0154489. **IF.:** ***2,806***

Bender B, Baranyi M, **Kerekes A**, Bodrogi L, Brands R, Uhrin P, Bösze Z (2015) „Recombinant human tissue non-specific alkaline phosphatase successfully counteracts lipopolysaccharide induced sepsis in mice." *Physiological research* 64:(5) pp. 731-738. **IF.:** ***1,646***

Catunda Lemos AP, Cervenak J, Bender B, Hoffmann OI, Baranyi M, **Kerekes A**, Farkas A, Bősze Z, Hiripi L, Kacskovics I (2012) „Characterization of the rabbit neonatal Fc receptor (FcRn) and analyzing the immunophenotype of the transgenic rabbits that overexpresses FcRn." *PLoS One* 7:(1) p. e28869. **IF.:** ***3,730***

Lichner Z, Pall E, **Kerekes A**, Pallinger E, Maraghechi P, Bosze Z, Gocza E (2011) „The miR-290-295 cluster promotes pluripotency maintenance by regulating cell cycle phase distribution in mouse embryonic stem cells." *Differentiation* 81:(1) pp. 11-24. **IF.:** ***2,807***

Összes **IF.:** ***19,527***

7.2. DOMESTIC, SCIENTIFIC ARTICLES WITH OUT IMPACT FACTOR

Fábián Renáta, Skoda Gabriella, Hiripi László, Hoffmann Orsolya Ivett, Daniela Ilie, **Kerekes Andrea**, Gócza Elen, Bodó Szilárd (2017) „Egy sejt PCR beállítása nyúlon preimplantációs genetikai diagnózis céljából." *Állattenyésztés és takarmányozás* 66:(2) pp. 174-179.

Balogh L, **Kerekes A**, Debnár V J, Ray K, Bodó Sz (2013) „Tej alapú higítók alkamazásának lehetősége nyúl sperma mélyhűtésénél.” *Animal welfare ethology and housing systems* 9:(3) pp. 69-72.

Kerekes Andrea, Hoffmann Orsolya Ivett, Skoda Gabriella, Barta Endre, Rocha Dominique, Lejard Véronique, Hiripi László, Bősze Zsuzsanna (2013) „A szarvasmarha prion fehérje szabályozó SNP vizsgálata transzgénikus egér modellben.” *Animal welfare ethology and housing systems* 9:(3 Klnsz) pp. 177-182.

Skoda Gabriella, **Kerekes Andrea**, Hoffmann Orsolya Ivett, Barta Endre, Rocha Dominique, Lejard Véronique, Hiripi László, Bősze Zsuzsanna (2013) „Szarvasmarha RCAN2 gén szabályozó régiójában található SNP vizsgálata transzgénikus egérmodellben.” *Animal welfare ethology and housing systems* 9:(Klnsz) pp. 321-327.

Kerekes Andrea, Baranyi Mária, Nagy Sándor, Kovács Péter, Bősze Zsuzsanna (2008) „Bétalaktoglobulin genetikai polimorfizmus vizsgálatok hazai awassi és racka juhokban.” *Animal welfare ethology and housing systems* 4:(2) pp. 437-444.

7.3. CONFERENCE ANNOUNCEMENTS

Antal Anita, Bender Balázs, Hiripi László, Bősze Zsuzsanna, Bodó Szilárd, **Kerekes Andrea**: A házinyúl ejakulált ondó és a mellékhere eredetű spermiumok feltételezett termékenyítő képességének összehasonlítása. 6th Scientific Day of Animal Breeding in Gödöllő - International Conference; VI. Gödöllői Állattenyésztési Tudományos Nap - Nemzetközi Konferencia: Book of abstracts of presentations and posters; Előadások és poszterek összefoglaló kötete. 71 p. Konferencia helye, ideje: Gödöllő, Magyarország, 2017.11.24 Gödöllő: Szent István Egyetem Mezőgazdaság- és Környezettudományi Kar, 2017. p. 35. 1 p. (ISBN: 978-963-269-689-8)

Kerekes Andrea, Hoffmann Orsolya Ivett, Lipták Nándor, Hiripi László, Kues Willfried, Bősze Zsuzsanna: A transzpozon alapú transzgenezis, mint új lehetőség a rekombináns fehérje tejben történő termeltetésére. 6th Scientific Day of Animal Breeding in Gödöllő - International Conference; VI. Gödöllői Állattenyésztési

Tudományos Nap - Nemzetközi Konferencia: Book of abstracts of presentations and posters; Előadások és poszterek összefoglaló kötete. 71 p. Konferencia helye, ideje: Gödöllő, Magyarország, 2017.11.24 Gödöllő: Szent István Egyetem Mezőgazdaság- és Környezettudományi Kar, 2017. p. 37. 1 p. (ISBN: 978-963-269-689-8)

Nándor Lipták, Osolya Ivett Hoffmann, **Andrea Kerekes**, Gergely Iski, Dávid Ernszt, Krisztián Kvell, László Hiripi, Zsuzsanna Bősze: The assessment of Venus transgenic cell migration during pregnancy in non-transgenic rabbits. Konferencia helye, ideje: Eger, Magyarország, 2017.03.31-2017.04.02. Eger:2017. 1 p.

Nándor Lipták, Orsolya Ivett Hoffmann, Gabriella Skoda, Elen Gócza, **Andrea Kerekes**, Zsuzsanna Bősze, László Hiripi: Mild focal segmental glomerulosclerosis in Venus transgenic rabbits. Konferencia helye, ideje: Halle (Saale), Németország, 2017.09.28-2017.09.29. Halle (Saale):2017. 1 p.

Tokár Alexandra, Stéger Viktor, Heltai Botond, Szepesi Kinga, Debnár Viktória Johanna, **Kerekes Andrea,** Antal Anita, Bodó Szilárd: Előkísérletek házi méh (Apis Mellifera) genotipizálására és spermium mélyhűtésére. 6th Scientific Day of Animal Breeding in Gödöllő - International Conference; VI. Gödöllői Állattenyésztési Tudományos Nap - Nemzetközi Konferencia: Book of abstracts of presentations and posters; Előadások és poszterek összefoglaló kötete. 71 p. Konferencia helye, ideje: Gödöllő, Magyarország, 2017.11.24 Gödöllő: Szent István Egyetem Mezőgazdaság- és Környezettudományi Kar, 2017. p. 40. 1 p. (ISBN: 978-963-269-689-8)

Fábián Renáta, Hoffmann Orsolya Ivett, Skoda Gabriella, Hiripi László, **Kerekes Andrea**, Gócza Elen, Altbäcker Vilmos, Bodó Szilárd: Development of methods for obtaining offspring of the desired sex in rabbit. Fiatal Biotechnológusok Országos Konferenciája. 109 p. Konferencia helye, ideje: Gödöllő, Magyarország, 2016.03.21-2016.03.22. Gödöllő: Szent István Egyetemi Kiadó, 2016. p. 22. 1 p. (ISBN: 978-963-269-536-5)

Kerekes Andrea, Hoffmann Orolya Ivett, Lipták Nándor, Hiripi László, Wilfried Kues, Bősze Zsuzsan: New opportunity for the recombinant protein production in milk. Fiatal Biotechnológusok Országos Konferenciája. 109 p. Konferencia helye,

ideje: Gödöllő, Magyarország, 2016.03.21-2016.03.22. Gödöllő: Szent István Egyetemi Kiadó, 2016. p. 55. 1 p. (ISBN: 978-963-269-536-5)

Skoda Gabriella, **Kerekes Andrea**, Hoffmann Orsolya Ivett, Bősze Zsuzsanna, Hiripi László: Célzott genom módosítás a nyúl miosztatin génjében. Fiatal Biotechnológusok Országos Konferenciája. 109 p. Konferencia helye, ideje: Gödöllő, Magyarország, 2016.03.21-2016.03.22. Gödöllő: Szent István Egyetemi Kiadó, 2016. p. 26. 1 p. (ISBN: 978-963-269-536-5)

Debnár Viktória Johanna, **Kerekes Andrea**, Torda Orsolya, Altbäcker Vilmos, Bodó Szilárd: Spermavétel és mélyhűtés üregi nyúlon. Vad- és egzotikus állatok szaporodásbiológiája, állatkerti tenyészprogramok – fiatal- és növendék állatok betegségei [Reproductive Biology of Wild and Zoo Animals, Conservation Breeding Programs; Diseases of Young and Growing Animals]. Konferencia helye, ideje: Budapest, Magyarország, 2015.03.27-2015.03.29. Budapest: Magyar Vad- és Állatkerti Állatorvosok Társasága; Fővárosi Állat- és Növénykert, 2015. pp. 64-65. (ISBN: 978-615-5392-06-1)

Kerekes A, Bodó Sz, Hoffmann O I, Hiripi L, Kulikova B, Chrenek P, Bősze Zs: Géntechnológiai módosítás hatása a nyúlsperma minőségére. 21. Szaporodásbiológiai Találkozó. 37 p. Konferencia helye, ideje: Visegrád, Magyarország, 2015.09.21-2015.09.22.p. 25.

Orsolya Ivett Hoffmann, **Andrea Kerekes**, Nóra Dobrosi, Elen Gózca, Szilárd Bodó, László Hiripi, Zsuzsanna Bősze: Analysis of the transmission of transposon-transgenic fluorofore protein in rabbit. Hungarian Molecular Life Sciences 2015: Programme & Book of Abstracts. 304 p. Konferencia helye, ideje: Eger, Magyarország, 2015.03.27-2015.03.29. Budapest: Diamond Congress Ltd., 2015. pp. 126-127. (ISBN: 978-615-5270-15-4)

Baczkó I, Juhász V, Major P, Kovács M, Hornyik T, **Kerekes A**, Hiripi L, Bősze Zs, Papp JGy, Varró A: LQT5 transgenic rabbits are characterized by increased repolarization instability and arrhythmia susceptibility. Cardiovascular research 103:(Suppl.1) p. S50. (2014)

Debnár Viktória Johanna, **Kerekes Andrea**, Altbäcker Vilmos, Torda Orsolya, Bodó Szilárd: az üregi nyúlsperma hosszú távú tárolásának lehetősége In: 20.

Szaporodásbiológiai Találkozó. Konferencia helye, ideje: Herceghalom, Magyarország, 2014.11.07-2014.11.08.p. 23. 1 p.

Hoffmann Orsolya Ivett, Barta Endre, Dominique Rocha, Veronique Lejard, **Kerekes Andrea**, Skoda Gabriella, Bősze Zsuzsanna, Hiripi László: In vivo examination of bovine rSNPs in transgenic mouse model. In: CTC2014: 13th Annual Meeting of the Complex Trait Consortium. Konferencia helye, ideje: Berlin, Németország, 2014.05.19-2014.05.22. Berlin: pp. 13-14.

Juhász V, Major P, Kovács M, Hornyik T**, Kerekes A**, Hiripi L, Bősze Zs, Varró A, Baczkó I :Egy új, LQT5 transzgenikus nyúl proaritmia model jellemzése. (Characterization of a novel LQT5 transgenic rabbit proarrhythmia model.) Cardiologia hungarica 44:(Suppl.E) pp. E28-E29. (2014)

Kerekes A, Skoda G, Hoffmann O I, Balogh L, Debnár V J, Bősze Zs, Hiripi L, Bodó Sz: Transzgénikus állatvonalak ex situ genetikai megőrzése. In: 20. Szaporodásbiológiai Találkozó. Konferencia helye, ideje: Herceghalom, Magyarország, 2014.11.07-2014.11.08.p. 25.

Skoda G, **Kerekes A**, Hoffmann OI, Barta E, Rocha D, Lejard V, Bosze Zs, Hiripi L: Examination of a regulatory SNP of bovine RCAN2 gene in transgenic mouse models. In: Transgenic Research. Konferencia helye, ideje: Edinburgh, Egyesült Királyság / Skócia, 2014.10.06-2014.10.08.Paper Volume: 23 Issue: 5 Pages: 889-889.

Skoda G, **Kerekes A**, Hoffmann O I, Iski G, Barta E, Dominique R, Véronique L, Bősze Zs, Hiripi L: Komplex módszerek kifejlesztése szarvasmarha szabályozó polimorfizmusok vizsgálatára. In: 20. Szaporodásbiológiai Találkozó. Konferencia helye, ideje: Herceghalom, Magyarország, 2014.11.07-2014.11.08.p. 27.

Kerekes Andrea, Hoffmann Orsolya Ivett, Skoda Gabriella, Barta Endre, Dominique Rocha, Véronique Lejard, Hiripi László, Bősze Zsuzsanna: a szarvamarha prion fehérje szabályozó snp vizsgálata transzgénikus egér modellben. IV. Gödöllői Állattenyésztési Tudományos Napok: Előadások és poszterek összefoglaló kötete. Konferencia helye, ideje: Gödöllő, Magyarország, 2013.10.24-2013.10.26. Gödöllő: Szent István Egyetem Egyetemi Kiadó, 2013. p. 52.

Major P, Hoffmann O, Bontovics B, **Kerekes A**, Hiripi L, Gócza E, Bősze Zs: A laboratóriumi nyúl mint betegség modellállat – tények és lehetiségek. 25. Nyúltenyésztési Tudományos Nap. Konferencia helye, ideje: Kaposvár, Magyarország, 2013.04.25 Kaposvár: Kaposvári Egyetem Állattudományi Kar, 2013. pp. 95-106. (ISBN: 9789639821606)

Orsolya Ivett Hoffmann, Endre Barta, Dominique Rocha, Veronique Lejard, **Andrea Kerekes**, Gabriella Skoda, Zsuzsanna Bősze, László Hiripi: In vivo validation of candidate cattle regulatory SNPs in indicatior transgenic mouse models. Hungarian Molecular Life Sciences 2013: programme & book of abstracts. 287 p.Konferencia helye, ideje: Siófok, Magyarország, 2013.04.05-2013.04.07. Budapest: Diamond Congress Ltd., 2013. p. 207. (ISBN: 978-615-5270-02-4)

Skoda Gabriella, **Kerekes Andrea**, Hoffmann Orsolya Ivett, Barta Endre, Dominique Rocha, Véronique Lejard, Hiripi László, Bősze Zsuzsanna: szarvasmarha rcan2 gén szabályozó régiójában található snp vizsgálata transzgénikus egérmodellben. IV. Gödöllői Állattenyésztési Tudományos Napok: Előadások és poszterek összefoglaló kötete. Konferencia helye, ideje: Gödöllő, Magyarország, 2013.10.24-2013.10.26. Gödöllő: Szent István Egyetem Egyetemi Kiadó, 2013. p. 61. (ISBN: 978-963-269-385-9)

Skoda Gabriella, **Kerekes Andrea**, Hoffmann Orsolya Ivett, Dominique Rocha, Veronique Léjard, Barta Endre, Bősze Zsuzsanna, Hiripi László: A szarvasmarha tenyésztésében potenciálisan használható PRNP és RCAN2 génekben található szabályozó polimorfizmusok jellemzése. In: MBK napok 2013. Konferencia helye, ideje: Gödöllő, Magyarország, 2013.11.21-2013.11.22. Paper 5.

Bodó Sz, **Kerekes A**, Kriczky N, Balogh L, Bősze Zs: Nyúlsperma mélyhűtési eljárás optimalizálása. A magyar mezőgazdaság - lehetőségek, források, új gondolatok. 34. Óvári Tudományos Nap. 777 p.Konferencia helye, ideje: Mosonmagyaróvár, Magyarország, 2012.10.05 Mosonmagyaróvár: Nyugat-magyarországi Egyetem Mezőgazdaság- és Élelmiszertudományi Kar, 2012. pp. 225-229. (ISBN: 978 963 9883 93 2)

Kerekes A, Kriczky N, Kása E, Bősze Zs, Bodó Sz: Nyúlsperma mélyhűtési protokollok vizsgálata. 24. Nyúltenyésztési Tudományos Nap. 120 p.

Konferencia helye, ideje: Kaposvár, Magyarország, 2012.05.30 Kaposvár: Kaposvári Egyetem Állattudományi Kar, 2012. pp. 47-52. (ISBN: 978 963 9821 51 4)

Bősze Zs, Hiripi L, Hoffmann O I, **Kerekes A**, Bender B, Kacskovics I: IgG binding FcRn transgenic rabbits created through BAC transgenesis. In: 4th International Rabbit Biotechnology Meeting, abstract, lecture, Worl Rabbit Sci. Konferencia helye, ideje: Budapest, Magyarország, 2011.06.30-2011.07.01.p. 13.

Gocza E, Maraghechi P, Bontovics B, Hiripi L, Hoffmann O I, **Kerekes A**, Bosze Zs: Expression pattern of stem cell specific markers and microRNAs during rabbit embryonic development and in embryonic stem cells. In: Regenerative Medicine, 6/Suppl.2. World Conference on Regenerative Medicine, abstract, poster. Konferencia helye, ideje: Leipzig, Németország, 2011.11.02-2011.11.04. Future Medicine Ltd., p. 205.

Kerekes A, Szikra D, Nagy Sz, Pribenszky Cs, Bősze Zs: Magas nyomású előkezelés hatása a fagyasztott nyúlsperma túlélőképességére. 23. Nyúltenyésztési Tudományos Nap. 109 p. Konferencia helye, ideje: Kaposvár, Magyarország, 2011.05.25 Kaposvár: Kaposvári Egyetem Állattudományi Kar, 2011. pp. 89-94.

Kerekes A, Kriczky N, Kása E, Bősze Zs, Bodó Sz: Nyúlsperma mélyhűtési eljárások technikai lépéseinek vizsgálata fénymikroszkópos festéssel. In: 17. Szaporodásbiológiai Találkozó: Az állattenyésztés és szaporodásbiológia helye és szerepe a magyar agrárstratégiában. Konferencia helye, ideje: Herceghalom, Magyarország, 2011.10.28-2011.10.29.p. 26.

Maraghechi P, Bontovics B, Hiripi L, **Kerekes A**, Hoffmann O I, Bősze Zs, Gócza E: Az embrionális fejlődés kezdeti szakaszára jellemző markerek expressziós mintázatának vizsgálata nyúl embriókban. 23. Nyúltenyésztési Tudományos Nap. 109 p. Konferencia helye, ideje: Kaposvár: Kaposvári Egyetem Állattudományi Kar, 2011.05.25 2011. pp. 99-104.(ISBN: 978-963-9821-30-9)

Kerekes Andrea, Baranyi Mária, Bősze Zsuzsanna: a béta-laktoglobulin tejfehérje polimorfizmus vizsgálata hazai awassi és racka juhokban. In: Akadémiai beszámolók. Konferencia helye, ideje: Budapest, Magyarország, 2010.01.27 pp. 10.

Mária Baranyi, **Andrea Kerekes**, László Hiripi, Zsuzsanna Bősze: Preliminary data on beta-lactoglobulin genetic polymorphisms in Hungarian Awassi and Racka sheep. In: DAGENE Anual Meeting. Konferencia helye, ideje: Brazi, Románia, 2010.04.15-2010.04.17.pp. 1-5.

Kerekes Andrea, Baranyi Mária, Bősze Zsuzsanna: Béta-laktoglobulin genetikai polimorfizmus vizsgálatok két hazai juh populációban. In: Magyar Genetikusok Egyesülete VIII. Magyar Genetikai Kongresszus; XV. Sejt- és Fejlődésbiológiai Napok. Konferencia helye, ideje: Nyíregyháza, Magyarország, 2009.04.17-2009.04.19. p. 122. 1 p.

Kerekes Andrea, Baranyi Mária, Bősze Zsuzsanna: Béta-laktoglobulin genetikai polimorfizmus vizsgálatok hazai awassi és gyimesi racka juhokban. In: MBK Napok, előadás, absztrakt. Konferencia helye, ideje: Gödöllő, Magyarország, 2008.11.17-2008.11.18. pp. 18.